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Global Food Security and Wellness

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Preface

This book is based on oral presentations delivered at the XVI IUFoST (International Union of Food Science and Technology) World Congress held in Foz do Iguaçu, Brazil, August 5–9, 2012. Selected speakers were invited to submit a full text of their presentations, which were then peer-reviewed. This IUFoST World Congress, the first held in Latin America, was a major success in terms of the number of international delegates representing more than 90 countries, as well as the participation of the most distinguished scientists in the field. A broad number of topics were covered in depth by colleagues from many diverse backgrounds. Gláucia M. Pastore, University of Campinas, Brazil, served as the Conference Organizer and she received invaluable support from a number of sponsors, international and local organizations, committees and individuals. The Congress Scientific Committee, which was comprised of world renowned scholars, was led by Delia Rodriguez-Amaya, University of Campinas and Rickey Yada, University of British Columbia, Canada. This Committee was instrumental in the success of the Congress; among many other duties, it was charged with inviting speakers, handling all of the abstracts, preparing the scientific program and corresponding with the chosen presenters.

This book contains 23 chapters, which are divided into five sections: Food Security; Nutrition and Health; Global Education; Food Science; and Food Engineering. The first three sections cover global and regional issues of great relevance and include such topics as: food safety in developing and developed countries; IUFoST distance-assisted training; undergraduate and graduate curricula in different parts of the world. The last two sections are dedicated to specific topics of current interest such as, religious slaughter of animals, multiphysics modelling, and microbial inactivation by microwave heating.

The editors are very thankful to Springer for facilitating the development and publication of this book, as well as to Jeannie Bagby at Washington State University for her exceptional work in copyediting all of the chapters. The authors are highly commended for their excellent contributions, and the reviewers for their insights and valuable comments.

We hope this IUFoST book to become a valuable addition to the body of knowledge of the food science and engineering scientific community.

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Biography of the Editors

Gustavo V. Barbosa-Cánovas is a Professor of Food Engineering at Washington State University, USA. His areas of research interest in the food domain include nonthermal and thermal processing, dehydration, physical properties, powder technology, edible films and water activity. He received his B.S. in Mechanical Engineering at the University of Uruguay and his M.S. and Ph.D. in Food Engineering at the University of Massachusetts. Dr. Barbosa-Cánovas is Editor-in-Chief of the Food Engineering Book Series as well as the Food Engineering Reviews Journal, both published by Springer. He is a past President of the International Society of Food Engineering (ISFE) and Chair of the Scientific Council of IUFoST.

Gláucia María Pastore is a Professor of Food Science at the University of Campinas (UNICAMP), Sao Paulo State, Brazil. She received a B.S. in Biology at Pontificia Universidade Catolica de Campinas (PUCCAMP) and an M.S. and a Ph.D. in Food Science at the University of Campinas. Professor Pastore was a Visiting Scholar at the Department of Food Science, Ohio State University, USA, working on producing flavor compounds from microorganisms. She is a Fellow of the International Academy of Food Science and Technology (IAFoST) and Vice-President for Research at UNICAMP. She was President of the Brazilian Society of Food Science and Technology (SBCTA) and President of ALACCTA (Latin American and Caribbean Association of Food Science and Technology). Professor Pastore focuses her research on the biochemistry of functional foods and bioactive compounds from tropical fruits, biotransformation of terpenes in aroma compounds, and the study of biosurfactants from microorganisms.

Kezban Candoğan is a Professor of Food Engineering at Ankara University, Turkey, where she received her B.S. and M.S. degrees, as well as a Ph.D. from Clemson University, USA. Her M.S. research was on poultry meat processing, whereas her doctoral work was on fermented sausage processing. In 2001 she joined the Food Engineering Department, Ankara University, as a postdoctoral fellow. She was promoted to Associate Professor in 2003 and to Full Professor in 2009. She is a certified evaluator for MÜDEK (Turkish Association for Evaluation and Accreditation of Engineering Programs). She is also a scientific consultant for the

Turkish food industry and is a scientific advisory committee member for SETBIR (Union of Dairy, Meat, Food Processors and Producers of Turkey). Professor Candoğan's current research interests include active packaging of muscle foods, development of functional and new meat products, characterization of PSE meats in poultry processing plants, and detection of meat adulteration.

Ilce G. Medina Meza is an Assistant Professor at the Department of Biosystems and Agricultural Engineering at Michigan State University, USA. She earned her Ph.D. in Food Science at the Instituto Tecnologico de Veracruz, Mexico, and her B.S. in Chemical Engineering at the Instituto Tecnologico de Orizaba, Mexico. She has recently been a Visiting Professor at the Center for Nonthermal Processing of Food (2012–2014) and an adjunct faculty member (2014–2016) at Washington State University. Her research interests are on food process engineering, metabolomics, and health engineering. She has devoted significant time exploring chemical changes induced by thermal and nonthermal oxidative processes in lipids and steroids, and evaluating their impact on food and health.

Suzana Caetano da Silva Lannes is Associate Professor III at the School of Pharmaceutical Sciences, University of São Paulo, Brazil. She is President of the Brazilian Society of Food Science and Technology (SBCTA), Vice President of the Brazilian Association of Rheology, Member of the Brazilian Association of Scientific Editors (ABEC), and Editor-in-Chief of Food Science and Technology Journal. Professor Lannes has been working on the following food science and technology areas: rheology, physical properties, development of special and nutritional food formulations, cocoa products, and the study of fat foods and colloidal systems.

Ken Buckle is Emeritus Professor of the Food Science and Technology Group, School of Chemical Engineering at the University of New South Wales (UNSW), Sydney, Australia. He has a B.Sc. (Hons 1, Medal) and Ph.D. in food technology from UNSW. Professor Buckle was Head of the Department of Food Science and Technology and the School of Applied Biosciences, as well as Associate Dean for International Development of Science Faculties at UNSW. His teaching and research interests have covered food preservation, traditional food products, food safety, hurdle technologies, food waste treatment and utilization, and food standards. He was Chair of the IUFoST Scientific Council and a member of its Governing Council (2003–2006), President (2001–2003) of the International Academy of Food Science and Technology, and is Chair since 2006 of the Editorial Advisory Board of the online IUFoST magazine *The World of Food Science.*

Rickey Y. Yada was appointed Dean of the Faculty of Land and Food Systems, and Professor of Food Science at the University of British Columbia (UBC), Vancouver, Canada in 2014. Prior to joining UBC, Dr. Yada was at the University of Guelph in a number of leadership roles, including Chair and Professor of the Department of Food Science, Assistant Vice President for Research, Canada Research Chair in Food Protein Structure, and Scientific Director of the Food Institute. He was also the Vice Chair of the Institute of Nutrition, Metabolism and Diabetes of the Canadian Institutes of Health Research. Among other activities, Dr. Yada is a Member of the Board of

Trustees of the International Life Science Institute (ILSI) North America, and the North American Editor for *Trends in Food Science and Technology*. Dr. Yada is a Past President and Fellow of the Canadian Institute of Food Science and Technology, IUFoST Past-President and Fellow of the International Academy of Food Science and Technology, as well as Fellow of the Institute of Food Technologists.

Amauri Rosenthal is a Scientific Researcher at EMBRAPA (Brazilian Agricultural Research Corporation) Food Technology Center, Rio de Janeiro, Brazil, where he was Director for 4 years. He represented his organization by spending 2 years at AgroSup Dijon and Ensbana Dijon, France, as part of EMPRAPA Labex, an International Program among Brazil and selected countries, working on emerging technologies to process foods, mainly focusing on bacterial spore inactivation. His areas of research interest are mostly in food engineering, where he has been spending a significant amount of time on high pressure and thermal processing, dehydration, hurdle technology, food safety and food quality. His B.Sc. in Food Engineering and M.Sc. in Food Science were completed at the University of Campinas (UNICAMP), Brazil, and his Ph.D. in Food Bioengineering at the University of Reading, UK. He is Chair of Section VI on Post Harvest and Bioprocess Engineering and Vice-Chair of the Food Safety Working Group of CIGR (International Commission of Agricultural and Biosystems Engineering), while also serving as the Brazilian Deputy Representative at the ISEKI Food Association. He is a Member of the Editorial Board of the Food Engineering Reviews Journal and the lead editor of the Fruit Processing book published by Springer. He was chair of the 2013 IFT/EFFoST (Institute of Food Technologists/European Federation of Food Science and Technology) Workshop on Nonthermal Processing of Food held in Florianopolis, Brazil.

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Chapter 1
Supporting Country-Driven Innovations and Agrifood Value Chains for Poverty and Hunger Reduction

Shenggen Fan

1.1 Introduction

According to the latest World Bank estimate, about one billion people live in poverty, despite tremendous progress made in the past two decades (World Bank 2014). Ruling out China, the developing world is not on track to halve poverty—the first Millennium Development Goal (Stevens et al. 2012; Chen and Ravallion 2012). Progress toward halving the prevalence of undernourishment is also off-track and even much slower if the number of undernourished people is considered. Today around 805 million people suffer from hunger (FAO 2012), and "hidden hunger," as micronutrient deficiencies are called, affects the lives of more than two billion people. According to IFPRI's 2014 Global Hunger Index, more than 50 countries had levels of hunger that were "serious," "alarming," or even "extremely alarming" (von Grebmer et al. 2014). The main hotspots for hunger and poverty are Sub-Saharan Africa and South Asia (see Figs. 1.1 and 1.2). It is clear that a "business as unusual" approach that is smarter, more innovative, better focused, and more cost-effective is urgently needed. Most importantly, this approach must be driven by the countries themselves (Fan 2010).

Large successes in agricultural development and food and nutrition security enhancement have been country-driven. Agricultural reforms in China and Vietnam, the Green Revolution in Asia, and the recent surge in agricultural production and productivity in Africa—induced by increased investment in agriculture—are some select examples (Spielman and Pandya-Lorch 2009). The success of country-owned

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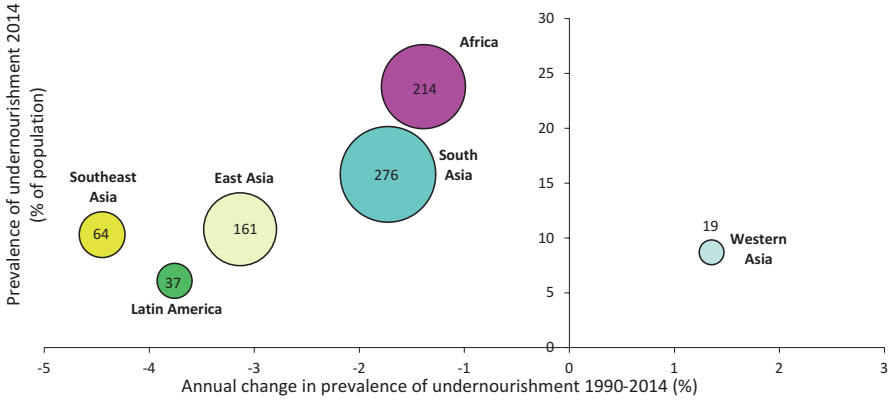

Fig. 1.1 Prevalence of global hunger, by region. *Source*: FAO (2012). *Note*: The size of the bubbles represents millions of undernourished people

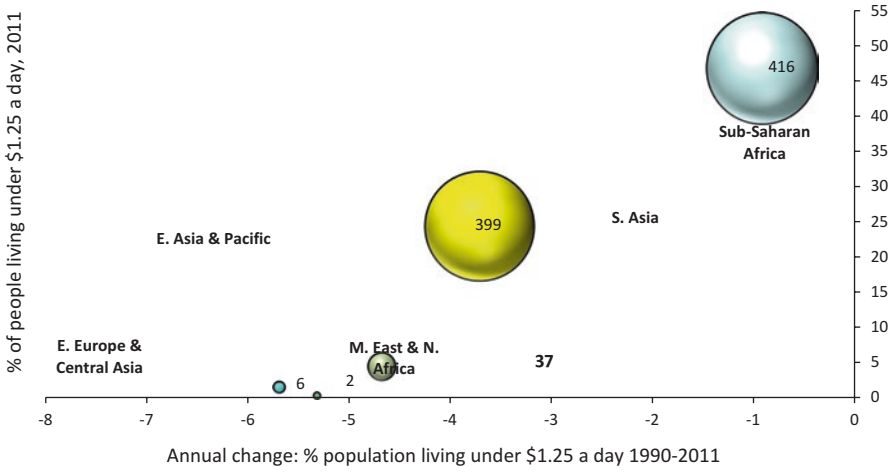

Fig. 1.2 Prevalence of absolute poverty in the world, by region. *Source*: Data from World Bank (2014). *Note*: The size of bubbles represents millions living under $1.25 a day. "Absolute poverty" is measured by the share of population living on less than US$1.25 (PPP) a day

and country-led processes hinges on a combination of good policies, increased agricultural investments, technological innovations, strong institutions, and good governance. This chapter focuses on country-led innovations, agrifood value chains, reduction in postharvest losses and food waste, as well as on related capacities that are needed in developing countries.

Table 1.1 Public investment impact in a sample of developing countries

Sector	Ghana	Uganda	Tanzania	Ethiopia	China	India	Thailand
	Returns to agriculture or rural income (local currency/local currency spending)						
Agriculture	16.8	12.4	12.5	0.14	6.8	13.5	12.6
Education	–0.2	7.2	9.0	0.56	2.2	1.4	2.1
Health	1.3	0.9	n.e.	–0.03	n.e.	0.8	n.e.
Roads	8.8	2.7	9.1	4.22	1.7	5.3	0.9
	Ranking in returns to poverty reduction						
Agriculture	n.e.	1	2	n.e.	2	2	1
Education	n.e.	3	1	n.e.	1	3	3
Health	n.e.	4	n.e.	n.e.	n.e.	4	n.e.
Roads	n.e.	2	3	n.e.	3	1	2

n.e. = no effect

Source: Fan et al. (2009)

1.2 Investments and Innovations

Increasing agricultural investments and setting right priorities are essential to broad-based growth and poverty reduction, since in many developing countries the agricultural sector accounts for a large share of national income and employment, and poverty, continues to be a largely rural phenomenon. As illustrated in Table 1.1, spending on agriculture represents a "win–win" strategy for development as it supports both growth and poverty reduction (Gulati and Fan 2007). The Comprehensive Africa Agriculture Development Program (CAADP), which is supported by IFPRI, is one program that promotes evidence-based policies and agricultural investments and has made considerable progress over the last few years (IFPRI 2011).

Investment and innovation must address growing resource constraints. Among other approaches, more efficient input and resource use can be achieved through improved storage, processing, and marketing facilities that reduce postharvest losses, through water conservation efforts, as well as through breeding crop varieties that are resistant to droughts and pests. In the case of the latter, innovations in biotechnology have the potential to increase crop productivity, nutritional impact, and environmental sustainability without the significant use of additional—and potentially harmful—inputs.

Given the need to mitigate and adapt to climate change, technological innovations should also aim at transforming agriculture into a low-carbon sector. This includes land-management practices—such as mixed cropping, cover crops, and integrated farming—that can optimize crop productivity while limiting greenhouse gas (GHG) emissions. In this context, new approaches are also needed to help measure, track, and map GHG emissions in order to better target and monitor the mitigation potential of agriculture (Nelson et al. 2010).

Cross-sectorial impacts such as the contribution of rural roads and telecommunication (ICT) to agricultural productivity also need to be exploited, and other

development outcomes, such as nutrition and health cannot be neglected either. In particular, ICTs can provide farmers with valuable information such as market prices and weather forecasts, and they can facilitate farmers' access to much needed financial services (Goyal 2010).

Ultimately, the exact priorities depend on country-specific needs, capacities, and resources. In addition, to reduce the vulnerability of the poor to shocks, countries also need to expand and better target their social protection systems (Geleta et al. 2012). Integrated programs, like Ethiopia's Productive Safety Net Program (PSNP), can tackle rural poverty by linking initiatives and helping poor farmers and herders build up assets and improve their productivity. Research has shown that households that benefitted from the PSNP (in combination with other social security programs) had a 10 % higher mean caloric availability than comparison groups, and their credit use as well as their fertilizer use was 12 and 10 percentage points higher, respectively. Moreover, these households were almost 7 % more likely to operate their own nonfarm businesses (Gilligan et al. 2008).

1.3 Agrifood Value Chains

Over the past years a value chain approach to development—ranging from inputs supply, extension, market services, financing, production, processing, and distribution to marketing (Fig. 1.3)—has increasingly been adopted by governments, donors, nongovernmental organizations, and development initiatives to promote market-oriented growth, and to reduce rural poverty in developing countries. At the same time, agrifood value chains in developing countries are changing rapidly as these countries' populations grow, get richer, and become more urban, with supermarkets, distribution, wholesaling firms, processors, and agro-exporters expanding and changing the marketing channels in which smallholders participate both as farmers and as consumers (Humphrey and Navas-Alemán 2010; Gómez et al. 2011).

Given that market failures often prevail, the value chain approach may not always be beneficial for all farmers, and so before taking action, policymakers should consider potential winners and losers in each case. Companies tend to contract with larger farmers first, and source from smallholders only where these dominate production—and then their preference is farmers with certain nonland assets, such as irrigation or access to paved roads. Yet, if deemed expedient, companies use "resource-provision contracts" to address credit, input, or extension constraints, which can help smallholders (Reardon et al. 2012).

The lessons of selected studies by IFPRI authors or affiliated researchers are summarized in Table 1.2. Apart from addressing input and infrastructure constraints, collective action by producers, public–private partnerships, and market deregulation are the measures that are most promising in helping integrate smallholders into agrifood value chains and in addressing other demands on modern food value chains, such as improving food quality and food safety, especially of perishable products.

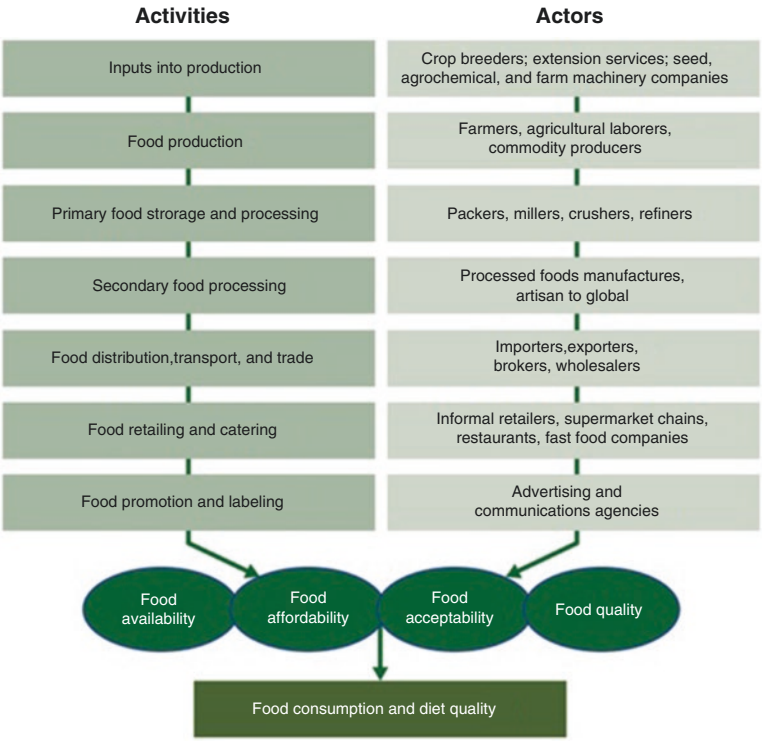

Fig. 1.3 Representation of a food supply chain. *Source*: Hawkes and Ruel (2011)

To date, only a limited number of impact assessments have been carried out to analyze poverty alleviation through value chain interventions, and often it is unclear whether the interventions were responsible for observed improvements, whether the interventions benefited the poor disproportionately, or whether they were more cost-effective than alternative approaches. Since most empirical studies of the welfare effects of agricultural value chains have struggled to establish causality, it is therefore necessary to carry out additional analyses and systematic impact assessments at the program level to develop a stronger evidence base, and to explore long-term effects of smallholder participation in value chains (Humphrey and Navas-Alemán 2010).

1.4 Postharvest Losses and Food Waste

One particular concern with current agrifood supply chains is the amount of food that is lost or wasted between farmers' fields and consumers' plates. According to the FAO, this is roughly one-third of the global food production (Gustavsson et al. 2011). The World Wildlife Fund (WWF) and others report estimates for current

Table 1.2 Agrifood value chains and smallholders

Authors	Focus	Product	Country	Beneficiaries, outcomes and, impact	Challenges
Saenger et al. (2012)	Contract farming	Dairy	Vietnam	Farmers: higher productivity. Processors: better milk quality with quality-dependent pricing, lower per-unit transaction costs with independent quality control.	Lacking incentive to invest in quality-improving inputs and to produce high-quality milk when asymmetry of information is given, e.g., when opportunistic buyers can report lower than actual output quality, which negatively affects farmers' compensation.
Hartmann (2012)	Pro-poor chains	General	General	Poor farmers: poverty alleviation through integration into value chains.	Access to markets, elasticity of demand when supply increases, regulatory requirements, market interference, market failures, competition among targeted value chain actors.
Chenevix Trench et al. (2011)	Food safety, food quality	Perishable food	General	Poor farmers: higher incomes from producing high-value food. Consumers: lower health risk. Labor: more employment.	Supply: low productivity, inadequate capacity, remote location, inability to comply with food safety requirements, lack of financial resources or credit, lack of recognition as producers of safe food, cost of certification, limited competitiveness vis-à-vis larger growers, economies of scale in ensuring food safety, lack of information on good agricultural practices, late payments by retailers. Demand: low accessibility to safe food and poor awareness of health risks of unsafe food.
Hawkes and Ruel (2011)	Improved nutrition	Micronutrient-rich food	General	Poor and marginal populations: Better nutrition through more available, affordable and acceptable food that is more nutritious.	Intersectorial barriers that create disincentives to closer cooperation between sectors, lack of knowledge about what influences demand for nutritious food, focus on quick fixes to address nutrition problems, trade-offs between economic returns and nutritional benefits of agriculture, affordability of value-added products.

Reardon et al. (2012)	Structural transformation	Rice, Potato	Bangladesh, China, and India	Farmers: higher farm prices. Urban consumers: greater food availability, safety and quality. Labor: more employment.	Weak infrastructure and high marketing costs, limited access to subsidized inputs, inefficient input supply chains, lack of upgrading of equipment, weak enforcement of intellectual property rights, poor incentives and low capacity to implement food safety measures and comply with standards.
Bernard and Spielman (2009)	Producer cooperatives	Grains	Ethiopia	Smallholders: positive spillovers from cooperative activities.	Concentration of decision making in management committees that are less inclusive of poor members, trade-off between inclusive membership, participatory decision making, and marketing performance.
Cunningham (2009)	National commodity development boards	Dairy	India	Small farmers and landless producers: access to markets and higher incomes. Urban consumers: more and better dairy products. Labor: female employment.	Lacking systems for procuring milk produced in rural areas, difficult and expensive transport of perishable products, ad hoc marketing of milk, lack of private sector confidence in dairying, lack of program funding.

Source: Author's compilation

postharvest losses to be between 20 and 50 % (Grethe et al. 2011). In industrialized countries the main problem is food waste: for instance, calculations for the United States show that food worth more than 1400 kcal per person per day is wasted, accounting for more than one-quarter of the total freshwater consumption and about 300 million barrels of oil each year (Hall et al. 2009). In developing countries the biggest problem is food losses—which are caused by poor infrastructure, low levels of technology, and low investment in the food production systems, and which occur in the production, harvest, postharvest, and processing phases. Weeds, pathogens, and animal pests alone can cause crop losses of 26–40 %; in Nigeria, 10–20 % of the total production of grains and tubers is lost due to poor storage alone (Oerke 2006; Phillip et al. 2009). According to the World Bank, in all of Sub-Saharan Africa the value of postharvest grain losses amounts to US$4 billion a year—or as much food as could feed 48 million people (Zorya et al. 2011).

Losses in the production phase occur through biotic and abiotic stresses, such as diseases, pests, and droughts. To reduce these losses, for instance, more stress-resistant crops can be developed, whether through conventional breeding, as in the case of fungi-resistant wheat, or through genetic engineering, as in the case of insect-resistant crops. Farmers can also pursue a crop diversification strategy where they replace local varieties with more drought-tolerant varieties (Pauw and Thurlow 2010). Alternatively, they can adopt improved cultivation practices—a process that can be enhanced by the build-up of social capital (Padmaja and Bantilan 2007).

Once harvested, the crops become part of the agrifood supply chain, and there the challenge for food scientists is to find solutions to reducing postharvest losses at various supply-chain stages (Fig. 1.4). One emerging technology that may present new opportunities to improve food security and the livelihoods of the poor—by increasing productivity, reducing postharvest loss, improving product quality, increasing the competitiveness of agricultural producers, and improving market access—is nanotechnology (Gruere et al. 2011). Another example that promotes food safety and reduces food loss is hurdle technology, an approach that combines

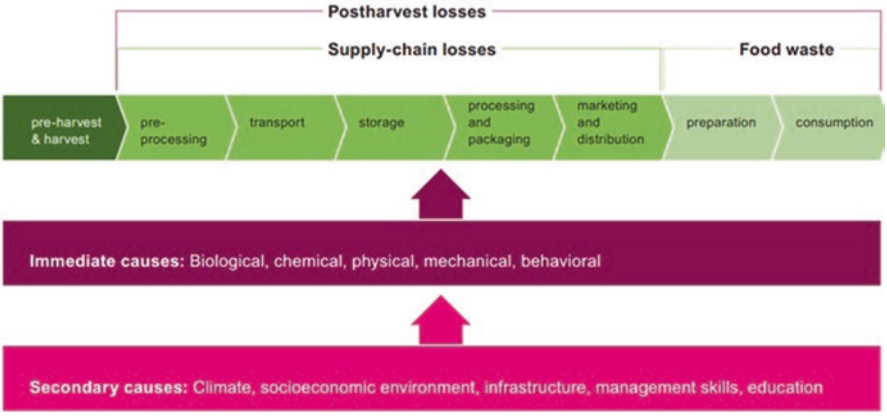

Fig. 1.4 Losses along the food chain. *Source*: Grethe et al. (2011)

preservation methods to control microbial growth, applied at various stages of the food supply chain (Barbosa-Cánovas et al. 2003).

More generally, to avoid losses in developing countries, the agrifood supply chain needs to be strengthened, which requires investments in infrastructure and transportation, as well as in food and packaging industries, with both the public and private sectors playing a role. In richer countries poor coordination along the supply chain, exaggerated quality and esthetic standards, and the relatively low cost of foodstuffs contribute to large amounts of loss and wastage (Gustavsson et al. 2011). There are promising signs that investments and human resource capacity in public agricultural research and development (R&D) are picking up in a number of developing countries—even if so far this is achieving little more than compensating for the neglect of the past decades (Beintema and Stads 2011). Similarly, there is increasing interest and commitment from the private sector to engage in the agrifood supply chain in developing countries, and private-sector innovation and research have already shown to be important sources of new agricultural technology in Sub-Saharan Africa—when and where they were facilitated through conducive government policies (Pray et al. 2011).

Strengthening the agrifood supply chain in developing countries can have wider benefits. For instance, when a more stable and sufficient supply of produce of the right quality allows food processing factories to operate reliably and profitably, it reduces the dependence of low-income countries on imported food products and thereby improves their foreign exchange reserves while providing employment and development opportunities in poor rural areas. Furthermore, reducing wastage is a key element in increasing resource-use efficiency, one of the tenets of the green economy; as such, efforts may streamline agrifood supply chains and reduce unnecessary use of energy, water, fertilizer, and land (Fan et al. 2012).

An emerging issue with negative consequences for postharvest losses and the agrifood supply chain is climate change. For instance, climatic fluctuations can favor the growth of mycotoxins, and more frequent extreme weather events can damage infrastructure such as warehouses and roads (Beddington et al. 2012).

1.5 In-Country Capacities and Scaling Up Successes

To speed up progress in the fight against hunger and poverty, a smarter, more innovative, better focused, and cost-effective approach is needed (Fan 2010). This includes:

- First, the international community and developing countries themselves have to invest in agricultural research and extension, rural development, and targeted social protection, as these investments have large positive impacts on agricultural productivity, poverty alleviation, and production capacity.
- Second, new actors in global development, such as the private sector and in particular the food industry, have important roles to play in reducing hunger in

developing countries. Given the right incentives and a conducive business environment, companies can provide effective and sustainable investment and innovation to upgrade local agrifood supply chains.

- Third, to be more effective, efficient, and sustainable, as well as better adapted to the local context, in many cases policies have to be country-led. Similarly, to be more inclusive and to increase "ownership," reforms have to build on bottom-up support, with local people acting as a driving force in the development process.
- Fourth, to improve the success rate of reforms, successful pilot projects and policy experiments need to be scaled up and unsuccessful policy options have to be eliminated. To generate the evidence needed for these decisions, policymakers need to allow impartial monitoring of these activities and to apply the lessons learned by adjusting their policies.
- Fifth, decision makers at all levels have to stand by their commitments to policies and investments for enhancing food security, not only by ensuring the disbursement of pledged funds, but also by supporting them with institutions, governance, and monitoring.

Some such approaches have already been successful in a few countries, but they need to be scaled up and extended to additional countries to have a real impact on the reduction of global hunger and poverty. To support developing-country governments and their partners in identifying areas for agricultural and rural investment and policy interventions, IFPRI established a "Strategic Analysis and Knowledge Support System" (SAKSS) to act as a network for governments, donors, research institutes, universities, the private sector, and nongovernmental organizations in Africa. To this end, SAKSS compiles, analyzes, and disseminates data, information, and tools that help better design, implement, and evaluate agriculture and rural development strategies. By having IFPRI's in-country strategy support programs implement SAKSS, capacity building in the target countries is not neglected, which is another important element for successful country-owned and country-led approaches for reducing hunger and poverty.

1.6 Conclusions

Despite progress, so far less than half of all developing countries have a reasonable chance of meeting the Millennium Development Goal of halving hunger by 2015, compared to the proportion of hungry people in 1990, at least if measured by the proportion of underweight children. Neither is the developing world on track to achieving the MDG of halving the 1990 poverty rate—if China is excluded. This is an alarming development and it clearly shows that more and continued efforts are required by global, national, and local actors to reduce hunger and poverty.

More and sustained investment in agriculture is one of the best bets in the fight against hunger and poverty, but related fields like rural infrastructure, as well as nutrition and health in developing countries, also need to receive more funding.

Technological innovations in food and agriculture that increase productivity and reduce losses—and that are compatible with the needs of smallholders—have to be promoted. In this context, the increasing interest of the private sector in engaging in developing countries can facilitate the expansion of the agrifood value chain and formal markets, and it can help in the development and dissemination of innovations. Government policies that strengthen the position of smallholders should support their integration into the agrifood value chain, which will be facilitated where their productivity and access to rural infrastructures is improved. Further down the value chain, food science and technology in particular can help reduce food losses and increase resource-use efficiency.

However, sound evidence is needed to determine which strategies, technologies, investments, institutions, and partnerships should be scaled up in-country to achieve enduring impact on hunger and poverty. Therefore, a framework—and an open mind set by decision makers—for evaluating pilot projects and experiments in developing countries is needed, as is a political and legal space to transform the lessons learned into large-scale initiatives. To this end stakeholders at all levels and from all sectors need to work together.

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Chapter 2
Preserving Food After Harvest is an Integral Component of Food Security

Malcolm C. Bourne

2.1 Introduction

The projections of increasing world population for the next 40 years and possible reductions in agricultural productivity because of climate change, water shortages, and other factors have propelled food security into the forefront of concern on the world stage. The first step in these discussions is to define exactly what is meant by the term "Food Security."

The Food and Agriculture Organization (FAO) of the United Nations definition is: "Food security exists when all people, at all times, have access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (FAO 1996).

The United States Department of Agriculture gives a more detailed definition as follows: "Food security for a household means access by all members at all times to enough food for an active, healthy life. Food security includes as a minimum (1) the ready availability of nutritionally adequate and safe food, and (2) an assured ability to acquire acceptable foods in socially acceptable ways (that is, without resorting to emergency food supplies, scavenging, stealing, or other coping strategies)" (USDA 2014).

Hundreds of millions of people do not have security as defined above and the number will increase in the future unless major investments are made to improve the situation. The major causes of food insecurity are:

1. Poverty
2. Losses in seasonal crops after harvest
3. Population and Urbanization—distance and time from farm to fork becomes longer

M.C. Bourne (Deceased)

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4. Disasters—floods, drought, pests that lower productivity
5. Health—sick people cannot work hard
6. Wars and Conflicts
7. Gender Inequity

Item number 2 in the above list—losses in seasonal crops after harvest—is the sector in which food technologists have the knowledge to make a major contribution to resolving the problem of food insecurity. The International Union of Food Science and Technology (IUFoST) at the 15th World Food Congress held in Cape Town in August 2010 explained the situation in the following words: "We accept that the problem of food insecurity has huge political and economic dimensions and will not be solved by food science and technology alone or even by science alone; but it will certainly not be solved without the contribution of science and of food science and technology" (IUFoST Cape Town Declaration 2010). Food technology is a central component but not the only component in resolving the problem of food insecurity.

2.2 Role of Food Technology

Food technology has two main branches:

1. PRESERVATION TECHNOLOGIES stabilize, safeguard, and maintain the harvest from land and sea in a condition suitable and safe for human consumption. Examples for foods that spoil quickly are drying, canning, refrigeration, freezing, and preservatives. For stable foods such as cereal grains, the main preservation technology is adequate drying to prevent mold growth. Control of insects and designing storage structures that prevent entry by rats, mice, and other vertebrate pests are also important technologies for stable foods.

   This is the sector where food technology can make a large contribution to reducing food insecurity. Huge quantities of food already harvested are not eaten because the food is lost or becomes inedible. The causes of the postharvest losses are well known. Technologies to prevent the spoilage vectors are also well known in the food science community.

   The causes of food losses depend on the type of food and are summarized below:

   CEREALS—fungi, insects, vertebrates, poor milling
   FRUITS and VEGETABLES—bruising, rotting, senescence, wilting
   ROOTS and TUBERS—bruising, rotting, senescence, wilting, sprouting, insects
   MEAT, MILK, POULTRY, FISH—growth of microbes
   DRY FISH—fungi, insects

2. PROCESSING TECHNOLOGIES convert edible food materials into another form with higher acceptability. People prefer to eat bread, spaghetti, cake, and many other delicious items rather than grains of wheat. They prefer to drink beer

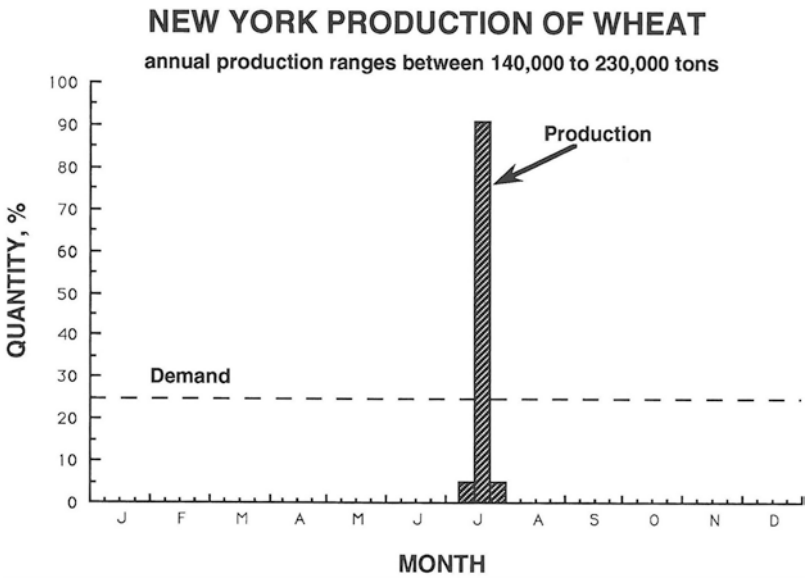

Fig. 2.1 Production of wheat in New York State. A year's supply is harvested within 3 weeks and most of it in 1 week

rather than eat barley grains. They prefer tough cuts of meat to be converted into some kind of sausage. They like foods that have been processed to give a crispy texture and blends of ingredients that create creamy sauces.

Because of economic forces food science departments in developed countries put great emphasis on food processing technologies. Preservation technologies are well covered for highly perishable foods such as milk and meat, but stable commodities such as cereal grains get less coverage even though the technologies to control fungi, insects, and vertebrates are well known.

2.2.1 Seasonal Crops

Within a given region the daily demand for food is constant over the course of a year, but the supply of food for that region is very uneven throughout the year. Food preservation, storage, and transportation are the mechanisms humankind uses to match the very uneven day-to-day supply of food with the even day-to-day demand for food. Matching the uneven food supply to the even demand is a problem the human race has had to face ever since the dawn of agriculture.

The nature of the problem is illustrated in Fig. 2.1 which shows the production of wheat in New York State for each month of the year. The farms in New York produce no wheat from January to June or from August to December. All the wheat is harvested in the month of July as shown by the vertical bar. In fact, 90 % of the

wheat is harvested in 1 week, usually the third week of July. However, the demand for bread, macaroni, donuts, and all the other nice foods made from wheat is very uniform throughout the year as shown by the dashed line parallel to the horizontal axis of Fig. 2.1.

Most of the world food supply is seasonal—cereals, legumes, fruits, vegetables, roots, and tubers produce a large crop over a short period of time and then nothing until the next crop matures. The time of year when the harvest is ready can be any month of the year depending on the climate and nature of the crop, but the pattern of a large harvest followed by a period of no harvest is the common feature. In some locations where climate and water supply permit there is more than one harvest per year. In those cases there are two or more peaks of production with nothing in between.

Foods from domestic animals are not seasonal. With good management the time of harvest can be controlled to become uniform throughout the year. Milk and eggs can be harvested daily. Animals and birds can be kept alive and slaughtered as needed. Some fish harvests are seasonal because wild fish cannot be managed like domestic animals.

Of the many factors that affect food security, one that is often overlooked is the need to prevent spoilage of food between the time it is harvested and the time it is consumed. This time can range from 1 day for highly perishable foods such as milk and meat to several years for stable crops such as cereal grains.

Many activities are required to convert mature agricultural products in the field into a form suitable for human consumption and to deliver it to the meal table in an acceptable form. There are many opportunities for food to be lost between harvest and consumption. These are known as *POSTHARVEST FOOD LOSSES*; they represent a loss of valuable nutrients and money, especially in developing countries where many are already undernourished and poor. Food must not only be produced, it must be delivered to the ultimate consumer in an acceptable form if it is to fulfill its nutritional destiny (Bourne 1984). It must also be fit to eat and safe to eat when mealtime comes.

2.3 Historical Development

How attention to reduction of food losses as an essential component of food security developed will now be discussed in three parts: (1) before 1975, (2) 1975–2010, and (3) 2011 and into the future.

2.3.1 Before 1975

The Tropical Products Institute founded in London in 1896 always had a strong interest in preservation and quality of foods in the colonies of the British Empire. It was renamed Natural Resources Institute in 1990 and moved from London to Greenwich

and is still a leading player in food security issues. Their library is probably the greatest single resource of knowledge on this subject.

The Food and Agriculture Organization (FAO) of the United Nations was founded in 1945 and has devoted much attention to food security issues. Its headquarters are in Rome and there are regional offices on every continent.

In the United States, the International Development Assistance Program, which began in 1950 during the Truman Administration, had food security as one of its high-priority objectives. The name was changed to United States Agency for International Development (USAID) in 1961 during the Kennedy Administration.

A number of other institutions, both public and private, established since 1960 have had food security as one of their major areas of interest. Reduction of postharvest losses of food, with emphasis on losses in developing countries where malnutrition and hunger are high has often been on their action agenda. However, most of these programs were sporadic and short-lived because they depended on the foresight and enthusiasm of one or two people on location in a developing country who saw the shocking rate of spoilage in food already harvested. The programs usually expired after the initiators of these programs completed their assignments and returned to their homeland. This piece-meal, localized approach occurred without much recognition at high policy levels.

2.3.2 1975–2010

The situation changed dramatically in September 1975 when the United Nations General Assembly meeting in New York passed the following resolution: "The further reduction of postharvest losses in developing countries should be undertaken as a matter of priority, with a view to reaching at least 50 % reduction by 1985. All countries and competent international organizations should cooperate financially and technically in the effort to achieve this objective."

This resolution drew the attention of the highest levels of governments and donor organizations around the world to the problem of food losses and the contribution that reducing these losses can make to the improvement of the nutritional status of the poor. The resolution is realistic; it recognizes that food losses will never be reduced to zero but calls for efforts to reduce sharply the high levels of loss that presently occur. Note the focus on improving the food supply in developing countries at the highest policy level.

A result of the 1975 UN resolution was that many governments and other agencies made it a POLICY to include a postharvest food loss component in their foreign aid programs. The US Agency for International Development (USAID) initiated a number of programs including commissioning a report from the US National Academy of Sciences. This report was the standard reference in the field for many years and is still a valuable resource.

Governments in the European Union, Japan, Canada, and Australia, and a number of nongovernment organizations also initiated postharvest food loss programs.

Table 2.1 Postharvest losses in the Philippines before the 1975 United Nations Resolution and 20 years later

	1974 (%)	1994 (%)
Range	10–37	11–32
Average	23.5	14.8

BPRE Newsletter, Vol. 3, No. 4, December 1997

FAO expanded its efforts in postharvest loss reduction. Many good programs were put in place, and substantial reductions in food losses were achieved in some developing countries.

One example is shown in Table 2.1. Postharvest losses of rice in the Philippines averaged 23.5 % in 1974. Thanks to the effort of many this figure fell to 14.8 % by 1994—a 37 % reduction from the 1974 figure. The efforts did not reach the goal of 50 % reduction called for by the UN resolution, but substantial progress was made.

2.3.2.1 Publications

A number of publications were produced that describe the problem and became a valuable resource to organizations planning a postharvest program. Some of the most notable are:

1. M. C. Bourne 1977 "Postharvest Food Losses—The Neglected Dimension in Increasing the World Food Supply" Cornell International Agriculture Mimeograph, No. 53, 69 pages. It is now available free online at: http://ecommons.library.cornell.edu/handle/1813/28900
2. US National Academy of Sciences 1978 "Postharvest Food Losses in Developing Countries," 199 pages. The Academy also published a 350 page bibliography on postharvest food losses comprising approximately 2100 entries. This is the most complete compilation of literature up to 1978.
3. Food and Agriculture Organization 1981, "Food Loss Prevention in Perishable Crops", 72 pages, FAO Agricultural Services Bulletin 43. This publication is the result of an expert consultation jointly organized by FAO and the United Nations Environment Program. It describes the importance of fruits, vegetables, roots, and tubers, the technologies that prolong shelf life and gives 14 recommendations for policy makers, planners, development corporations, and potential investors in developing countries.
4. United Nations Environment Programme 1983, Industry and Environment Guideline Series "Guidelines for Postharvest Food Loss Reduction Activities," 47 pages. The Guidelines synthesized information and experiences in a concise form as an aid to policy formulation. It includes an appendix that lists 12 international organizations and 55 national organizations in 31 countries that have active programs in food loss reduction. Published by UNEP Industry and Environment Office, Paris.

5. US National Academy of Sciences and China State Science and Technology Commission, "Postharvest Food Losses in Fruits and Vegetables," 1986, 188 pages. Published by National Academy Press, Washington, D.C.

The above are a sampling of the most influential documents resulting from the 1975 United Nations Resolution. There are many more including some in languages other than English.

2.3.2.2 Education

A number of programs to train people on the causes of postharvest losses and the technologies available to prevent these losses were put in place in many countries.

In 1977, Cornell University instituted the course Food Science 447 "International Postharvest Food Systems." This interdisciplinary course described the causes of postharvest food losses and methods available to reduce the losses. It was designed for all seniors and graduate students who were interested in storage and preservation technologies for unprocessed or minimally processed food commodities (cereals, dry legumes, roots, tubers, vegetables, fruit, fish) in the U.S. and overseas. The course was of special interest to students who had worked in, or planned to work in developing countries. This course was offered for 25 years.

2.4 2010 and the Future

More than 35 years have passed since that 1975 UN Resolution was adopted. The scientists who carried out those postharvest activities in the field have retired as have the policy makers of that generation. Most of the excellent programs of the late 1970s and 1980s have expired and not been renewed. Policy has moved away from the initial focus on preserving raw agricultural commodities to include all activities from harvest to consumption with greater attention given to processing technologies that convert raw products into foods ready to eat. The programs that are still active have generally shifted emphasis from reducing losses to improving the quality and safety of foods exported from developing countries to developed countries. It seems that postharvest has fallen from the policy agenda of most governments and donor agencies.

An International Congress entitled "SAVE FOOD" was organized by the Food and Agriculture Organization, May 16–17, 2011, at the International Packaging Industry Fair Interpack 2011 in Düsseldorf, Germany and the proceedings published by FAO with the title "Global Food Losses and Food Waste." It is based on studies carried out by the Swedish Institute for Food and Biotechnology (SIK) at the request of FAO. The study highlights the losses occurring along the entire food chain, identifies causes of food losses and possible ways of preventing them. Considerable data on losses from agricultural production, postharvest handling and storage, processing and packaging, to distribution and consumption are given.

Table 2.2 Comparison of United Nations 1975 resolution and the Food and Agriculture Organization Congress 2011 report

UN 1975	FAO 2011
Postharvest food loss	Food loss and waste
Developing countries	Developed and developing countries
Motivation	Motivation
1. Improve nutrition of poor	1. Reduce stress on environment
Seasonal crops (cereals, roots, tubers, vegetables, fruits, fish)	2. Improve nutrition of poor
	All foods (meat, poultry, fruits, vegetables, fish, roots, tubers, cereals, oilseeds, dairy)

It is noteworthy that "food waste" was added to the established phrase "food loss." The differences between the 1975 UN resolution and the 2011 FAO report are summarized in Table 2.2. The major changes are that great attention is now given to reducing waste, especially in developed countries, that all foods get attention, and the main motivation is to reduce stress on the environment.

The major causes of food waste are:

1. Demand for perfect appearance and convenience.
2. People in developed countries can afford to discard food because it is abundant and low in cost relative to income.
3. Production exceeds demand.
4. Food is unsafe or suspected of being unsafe.

These causes are completely different from the causes of food losses shown in Sect. 2.2, point 1. Unlike food loss reduction technologies where food science has the major role, reducing waste requires modification of human behavior, an area in which food scientists have no expertise except for item No. 4, food safety.

Also, the emphasis has moved from staple seasonal crops such as cereals to foods of animal origin. A rough comparison of this change is shown in Table 2.3 where the number of references cited in the 2011 FAO report is compared with the number of pages devoted to each food in the 1978 National Academy of Science report as mentioned in Sect. 2.3.2.1. Although this is an "apples versus oranges" type of comparison it clearly demonstrates the change in emphasis between 1975 and 2011.

Extensive tables in the 2011 FAO report show that the highest food *losses* occur in developing countries and the highest food *waste* in developed countries. Food waste is low in developing countries and food losses are low in developed countries. This is demonstrated in Table 2.4, which summarizes extensive data prepared by SIK and published in the FAO report.

While we need to reduce food waste in rich countries, we must not forget the hundreds of millions who are malnourished and poor. In October 2012 FAO reported that 868 million people are hungry, that is 12.5 % of the world's population. The mortality and morbidity rates are still very high in LDCs and stunted growth is considered normal.

Table 2.3 Comparison of commodity emphasis between 2011 Food and Agriculture Organization Congress and 1978 National Academy of Sciences report

	Number of references in 2011 FAO report	Number of pages in 1978 NAS report
Meat and poultry	30	0
Fish	16	17
Dairy	3	0
Cereals	6	60
Fruits, vegetables, roots, tubers	31	27
Oilseeds	2	0
Unclassifiable	38	

Table 2.4 Summary of food loss and waste from Food and Agriculture Organization Congress 2011

	Postharvest and storage (%)	Distribution (%)	Consumption (%)
Europe and Russia	4–9	2–10	17–25
N. America and Australia	2–10	2–12	27–30
S and SE Asia	7–19	2–11	1–7
Sub-Saharan Africa	8–18	2–17	1–5

Figure 2.2 shows the tragedy of severe malnourishment, a 30-month-old girl who weighs 5.5 kg. Note the skinny legs and arms, distended abdomen, and lethargy. There are still many thousands of children such as this who die every year.

A more serious problem of malnourishment is the stunted growth that results from insufficient food. Figure 2.3 shows two boys the same age. The boy on the left grew up in a developed country and weighs 33.6 kg (74 lb), while the boy on the right weighs 25.4 kg (56 lb) and is not as tall as the boy on the left. There are many millions of people like this whose stunted growth affects their whole life span.

How did that 1975 UN Resolution (shown in Sect. 2.3.2) that resulted in many postharvest loss reduction activities, come to be passed?

The answer is that the US Secretary of State, Dr. Henry Kissinger, in an address presented at the 7th special session of the United Nations General Assembly on September 1, 1975 in New York said, “Another priority in the poorest countries must be to reduce the tragic waste of losses after harvest from inadequate storage, transportation, and pest control. There are often simple and inexpensive techniques to resolve these problems. Investment in such areas as better storage and pesticides can have a rapid and substantial impact on the world’s food supply; indeed, the savings could match the total of the food aid being given around the world. Therefore, we urge that the Food and Agriculture Organization, in conjunction with the UN Development Program and the World Bank, set a goal of cutting in half these postharvest losses by 1985, and develop a comprehensive program to this end.”

The resources allocated to postharvest food loss reduction from the mid-1970s to 1990s were a direct result of the speech Dr. Kissinger made to the United Nations

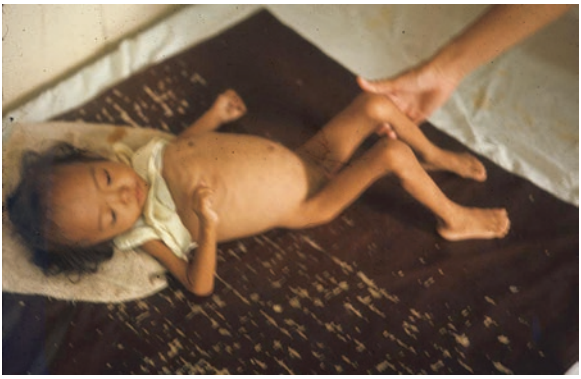

Fig. 2.2 A 30-month-old child suffering from severe malnutrition (Photo from M. C. Bourne file)

Fig. 2.3 Height and weight of a boy who grew up in a developed country (LHS) is significantly greater than for a boy of the same age who grew up in a developing country (RHS) (Photo from M. C. Bourne file)

General Assembly in 1975. Significant resources for postharvest food loss reduction in 2012 and forward are unlikely to be assigned until they become government policy again. I fear that resources devoted to improve the nutritional status of the poorest who suffer the most from food insecurity will not increase until postharvest food loss reduction becomes a high-priority issue for world leaders again.

Where do we find a prominent person who will make a similar speech to the one made by Dr. Kissinger to the United Nations or a similar prestigious organization that will trigger the allocation of significant resources by governments and donor organizations to reducing postharvest food losses in developing countries? Efforts to increase food security will be crippled unless a postharvest component is included in the programs.

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Chapter 3
Microbiological Safety of Fruit and Vegetables in the Field, During Harvest, and Packaging: A Global Issue

Santos García and Norma Heredia

3.1 Introduction

Worldwide produce consumption has grown due to increased availability, affordability, and public education and healthy trends. Global improvements in agricultural practices have enhanced the world's capacity to produce more food. Furthermore, a diversity of foods, international trade, electronic communication, rising income levels, urbanization, and improved transportation types and routes has resulted in important changes in food consumption worldwide (Kearney 2010).

Following the 1980s, global fruit and vegetable trade increased at a higher rate than any other agricultural commodity. The European Union, the North American Free Trade Agreement region, and Asia are the major fruit and vegetable supply destinations and sources. However, developing countries are important produce sources, because preparing products for market is labor-intensive, and labor costs are lower, and many developing countries possess large cultivation areas and suitable climates to grow a diversity of greens (Avery et al. 2011; Huang 2004).

Unfortunately, corresponding with increased produce cultivation has been a rise in foodborne disease outbreaks resulting from contaminated produce consumption. The level of documented disease has been of such magnitude that produce food safety has been a priority for many countries.

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3.2 Produce Benefits

Fruit and vegetable consumption is an integral component of a healthy diet. Low fruit and vegetable intake is among the top 10 selected risk factors in global mortality. Sufficient daily fresh fruit and vegetable consumption reduces the risk for many leading causes of death, including prevention of certain cancer types, reduced cardiovascular disease, healthy weight maintenance, and decreased risk of obesity (Grimm et al. 2010).

For a diet that contributes to health benefits and prevents chronic diseases and micronutrient deficiencies, the World Health and Food and Agriculture Organizations of the United Nations (WHO/FAO) recommend a minimum of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers). It is estimated that low fruit and vegetable consumption leads to 16.0 million (1.0 %) disability adjusted life years (DALYs, a measure of the potential life lost due to premature mortality, and years of productive life lost due to disability), and 1.7 million (2.8 %) deaths worldwide (WHO 2012). At a global level, it is estimated that 14 % of gastrointestinal cancer deaths, 11 % of ischemic heart disease deaths, and approximately 9 % of stroke deaths are caused by poor fruit and vegetable consumption (Grimm et al. 2010; WHO 2012). Consequently, in 2003 WHO and FAO launched a joint initiative to increase human consumption of fruits and vegetables worldwide. This effort has resulted in specific programs, including the US program "Healthy People 2010 and 2020." One of the program objectives for fruits and vegetables is to increase the proportion of people who consume two or more servings of fruit daily (Healthy People 2012).

3.3 Microbial Contamination

In our current social climate, the public is more educated, possesses an awareness of the benefits of a healthy diet, and consumers are in a mid- to high-income bracket. Therefore, this global consumer demands quality and safety in the products they consume. Produce can become contaminated with foodborne pathogens at various points during production, handling, and the packing process. Contaminated produce consumption will not only abolish the health benefits, but poses a health risk. Leafy greens, tomatoes, cucurbits, peppers, and nuts are among the foods commonly linked to outbreaks of gastrointestinal illnesses caused by *Escherichia coli* O157:H7 and *Salmonella* (FAO/WHO 2008).

According to a study conducted by the WHO/FAO, leafy green vegetables are the commodity group of highest concern as the cause of foodborne outbreaks; these data are supported by the reported number of outbreaks, the large production volume, the substantial commercial trade, and the complexity and variability of the production and distribution chain (FAO/WHO 2008). This group includes all leafy vegetables, where the leaf is the plant part intended for consumption, including lettuce (all varieties), spinach, cabbages, chicory, leafy fresh herbs (e.g. cilantro, basil, parsley), and watercress. These products are often contaminated with the

Table 3.1 Common commodities and the microorganisms involved in outbreaks (Modified from FAO/WHO 2008)

	Bc	Camp	Ec-D	Lm	Salm	Shig	Yers	Norov	VHep	Cycl	Cript	Helm
Almonds					X							
Baby Corn						X						
Berries								X	X	X	X	
Carrots			X			X	X	X	X		X	X
Celery								X	X			
Cucumber		X	X		X							X
Garlic												
Green onions			X		X	X			X			
Leafy green, fresh herbs		X	X	X	X	X	X	X	X	X	X	X
Mangoes					X							
Melons			X		X			X				
Onions			X		X							
Papaya												
Paw paw					X							
Sprouted seeds	X		X		X							
Tomatoes					X				X			

Bc = *Bacillus cereus*; Camp = *Campylobacter* spp. Ec-D = Diarrheagenic *Escherichia coli*. Lm = *Listeria monocytogenes*. Salm = *Salmonella enterica*. Shig = *Shigella* spp., Yers = *Yersinia* spp., Norov = Norovirus, VHep = Hepatitis Virus. Cycl = *Cyclospora* spp. Cript = *Cryptosporidium* spp. Helm = Helminthes

pathogens that are most commonly responsible for foodborne disease outbreaks at the global level (Table 3.1) (FAO/WHO 2008; Erickson 2010).

Bacillus cereus, *Campylobacter jejuni*, *E. coli* O157:H7, *Listeria monocytogenes*, *S. enterica*, *Shigella*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Cyclospora cayetanensis*, *Giardia lamblia*, *Cryptosporidium parvum*, Hepatitis A virus (HAV), and Norovirus (NoV) are the most common pathogens involved in outbreaks resulting from contaminated produce consumption. A brief description of these microorganisms, the disease caused by the pathogen, and the most commonly implicated commodities follows.

3.4 Bacterial Pathogens

Campylobacter. This microorganism is reported as the most common cause of foodborne bacterial gastroenteritis in humans in many countries, and possibly worldwide (Garcia and Heredia 2009). The bacterium is a significant public health hazard due to its low infective dose in humans, and its potentially severe sequelae. Campylobacters are unable to grow below 30 °C, are very sensitive to desiccation,

and do not survive well on dry surfaces (Butzler 2004; Snelling et al. 2005). Lettuce, sweet potatoes, cucumber, and orange juice are known sources of infection (Heaton and Jones 2008).

The infective dose of *Campylobacter* sp. ranges from 500 to 10,000 organisms that can cause, following an incubation period of 1–7 days, asymptomatic infections, watery diarrhea, or dysentery-type illnesses in humans. Fortunately, most infections are self-limiting and rarely cause death; however, some are associated with chronic, debilitating sequelae, such as arthritis, Reiter syndrome, or Guillain-Barré syndrome (Butzler 2004; Snelling et al. 2005). Diarrhea, fever and abdominal cramps, headache, asthenia, and anorexia are usual symptoms of the gastrointestinal illness (Garcia and Heredia 2009).

C. jejuni is generally found in the gastrointestinal flora of avians, swine, and cattle, and it is suggested that wild and domestic birds, and domestic swine and cattle are reservoirs for strains that infect humans. The primary reservoir for *C. coli* is swine; however, *C. coli* constitutes only a minimal *Campylobacter* percentage of isolates from chicken and cattle (Butzler 2004). Fecal droppings from these animals are a source of produce contamination by *Campylobacter*.

Escherichia coli. *E. coli* is a facultative anaerobe, and a natural component of the human and animal lower intestinal microflora. However, some strains are virulent, and cause disease ranging from mild to cholera-like diarrhea, and are conducive to potentially fatal complications, including hemolytic uremic syndrome (HUS). Food vehicles, such as fruit salad, cantaloupe, sprouted seeds, and unpasteurized juice (e.g. cider) have been involved in disease outbreaks caused by this bacterium (Betts 2000; Garcia and Heredia 2009).

The most diarrheagenic *E. coli* are classified into at least six groups, which exhibit distinct pathogenic characteristics. The six groups include diffuse-adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (ETEC) (Nataro and Kaper 1998; Garcia and Heredia 2009). Of these, only the latter four groups have been implicated in food or water borne illness (Feng et al. 2011).

EPEC is a major cause of human infantile diarrhea in less-developed countries, and the most widespread diarrheagenic *E. coli*. The infection is an acute or watery, nonbloody or mucoid diarrhea, frequently with fever and vomiting (Nataro and Kaper 1998; Clarke et al. 2002).

EHEC, also referred as Shiga-toxin producing *E. coli* (STEC) is responsible for serious human infections, including uncomplicated diarrhea, hemorrhagic colitis, and HUS. The EHEC strains produce Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) that resemble *S. dysenteriae* (Feng et al. 2011; Betts 2000). Although serotype O157:H7 is the pathogen most frequently implicated in foodborne outbreaks worldwide, more than 100 STEC serotypes (e.g. serogroup members O26, O91, O103, O111, O118, O145, and O166) are known to cause human illnesses, including HUS (Feng et al. 2011; Betts 2000).

Initial infection symptoms include nonbloody or bloody diarrhea, and abdominal pain. Fever and vomiting occur in many patients; in some individuals, the illness

progresses to HUS, with hemolytic anemia, thrombocytopenia, and renal failure. Nataro and Kaper (1998) indicated heightened mortality rates occur in children.

EIEC is subdivided into 11 known serogroups based on serological characteristics. Disease in healthy humans requires an infectious dose of 10^6 cells or more. The infection occurs as watery diarrhea or dysentery, the latter manifested as blood, mucus, and leukocytes in the stool, tenesmus, and fever (Nataro and Kaper 1998).

ETEC is a primary etiologic agent of diarrhea in infants and travelers. ETEC strains produce enterotoxins; one a heat-labile toxin (LT, very similar to the cholera toxin), one a heat-stable toxin (ST), or both, and surface adhesins known as colonization factors (Nataro and Kaper 1998; Feng et al. 2011). The ETEC infective dose in otherwise healthy adults is estimated to be approximately 10^8 CFU. The illness is characterized by watery diarrhea with little or no fever (Nataro and Kaper 1998; Feng et al. 2011).

A new pathotype, Entero-Aggregative-Haemorrhagic *E. coli* (EAHEC) has been suggested. The pathotype was based on a unique combination of genomic features in one strain that caused an outbreak that originated in Germany with 3368 cases, including 36 deaths. The strain contains traits from pathotypes EAEC and EHEC (Brzuszkiewicz et al. 2011).

In general, *E. coli* survives freezing to −20 °C, and can withstand cold storage, exhibiting growth at a minimum temperature of 6.5 °C. *E. coli* normally live in the intestines of warm-blooded animals. Cattle serve as a main reservoir for *E. coli* O157:H7 strains. Other species, including horses, deer, sheep, goats, pigs, cats, dogs, chickens, gulls, birds, and flies have been reported as sources of these organisms (Nataro and Kaper 1998). *E. coli* can contaminate a wide variety of produce by different means, including contaminated hands, contaminated fomites serving as transmission vehicles, indirect contamination via polluted water, manure, and during product handling and packaging (Nataro and Kaper 1998; Clarke et al. 2002; Feng et al. 2011).

Listeria. The eight species comprising the genus *Listeria* are facultative anaerobic rods that can grow over wide temperature (−1.5 °C to 45–50 °C) and pH (4.3–9.6) ranges (Vázquez-Boland et al. 2001; Lianou and Sofos 2007; Kathariou 2002). *L. monocytogenes* is a ubiquitous Gram-positive species that causes high mortality rates, particularly in high-risk groups, such as the elderly and immunocompromised, as well as pregnant women and their neonates. In the United States, *L. monocytogenes* is responsible for less than 1 % of foodborne illnesses, but approximately 28 % mortality (Vázquez-Boland et al. 2001; Kathariou 2002).

L. monocytogenes is widely distributed in the natural environment, and has been isolated from a variety of sources, including surface water, soil, sewage, vegetation, human and animal feces, and food-processing plants. Listeriosis has been associated with contaminated vegetables, such as (but not limited to) mushrooms, vegetable rennet, coleslaw, and corn salad (Garcia and Heredia 2009; Cocolin et al. 2002; Kathariou 2002).

The primary transmission mode of this pathogen to humans is contaminated food consumption. Contamination levels as low as 10^2–10^4 cells per gram of food have been associated with human listeriosis. Vázquez-Boland et al. (2001) described the

clinical features of systemic listeriosis, which include late-term spontaneous abortion, prenatal infection, meningitis, encephalitis, septicemia, and gastroenteritis.

Refrigerated ready-to-eat foods are the main concern for this pathogen, because such food products are typically not heated prior to eating. Currently in the United States, a "zero tolerance" is in effect for this bacterium in ready-to-eat foods, but the European Union regulations have established a 100 CFU/g limit in ready-to-eat foods unable to support *L. monocytogenes* growth. Common *L. monocytogenes* contamination sites include filling and packing equipment, conveyors, chill solutions, slicers, dicers, shredders, and blenders (Vázquez-Boland et al. 2001; Cocolin et al. 2002; Kathariou 2002).

L. monocytogenes presence in many fresh fruits and vegetables has frequently been reported in the literature. Alfalfa and bean sprouts, strawberries, frozen vegetables, potatoes, mushrooms, and radishes have been identified as sources of *L. monocytogenes* contamination during retail food surveys (Aytac et al. 2010; Lianou and Sofos 2007). Additional studies have confirmed *L. monocytogenes* growth and survival on asparagus, broccoli, cauliflower, corn, green beans, lettuce, and radishes (Aytac et al. 2010; Lianou and Sofos 2007).

Recent product recalls reported by the United States Food and Drug Administration (USFDA) included romaine lettuce, spinach, apples, prepackaged salad mixes, cantaloupes, different sprout varieties, precut and packaged fruit salad, mushrooms, and onions. A recent lethal listeriosis outbreak in 2011 was caused by consumption of whole cantaloupe contaminated with *L. monocytogenes*. The fruit was distributed to 28 US states, and resulted in 146 illnesses, 30 deaths, and one miscarriage (CDC 2011; Olaimat and Holley 2012).

Salmonella. Salmonellosis is a leading cause of foodborne disease throughout the world. There are three recognized species of *Salmonella*, *S. enterica*, *S. bongori*, and *S. subterranea*n. Most of the 2500 identified serovars have the potential to infect a wide variety of animal species and humans (D'Aoust 2001; Hendriksen et al. 2011). *Salmonella* is a Gram-negative mesophilic bacterium that grows rapidly between 25 and 43 °C, and has the capacity to grow under refrigerated conditions (4–10 °C), although members of the genus are typically sensitive to temperatures above 55 °C (D'Aoust 2001).

Salmonella is frequently present in the gastrointestinal tracts of cattle, pigs, poultry, rodents, turtles, fish, and other animal species, and is transferred to humans via the food chain. Fresh fruits and vegetables, including fresh tomatoes, lettuce, alfalfa, melons, unpasteurized juices, and ciders have been associated with *Salmonella* infections in humans. *Salmonella* can survive in nuts or low-Aw (water activity) foods for long periods of time. Furthermore, *Salmonella* survival and growth has been reported in acid foods, such as apple juice and tomato core tissue (Goverd et al. 1979; Shachar and Yaron 2006).

S. enterica is implicated in two main clinical syndromes in humans: enterocolitis and enteric fever. Self-limited enterocolitis is the most common and characteristic disease caused by *S. enterica* in humans. Disease symptoms appear 8–72 h following exposure to non-typhoid salmonellae with remission within 4–5 days following

disease onset. Severe abdominal pain, diarrhea, vomiting, and fever typically characterize enterocolitis. Following exposure and invasion by *S*. Typhi or *S*. Paratyphi to human host tissues, enteric fever, an acute gastrointestinal disease originates. The individual experiences watery diarrhea, fever, nausea, and abdominal pain 7–28 days following exposure to the infectious agent (D'Aoust 2001).

Shigella. *Shigella* are Gram-negative, nonmotile, nonspore forming rod-shaped bacteria. The genus is divided into the following four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei. Shigella* caused dysentery is a common infectious disease in developing countries, and in travelers to tropical countries. Several foodborne shigellosis outbreaks have been associated with the consumption of *Shigella*-contaminated vegetable products, including lettuce, parsley, green onion, cilantro, unpasteurized orange juice, salads, and dips (Zaika 2002).

Shigella sp. survive at low pH for several hours, and in acidic foods for extended periods; for example, *S. flexneri* survives at 48 °C for at least 11 days in carrot salad (pH 2.2–2.9), potato salad (pH 3.3–4.4), and coleslaw (pH 4.1–4.2) (Zaika 2002). After a low infective dose (10–100 cells), *Shigella* can cause acute inflammatory colitis, which in its severe form is characterized by intestinal cramps, bloody diarrhea (dysentery), intense headache, and convulsions. During outbreaks in developing countries, the severe form can lead to a 10–30 % mortality rate in children under 5 years of age.

Other Pathogenic Bacterial Species. *Bacillus cereus*, *Staphylococcus aureus*, and *Yersinia* sp. cause illness by fruit and vegetable consumption. ***B. cereus*** is ubiquitous in nature, and is an opportunistic pathogen. Two forms of human food poisoning are often associated with *B. cereus* due to the production of different toxins, and disease is characterized by diarrhea and abdominal distress, or nausea and vomiting (Dierick et al. 2005; Rasko et al. 2005). The bacterium exhibits enhanced resistance and survival in soil, dust, water, and diverse foods due to the production of endospores. Its presence in spices, raw vegetables, and salad dressings has been reported (Dierick et al. 2005; Rasko et al. 2005; Rajkovic et al. 2006). ***S. aureus*** is a ubiquitous bacterium, which produces heat-stable enterotoxins that contribute to foodborne poisonings worldwide (Argudín et al. 2010; Dinges et al. 2000). A broad variety of foods support growth of the enterotoxigenic staphylococci. Foods are contaminated during preparation, and the toxin will form if these foods are subsequently mishandled prior to consumption. *S. aureus* has been isolated from the following foods: potato salad, canned mushrooms, carrots, lettuce, parsley, radishes, seed sprouts, and salad greens (Argudín et al. 2010; Beuchat 1996). ***Yersinia*** is a genus of Gram-negative, facultative anaerobic foodborne pathogenic species, which have the capacity to grow under refrigerated conditions. *Yersinia* is widely distributed in nature and in animal hosts. The disease can range from a self-limiting gastroenteritis to a potentially fatal septicemia (Fredriksson-Ahomaa 2009). Pork and pork products are considered the primary vehicles for *Y. enterocolitica* infection; however, drinking water and raw vegetables have also been reported (Fredriksson-Ahomaa 2009; MacDonald et al. 2011).

3.5 Pathogenic Protozoa

Cryptosporidium. *C. parvum* and *C. hominis* are parasitic protozoa causing disease in humans. The oocyst, which is the transmissible stage of *C. parvum* is found in human and animal feces, and contaminates surface water (Kniel and Jenkins 2005). *Cryptosporidium* infections are common in livestock animals, particularly cattle and sheep, although pigs, goats, and horses are also infected.

Cryptosporidiosis outbreaks have been associated with different vegetables, including apple cider, basil, and green onions (Casemore 1990; Yoder et al. 2012). Ingestion of just a few oocysts can result in acute gastrointestinal illness that lasts 1–2 weeks in healthy individuals, or indefinitely in immunocompromised individuals. Following approximately 3–11 days, clinical manifestations appear, and range from asymptomatic infections to severe, life-threatening illness. Symptoms include watery diarrhea, vomiting, fever, nausea, anorexia, malaise, abdominal pain, and weight-loss (Casemore 1990; Yoder et al. 2012).

Cyclospora. *Cyclospora cayetanensisis* is a protozoan found in humans that causes cyclosporiasis (Erickson and Ortega 2006). Ingestion of contaminated food or water results in disease. Fruits and vegetables including (but not limited to) fresh raspberries, lettuce, and basil contaminated with feces of sick individuals are potential sources of infection (Werker 1997; Erickson and Ortega 2006).

Ingested *Cyclospora* in the form of oocysts excyst in the gastrointestinal tract freeing the sporozoites, which invade the epithelial cells of the small intestine (Erickson and Ortega 2006). Cyclosporiasis includes watery stools, flu-like symptoms, and other gastrointestinal disorders, e.g. flatulence and burping. In untreated patients, symptoms may last from few days to a month or longer (Werker 1997).

Giardia lamblia. *G. lamblia* is a flagellated pathogenic protozoan with human specificity. Giardiasis is possibly the most common human parasitic infection worldwide (Kucik et al. 2004; Casemore 1990). It is a pear-shaped, binucleate, flagellated organism with trophic (feeding) and cystic (resting) stages. Infected persons can excrete cysts intermittently in the stools for weeks or months. Cysts are detected in sewage effluents, surface waters, and some potable water supplies.

Cases of food-associated outbreaks have been linked with consumption of *Giardia* cysts in lettuce, carrots, cucumbers, green onions, tomatoes, fruit salads, and other crops (Casemore 1990; Mota et al. 2009). Infection results after ingestion of at least 10–25 cysts in contaminated water or food, or person-to-person contact. Giardiasis may be asymptomatic or with nausea, vomiting, malaise, flatulence, abdominal cramps, diarrhea, steatorrhea, fatigue, and weight loss (Casemore 1990).

3.6 Viral Contaminants

Norovirus. Noroviruses (NoV) are a genetically diverse group of single-stranded RNA. NoVs are small, non-enveloped, spherical viruses that are the most widely recognized causative agent of food- and waterborne viral gastroenteritis outbreaks

(Baert et al. 2011; Koopmans et al. 2002). Foodborne disease due to NoVs have been associated with consumption of fresh fruits (or frozen fresh fruits), such as grapes, raspberries, and strawberries; and prepared foods, including prepackaged salads, sandwiches, and cold foods (Koopmans et al. 2002; Chancellor et al. 2006).

Fewer than 100 viral particles and an incubation period of 1–3 days may develop into fever, diarrhea, nausea, vomiting, and headache in infected individuals. A large viral particle count is found in stools and vomitus. The illness is generally considered mild and self-limiting, lasting 1–3 days. However, NoV infections are highly contagious, resulting in a high transmission rate (Koopmans et al. 2002; Chancellor et al. 2006).

NoV contamination sources include infected food handlers, food preparation surfaces, and crop irrigation and fertilization with animal manure sewage containing NoV (Baert et al. 2011). Infected food handlers can transmit infectious viruses during the viral incubation period, and following recovery from illness (Chancellor et al. 2006).

Hepatitis A and E viruses. Hepatitis A (HAV) and E (HEV) viruses cause hepatitis in humans. HAV is the source of hepatitis A, which is a common form of acute viral hepatitis in many parts of the world, and rarely results in human death (Koopmans and Duizer 2004). Hepatitis A is an acute liver infection, with fever, nausea, headache, abdominal discomfort, and jaundice. The virus enters via the intestinal tract, and is transported to the liver where it is shed through the bile (Koopmans et al. 2002). The illness is relatively self-limiting, although it lasts up to several months. Typically hepatitis A among children younger than 6 years of age is asymptomatic, and the children rarely jaundice. However, among older children and adults, infection is usually symptomatic with jaundice (Koopmans et al. 2002; Chancellor et al. 2006).

The primary source for HEV infection is fecally polluted water that contaminates fruits and vegetables (Koopmans et al. 2002). HAV is transmitted by the fecal–oral route, either by direct contact with an infected person, or by ingestion of contaminated food (Chancellor et al. 2006). Currently, food contaminated by an infected food-service worker is the primary cause of foodborne outbreaks (Chancellor et al. 2006).

3.7 Trade, Outbreaks, and Cooperation

The number of documented disease outbreaks with the source of illness consumption of contaminated produce has increased in recent years. Several factors have contributed to this situation; globalization of the produce industry, new methodologies to detect microorganisms, improved surveillance, new healthy diet trends, and higher consumer income levels. However, several countries do not possess surveillance programs, and have deficiencies in their agricultural practices. Even with safe and well-established agricultural practices, accidents do occur. Therefore, contaminated vegetables produced in one country can affect other countries due to international trade.

Table 3.2 Selected recent multinational foodborne outbreaks due to contaminated produce items

Year	Pathogen	No. of cases	No. of countries	Affected regions	Implicated food
2011	*Escherichia coli* O104:H4	3842	6	Europe/North America	Fenugreek seeds (sprouts)
2008	*Salmonella* Saintpaul	1442	2	North America	Fresh peppers, tomatoes
2007	*Salmonella* Senftenberg	51	5	Europe, North America	Fresh basil
2007	*Shigella sonnei*	175	2	Australia, Europe	Raw baby com
2007	*Salmonella* Weltervreden	45	3	Europe	Alfalfa sprouts
2006	*Escherichia coli* 0157:H7	206	2	North America	Fresh spinach
2006	*Salmonella* Thompson	20+	3	Europe	Ruccola (arugula)

Modified from Lynch et al. Epidemiol. Infect. (2009), 137, 307–315

Several examples of multinational outbreaks have been reported resulting from commercial distribution of a contaminated product. In recent years, various multinational outbreaks have occurred (Table 3.2). As examples, the following outbreak summaries provide an overview of the extent of produce trade globalization, the vital importance of international communication and cooperation to limit and halt outbreaks, characterize isolates, identify the implicated vehicle(s), and properly treat patients.

In 2004, a multinational outbreak associated with imported Italian rucola lettuce occurred in Sweden, Norway, and the United Kingdom (Nygård et al. 2008). The outbreak was caused by *Salmonella* serotype Thompson, and resulted in at least 21 reported cases. *Salmonella* Thompson isolates from products and patients exhibited high similarity shown by pulsed-field gel electrophoresis, but significant differences were detected in some isolates. Other pathogens were identified, indicating a massive contamination, possibly caused by irrigation with nonpotable water.

In 2007, an outbreak of *Salmonella* serotype Weltevreden infections occurred in Norway, Denmark, and Finland associated with alfalfa sprouts from a Danish producer. The seed batch used in Denmark and Norway was traded, via retailers in Germany and the Netherlands to Denmark, and likely originated from Italy (Emberland et al. 2007).

An outbreak of *S. sonnei* infections occurred in Denmark and Australia associated with imported baby corn. Epidemiological and food trace-back analyses strongly implicated baby corn from one packing shed in Thailand. The supplier, which provided baby corn to Denmark and Australia, had deficiencies in hygienic practices in the supply chain. More than 215 cases were laboratory-confirmed in Denmark, and 12 in Australia (Lewis et al. 2007).

In January through June 2007, England, Wales, Denmark, the Netherlands, and the United States were affected by an outbreak of *Salmonella* serotype Senftenberg

after consuming fresh basil. At least 51 cases were recorded, and basil imported from Israel tested positive for the suspected strain (Pezzoli et al. 2008).

In 2008, a multistate outbreak in the United States and Canada was caused by *Salmonella* serotype Saintpaul. Among the 1500 case subjects, 21 % were hospitalized, and two died. Jalapeño peppers were implicated in the outbreak, but serrano peppers and tomatoes were also suspected. The outbreak strain was detected in jalapeño peppers collected in Texas, and in water and serrano peppers on a Mexican farm. Tomato tracebacks did not converge on a specific source (Barton-Behravesh et al. 2011).

The second largest documented foodborne *E. coli* outbreak in history occurred in May 2011. An EHEC outbreak originated in Germany, and was the source of at least 3368 cases, including 818 HUS cases and 36 deaths. Thirteen European Union Member States, the United States, and Canada were each impacted by the outbreak. More than 95 % of cases were reported from Germany. The EHEC strain O104:H4 was identified as the causative agent of the infection outbreak. This strain, although previously detected in humans, was not related to other documented EHEC outbreaks (Struelens et al. 2011). A high probability existed that the infection vehicle was sprouts from an organic farm in Germany that used fenugreek seeds imported from Egypt, which were contaminated by EHEC O104:H4 (Brzuszkiewicz et al. 2011; Struelens et al. 2011).

3.8 Microbial Ecology and Contamination

Currently, limitations in our understanding of foodborne pathogen introduction routes onto produce limit the application of suitable interventions to control contamination. Furthermore, at the grower's level, each specific farm has a high number of variables to address, including crop type, location, region, farm worker behavior, irrigation type, among other factors. These are all challenges to establish a viable intervention system (Brandl 2006; Olaimat and Holley 2012).

Produce contamination can occur at various steps from farm to table. In particular, preharvest and harvest contamination is associated with an increasing number of foodborne pathogen outbreaks (Brandl 2006; Olaimat and Holley 2012). The primary plant contamination sources at preharvest and harvest include contaminated soil, poor water quality, animal fecal droppings (e.g. cattle, birds, reptiles, and domestic animals), poor worker hygiene, inadequately composted or raw animal manure or sewage, and insect infestations from fields adjacent to cattle rangeland. As expected, not all these possibilities apply to a particular farm or crop. These depend on the farm characteristics (Brandl 2006; Olaimat and Holley 2012).

Contaminated soil. Pathogens contaminate soils in a variety of ways; contaminated irrigation water, fecal droppings, use of animal manure as fertilizer, discarded sewage, among others are potential sources (Heaton and Jones 2008; Olaimat and Holley 2012). Pathogen viability in soil has an impact on produce contamination. *E. coli* O157:H7 and *Salmonella* persist in soils for extended periods of time when introduced by compost or irrigation water. *S.* Typhimurium inoculated in irrigation

water survived for 203 days in soil post application (Islam et al. 2004). The longer a pathogen survives in soil, the higher the probability of produce contamination, particularly if survival occurs near harvest (Heaton and Jones 2008).

Water quality. Primary sources of irrigation water include rain, rivers, streams, reservoirs, springs and well water. Water from rivers and reservoirs is susceptible to contamination with wild animal or grazing cattle feces, flood and runoff from nearby farms, overflow of animal lagoons, and leakage of septic tanks or sewage pipes (Brandl 2006; Heaton and Jones 2008). *E. coli* and *S. enterica* also survive well in water sediments; therefore flooding fields with overflowing contaminated water poses a risk (Brandl 2006).

Some regions, particularly arid areas and in the developing world, use untreated wastewater for irrigation. Care must be taken to avoid cultivating leafy greens and fruits in these regions. Wastewater used to irrigate various crops has been the cause of outbreaks (Heaton and Jones 2008).

Worker hygiene. Foodborne pathogens are common in human intestines, skin, and excretions. Individuals suffering from foodborne disease are a high risk of transmitting foodborne illness. Many human enteric pathogens are shed in human feces, and are relatively stable in the environment (Cliver 2009). Other sources of pathogen release and potential transmission to foods include nasopharyngeal secretions, respiratory inhalation (vomitus aerosolization), and fomites. Pathogens can be transferred to foods via a handler's unwashed fecally contaminated hands (Cliver 2009; Greig et al. 2007; Todd et al. 2007). Although ill workers must stop handling food, this situation can often be difficult to control. Employees are often afraid to be fired if they reveal their condition, they do not understand the risk to the public or their cohorts, or there is no one to replace them on the job. In addition, poor hygiene practices by field workers are exacerbated by a lack of toilet rooms and on-site sanitation facilities.

On the other hand, asymptomatic infected workers excrete pathogens unknowingly, and consequently risk contaminating food. A food service to oversee worker health and food handling regulations can prevent asymptomatic spread of infection among workers (Todd et al. 2007). According to the Centers for Disease Control and Prevention (CDC 2011), approximately 30 % of NoV infections are asymptomatic, with the infected individuals actively shedding the virus while appearing healthy, and continuing to spread infection in outbreak areas (Li et al. 2012; Todd et al. 2007; Tuan Zainazor et al. 2010).

Animal manure. Manure is commonly applied to agricultural fields as a means of improving soil chemical and physical properties, and provides nutrients for plant growth (Brandl 2006). Manure provides an economic and practical solution for improving soil quality; however, the use of these organic fertilizers can introduce pathogens directly to the field (Heaton and Jones 2008). Manure may contain pathogenic bacteria, including *E. coli*, *Salmonella* sp., *Campylobacter* sp., NoV, and hepatitis virus. Enteric pathogens survive long periods of time in manure, and contaminate crops in the field (Brandl 2006). Hepatitis E virus is sometimes transmitted to humans by swine manure (Cliver 2009). *E. coli* and *Salmonella* sp. from raw manure are capable of colonizing the root zone and above ground plant parts (Brandl 2006; Teplitski et al. 2009).

Comprehensive guidelines are available to growers that advise on sufficient composted waste treatment and correct application timing of composted manure to avoid or decrease the possibility of contamination by improperly composted manure. It has been suggested that raw manure can be applied to soils 120 days prior to harvesting a food crop with direct soil contact, or 90 days for crops with no soil contact. Some practices, such as the California Leafy Green Products Handler Marketing Agreement (LGMA) recommend that raw manure not be used for leafy green and lettuce production, and composted manure should not be applied following plant emergence (LGMA 2012; Wei and Kniel 2010).

Sewage. Similar to animal manure, use of other organic fertilizers, such as sewage sludge, introduces pathogens directly to the field. Although it is considered an economic and practical means to fertilize soil, wastewater may contain bacterial pathogens such as *Salmonella* sp., *Shigella* sp., *Yersinia* sp., *Vibrio cholerae*, *C. jejuni*, pathogenic *E. coli*, viruses, and parasites (Cliver 2009; Jacobsen and Bech 2012). Unfortunately, in many regions of the world, raw sewage application is a common fertilizer practice for any crop, regardless of existent regulations that prohibit its use (Cerna-Cortes et al. 2012).

Biosolids, which are nutrient-rich organic materials from domestic sewage treatment in a treatment facility, can be used to fertilize agricultural fields to raise crops. Biosolids result in significant improvements in crop growth and yield. Biosolids use reduces production costs and replenishes organic matter depleted over time. According to the US-EPA, class A biosolids contain no detectable pathogens. Class B biosolids are treated, but contain detectable levels of pathogens. In general, exceptional quality (Class A) biosolids applied in small quantities by the general public have no buffer requirements, crop type, crop harvesting, or site-access restrictions (USEPA 2012).

Class B biosolids are more often applied as fertilizers for feed and fiber crops. In some parts of the word, food safety practices do not allow the use of biosolids as soil amendments in leafy green production (LGMA 2012; Wei and Kniel 2010).

Postharvest contamination sources. After harvest, produce is subjected to a series of packing shed operations that potentially contribute to microbial contamination if unsuitable processing is conducted. Depending on crop types, and other factors prior to distribution, i.e. transport to packing sheds, handling, washing, chill tanks, selection, peeling, cutting, shredding, use of shipping ice, and packaging, produce has increased potential for contamination. Furthermore, produce is susceptible to contamination by contact with hands, equipment surfaces, utensils, and water (Doyle and Erickson 2008; León et al. 2009).

3.9 Microbial Ecology and Contamination

It has been shown that *Salmonella* and enterovirulent *E. coli* survive as plant-associated endo- or epiphytes (Brandl 2006; Teplitski et al. 2009). Enteric bacteria use cellulose and aggregative fimbriae to attach to plant surfaces. In field studies,

E. coli and *Salmonella* from raw manure colonize the root zone and above ground plant parts (Islam et al. 2004; Teplitski et al. 2009).

Bacterial biofilms are structured surface-associated actively dividing bacterial communities enclosed in an amorphous, extracellular matrix that adhere to biological or nonbiological surfaces, and are difficult to remove. Attachment and biofilm formation by *Salmonella* in different food environments increases resistance to environmental stresses, and some cells may not be eliminated during cleaning and disinfection procedures (Kim and Wei 2007). Therefore, the most effective disinfectants against bacterial cells in suspension may not be as effective when treating a cellular bacterial matrix biofilm (Xu et al. 2011). O'May and Tufenkji (2011) indicated that swarming is the most rapid bacterial mode of surface translocation, and enables accelerated colonization of nutrient-rich environments. This form of motility contributes to biofilm formation (Costerton et al. 1999). *Salmonella* rapidly attaches and colonizes plant tissues, and can reach large biofilm populations, similar to plant-associated bacteria (Barak et al. 2009; Patel and Sharma 2010). Biofilm formation has been involved in *Salmonella* colonization and persistence in leafy greens (Patel and Sharma 2010). Barak et al. (2009) reported swarming motility was also involved in plant colonization, independent of biofilm formation.

Jeter and Matthysse (2005) demonstrated that cellulose and aggregative fimbriae were required for *E. coli* or *Salmonella* attachment to plant seedlings. *E. coli* O157:H7 can form colonies around and inside leaf stomata. *Salmonella* also grows in the soil rhizosphere, at lateral root emergence sites (Dong et al. 2003). Various reports have demonstrated pathogen internalization into cavities or plant tissues; however, the consequences of this process as a cause of disease remains to be determined. Deering et al. (2012) reported that bacterial internalization into plant cavities or tissues can be achieved via the following two means: (1) bacteria enter through natural openings in the plant surface (e.g. stomata, lenticels, and lateral root emergence sites), and/or through physical damage sites, and (2) bacteria accompany water via xylem tissue into internal plant tissues. For example, washing produce under conditions where a differential temperature exists between the produce and the contaminated wash water facilitates the internalization of pathogens into tissues (Penteado et al. 2004). Exposure of a warm fruit or vegetable to cold water causes the internal cavities to contract, capture, and absorb water and associated pathogens into the fruit or vegetable. Internalized pathogens are consequently protected from washing and sanitizing procedures used to eliminate or decrease the microbial load.

Enteric viruses can survive on the fruit or vegetable surface for weeks, depending on environmental conditions (i.e. temperature and moisture) (Cliver 2009). HAV exhibited increased viability under dry conditions on lettuce leaf surfaces (Stine et al. 2005), and NoV surrogates survived more than 6 months in frozen onion and spinach (Baert et al. 2008). Survival in soil and water are other means to maintain produce contamination sources. Murine NoV and HAV have been shown to maintain infectivity in manure and biosolids following months of storage, and Human NoV persists and remains viable in water for months (Cliver 2009; Wei and Kniel 2010).

3.10 Control and Prevention

Mechanisms to control and prevent produce contamination with microbial pathogens require measures during the production chain. The following should be standard policy: agricultural practices designed to reduce contamination by pathogenic agents, good hygienic practices with education and standards set for food handlers, good manufacturing practices, and the Hazard Analysis and Critical Control Point (HACCP) system, which set expected food safety standards required by a general legislation in most countries (EC 2004, 2005).

Food handler/worker instruction on good hygienic habits is vital. This must be performed in the workers' native language to facilitate a thorough understanding. Exclusion of suspected or ill food handlers, and maintenance of strict personal hygiene and good handling practices are required to minimize contamination risks (Koopmans et al. 2002; Chancellor et al. 2006). Antibacterial soap minimizes pathogen transfer (Fischler et al. 2007), but it requires that all handlers commit to proper hygiene. Good handling practices are critical in preventing foodborne infections, and include frequent hand washing and wearing gloves (Koopmans et al. 2002; Chancellor et al. 2006).

Testing irrigation water for coliform bacteria characteristics can prevent contamination. However, this is not typically undertaken in many parts of the world, and contaminated irrigation waters continue to be used, and are often the only irrigation source. Therefore, produce washing procedures are designed to remove soil at harvest, and aid in preventing cross-contamination between each harvest batch. Special care regarding the microbiological water quality used to wash vegetables, fruits, or other kinds of foods, and appropriate conditions for storage or distribution must be applied (Chan and Blaschek 2005). Washing fresh fruit and vegetables using potable water reduces the likelihood of pathogen transmission. Chemicals, including chlorine, T-128 (phosphoric acid + propylene glycol), Tsunami 100 (peroxyacetic acid), and other intervention measures have been examined for their efficacy in eliminating pathogenic bacteria from different food products. Chlorine is the most common chemical applied in the food industry for disinfecting fresh produce (Nou et al. 2011); however, chlorine and other sanitizers cannot completely remove or inactivate microorganisms. Chlorine (200 ppm) reportedly removes approximately 1.5–2 logs of background or pathogenic microflora on lettuce, cilantro, and parsley (Foley et al. 2004; Beuchat et al. 1998). Due to economic and practical characteristics, these synthetic additives have been widely employed by the food industry; however, consumer trends have shifted toward fewer synthetic food additives, so that products can be consumed in a more natural or all-natural state (Conte et al. 2007). Consequently, natural products such as essential oils have been explored with similar efficacy (Burt 2004). The majority of the essential oils are classified as "generally recognized as safe" (GRAS), and together with other edible plant extracts, can be used as alternatives to control or decrease pathogen loads in produce (Orue et al. 2013).

If contamination is detected, tracking contamination is an integral step to avoid future recontamination. In addition to the current fecal indicator bacteria (FIB) used to determine fecal pollution and to predict the risk of pathogenic microorganisms in aquatic environments, microbial source tracking is an emerging tool developed to detect fecal pollution sources in water (Tambalo et al. 2012). It involves detection of the Order Bacterioidales, which is a taxonomic order of environmental bacteria comprised of four families (the *Bacteroidaceae*, *the Prevotellaceae*, the *Porphyromonaceae*, and the *Rikenellaceae*) with a high degree of host specificity, and abundant in human and animal feces. Bacterioidales detection and DNA analyses of the group could provide data regarding the contamination source, and appropriate measures to stop contamination could be implemented (Tambalo et al. 2012). The application of this tool for use in vegetables (Ravaliya et al. 2014), or the choice of other bacteria, i.e. *Faecalibacterium*, is currently under study (Shen et al. 2012; Zheng et al. 2009).

3.11 Conclusion

Worldwide produce consumption has increased due to more availability, diversity, affordability, international trade, electronic communication, urbanization, improved transportation, public education and health trends, and improvements in agricultural practices. The WHO and FAO joint initiative to increase human fruit and vegetable consumption worldwide, which serves to reduce risk factors for global mortality, will heighten consumer safe crop demands.

Contaminated produce consumption would obviously abolish health benefits, and pose health risks. Produce can be contaminated with foodborne pathogens at various points during production, handling, and packing processes. Leafy greens, tomatoes, cucurbits, peppers, and nuts are among the foods commonly associated with outbreaks of gastrointestinal illnesses. At a global level, *B. cereus*, *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes*, *S. enterica*, *Shigella* sp., *S. aureus*, *Yersinia* sp., *C. cayetanensis*, *G. lamblia*, *C. parvum*; Hepatitis A virus (HAV), and Noroviruses (NoV) are the most common and important pathogens involved in outbreaks resulting from contaminated produce consumption.

Because the number of outbreaks due to contaminated produce consumption has increased in recent years, the key factors responsible for these outbreaks need to be identified. Currently, limitations in understanding foodborne pathogen entry routes onto produce limit the application of proper intervention to control contamination. Furthermore, the high number of variables involved in each particular farm, including crop type, location, region, farm worker behavior, and irrigation type, among others complicate this problem. Each farm is unique, and must be characterized to devise an appropriate intervention strategy.

Produce preharvest and harvest contamination are associated with a large number of foodborne pathogen outbreaks. Irrigation water from rivers and reservoirs are susceptible to contamination by wild animal and grazing cattle feces, flood and run-

off from nearby farms, animal waste lagoon overflow, and septic tank and sewage pipe leakage. Some regions, particularly arid areas and those in the developing world, use untreated wastewater for irrigation, and this enhances polluted water problems. Soil can be contaminated with irrigation water, fecal droppings, and the use of improperly composted manure or sewage. Many pathogens can maintain long-term viability in soil, and contribute to produce contamination.

Pathogens can be transmitted to foods by direct contact of a food handler's fecal contaminated unwashed hands, or contact with equipment surfaces, utensils, and water. Poor hygienic practices by field-workers, asymptomatic infected workers excreting a pathogen unknowingly, the absence of toilet rooms and on-site sanitation facilities, are all risks associated with produce contamination.

Pathogen biofilm formation is a novel evolutionary adaptation. The capacity to adhere, survive, and reproduce on different food environments, increased environmental stress tolerance, and resistance to cleaning and disinfection procedures has favored biofilms.

Mechanisms to control and prevent produce contaminated with microbial pathogens require measures during the production chain. Agricultural practices designed to reduce contamination by pathogenic agents, good hygienic practices with education and standards set for food handlers, anti-microbial manufacturing practices, and the Hazard Analysis and Critical Control Point (HACCP) system should be implemented. Furthermore, quality controls systems that determine the efficacy of these practices and corrective actions, if necessary, should be put into action.

If contamination has been detected, contamination tracking is an integral step to avoid future recontamination. International produce trade is a growing industry, and many countries have deficiencies in produce growing practices. Even under the best of conditions, accidents can and do occur. Consequently, contaminated fruit and vegetables produced in one country will affect other countries. Communication and cooperation between government agencies is vital to prevent, detect, and control risks or outbreaks due to contaminated produce.

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Chapter 4
Ensuring Food Safety in Developing and Developed Countries: Aspects Associated with the Use of Veterinary Drugs in Fish Farming in Brazil

Felix G.R. Reyes and Katia S.D. Nunes

4.1 Introduction

Food safety is a public health issue and microbiological and chemical contamination of food is an important cause of disease. In addition to improving public health, effective food safety systems are also essential to maintain consumer confidence in the food system, as well as to provide a sound basis for regulating the domestic and international trade of food, which serves to support economic development (FAO/WHO 1991).

It is worth mentioning that food safety is the responsibility of everyone involved in the food chain. However, governments are responsible for providing an institutional and regulatory environment for the control of food. In this regard, developed countries have well-defined procedures for assessing, managing, and communicating risks related to food safety, from which best practices can be derived aiming at food safety (Barlow et al. 2002). On the other hand, developing countries are taking steps to improve and strengthen their food safety systems, based mainly on the guidelines of the Codex Alimentarius Commission (CAC) (FAO/WHO 2005a, 2006b; Hanak et al. 2002). Furthermore, the Codex standards for food safety are recognized by the World Trade Organization (WTO) on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and the Agreement on Technical Barriers to Trade (TBT Agreement). Thus, adopting Codex international standards as a basis for national regulations, which is recommended, helps to harmonize global application of food safety measures.

The CAC is supported in its decisions on the principles of risk assessment (FAO 2000; FAO/WHO 2008). The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have played a leadership

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role in the development of risk analysis in food safety, which has demonstrated its capacity to improve the processes of decision making regarding food safety and the development of improvements in public health. Nevertheless, it should be mentioned that the paradigm of risk analysis is only part of an effective food safety system (FAO/WHO 2005b). Other aspects that must be considered include:

- Food safety policies
- Food legislation (food law, regulations, and standards)
- Food inspection
- Laboratory analysis
- Epidemiological surveillance of food-borne diseases
- Monitoring systems information
- Education
- Communication

In addition to the growing concern over food safety, there has also been an increase in consumer preference for healthier food (FAO 2012). In this respect, in search of a healthier diet with appropriate nutritional profile, demand for fish consumption is increasing worldwide. Therefore, an area of great interest in the production of food of animal origin is fish farming, which has experienced significant development since the 1990s. Approximately 40 % of the world's fish production comes from fish farming, and most intensive production systems of fish are established in Asian countries. In these countries, antimicrobials are widely used in aquaculture, and few data on the type and quantity of veterinary drugs used are available. In addition, regulations on the use of antimicrobial agents in this sector are limited or do not exist in developing countries that have high activity in aquaculture. Thus, various authors have warned about the potential risk to human health from the use of veterinary drugs in this sector (Serrano 2005; Torres et al. 2010).

Complicating factors in the practice of aquaculture are the substantial differences between developed and developing countries, making it difficult to generalize findings and information taking into account the two groups. Moreover, while most of the global aquaculture production is in developing countries, the limited data currently available regarding the impact of fish farming in food and environmental safety are generated by developed countries (Sapkota et al. 2008).

In this chapter, aspects of risk assessment related to veterinary drugs are presented. In particular, aspects associated with the current status of the use of veterinary drugs in fish farming, with emphasis on Brazil, will be addressed.

4.2 Risk Assessment

As defined by the CAC, risk assessment is a component of the risk analysis paradigm, which is a process that provides information about the dangers in foods that could be directly related to the data on the risk to human health. In this regard, risk analysis

Fig. 4.1 Components of risk analysis (FAO/WHO 1997)

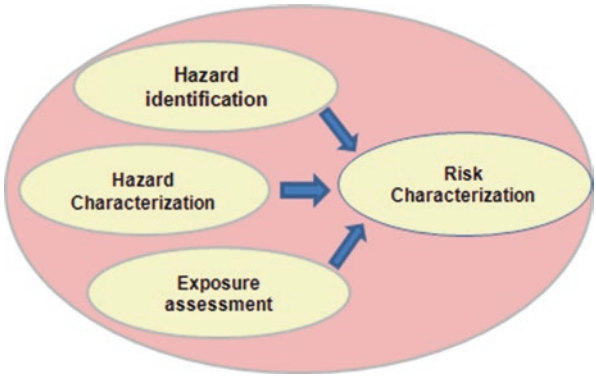

Fig. 4.2 Steps of risk assessment (FAO/WHO 1997)

is a process consisting of three components: assessment, management, and communication of risk (FAO/WHO 1995, 2005b) (Fig. 4.1).

Risk assessment is the stage in which toxicological data available are evaluated to assess the safety of chemicals in food. Known or potential adverse effects resulting from human exposure to food-borne hazards are scientifically evaluated. Thus, risk assessment provides a scientific basis for decisions that may be taken in the stage of risk management necessary to protect human health, and also takes into consideration all relevant scientific data (in the case of veterinary drug residues in foods this includes toxicological, pharmacological, and microbiological data), as well as identifies uncertainties inherent to the food safety evaluation. It consists of four steps: hazard identification, hazard characterization, exposure assessment and risk characterization (Fig. 4.2) (FAO/WHO 1995, 2005b).

Assessment of risk to human health resulting from exposure to veterinary drug residues in foods is part of the central activity performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO 1999). As a result of the evaluation of all relevant data available to the committee, JECFA set health-based guidance values, such as the Acceptable Daily Intake (ADI), which is a level of exposure to a substance considered to be "without appreciable health risk." The setting of

this parameter provides quantitative information of risk assessment used for risk management (IPCS 2009). ADI is defined as the amount of a substance, expressed in mg/kg body weight and as a range of zero to an upper limit, which may be ingested daily over a lifetime, without damage to human health, based on toxicological data available at the time of the evaluation (Vettorazzi 2001).

Also, to protect consumers against possible risks associated with the presence of veterinary drug residues in products of animal origin, maximum residue levels (MRLs) are established to ensure the quality and safety of consumer products. Residues of veterinary drugs include the parent compounds and/or their metabolites in any edible portion of the animal product, and include residues of associated impurities of the veterinary drug concerned.

The MRL for veterinary drugs is defined by CAC as the maximum concentration of residue resulting from the use of a veterinary drug (expressed in mg/kg or μg/kg on a fresh weight basis) that is recommended by the CAC to be legally permitted or recognized as acceptable in or on a food (CAC 2012). MRLs are established for all veterinary drugs approved for use in animals intended to produce foods that when ingested by humans pose no health risks, and they are differentiated between animal species and tissues (JECFA 2007).

The MRL is based on the type and amount of residue considered to be without any toxicological, pharmacological, or microbiological hazard for human health as expressed by the ADI, or on the basis of a temporary ADI that utilizes an additional uncertainty factor. It also takes into account other relevant public health risks as well as food technological aspects and estimated food intakes (Vettorazzi 2001). When establishing MRL, consideration is also given to residues that are present in foods of plant origin and/or the environment. Furthermore, the MRL may be reduced to be consistent with good practices in the use of veterinary drugs (GPVD) and to the extent that practical analytical methods are available. GPVD is the officially recommended or authorized usage, including withdrawal periods, approved by national authorities of veterinary drugs under practical conditions (FAO/WHO 2006a).

Although MRLs are linked to the ADI there is some flexibility to recommend MRLs depending on factors such as consumption, veterinary use practices, and availability of suitable analytical methods for determining residues in food animal tissues. JECFA has devoted significant effort to analytical methods performance because of the strong role it has in recommending MRLs. Therefore, recommended MRLs may be reduced to more conservative values than full use of the ADI (FAO/WHO 2006a).

Regarding the impact on human health, intake of veterinary drug residues above the limit that is considered safe is an important public health issue because it can cause possible adverse effects, such as allergies and problems with human intestinal flora (FAO 2010; FAO/OIE/WHO 2006; Lunestad 1992). Thus, organizations such as the Codex Alimentarius and the European Medicines Agency (EMA) regulate the use of these substances in animal production.

4.3 Health-Based Guidance Values

Health-based guidance values (ADI) arise from dose–response evaluation for the most relevant adverse effect of concern (*endpoint*) on the more sensible species in preclinical studies conducted to determine potential hazards of a substance. The studies are mainly conducted in laboratory animals, and should be adequate to allow for determining a reference point for hazard characterization, also known as point of departure (POD), such as a no-observed-adverse-effect level (NOAEL: the highest concentration of a toxicant expressed as mg/kg body weight/day, which does not cause dysfunctions in animals) or, sometimes, the LOAEL (lowest observed adverse effect level) or a Benchmark dose (BMD—a dose that produces a low but measurable adverse response). For veterinary drug risk assessment the POD is derived from the toxicological, pharmacological, and microbiological data available at the time of the compound evaluation, and ADI values are only established for substances that produce adverse effects that exhibit a threshold of toxicity (IPCS 2009).

When calculating a health-based guidance value (ADI) an "uncertainty factor" is applied to the POD, in order to provide a conservative margin of safety due to uncertainties inherent in the extrapolation of toxicity data obtained with experimental animals for potential effects on humans, and due to variability among humans. In the derivation of "uncertainty factors" based on data, instead of using standard factors, the concept of adjustment factors specific to a chemical has been introduced to allow, when possible, the use of specific data on species differences, or human variability, in toxicokinetics or toxicodynamics (Renwick et al. 2003; IPCS 1994, 2009).

In setting the IDA of a veterinary drug, the toxicity of the parent compound and its major biotransformation products are considered. If a veterinary drug affects human intestinal microflora in exposures below those that cause toxicological effect, then this effect (*endpoint*) is used as the basis for the establishment of the ADI (Vettorazzi 2001).

4.4 Risk Characterization

In risk characterization of veterinary drugs, ADI values are compared with estimated or determined human exposure. This stage of risk assessment integrates information from hazard characterization and exposure assessment.

Where exposures exceed ADI values, this situation by itself does not allow inference of the extent of risk to those individuals exposed to these higher levels, since ADI values incorporate "uncertainty factors." Exposure that occasionally exceeds the ADI value does not necessarily imply that adverse health effects will occur in humans (IPCS 1987, 2009). The risk characterization considers both the uncertainty and the variability. Uncertainty refers to the limitations of the information available to the risk assessor about the data and models used. Variability reflects the inherent biological heterogeneity, either in exposure or in response. Uncertainty can

be reduced when the quantity or quality of the information is improved. The characterization of exposure variability via diet may be improved by better information, but the variability cannot be eliminated.

It should be emphasized that data on exposure to veterinary drug residues in food intended for human consumption are needed to characterize the risk that these compounds offer to human health. Nonetheless, in the case of fish and fish derivatives available to consumers, such data are not available in Brazil. In particular, data related to fish from "fish and pay" places are scarce.

4.5 Analytical Methods

Development of an analytical method for determination of veterinary drug residues in foods and biological matrices is essential to ensure that products are safe for consumer health. Thus, to guarantee attainment of adequate results, the method must go through a procedure called analytical validation, which aims to evaluate and verify whether the results are suitable for the intended analysis (Paschoal et al. 2008). Specific MRLs for each target analysis must be taken into account in establishing the protocol for validation of the analytical method to be used in determining these residues in any matrix.

In cases of prohibited or unauthorized substances without MRL standards, the European Community has established the Minimum Required Performance Limits (MRPLs), which is recognized as the minimum amount of a substance(s) present in a sample to be detected and confirmed by a determined analytical method (EC 2002).

Validated analytical methods are also necessary for determining the concentration of a veterinary drug and its biotransformation products in pharmacokinetic and toxicokinetic studies (IPCS 1987, 1990, 2009; Paschoal et al. 2008).

When necessary, the form of application and distribution of residues that result from each application mode of a veterinary drug should be determined. In the case of veterinary drugs, the depletion of residues in each species should be studied in the edible tissue. Furthermore, the consequence of processing of foods of animal origin on the veterinary drug residue level needs to be examined (FAO/WHO 1985; IPCS 2009).

Data generated in Brazil about development and validation of analytical methods for the determination of veterinary drug residues in foods are rarely available. Nonetheless, a special issue of the journal Food Additives and Contaminants (FAC) was dedicated to Brazil and to the strategies implemented by the Ministry of Agriculture, Livestock and Food Supply (MAPA), to tackle chemical food safety issues. This (FAC) issue features a selection of papers arising mainly from work conducted within laboratories connected to MAPA. The papers deal with the determination of chemicals, such as heavy metals, polycyclic aromatic hydrocarbons, phytosanitary products, mycotoxins, and veterinary drugs or dyes that are introduced into foods either as a result of their occurrence in the environment, natural infection by fungi, or other human activities (MAPA 2012).

Table 4.1 presents a list of papers related to work conducted in Brazil associated with the development and validation of analytical methods for the determination of antimicrobial residues in fish and determination of the withdrawal period under Brazilian environmental conditions. Although commercially available samples have not shown a positive result for the presence of the studied compounds, the number of samples analyzed was too low to draw a conclusion about the use of antimicrobials in fish farming in Brazil.

4.6 Fish Farming

China is the largest fish producer in the world, followed by Indonesia, India, and Peru. Nevertheless, Brazil has significant natural potential for aquaculture development. It has 7367 km of coastline and nine million hectares in water and hydroelectric reservoirs; the climate is predominantly tropical, which favors the production of several aquatic species, and concentrates, about, 12.8 % of the world's available surface freshwater. Brazil also has a significant extraction and industrial processing infrastructure, with fishing plants that are qualified according to the HACCP concept (Hazard Analysis and Critical Control Points). Thus, Brazil has the potential to become the largest fish producer in the world (Ostrensky et al. 2008).

In Brazil, fish farming presents the highest growth among producers of food of animal origin. According to FAO and the Brazilian Ministry of Fisheries and Aquaculture (MPA), in 2011 Brazil occupied the third place among American aquaculture producers, and Brazil's total annual fish farming production was 628,704 t, increasing by 31.1 % in relation to the production from 2010. In 2011 the continental fish production represented 86.6 % of total production, compared to 82.3 % in 2010, and 81.2 % in 2009. In contrast, marine aquaculture decreased compared to previous years (Table 4.2) (FAO 2012; MPA 2010, 2011).

The FAO recommends the consumption of fish due to its outstanding quality as a source of animal protein. In 2008, per capita world consumption of fish was 17.1 kg/year, with a projection of 22.5 kg/year by 2030, which represents more than 100 million tons/year. In 2010, according to data provided by the MPA (2010), per capita consumption of fish in Brazil was 9.75 kg/year, an increase of 8 % over the previous year. Thus, Brazil has an excellent opportunity for the expansion of its fish farming.

Brazilian aquaculture is mainly based on semi-intensive production regimes, and most fish culture is done in excavated ponds. Larvae and fingerlings are usually stocked in the ponds and fed during the entire cultivation period. It is also common to use fish cages installed in reservoirs and big ponds. According to MPA (2011), among exotic species, tilapia (*Oreochromis niloticus*) is the most cultivated in Brazilian continental fish farming (46.62 %), followed by Chinese and common carp (*C. carpio*, *C. idella*, *Hypophthalmichthys molitrix,* and *C. nobilis*) (6.99 %). Moreover, among native species, tambaqui (*Colossoma macropomum*) (20.40 %), pacu

Table 4.1 Work conducted in Brazil related to the development and validation of analytical method for the determination of veterinary drug residues in fish and determination of the withdrawal period under Brazilian environmental conditions

Title	Comments	References
Depletion study and estimation of the withdrawal period for enrofloxacin in pacu (*Piaractus mesopotamicus*)	The results allowed the estimation of a half-life for ENR of 2.75 days and a withdrawal period of 23 days. The results obtained in this study are important for the farming of pacu in tropical regions	Paschoal et al. (2013)
Determination and confirmation of chloramphenicol in honey, fish, and prawns by liquid chromatography–tandem mass spectrometry with minimum sample preparation: validation according to 2002/657/EC Directive	The method is suitable for the routine analysis in the National Residues and Contaminants Control Plan	Barreto et al. (2012)
Validation of an LC-MS/MS method for malachite green (MG), leucomalachite green (LMG), crystal violet (CV), and leucocrystal violet (LCV) residues in fish and shrimp	The findings demonstrate the suitability of the method to detect simultaneously MG, CV, and their metabolites (LMG and LCV) in fish and shrimp	Ascari et al. (2012)
A Simple Method for the Determination of Malachite Green and Leucomalachite Green Residues in Fish by a Modified QuEChERS Extraction and LC-MS/MS	Method attends the EC validation guide (EC 2002) which establishes that must attain, at least, a minimum required performance limit of 2 ng/g. Tilapia samples ($n = 20$) commercialized in Campinas, SP, Brazil were analyzed. None of them presented detectable levels of MG or LMG residues.	Hashimoto et al. (2012)
Depletion study and estimation of the withdrawal period for oxytetracycline in tilapia cultured in Brazil	The elimination half-life for OTC in tilapia fillet and the withdrawal period were 1.65 days and 6 days, respectively. Even though the study was carried out in the winter under practical conditions where water temperature varied, the results are similar to those obtained under controlled temperature.	Paschoal et al. (2012)
A high-throughput method for determining chloramphenicol residues in poultry, egg, shrimp, fish, swine, and bovine using LC-ESI-MS-MS	The advantages of the method include the short time to accomplish the analysis, and the extraction procedure is very effective, simple, and fast, with no further cleanup step. The method was sufficiently efficient for routine quality control operations. None of the fish samples analyzed ($n = 16$) presented levels of CAP above the LOQ (0.1 ng/g).	Siqueira et al. (2009)

(continued)

Table 4.1 (continued)

Title	Comments	References
Determination of quinolone residues in tilapias (*Orechromis niloticus*) by HPLC-FLD and LC-MS/MS QToF	The LOQ was below the MRL established by JECFA, which indicates that the method is appropriate for the determination of quinolones in fish fillet.	Paschoal et al. (2009a)
Quantitation and identity confirmation of quinolones residues in fish fillets by LC-ESI-MS/MS QtoF	The method is suitable for the determination of quinolone residues in fish fillets and the QToF technique made it possible to obtain *m*/*z* ratios with less than 10 ppm of error for each analyte.	Paschoal et al. (2009b)

LOQ = limit of quantification; MRL = maximum residue level; JECFA = FAO/WHO Joint Expert Committee on Food Additives

Table 4.2 Brazilian annual fish production (tons) from continental and marine aquaculture from 2008 to 2011

	2008		2009		2010	
Production	(t)	(%)	(t)	(%)	(t)	(%)
Continental	282,008	77.2	337,352	81.2	394.340	82.3
Marine	83,358	22.8	78,296	18.8	85.058	17.7
Total	365,366		415.648		479.398	

Source: MPA (2010, 2011)

(*Piaractus mesapotamicus*) (3.98 %), and tambacu hybrid (*Piaractus mesopotamicus male* × *Colossoma macropomum* female) (9.15 %) are becoming prominent.

The most common diseases affecting fish farming are caused by facultative pathogens. These diseases are especially common in fish submitted to chronic stress. The major causes of stress are directly related to inadequate handling practices, poor water quality, and low quality feeds that do not meet the nutritional requirements of the different species of fish. Thus, low quality feeds increase the chance of disease occurrence and high mortality rates. The use of high fish densities in cages is another factor of stress in such systems and facilitates the spread of pathogens, thus producing high mortality levels (Ostrensky et al. 2008). The degree of stress resulting from these conditions is characterized by modifications in biochemical, physiological, and behavioral mechanisms, with an increase in susceptibility to infectious processes caused by opportunistic pathogens such as viruses, bacteria, fungi, and parasites (Santos 2007). Bacterial diseases stand out as being identified as the main factors limiting productivity, with the most common diseases including columnaris (or gill necrosis), mobile septicaemia, vibrio, streptococcal (spiral swimming disease), edwardsiellosis, and visceral granuloma (Kubitza 2005a, b; Ranzani-Paiva et al. 1997).

Among environmental issues, it is important to consider the following factors: decrease in dissolved oxygen levels; increasing levels of carbon dioxide (CO_2), ammonia (NH_3), and nitrite (NO_2); sudden changes in temperature; excessive accumulation of organic material; and other physical–chemical changes in the water. Another important factor to consider is quick transportation of the fish, which facilitates the spread of disease when purchasing care and quarantine guidelines are not respected (Pavanelli et al. 2002; Rotta and Queiroz 2003).

Few studies have been carried out in Brazil with the objective of testing the efficiency and secondary effects of drugs used for the treatment of fish diseases, especially for fish produced in intensive culture systems (Pavanelli et al. 2002; Pizzolatti 2000). Furthermore, Souza (2003) affirmed that indiscriminate use of chemical products to control parasites in fish culture systems is an issue of great concern, and that these conditions still persist. As a matter of fact, in Brazil, few data are available on the monitoring of veterinary drug residues in fish and fish products. In 2006, a study was conducted based on the application of questionnaires to fish farmers in the Mogi-Guaçu (SP) hydrographic basin; obtained results showed that 13 of 84 fish farmers used malachite green on their properties for prophylaxis and/or disease treatment. Among other compounds mentioned, only calcium oxide (lime), pesticides (organophosphates, benzoylphenylurea, carbamates, and pyrethroids) and common salt, presented higher frequency of use when compared to malachite green (Santos 2007). Thus, this study indicates that fish farming therapy for the treatment of disease, control and prevention of infection to increase production efficiency is conducted without conformity, and that sound veterinary practices are not followed.

4.7 Use of Veterinary Drugs in Fish Farming

Inevitably, disease is present in all animal operations, and fish farming is no exception. All intensive animal production systems are favorable environments for the spread of disease due to the higher population density of animals, with the aggravating factor that in fish farming the aquatic environment favors the proliferation of pathogens. Intensification of this activity contributes to the spread of diseases, which is linked to inadequate management and poor environmental conditions in fish farming (Pavanelli et al. 2002; Roberts and Bullock 1980; Rotta and Queiroz 2003; Schalch et al. 2005).

In fish farming systems the use of veterinary drugs, such as antimicrobials, could be necessary under certain circumstances. However, globally, there is great concern about the indiscriminate use of antimicrobials in the livestock industry, especially regarding the issue of antimicrobial resistance and the potential impact on the animal production system and human and environmental health (OIE 2003; WHO 1998). In the animal production system, antimicrobial resistance can decrease the efficiency of veterinary drugs, thereby increasing the risk of economic loss. With respect to human health, several studies have shown that the use of antimicrobials in

aquaculture generates the appearance of resistant bacteria, and there is epidemiological and molecular evidence indicating that the genes that mediate this resistance can be transmitted from aquatic bacteria to bacteria capable of producing infections in humans and land animals (Cabello 2004; Serrano 2005; Sørum and L'Abee-Lund 2002; Sørum 1998).

In fish farming, veterinary drugs are used for the following purposes: therapeutic (the administration of medication to treat sick animals); and metaphylactic (the use of mass medication to eliminate or minimize an expected outbreak of a disease). In Brazil, due to the lack of alternative veterinary drugs approved for use, associated with the lack of scientific information about alternative treatments to control fish diseases, and the high cost of available drugs, there is the suspicion that fish farmers, and in particular "fish and pay" locals, make irregular use of veterinary drugs approved for other animal species, or even use illicit substances (Carneiro et al. 2005; FAO 2010; Hashimoto et al. 2011, 2012; MAPA 2012). The producers' attempt is to avoid economic losses. The improper and uncontrolled use of these products, however, increases the possibility of the presence of residues higher than the MRLs of these products, the risk of residues affecting the fish farming production, the environment, and consequently, human health. These residues are directly related to the issue of antimicrobial resistance (FAO 1997; FAO/SEAFDEC/CIDA 2000; OIE 2003; WHO 1998).

The development of microbial resistance is dependent on various circumstances. It is a proven fact that the bacterial strain that colonizes fish intestines may be more susceptible or resistant to antimicrobials such as oxytetracycline and quinolones (flumequine and oxolinic acid) depending on the aquatic environment (fresh or salt water) (FAO/RCAAP/OMS 1999).

Regarding the risk to human health through the development of microbial resistance in aquaculture, the FAO/OIE/WHO (2006) highlights two important aspects: (1) The development of antimicrobial resistance acquired by aquatic bacteria, which can be regarded as a direct spread to humans; (2) The development of microbial resistance, acquired in aquatic bacteria through bacteria that act as a reservoir of resistance genes that are disseminated to the pathogenic bacteria and then humans. This is considered as indirect spread to humans, caused by horizontal gene transfer.

Currently, there is evidence to indicate that the genes that mediate this resistance can be transmitted from aquatic bacteria to bacteria capable of producing infections in humans and land animals. This shows that there are no boundaries with respect to the flow of microbial resistance genes and that this phenomenon is global because the use of antimicrobials in one environment will impact other environments that are seemingly distant (Cabello 2004).

It must be mentioned that the use of antibiotics in aquaculture depends on local regulations, which varies widely between countries. As a result of a growing awareness that antibiotics should be used more carefully, there is a trend toward more restrictive regulations of these compounds in the aquaculture sector, and in the presence of antibiotic residues in food products originating from aquaculture (Serrano 2005). In some developed countries, regulations on the use of antibiotics

are severe and few compounds are licensed for use in aquaculture. However, a large portion of global aquaculture production is in developing countries that do not have, or have limited regulation regarding the use of veterinary drugs.

4.8 Regulatory Aspects of Veterinary Drugs

The establishment and application of MRL standards for veterinary drugs used in animal production is indispensable, and currently this is done by developed countries in relation to veterinary drugs used in fish farming. Regulations not only determine the types of antimicrobial agents that can be used, but they also specify the species for which it is intended, the diagnosis, the dosage and duration of treatment, as well as the interruption period to be observed between the last dosing and the slaughter (withdrawal period) when the veterinary drug (i.e. antimicrobial) is to be used as a therapeutic agent. Compliance with these conditions and regulations ensures that residues are below the established MRL standards, and the risk of pathogenic bacteria developing resistance is negligible or at least acceptable. In developed countries, the majority of approved antimicrobials are available by prescription only and under the supervision of a qualified professional (FAO 2002).

On the other hand, developing countries have adopted CAC recommendations to establish sanitary monitoring measures. As a member of Codex, the Brazilian Ministry of Health adheres to MRL standards for veterinary drugs established by CAC for food of animal origin. Nevertheless, in Brazil, some of the difficulties related to preventing and treating fish diseases is due to the deficiency of specific legislation and approved veterinary drugs for use by fish farmers. Currently, there are regulations for only three veterinary drugs: two antimicrobials (florfenicol and oxytetracycline) and a parasiticide based on trichlorfon (Fig. 4.3).

For inspection purposes, in case MRLs are not set by the CAC or by the Ministry of Health, MAPA uses those established in the Southern Common Market (MERCOSUR), or those adopted by the Commission of the European Union or by the US FDA. In this regard, MAPA created the National Plan for Control of Residues and Contaminants (PNCRC). PNCRC aims to verify the presence in foods of residues of chemical compounds potentially hazardous to consumer health, such as pesticides and veterinary drug residues, environmental contaminants, and inorganic contaminants. This plan is intended for products of vegetal (PNCRC/Vegetal) and animal origin (PNCRC/Animal). One of the food matrices evaluated under the PNCRC/Animal is fish produced by fish farmers (PNCRC/fish) (MAPA 1999).

One of the purposes of the PNCRC/Animal subprogram is to evaluate the “no compliance” to the MRL of the veterinary drugs, as well as the monitoring of the use of prohibited substances. Thus, PNCRC/Animal constitutes a tool of “risk management” with the primary objective of promoting quality assurance on the production system of foods of animal origin along the supply chains. One of the concepts adopted by PNCRC is the “equivalence of systems.” That is, products exported by Brazil must meet the requirements of quality and safety practiced by importing markets,

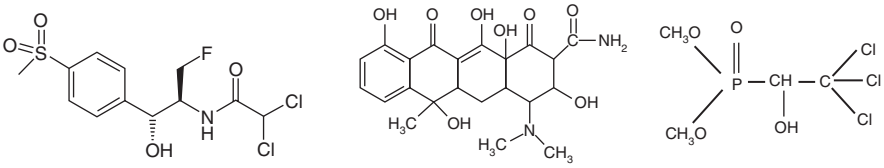

Fig. 4.3 Structures of veterinary drugs officially approves in Brazil for use in fish farming

according to the precepts of SPS (Sanitary and Phytosanitary Measures) and CAC parameters, in order to provide assurance and mutual recognition (MAPA 2013b).

In 2007 MAPA started to publish results obtained from the analyses conducted through PNCRC/fish. All data reported indicated residual absence of the determined antimicrobials, or of the food dye malachite green, in the analyzed samples (MAPA 2007, 2008, 2009, 2010, 2011, 2013a). However, it must be mentioned that the samples analyzed under the PNCRC/Animal are from establishments that have Federal Inspection Service (SIF). Nonetheless, a popular fish production system in Brazil, not only as a fish supply but for recreation purposes, is the so called "fish and pay" system. Due to the stressful condition of the fish produced at this system, and the fact that they are not inspected by SIF, there is the suspicion of nonconformity in the use of veterinary drugs, in particular antimicrobials, at those systems. Nevertheless, no data are available related to veterinary drug residues on fish from such "fish and pay" establishments.

In 2003, the Brazilian National Sanitary Surveillance Agency (ANVISA) created the National Program for the Analysis of Veterinary Drug Residues in Foods of Animal Origin (PAMVet). This program contemplates sample collection actions on the retail market and the determination of residues, with the objective of evaluating the exposure of the consumer to veterinary drugs through the consumption of foods of animal origin. In the initial schedule of the program, analyses of fish samples were planned for the fourth year of activity after its implementation (ANVISA 2003). However, those analyses have not yet been implemented (ANVISA 2006).

Unfortunately, in Brazil surveillance and risk management regarding the presence of contaminant residues in foods have occurred primarily as a result of noncompliance with the requirements of the international market, leaving in the background the protection of the health of its population (Spisso et al. 2009).

4.9 Final Considerations

Codex standards are established based on scientific criteria grounded in risk analysis, as an important part of the food safety system. JECFA, the Codex advisory committee, has played a leading role in the development of this paradigm. The adoption of Codex standards as a basis for national regulations helps to harmonize

the global application of food safety measures. Thus, it is recommended that developing countries continue to harmonize their laws based on Codex standards and to adopt the criteria of risk analysis. However, exposure data related to chemical substances present in food (i.e., veterinary drug residues in fish) is needed to characterize the risk that these substances provide to population health, though, in many situations, developing countries lack data on the quality of food products, either due to lack of appropriate analytical methods, or to lack of qualified technicians to perform the analyses, resulting in deficiency or lack of key data in the risk assessment.

On the other hand, aquaculture is the fastest growing food production system in the world, and Brazil has a high potential for fish production. Under some circumstances, like other agribusiness activities, the use of veterinary drugs is a tool to increase the efficiency in fish farming. However, Brazil faces a shortage of veterinary drugs officially approved to be used by fish farmers in this activity. Thus, there is the suspicion that veterinary drugs approved for other species, and even illicit substances are being used in fish farming, in particular in "fish and pay" establishments. Nevertheless, in Brazil there are two programs aimed at the monitoring of veterinary drug residues in foods; the PNCRC (MAPA 2013b) and the PAMVet (ANVISA 2003) that belong to MAPA and the Ministry of Health, respectively. Only PNCRC is currently monitoring veterinary drugs residues in fish (PNCRC/fish).

Veterinary drugs ought to be administered in a responsible and prudent manner, always respecting good veterinary practices, in order to avoid affecting the efficiency of fish production and the establishment of exportation barriers, as well as the risk to human and environmental health the exposure to those substances may cause. In particular it is important to manage the issue of antimicrobial resistance, which is currently being discussed, taking into consideration the impact on aquaculture production, human and environmental health. For this reason, monitoring of the presence of veterinary drug residues in fish products is of fundamental importance to protect consumers' health, and also for the development of fish farming activity. In this regard, the analytical method is a vitally important tool to ensure that the products are presented in conformity with food laws.

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Chapter 5
Functional Dairy Products

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5.1 Introduction

From the point of view of food processing, the dairy industry is probably one of the most traditional industries in the world. Most dairy products manufactured today have been produced in roughly the same way for hundreds, if not thousands of years and the taste and preference for these products has been strongly set in most consumers around the world for a very long time. However, in the past three decades, the introduction of the concept of "functionality" has produced important changes in the type of dairy products that consumers expect and demand. Driven by important socioeconomic changes around the world, this tendency to expect "more" from food is derived firsthand from the increasing awareness of consumers about the link between nutrition and health, and second by the ever rising cost of health services, which makes it necessary to find less expensive ways to recover health, and especially to maintain it through the use of preventive strategies and smart lifestyle decisions. In this context, the dairy industry has taken a strong stand, developing new dairy products that deliver "health" as a result of taking advantage of either the natural functional benefits of milk or by using milk and dairy products as a vehicle to achieve such benefits. In the mind of most consumers, dairy products are healthy and safe to consume, especially for children, making dairy one of the most successful ways to deliver functionality through food. Various approaches have been successfully employed in this regard at a commercial scale (Table 5.1). Among these approaches, the addition of probiotic bacteria, prebiotic oligosaccharides and fiber, conjugated linoleic acid, omega-3 fatty acids, and the addition of phytosterols and phytostanols are without a doubt some of the most broadly disseminated strategies

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Table 5.1 Some examples of commercially available functional dairy products

Brand name	Manufacturer	Type of product	Claimed active ingredient	Claimed health function
Probiotics				
Yakult	Yakult Honsha Co., Japan	Fermented milk	*Lactobacillus casei* Shirota	Prevents gastrointestinal infections and constipation
Valio Gefilus (LGG)	Valio Ltd, Finland	Fermented milk beverages	*Lactobacillus rhamnosus GG*	Prevents gastrointestinal infections
Biobalance	Dos Pinos R.L., Costa Rica	Fermented milk	*Bifidobacterium lactis*	Facilitates intestinal transit
NAN 2/NAN 3	Nestle, Spain	Infant formula	*Lactobacillus reuteri*	Reduces colic in infants
Activia	Groupe Danone, France	Yogurt	Bifidobacterium animalis DN-173, Bifidus regularis®, Bifidus activo®	Controls irritable bowel syndrome
Gastro Protect	Nestle, Mexico	Fermented milk	*Lactobacillus johnsonii LA1*	Controls gastritis and peptic ulcer disease
Vaalia Innergy	Parmalat, Australia	Yogurt	*Lactobacillus rhamnosus GG*	Prevents gastrointestinal infections
DanActive	Groupe Danone, France	Fermented milk	*Lactobacillus casei DN-114001*, *Lactobacillus casei defensis*®, *Lactobacillus casei immunitas*®	Fortifies immune system
BioPLUS	Dos Pinos R.L., Costa Rica	Fermented milk	*Lactobacillus casei CRL 431*, *Lactobacillus casei Vitalis*®	Fortifies immune system
R-1	Meiji Co., Ltd. Japan	Fermented milk	*Lactobacillus delbruecki* ssp. *bulgaricus OLL1073R-1*	Prevents viral infections
Actimel	La Serenisima, Argentina	Fermented milk	*Lactobacillus casei defensis*	Reduces cortisol levels and stress
Bioactive peptides				
BioPURE-GMP	Davisco Foods International, Inc., USA	Hydrolysate	Glicomacropeptide	Anticarcinogenic and antimicrobial effect
Calpis/Ameal S	Calpis Co., Japan	Sour milk	Hypotensive tripeptides	Reduces blood pressure
VitaTEN	Kaiku Corporación Alimentaria, Spain	Fermented milk	Hypotensive tripeptides	Reduces blood pressure
BioZate	Davisco, USA	Milk hydrolysate	Whey protein hypotensive peptides	Reduces blood pressure

(continued)

Table 5.1 (continued)

Brand name	Manufacturer	Type of product	Claimed active ingredient	Claimed health function
Lactium	Ingredia Nutritional, France	Milk hydrolysate	Casein anti-stress peptides	Prevents and regulates problems due to stress
Recaldent	Cadbury Co. UK	Ingredient	Caseinophosphopeptides and amorphous calcium phosphate	Anticariogenic activity
Prebiotics				
Orafti	BENEO Group, Belgium	Soluble powder	Inulin/oligofructose	Improves intestinal flora
Fibra con Regulaplus	Central Lechera Asturiana, Spain	Enriched milk	Soluble fiber	Enhances proliferation of intestinal and prevents constipation
Others				
Puleva OMEGA 3	Lactalis Puleva, S.L. Spain	Enriched milk	Oleic acid, eicosapentaenoic acid, docosahexaenoic acid, vitamin E	Improves cardiovascular health
Benecol	Kaiku Corporación Alimentaria, Spain	Fermented milk, yogurt	Sterol, plant stanol ester	Reduces cholesterol and cardiovascular diseases
Safflower Power	Old Home Foods, USA	Yogurt	Conjugated linoleic acid (CLA)	Reduces body fat mass
Danacol	Groupe Danone, France	Fermented milk	Sterol, plant stanol ester	Reduces cholesterol and cardiovascular diseases
Horizon Organic DHA Omega 3	WhiteWave Foods, Inc. USA	Enriched milk	Docosahexaenoic acid	Improves neurological function and brain health
Puleva Calcio	Lactalis Puleva, S.L. Spain	Enriched milk	Soy isoflavones	Reduces symptoms of menopause
Densia Forte	Groupe Danone, France	Yogurt	Calcium	Prevents osteoporosis

to produce functional dairy products around the world. Additionally, in recent years, research in the area of dairy peptides has produced interesting results, making the use of these milk components an emergent area of opportunity in the development of functional dairy products. The objective of this chapter is to introduce readers to the area of functional dairy and to give them a better understanding of the variety of

functional ingredients used in dairy products commercially available, as well as some novel potential applications. More details regarding the properties of these ingredients and their impact on human health are provided in the following sections.

5.2 Probiotics

Probiotics are recognized as friendly bacteria or good bacteria, and are defined as microorganisms that provide health benefits to their host. Probiotics are defined by the World Health Organization and the Food and Agriculture Organization of the United Nations as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host." The first observation on the benefit conferred by some bacteria is attributed to the Russian microbiologist Elie Metchnikoff, who asserted that the dependence of intestinal microbes with respect to food makes it possible to take measures to modify the flora in our body and to replace harmful microbes by useful microbes (Metchnikoff 1907). In 1965, Lilly and Stillwell were the first to use the term "probiotic" to designate those substances secreted by a microorganism that promoted the growth of other microorganisms. Since then, probiotic research has increased exponentially, and the evidence suggests that the intake of probiotic strains have a positive effect on the health of the host, as they have shown to be effective in the control of/battle against various pathological conditions.

5.2.1 Uses of Probiotics to Prevent and Treat Diarrhea

The use of probiotics has focused mainly on the treatment of gastrointestinal disorders and has been shown to be effective in treating and preventing different types of diarrhea (i.e., infectious diarrhea, antibiotic-associated diarrhea, traveler's diarrhea) (Table 5.2). Worldwide, *diarrheal diseases* are the second leading cause of death in children under 5 years old, and responsible for killing 1.5 million children every year. In animal models, the use of probiotics has demonstrated a protective effect against intestinal infections (Ogawa et al. 2001). The mechanisms that may be involved include the production of hydrogen peroxide, acids and antimicrobial compounds, competition for nutrients and adhesion receptors, the action of antitoxins, and immune system stimulation (Marteau et al. 2001). Several studies have shown that *Lactobacillus* GG provides the best probiotic effect, preventing and shortening the duration of diarrhea caused by rotavirus (Raza et al. 1995; Szajewska et al. 2001). This microorganism has proved to be efficient even when heat inactivated and subsequently administered, although live probiotic is by far more effective in inducing the IgA response on rotavirus (Kaila et al. 1995). *Lactobacillus reuteri*, *Enterococcus faecium* SF68, *Bifidobacterium lactis* BB-12, and *Saccharomyces boulardii* have also proven to be effective in preventing and shortening the duration

Table 5.2 Studies of probiotics showing a significant therapeutic effect to prevent or shorten the duration of acute gastroenteritis

Effect	Probiotic	Reference
Reduction of duration of gastroenteritis in infants	*Enterococcus faecium* SF68	Bellomo et al. (1980)
	Saccharomyces boulardii	Chapoy (1985)
	Lactobacillus rhamnosus GG	Raza et al. (1995)
	Lactobacillus reuteri	Shornikova et al. (1997)
Reduction of duration of gastroenteritis in adults	*Enterococcus faecium* SF68	Buydens and Debeuckelaere (1996), Camarri et al. (1981)
Reduction of duration of rotavirus diarrhea in infants	*Lactobacillus rhamnosus GG*	Guandalini et al. (2000), Isolauri et al. (1994)
	Lactobacillus casei Shirota	Sugita and Togawa (1994)
Prevention of antibiotic-associated diarrhea	*Lactobacillus rhamnosus GG*	Vanderhoof et al. (1999)
	Enterococcus faecium SF68	Borgia et al. (1982)
	Saccharomyces boulardii	Wunderlich et al. (1989)
		McFarland et al. (1995)
Prevention of traveler's diarrhea	*Lactobacillus rhamnosus GG*	Oksanen et al. (1990)
	Mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*	McFarland (2007)
	Saccharomyces boulardii	McFarland (2007)

of diarrhea (Buydens and Debeuckelaere 1996; Camarri et al. 1981; Chapoy 1985; Shornikova et al. 1997). *Bifidobacterium lactis* BB-12 has also been reported as effective in preventing diarrhea caused by rotavirus in children (Isolauri et al. 1994; Szajewska et al. 2001).

Lactobacillus, *Bifidobacterium*, *Streptococcus*, and *Saccharomyces* have been shown to be beneficial by reducing the occurrence of traveler's diarrhea (Hilton et al. 1997; Scarpignato and Rampal 1995). A meta-analysis involving 8014 patients, based on 63 controlled studies concluded that the use of probiotics reduced the risk of suffering more than 4 days of diarrhea (relative risk 0.41) and the mean duration of diarrhea was 24.76 h. The authors concluded that probiotics appear to be safe and have a beneficial effect, shortening the duration and frequency of bowel movements (Allen et al. 2010). In another study that used the yeast *Saccharomyces cerevisiae* as a probiotic, it was observed that the probiotic effect was dependent on the dose administered. Pathogen infection rates were 33.6% and 31.8% in groups receiving 250 mg or 500 mg of *S. cerevisiae*, respectively, compared with 42.6% in the group receiving a placebo (Kollaritsch and Wiedermann 1992). Moreover, combinations of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, as well as the yeast *Saccharomyces boulardii*, have also demonstrated their effectiveness (McFarland 2007).

A study that used *L. rhamnosus* GG in children with antibiotic-associated diarrhea showed a significant reduction in the number of bowel movements and increased stool consistency (Vanderhoof et al. 1999). In another study conducted in children, the success of *L. rhamnosus* GG was also reported in the treatment of

recurrent diarrhea caused by *C. difficile* (Biller et al. 1995). *Enterococcus faecium* SF68 was more efficient than a placebo in preventing antibiotic-associated diarrhea in two double-blind randomized controlled trials (Wunderlich et al. 1989). A study conducted in elderly patients with colitis associated with *C. difficile* showed that *Saccharomyces boulardii* was able to reduce the recurrence of disease in patients with more than one sequential infection (Lewis et al. 1998) and decrease the risk of diarrhea (McFarland et al. 1995).

5.2.2 *Uses of Probiotics to Treat* Helicobacter pylori *Infections*

Helicobacter pylori is a gram-positive bacterium and is a major cause of chronic gastritis, gastroduodenal ulcers, gastric adenocarcinoma, lymphoma, and other non-gastrointestinal disorders. Treatment for the eradication of *H. pylori* is based on antibiotics and is 90 % effective. However, such treatment is expensive and carries the possibility of inducing resistance to antibiotics. Clinical and experimental models have shown that *Lactobacillus acidophilus* secretes products that inhibit the growth in vitro and in vivo of *H. pylori. Lactobacillus gasseri* and *Lactobacillus johnsonii* have proven to be effective in suppressing the growth of *H. pylori* and reducing gastric inflammation (Felley et al. 2001; Sakamoto et al. 2001). Generally, probiotics are used as a complement to antibiotics (Kim et al. 2008). In a study involving 347 patients infected with *H. pylori*, 168 patients were treated with a triple therapy of antibiotics and yogurt; the other 179 received the triple therapy only. The eradication rate in the yogurt treated group was 87.5 %, being higher than the control group, 78.7 %; however, there was no reduction in side effects associated with the antibiotics. In another study it was observed that *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 contained in a yogurt not only increased the percentage of eradication but also decreased the side effects of triple therapy (Sheu et al. 2006). It has been suggested that regular consumption of probiotics does not eradicate *H. pylori*; however, its consumption may be used as an adjuvant to conventional antibiotic therapy, since the effects of probiotics are explained by their indirect immunomodulatory properties and their ability to survive in the gastrointestinal tract, despite the use of antibiotics (Myllyluoma et al. 2005).

5.2.3 *Uses of Probiotics to Treat Inflammatory Bowel Disease*

Inflammatory bowel disease (IBD) is a disorder of unknown cause characterized by recurrent or chronic inflammation of the gastrointestinal system. These disorders include Crohn's disease (CD) and pouchitis, and they probably contribute to ulcerative colitis (UC) as well. Clinical and experimental observations involve an imbalance in the intestinal mucosa with a relative predominance of aggressive bacteria

and a decrease of protective bacteria (Mitsuyama et al. 2002), as well as stimulation of pro-inflammatory immune mechanisms (Gupta and Garg 2009). In preliminary studies, the administration of probiotics to patients suffering from inflammatory bowel disease suggests a positive response to the disease, causing an increase in the immune response (Malin et al. 1996), a reduction in the expression of inflammatory markers ex vivo (Borruel et al. 2002) and improves the functions of the gastrointestinal tract (Gupta et al. 2000). In an open trial that included patients with UC and on treatment with 5-ASA, they also were treated with *Saccharomyces boulardii* during 4 weeks; 71 % of patients maintained remission during treatment (Guslandi et al. 2003). *Escherichia coli* Nissle was also shown to be as effective as low-dose mesalamine for preventing relapse in UC (Kruis et al. 2004). In a study that included only four patients with CD, the efficacy of *Lactobacillus rhamnosus GG* ($>10^{10}$ CFU) was investigated for 4 weeks. The study showed a 100 % remission (Gupta et al. 2000). Most evidence on the efficacy of probiotics in IBD has been shown in randomized double-blind placebo controlled trials of a product called VSL # 3 (VSL Pharmaceuticals Inc.), which is a mixture of four species of lactobacilli, three species of bifidobacteria and *Streptococcus thermophilus*. In this study, 40 patients with relapsing chronic pouchitis were treated with VSL # 3 (6 g/day) after antibiotic-induced remission. After 9 months of treatment, there was a marked difference in the rates of relapse: 15 % relapse in the group treated with probiotics versus 100 % of relapse in the placebo-treated group (Gionchetti et al. 2000). Promising results have been obtained using probiotics in relapses and in the treatment of UC; however, the studies that have been conducted to treat CD using probiotics are less clear because of conflicting and limited data (Heczko et al. 2006). Therefore, more and new studies are required to demonstrate the beneficial effect of probiotics in people with inflammatory bowel disease.

5.2.4 *Uses of Probiotics to Treat Infections of the Urogenital Tract*

Lactobacilli predominate in the normal vaginal flora of premenopausal women and an imbalance in the flora can cause vaginal or bladder infections. The most common problems are: bacterial vaginosis, yeast vaginitis, and recurrent urinary tract infections. *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* GR-1 have shown effectiveness in colonizing and protecting the urogenital tract (Gupta and Garg 2009; Saxelin et al. 1995). During metabolism, lactobacilli produce H_2O_2, acids, and bacteriocins that inhibit the growth of pathogens, as well as biosurfactants that avoid their adherence (Reid 2001). In a double-blind, placebo-controlled clinical trial, 39 women with bacterial vaginosis were studied. One group of patients was treated with lactobacillus-containing vaginal tablets and another group with a placebo. The treatment consisted of one tablet daily for 7 days. At the end of therapy, all patients in the group treated with the probiotic ($n=18$) were free of bacterial vaginosis; 83 % showed a normal vaginal flora and 17 % intermediate vaginal flora,

while in the control group ($n = 16$) only two patients were free of BV with intermediate vaginal flora (12 %). Additionally, in the treated group, the symptoms and the intensity of symptoms, particularly vaginal malodor, were significantly lower in the two follow-up visits (Mastromarino et al. 2009). Moreover, one study suggests that oral treatment of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 can restore urogenital flora and alleviate infections in females with urogenital infections (Reid 2001). It has also been suggested that by reducing the risk of bacterial vaginosis, probiotics might also reduce spontaneous abortions and infant mortality (Gupta and Garg 2009).

5.2.5 Potential Uses of Probiotics to Treat Cancer

The immune system and endogenous flora play an important role in the modulation of carcinogenesis. Both can be influenced by probiotics, and this has led to the investigation of the role of probiotics in the prevention and cure of cancerous tumors. Several animal studies have focused on the effect of probiotics on intestinal microecology and cancer. *Lactobacillus rhamnosus* GG, *Lactobacillus casei* Shirota, and *Lactobacillus acidophilus* have been shown to have properties that inhibit tumors induced chemically in animals. One study conducted in rats with chemically induced colon cancer showed that the oral intake of *Lactobacillus casei* Shirota inhibited colon carcinogenesis in the rats and also showed a significantly decrease in the number of rats with colon cancers and the number of colon cancers per rat (Yamazaki et al. 2000). There is some evidence that probiotic microorganisms may prevent or delay the onset of intestinal tumors. It has been suggested that *Lactobacillus* strains have the ability to reduce the risk of cancer due to their ability to modify intestinal flora, and to reduce levels of β-glucuronidase and other carcinogens (Ling et al. 1994; Wollowski et al. 2001). Moreover, there is evidence that internal instillation of *Lactobacillus casei* Shirota may decrease cancer recurrence elsewhere, such as in the urinary bladder (Aso et al. 1995). In a prospective randomized, controlled trial, the effect of oral intake of *Lactobacillus casei* on bladder cancer was evaluated in patients who had undergone surgery for their condition. 74.6 % of patients who took 3 g *Lactobacillus casei* per day for a year remained cancer free 3 years after surgery, compared to only 59.9 % of those who had not taken the probiotic (Naito et al. 2008). However, all of this evidence requires more studies to confirm and understand the potential role of probiotics in the treatment of cancer.

5.2.6 Additional Benefits of the Use of Probiotics

The use of probiotics is also being investigated in other diseases where there is some evidence as to the beneficial effects they provide. For example, probiotic

microorganisms have been used for prevention of allergies (Schmidt 2004). In one study, the administration of *Lactobacillus rhamnosus* GG prenatally and postnatally during 6 months to children with a high risk of atopic disease, reduced by 50 % the prevalence of atopic eczema, when compared with children who received a placebo (Kalliomäki et al. 2001). Studies also have been conducted to see the effect of probiotics on respiratory allergies such as asthma (Cario et al. 2002). These studies raise the possibility of using probiotics to modulate the immune response and to prevent allergic diseases. Studies have also shown that probiotics can help with oral health. For example, *Lactobacillus reuteri* has been reported to decrease gum bleeding and reduce gingivitis (Krasse et al. 2006). There is increasing evidence suggesting that probiotics may confer benefit in cardiovascular diseases (Oxman et al. 2001), in the treatment of surgical infections (Gan et al. 2002), pancreatitis (Pezzilli and Fantini 2006), lactose intolerance (de Vrese et al. 2001), necrotizing enterocolitis (Stricker and Braegger 2006), among others. The evidence contained in a large number of studies suggests that specific probiotics represent an additional benefit to the already proven therapies for some diseases. The efficacy of some probiotic strains has been demonstrated by numerous studies in vivo and in vitro conducted in various countries. Promising results have been obtained in the use of probiotics to treat various inflammatory, allergic and infectious diseases, mainly gastrointestinal diseases. However, studies focused on understanding the mechanisms and functionality of probiotics are still needed. A further important point is to select the most suitable probiotic for a particular disease, as well as to determine the dose to be administered for treatment. In some cases, carrying out well-designed clinical studies, using double-blind and placebo controlled trials, is also necessary to confirm the health benefits of probiotics.

5.3 Prebiotics

Prebiotics are nondigestive food ingredients that improve the host's health by promoting the selective growth of beneficial flora in the intestinal tract. The most known prebiotics are dietary fibers, including nondigestible ingredients such as oligosaccharides, including fructo-oligosaccharides and galacto-oligosaccharides, inulin, and starch-based materials. Carbohydrates considered as prebiotics are digestible only for probiotic microorganisms, hence providing a selective microenvironment for them. Prebiotics are capable of passing the acidic stomach intact, being resistant to bile secretion and mammalian enzymes, and being able to produce a selective environment in the lower gut. Fermentation of prebiotics to produce short chain fatty acids reduces the pH in the colon, thus inhibiting colonization of pathogenic bacteria like *Escherichia coli*. Some nondigestible oligosaccharides have shown prebiotic properties due to their ability to selectively confer the required degree of fermentation into the gut microenvironment. Among these, fructo-oligosaccharides, lactulose, and trans-galacto-oligosaccharides, as well as

xylo-oligosaccharides, soybean-oligosaccharides, and isomalto-oligosaccharides, are notable (O'Hara et al. 2007; Rastall 2003).

5.3.1 Oligosaccharides

Oligosaccharides are defined by UIPAC as carbohydrates with up to ten monomer units; however, in practice, the boundary between oligosaccharides and polysaccharides is not precisely defined. These compounds are frequently found forming part of bacteria, fungi, plants and in the milk of placental mammals. They derivate from the hydrolysis of polysaccharides during digestion and can also be extracted from natural sources or synthesized using monomers.

Human milk contains an important quantity of oligosaccharides (0.7–1.2 g/l) in its composition, with this fraction being the largest contributor to the establishment of the microenvironment in the intestine of neonates, stimulating the immunological system to improve the defense of the organism against bacteria and viruses, and enhancing the bioavailability of minerals as well. Although the most studied health effect of milk oligosaccharides has been their ability to promote the growth of beneficial bacteria, some inherent health-promoting properties have been also discovered. Milk oligosaccharides are formed by monomers such as D-glucose, D-galactose, *N*-acetylglucosamine, L-fucose, and sialic acid. Interestingly enough, from an evolutionary point of view, human milk possess a higher content of oligosaccharides than the milk of domestic mammals such as cow, horse, goat, and sheep. Additionally, the structure and composition of oligosaccharides present in the milk of domestic mammals is different, with fucosylated oligosaccharides being the principal components in human milk, which is a compound absent in the milk of domestic mammals (Boehm and Stahl 2003).

The oligosaccharides of non-milk origin are important prebiotics as well. Most studies regarding this type of oligosaccharides focus on fructans, which are polymers of fructose that can be linear or branched. One of these fructans is inulin. This oligosaccharide can be easily extracted from plants such as garlic and onions, and is frequently used as an ingredient in food products. Others important non-milk oligosaccharides are galacto-oligosaccharides, which are synthesized from individual molecules of galactose in an enzymatic reaction using β-galactosidase, isolated from bacteria (*Bacillus circullans*). Fructo-oligosaccharides and inulin are extracted from natural sources and prepared by hydrolysis of polymers. Palatinose and isomaltulose, as well as lactosucrose and xylo-oligosaccharides, can be also mentioned as important non-milk oligosaccharides that can play an important role in improving human health (Gürayan et al. 2010). Although many different structures of oligosaccharides are known to contribute to human health, information about the relationship between these structures and their specific function in human health is very limited. In general, it can be said that oligosaccharides play a role in the modulation of intestinal flora, improvement of mineral

absorption and lipid metabolism, brain development and prevention of infection (Boehm and Stahl 2003).

5.3.1.1 Role of Oligosaccharides in Brain Development

Some studies suggest that dietary oligosaccharides play an important role in the development of the brain, due to the presence of molecules of galactose that could be used as precursors to synthetize galactocerebrosides, which are the most important glycolipids in myelin (Boehm and Stahl 2003). It has been suggested as well that sialyl-oligosaccharides from human milk are a source of sialic acid that is a precursor of brain gangliosides and sialyl glycoproteins in infants (Asakuma et al. 2007) which play a vital role in nerve cell-transmission, memory formation and cell to cell communication (Miller and McVeagh 1999).

5.3.1.2 Role of Oligosaccharides in the Prevention of Infections

Oligosaccharides are important as well in the defense of newborn children against infection. The anti-infectious effect of milk oligosaccharides has been explained by the structural similarities that free oligosaccharides and glycol-conjugates have with some pathogen receptors. The proposed mechanism establishes that oligosaccharides could act as pathogen-analogues restricting the pathogen attachment to epithelial cells. It has been proven that cow milk oligosaccharides can inhibit the adhesion of enterotoxic *Escherichia coli* strains in calf intestines, while some additional studies have demonstrated the effectiveness of fructans to interfere with the adhesion of *Salmonella enteritidis* into the cells of pig small intestines. Pectin hydrolysate causes the same effect, since it inhibits pathogen adhesion to epithelial cells of the intestine, as has been proven by in vitro and in vivo studies (Boehm and Stahl 2003).

5.3.1.3 Role of Oligosaccharides as Prebiotics

Probably the most important and well known property of oligosaccharides is their capacity to positively influence the growth of health-promoting bacteria such as bifidobacteria and lactobacillus. The human intestine cannot hydrolyze the β-glycosidic linkage present in oligosaccharides because the intestine does not have the necessary enzymes for this purpose. For this reason, oligosaccharides are considered to be nondigestible, hence remaining available to be utilized by probiotic bacteria (Boehm and Stahl 2003). Human milk oligosaccharides are important prebiotics, especially in infants, because they promote the growth of bifidobacteria, which is probably the most important bacterial genera in the infant intestine (Rockova et al. 2012). Fructo-oligosaccharides and inulin are the most important prebiotics that are utilized to produce fermented milk products, with the goal of preserving the life of probiotic bacteria, and increasing the viability of probiotic

bacteria (Gustaw et al. 2011). Prebiotics can be used in a wide range of food products such sweeteners, yogurt, nutritional bars, and baby formula (Sangwan et al. 2011). Prebiotic supplementation of infant formula is able to increase intestinal colonization by bifidobacteria and lactobacillus; such early colonization of beneficial bacteria in the intestine has lifelong lasting effects (Salvini et al. 2011).

5.3.1.4 Role of Oligosaccharides in Mineral Absorption

Milk oligosaccharides play an important role in improving calcium absorption. It has been suggested that oligosaccharides are able to indirectly improve mineral absorption because they influence the growth of probiotic bacteria, which synthesize short chain fatty acids that promote calcium absorption through reduction in the formation of calcium soaps in the colon. In a study with postmenopausal women, dietary galacto-oligosaccharides were capable of significantly improving calcium absorption. In the same way, in another study with infants, calcium absorption was higher in infants fed with milk supplemented with galacto-oligosaccharides and inulin, resulting in higher renal excretion of calcium, suggesting higher absorption in comparison with infants that are fed with non-supplemented milk. Some reports indicate that oligosaccharides can also improve the absorption of other minerals such as magnesium, iron and iodine, although such results are not as conclusive as those found in calcium studies (Boehm and Stahl 2003).

5.3.1.5 Role of Oligosaccharides in the Immune System

Oligosaccharides may play an important role in the modulation of the immune system. Studies suggest that this effect may be caused by the establishment of balanced microflora in the gastrointestinal tract. However, since some inherent properties have also been observed, more information is needed to settle this issue. Some studies have shown that human milk oligosaccharides are directly involved in the process of interleukin-10 production, as well as in the production of anti-inflammatory cytokines (Boehm and Stahl 2003). It has been demonstrated that human milk oligosaccharides improve the development of the neonate immune system, resulting in better protection against enteric and respiratory infection in infant breastfeeding (Rijnierse et al. 2011). Additional studies suggest that oligosaccharides can also inhibit acute allergic reactions by a mechanism that includes induction of a regulatory T-cell CD25, required for immunological tolerance (Schouten et al. 2010). The role of oligosaccharides in the immunity of neonates is also supported by the fact that infants who are not breast-fed present higher incidence and severity of diarrhea and respiratory diseases than their breast-fed counterparts, while it is also known that oligosaccharides diminish inflammatory activity in the intestinal mucosa (Newburg 2009). Studies conducted with non-milk oligosaccharides suggest that the properties observed in milk oligosaccharides may be present in oligosaccharides from alternative sources. In a study conducted with oligosaccharides extracted from

Yacon root, the results indicate a beneficial effect over the immune system through modulation of inflammatory process (decreasing cytokine pro-inflammatory interleukin-1 (IL-1), which could be beneficial in some autoimmune diseases). In this study, oligosaccharides also influenced the improvement of mucosal immunity by incrementing IgA production (Choque-Delgado et al. 2012). Much more research on this topic is required.

5.3.1.6 Role of Oligosaccharides on Cancer Prevention

In addition to promoting the synthesis of bacterial metabolites such as butyrate (i.e., by clostridia and eubacteria) that can reduce the risk of some types of cancer due to the promotion of apoptosis of cancerous cells (Boehm and Stahl 2003), oligosaccharides have shown some cancer inhibiting properties of their own. The oligosaccharides extracted from a green alga (*Caulerpa lentillifera*) are capable of inducing apoptosis of breast cancer cells in vitro because induce chromatin condensation and the degradation of the enzyme PARP (poly ADP-ribose polymerase) responsible for DNA repair in cell line MCF-7 (Maeda et al. 2012). Epidemiological studies also suggest that prebiotic oligosaccharides and probiotics could reduce the risk of cancer by inhibiting the toxicity of carcinogenic substances. Studies conducted in animal models suggest that cancer therapy is more effective when used in combination with probiotics and prebiotics; however, more information is required to produce sound conclusions regarding the precise role of oligosaccharides in cancer prevention. Yogurt is a food product that has been studied for cancer prevention because it is an ideal vehicle to deliver both probiotics and prebiotics to consumers (Burns and Rowland 2000).

5.3.2 Dietary Fiber

Dietary fiber is formed by polysaccharides that are components of the plant cell wall or intercellular structure. Dietary functional fiber is a term that refers to nondigestible carbohydrates that confer some beneficial effect to the consumer. Among the main health benefits that can be conveyed by fiber consumption, reduction of the levels of glucose and lipids in blood, improvement in calcium absorption, promotion of a healthy intestinal flora (as prebiotic fiber) and relief of constipation, may be listed as some of the most important and broadly studied. Fiber is found in foods such as grains, vegetables and fruits (Slavin and Jacobs 2010; Bodner and Sieg 2009). Some soluble fibers that have been added to milk and dairy products in an effort to improve their functional characteristics are polydextrose, inulin, fructo-oligosaccharides, galacto-oligosaccharides, pectin, beta-glucan, guar gum, xanthan gum, carrageenan, and carob powder, among others (Boehm and Stahl 2003). Dietary fiber as a broad term includes resistant starch, non-starch polysaccharides, cellulose, hemicellulose, pectin, gums, beta-glucans, inulin, oligosaccharides,

fructans, and lignin. Some disadvantages of dietary fiber intake may include reduced absorption of vitamins and nutrients, flatulence, diarrhea, and abdominal discomfort (Slavin and Jacobs 2010).

5.3.2.1 Role of Dietary Fiber on Heart Health

The daily intake of dietary fiber (2–10 g) can diminish blood cholesterol, especially LDL (low density lipoproteins) (Slavin and Jacobs 2010). Beta-glucans from oats, barley, and psyllium husk are approved by the FDA as dietary fiber intended for the reduction of heart disease risk and to diminish blood pressure. Dietary fiber from oats is capable of reducing LDL cholesterol and improving cardiovascular health. A study with 85 subjects with moderate hypercholesterolemia who consumed 100 g of oat cereal daily for a period of 6 weeks, resulted in a significant decrease of blood cholesterol levels in comparison with a control group treated without dietary fiber (Zhang et al. 2012a). Several mechanisms to explain the reduction of blood cholesterol caused by dietary fiber have been proposed. Among them, the interference caused by fiber on cholesterol and lipid absorption through the inhibition of the formation of micelles, the increment in cholesterol conversion to produce bile acids in the liver promoted by fiber, and the inhibition of the biosynthesis of cholesterol insulin-dependent indirectly caused by the diminished glucose absorption and insulin liberation promoted by fiber are some of the most broadly accepted. Finally, it may be also considered that fermentation of dietary fiber in the colon produces propionic acid that inhibits cholesterol formation; however, how this could influence plasmatic cholesterol remains unclear (Borderías et al. 2005).

5.3.2.2 Role of Dietary Fiber on Body Weight and Metabolic Disorders

Foods rich in fiber tend to have low energy density, conferring satiety at low energy levels (Slavin 2008). Studies conducted on animal models have proven that fiber consumption can significantly prevent body weight gain (60 %) when administered as a supplement to a normal diet for 4 weeks (Jiménez-Escriq et al. 2008). From this point of view, supplementing yogurt and dairy products with fiber represents an excellent opportunity to increase fiber consumption since it has been observed that regular consumers do not meet the required dietary daily intake of fiber, even those who exercise and control their body weight, leading to a condition that increases abdominal fat accumulation and risk of cardiovascular disease (Monteiro et al. 2012). Overweight and increased risk of developing chronic disease in newborns have also been linked to low intake of fiber from fruits and vegetables during pregnancy (Perälä et al. 2012).

Additionally, it has been demonstrated that dietary fiber ingestion (30 g/day) decreases the risk of type 2 diabetes by 21 % (Slavin and Jacobs 2010). Dietary fiber also plays an important role in glucose regulation in patients who have already developed type 2 diabetes, resulting in good control of blood glucose levels that

prevent diabetes complications (Jiang et al. 2012). Consumption of dietary fiber in subjects with diabetes mellitus also decreases the risk of mortality by cardiovascular disease due to the lowering of blood lipids, diminution of blood pressure and decrease of insulin resistance (Burger et al. 2012).

5.3.2.3 Role of Dietary Fiber on Intestinal Health

Studies suggest that ingestion of food with high quantities of dietary fiber improve intestinal health. It has been proven that dietary fiber promotes colonization of the lower intestinal tract by beneficial bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp., which contribute to maintaining optimum function of the intestinal epithelium (Paturi et al. 2012). Fiber is also capable of mitigating constipation symptoms by reducing intracolonic pressure, which also may diminish pain in patients with irritable bowel disease (IBD), as shown in a study where 60 % of patients with IBD improved their condition when dietary fiber consumption was increased in comparison with the placebo group (Bijkerk et al. 2004). In a study of 86 children, increase of dietary fiber and water consumption resulted in mitigation of the symptoms of constipation in all patients (Karagiozoglou-Lampoudi et al. 2012).

5.3.2.4 Role of Dietary Fiber on Cancer Prevention

The increase of dietary fiber intake to about 33 g/day may reduce the risk of colorectal cancer by 25–40 %, and possibly also play a protective role in breast cancer prevention (Slavin and Jacobs 2010). Studies conducted on humans for periods as long as 11 years have supported this conclusion, demonstrating that dietary fiber intake as low as 10 g a day may reduce the incidence of colorectal cancer by up to 11 %, independently of sex, age, smoking, intake of alcohol and consumption of red meat (Murphy et al. 2012). Studies with large numbers of human subjects ($n = 57{,}053$) have produced important information related to the role played by dietary fiber on anti-inflammatory regulation, showing that dietary fiber interacts with interleukin-10, preventing colorectal cancer in patients with genetic predisposition (Andersen et al. 2012). Additionally, it has also been suggested as a primary mechanism that dietary fiber could prevent colorectal cancer by reducing the time of exposure to carcinogenic substances in the intestinal lumen (Kaczmarczyka et al. 2012).

5.4 Conjugated Linoleic Acid (CLA)

Conjugated linoleic acid (CLA) is the term commonly employed to refer to all geometrical and positional isomers of linoleic acid that contain two double bonds in conformation cis or trans separated by one single bond. These isomers that are

naturally found in the meat and milk of ruminants are formed in a process of biohydrogenation of polyunsaturated fatty acids that occurs in the rumen, which is catalyzed by enzymes from anaerobic microorganisms such as *Butyririvibrio fibrisolvens*. In its natural form, milk contains CLA in concentrations that may vary from 2.9 to 8.2 mg/g, with the most abundant isomer being the 9 cis, 11 trans octadecadienoic acid (rumenic acid) which represents the 73–93 % of the total amount. The quantity of CLA in milk is variable depending on feeding, season of the year, and number of times that the animal has lactated. Depending on initial concentration and processing strategies, it is possible to produce milk, butter, yogurt, and cheese rich in CLA on a commercial scale; CLA can also be artificially synthesized through selective hydrogenation of linoleic acid from vegetal sources (Hennessy et al. 2007).

5.4.1 Use of Conjugated Linoleic Acid on Cancer Prevention

Some studies conducted on animal models have documented the capacity of CLA to inhibit carcinogens, protecting cells against toxic compounds such as 7,12-dimethylbenz(a)anthracene (DMBA) that induce epidermal tumors, benzo(a) pyrene that induce the formation of stomach tumors, and methylnitrose urea which induce mammary tumors. Additionally it has been shown that dietary supplementation with CLA may inhibit the formation of colon cancer tumors due to protection against 2-amino-3methylimidazo(4,5-f) quinoline and 2-amino-1-methyl-6--phenylimidazo(4,5-6)pyridine (PhIP) adduct formation. CLA has also shown the capability to inhibit the proliferation of human cancerous cells in vitro by 18–100 % in cases such as melanoma, human mammary cancer, gastric, colorectal, prostate, and lung cancer, as well as hepatoma, but the mechanism of action is still unknown. Studies have shown that CLA may inhibit carcinogenesis at stages of initiation, promotion, progression, and metastasis. CLA at dietary concentrations of 1 % is capable of reducing mammary cancer, as it exhibits high retention in adipose tissue. The retention of CLA in mammary tissue is related with tumor inhibition in the post-initiation stage. On the other hand, dietary concentrations of CLA of 0.5 %, 1 %, and 1.5 % can inhibit the number of tumors by 32 %, 56 %, and 60 % respectively. CLA consumed as part of the diet of animal models has also been shown to reduce tumor incidence up to 30 % in comparison with the control group. CLA consumption has also demonstrated effectiveness in inhibiting tumor growth, and impeding the spread of breast cancer cells to the lungs, peripheral blood, and bone marrow (Hennessy et al. 2007). The capacity of CLA to inhibit cancer development has been attributed to its antioxidant capacity. However, CLA is antioxidant only at low concentrations, while at high concentrations it has a contrary effect and acts like a pro-oxidant. Another suggested mechanism of action is that lipid peroxidation induced by CLA plays an important role in cytotoxicity over cancer cells. Furthermore, some reports suggest that CLA could induce apoptosis in cancerous cells through regulation of the expression of genes that control this process (Gnädig et al. 2003). Although at least some cancer-inhibiting capacity has been observed on

several CLA isomers and their combinations, the antiproliferative activity of CLA has been attributed chiefly to the 9 cis, 11 trans isomer (Hennessy et al. 2007).

5.4.2 *Use of Conjugated Linoleic Acid on the Reduction of Body Fat*

Dietary supplementation with CLA at concentrations of 0.5 % on animal models modifies corporal composition, reducing body fat, especially in females. A similar effect may be observed in humans, where a decrease in body fat is observed when CLA is administered at 6.8 g per day after 3 months of intake. CLA consumption also decreases the level of total cholesterol and LDL (low density lipoprotein) preventing accumulation in arteries and atherosclerotic lesions in animal models. CLA isomer 10 trans, 12 cis is capable of decreasing triacylglycerols and total cholesterol; however, some reports indicate that CLA could also increase atherosclerosis, so more information is required to have a conclusive position on this issue (Gnädig et al. 2003).

5.4.3 *Role of Conjugated Linoleic Acid on the Immune System*

It has been documented that CLA consumption may play an important role in the development of the immune system in humans through its participation in the formation of antibodies. Two CLA isomers have been pointed out as being mainly responsible for this activity (cis 9, trans 11 and trans 10, cis 12) (Gnädig et al. 2003). CLA is capable of suppressing inflammatory response in animal models through inhibition of the synthesis of eicosanoids such as prostaglandins, also decreasing the ability of CD4 and CD8 T cells to produce tumor necrosis factor α (TNFα), interferon γ, and IL-17. Through the activity of CLA, inflammation is inhibited, resulting in the lessening of symptoms of some conditions such as irritable bowel disease and colitis (Bassaganya-Riera et al. 2012). Studies on subjects with Crohn's disease treated with 6 g/day of CLA for a period of 12 weeks also showed a decrease in the synthesis of necrosis factor α (TNFα), interferon γ, and IL-17, which suggests that CLA is capable of suppressing the ability of T-cells to produce pro-inflammatory cytokines (Yamamoto and Shiraki 2012). Finally, additional studies on animal models have shown that dietary intake of CLA (cis 9, trans 11) during gestation and early infancy promotes the development of intestinal immunity (Pérez-Cano et al. 2009).

5.5 Omega-3 Fatty Acids

Omega-3 fatty acids are polyunsaturated fatty acids that feature a double bond after the third carbon counting from the end of the carbon chain. These fatty acids are extracted predominantly from fish oil and in minor fashion from vegetables such as

corn, sunflowers, and soy beans. Omega-3 fatty acids are considered an essential nutrient because eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are not synthetized in the human body, are essential for brain development, learning, and heart health (Gürayan et al. 2010). Omega-3 fatty acids improve cardiovascular system function and are implicated in cancer prevention and control of inflammatory and immune diseases. These compounds have been extensively employed in the dairy industry for the manufacture of functional products such as fluid milks (80 mg in 100 ml of milk), yogurt, butter, cheese, and ice cream. Although the fishy taste and aroma of omega-3 fatty acids was initially difficult to deal with in this type of products, microencapsulation has successfully overcome this issue (Saxelin et al. 2003). The current recommendation for dietary intake of omega-3 fatty acids is 3 g/day (de Lorgeril and Salen 2012).

5.5.1 Role of Omega-3 Fatty Acids on Cardiovascular Health

Consumption of omega-3 fatty acids from fish and plants is a good strategy to prevent cardiovascular disease, as they contribute to the improvement of cardiovascular health (de Lorgeril and Salen 2012). These fatty acids are capable of increasing HDL cholesterol and decreasing triglycerides in blood, inflammatory markers, resistance to insulin, and arterial stiffness. The decrease of these parameters implies a diminution on the risk of atherosclerosis (Turunen et al. 2013). A study conducted on large populations showed that consumption of milk with higher omega-3 fatty acids content reduced the prevalence of cardiovascular disease in comparison with populations that consumed milk containing less omega-3 (Thorsdottir et al. 2004). Omega-3 fatty acids also have the capacity of modulating blood pressure through modification in the quantities of vasoconstrictive prostaglandins and increasing vasodilator prostacyclin. Additionally, some studies suggest that since omega-3 fatty acids increase membrane fluidity in cardiac cells, they can also play a role in the prevention of arterial fibrillation (Riediger et al. 2009).

5.5.2 Role of Omega-3 Fatty Acids on Cancer Prevention

Epidemiological data indicates that the dietary intake of omega-3 fatty acids has the capacity to decrease the risk of breast cancer (de Lorgeril and Salen 2012). Omega-3 fatty acids inhibit metastatic invasion in breast cancer, probably due to inhibition of progestin that is known to stimulate breast cancer progression (Moore and King 2012). It is also suggested that omega-3 fatty acids are capable of attenuating the proliferation and migration of breast cancer cells through inhibition of calcium channels, which are indispensable for cell proliferation, by a mechanism that is still unknown (Zhang et al. 2012c). Additionally, another mechanism proposed for the antiproliferative activity of omega-3 fatty acids over breast cancer cells is that

consumption of this type of fatty acids modifies fatty acid composition of cell membranes and the proportion of saturated acids in their structure, changing the features of the membrane and causing a modification in key protein distribution (Corsetto et al. 2012). On the other hand, omega-3 fatty acids produce a 3–7-fold increase in the process of apoptosis compared with controls and possess antitumoral effects over colon cancer cells in vitro by a mechanism that includes the inhibition of expression of the protein GRP78 which has anti-apoptotic activity in tumoral cells (Fasano et al. 2012). Some studies have also evaluated the relationship between the intake of omega-3 fatty acids and the risk of endometrial cancer, finding that consumption of omega-3 fatty acids from food and supplements diminishes the incidence of this disease (Arem et al. 2013). Additional mechanisms proposed to explain the effect of omega-3 fatty acids on cancer inhibition include the modification of hormonal profiles, the modification of transduction signal pathways, and the promotion of changes in gene expression and immune system function. However, more studies are necessary to conclude the role of omega-3 fatty acids in cancer prevention/inhibition (Riediger et al. 2009).

5.5.3 Role of Omega-3 Fatty Acids on Brain Development

Many studies suggest that consumption of omega-3 fatty acids has beneficial effects on brain development and functionality; however, available studies are very different in sample size, omega-3 fatty acid dosage, combination with other treatments, study duration, and methodology, thus making it difficult to obtain a unified conclusion (Van de Rest et al. 2012). Some experimental, biochemical, and clinical studies show evidence of importance of omega-3 fatty acid consumption in memory and cognition, stress response, and depression, but the best dosage and form of administration is not clearly defined (Rombaldi Bernardi et al. 2012). Data from several studies indicate that omega-3 fatty acids should be consumed at adequate levels in pregnant woman and young children because they play an important role in brain development and cognition (Luchtman and Song 2013). Some studies have also demonstrated that supplementing infant formula with omega-3 fatty acids improves neurocognitive and visual functions, contributing to the development of children (Gianní et al. 2012). It has also been suggested that omega-3 fatty acids play an important role in the functionality of the brain by modulating the production and function of neurotransmitters such as serotonin and dopamine (Riediger et al. 2009).

5.5.4 Role of Omega-3 Fatty Acids on the Immune System

Some studies suggest that dietary supplementation with omega-3 fatty acids is helpful for the treatment of Alzheimer's disease, because of their capacity to change the gene expression involved in inflammation regulation and neurodegeneration.

Treatment with supplements of omega-3 fatty acids for 6 months in 16 subjects with Alzheimer's disease has been able to cause a reduction in symptoms in comparison with the use of a placebo (Vedin et al. 2012). Additionally, it has been proven that the presence of omega-3 fatty acids modulates lymphocyte T production and cytokine synthesis, suggesting that the anti-inflammatory properties of these fatty acids could play a role preventing autoimmune disease (Das 2004). It has also been suggested that ingestion of omega-3 fatty acids during pregnancy has the potential to reduce infant atopic eczema in newborns, although levels of immunoglobulin-E associated allergies remain unaffected and are independent of supplement intake (Palmer et al. 2012). Finally, studies have found that 2-week-old infants fed with formula containing omega-3 fatty acids show development of the immune system similar to that found in breast-fed infants, but significantly different from the immunity of infants fed with formula only. Infants fed with omega-3 fatty acids showed lower percentages of CD14 cells and higher percentages of CD3, CD4, and CD28 cells, as well as an increase of interferon γ production (Field et al. 2008).

5.6 Phytosterols and Phytostanols

Phytosterols are a group of steroid alcohols naturally found in plants. They are lipophilic compounds structurally related to cholesterol, but different in the side chain substitutions in carbon 24. The most important sources of plant sterols are vegetable oils from corn, sunflower, soybean, and rapeseed oil. Amongst phytosterols, β-sitosterol, campesterol, stigmasterol, and avenasterol may be differentiated. Phytosterols that have saturated sterol chains are called phytostanols, which are less abundant in nature, but can be synthetized, by a 5-α-hydrogenation, yielding products such as sitostanol and campestanol, among others (Varzakas and Arvanitoyannis 2010). Isolated plant stanols, hydrated forms of sterols, and crystallized particles are capable of binding cholesterol and inhibiting the absorption of cholesterol in the human body. Plant sterols and stanols have been employed as a food ingredient intended to diminish the assimilation of dietary cholesterol since the late 1990s in products such as margarine, butter, cheese, yogurt, mayonnaise, and salad dressing, among others (Saxelin et al. 2003). The Scientific Committee on Food and the European Food Safety Authority recognize phytosterols as safe to consume and approve their addition in food for cholesterol-lowering purposes. However, their use is limited to 3 g per day, as higher doses have not been shown to be able to provide additional benefits, and could lead to undesired effects such as interference with lipo-soluble vitamins absorption. In this regard, the effect of free sterols or esterified sterols on the inhibition of cholesterol absorption has been shown to be similar; however, the interference they cause with the absorption of tocopherol and beta-carotene is reduced when employing free sterols, a quality that increases their value as ingredients in functional food products (Clifton 2007).

5.6.1 Role of Phytosterols and Phytostanols on Cholesterol Regulation

The addition of 1.7 g per day of sterols in the diet of hypercholesterolemic men has been shown to be effective in diminishing blood cholesterol levels. Consumption of 1 g/day of sterols added in yogurt by men and women with high levels of LDL cholesterol for a period of 4 days resulted in a 6.2 % reduction of LDL cholesterol in comparison with those subjects that consumed yogurt only. A similar study showed a 13.7 % reduction in the LDL cholesterol of 60 subjects who consumed 1 g/day of stanols in yogurt when compared to the placebo group (Varzakas and Arvanitoyannis 2010). Interestingly enough, another study including 185 subjects evaluated the effectiveness of plant sterols in diminishing LDL cholesterol with a single shot of yogurt containing 2 g of sterols administered either with or without a meal, resulting in a more effective treatment when yogurt was consumed with food, reducing LDL cholesterol by 9.3–9.5 % in comparison with the yogurt administered without a meal that diminished LDL cholesterol by 5.1–6.9 % (Clifton 2007).

5.7 Dairy Peptides

Bovine milk contains approximately 33 g of proteins per liter. Around 80 % of these proteins are caseins (α_{s1}-casein, α_{s2}-casein, β-casein, and k-casein), and the remaining 20 % are known as serum proteins (α-lactalbumin, β-lactoglobulin, immunoglobulins, lactoferrin, and enzymes, among others) (Walstra et al. 2006). Since proteins available in bovine milk are of high biological quality, the consumption of milk proteins represents an important nutritional contribution to the human body. However, most milk proteins have additional physiological functions relevant to the maintenance and recovery of health. Some of these functions include: ion carrier (α_{s1}-casein, α_{s2}-casein, β-casein, k-casein, and α-lactalbumin), lactose synthesis (α-lactalbumin), immunomodulation (α-lactalbumin, lactoferrin), anticarcinogenic (α-lactalbumin, lactoferrin), retinol carrier (β-lactoglobulin), antioxidative (β-lactoglobulin, lactoferrin), immune protection (immunoglobulins A, M, and G), fatty acids binding (β-lactoglobulin), antimicrobial (glycomacropeptide, lactoperoxidase, lactoferrin, lysozyme), toxic binding (lactoferrin), and iron absorption (lactoferrin), among others (Korhonen et al. 1998).

Additionally, milk proteins have the ability to function as precursors to biologically active peptides (Table 5.3). In fact, milk proteins have been highly recognized as the most important source of bioactive peptides (Korhonen and Pihlanto 2006). Bioactive peptides are specific fragments of proteins, which have a positive effect on health (Karagül-Yüceer and Avşar 2010). These peptides are formed by peptide chains from 2 to 20 amino acids, with molecular weights not greater than 1500 Da (Meisel and FitzGerald 2003). Peptides with biological activity may be formed naturally by hydrolytic enzymes (mainly trypsin and pepsin) released in the gastro-

Table 5.3 Bioactive peptides derived from milk proteins

Bioactive peptide	Protein precursor	Bioactivity
Casocidin	α_{s2}-casein	Antimicrobial
Casokinins	α_{s1}- and β-casein	Antihypertensive
Casomorphins	α_{s1}- and β-csein	Opioid agonists
Casoplatelins	k-casein, and transferrin	Antithrombotic
Casoxins	k-casein	Opioid antagonists
Glycomacropeptide	Caseins	Anti-stress
Immunopeptides	α_{s1}-, β-, and k-casein	Immunostimulants
Isracidin	α_{s1}-casein	Antimicrobial
Lactoferricin	Lactoferrin	Antimicrobial
Lactoferroxin	Lactoferrin	Opioid antagonists
Lactokinins	α-lactalbumin, β-lactoglobulin, and serum albumin	Antihypertensive
α-Lactorphin	α-lactalbumin	Opioid agonists

Adapted from Fitzgerald and Meisel (2003), Saxelin et al. (2003), Pihlanto-Leppälä (2001)

intestinal tract when dairy products are consumed (Korhonen et al. 1998; Korhonen and Pihlanto 2006). Nevertheless, bioactive peptides are principally released by processing milk and dairy products using artificial methods, such as chemical processes (alkali or acid treatments), physical processes (thermal treatments), by the hydrolytic activity of proteases, usually from microbial or plant sources, and by fermentation processes produced by different cultures, mainly lactic acid bacteria (LAB) (Chryssanthopulos and Maridaki 2010; Korhonen and Pihlanto 2006; Fitzgerald and Meisel 2003). Studies have shown that bioactive peptides consumption has an important influence on the regulation of several systems of the body, such as regulation of the nervous system (opioid agonists and antagonists peptides); regulation of the immune system (antimicrobial, immunomodulatory and cytomodulatory peptides); regulation of the cardiovascular system (antihypertensive, antioxidant, antithrombotic, and hypocholesterolemic peptides); and regulation of the gastrointestinal system (mineral-binding, satiety-inducing, and antimicrobial peptides) (Fitzgerald and Meisel 2003; Korhonen and Pihlanto 2006) (Table 5.4).

5.7.1 Mineral-Binding Peptides

Milk proteins and their hydrolysates are an excellent source of minerals due to their ability to carry ions within the peptide structure through the gastrointestinal tract, thus increasing mineral bioavailability. The solubility and bioavailability of minerals depend mainly on two factors: peptide characteristics and the oxidation state of the ion (Vegarud et al. 2000). Currently, phosphorylated peptides from caseins, i.e., caseinophosphopeptides, are the main mineral-binding peptides commercialized by

different international companies. These biomolecules are capable of binding different divalent ions, such as calcium, iron or zinc, to a specific acidic domain. This domain, composed by three phosphorylated serines followed by two glutamic acids, is present in all α_{s1}-, α_{s2}-, β-, and k-caseins in some cases more than one time (Bouhallab and Bouglé 2004). Calcium is the principal mineral that binds phosphopeptides. Mellander (1950) showed that rachitic infants, fed with caseinophosphopeptides, improved their bone calcification without vitamin *D*. Iron has also proved to bind successfully to phosphopeptides. Aït-oukhatar et al. (2002) showed that rats fed with an iron phosphopeptide complex from β-casein, f(1–25), had a better absorption of iron compared to animals fed with inorganic iron, iron gluconate and the iron β-casein complex. Whey proteins, α-lactalbumin, β-lactoglobulin, and lactoferrin, are also capable of binding minerals. However, since they are not phosphorylated, they have other sites to bind minerals in a different way than caseins (Vegarud et al. 2000). Kim and Lim (2004) revealed that peptides obtained from the hydrolysis of cheddar cheese whey by tryptic hydrolysis were capable of binding calcium, due to the presence of carbonyl, carboxyl, and alcohol functional groups in their amino-acid residues. Likewise, whey protein peptides are capable of effectively binding iron. Kim et al. (2007) hydrolyzed whey protein concentrate with nine different proteolytic enzymes and found that alcalase was the most effective enzyme to produce iron-binding peptides, which showed high concentrations of Lys, Ala, and Phe. These results suggest that whey protein peptides derived by alcalase hydrolysis potentially may be used in food products for the treatment of anemia.

5.7.2 Antimicrobial Peptides

Antimicrobial peptides have been identified and isolated from different milk proteins. The most studied peptide in terms of antimicrobial activity has been lactoferricin, the N-terminal fragment f(17–41) of lactoferrin. This peptide exhibits high antifungal, antiviral, and antibacterial activity against both gram-positive and gram-negative bacteria. Studies in vitro (Mistry et al. 2007) showed that lactoferricin had an inhibitory effect on human papillomavirus in epithelial cells, suggesting that lactoferricin could also be useful in the treatment of warts. Similarly, Shestakov et al. (2012) showed that lactoferricin was capable of inhibiting herpes simplex virus type 2 in mice. The administration of this peptide to mice completely stopped herpes development, which proved the in vivo use of lactoferricin for this disease. On the other hand, Zweytick et al. (2008) studied different fractions of human lactoferricin on *Escherichia coli*. Fraction VS1-24 showed a minimal inhibitory concentration (MIC) for *E. coli* of 8 μg/ml without inducing hemolytic activity, which implies the potential use of this fragment for the development of an antimicrobial against *Escherichia coli*. A few antimicrobial peptides have also been identified from α_{s1}- and α_{s2}-casein. The effect of casein peptides has been proven against different yeasts, molds, and bacteria, such as *Helicobacter*, *Listeria*, *Salmonella*, and

Table 5.4 Commercially available functional dairy products and dairy ingredients based on bioactive peptides

Brand name (Manufacturer)	Type of product	Peptide	Precursor protein	Bioactivity
BioPURE-GMP (Davisco, USA)	Hydrolysate	Glicomacropeptide	k-casein	Anticarcinogenic, Antimicrobial, Antithrombotic
BioZate (Davisco, USA)	Hydrolysate	Whey protein derived peptides	β-lactoglobulin	Hypotensive
C12 Peption (DMV, Netherlands)	Ingredient	Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys	Casein	Hypotensive
Calpis/Ameal S (Calpis, Co., Japan)	Sour milk	Val-Pro-Pro, Ile-Pro-Pro	β- and k-casein	Hypotensive
Capolac (Arla Foods, Denmark)	Ingredient	Casein phosphopeptide	Casein	Mineral absorption
Casein DP Peptio Drink (Kanebo, Japan)	Soft drink	Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys	Casein	Hypotensive
CE90CPP (DMV, The Netherlands)	Ingredient	Casein phosphopeptide	Casein	Mineral absorption
Cysteine peptide (DMV, The Netherlands)	Hydrolysate	Casein peptide	Casein	Reduces blood pressure
Evolus (Valio, Finland)	Fermented milk	Val-Pro-Pro, Ile-Pro-Pro	β- and k-casein	Hypotensive
Festivo (MIT Agrifood Research, Finland)	Low-fat cheese	Arg-Pro-Lys-His-Pro-Ile, Arg-Pro-Lys-His-Pro-Ile-Lys, Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln	α_{s1}-casein f(1–6), α_{s1}-casein f(1–7), α_{s1}-casein f(1–9)	ACE inhibitory
Kotsu Kotsu calcium (Asahi Soft Drinks Co., Japan)	Soft drink	Casein phosphopeptide	Casein	Mineral absorption
PeptoPro (DSM Food Specialities, Netherlands)	Hydrolysate	Casein peptide	Casein	Improves muscle recovery
Praventin (DMV, The Netherlands)	Lactoferrin-rich hydrolysate	Whey protein derived peptides	Whey proteins	Reduces symptoms of skin infections
ProDiet F200 (Ingredia, France)	Milk drink, confectionery	Try-Leu-Gly-Tyr-Leu-Glu-Gln-Leu-Leu-Arg	α_{s1}-casein f(91–100)	Reduces stress
Recaldent (Cadbury Enterprises)	Chewing gum	Casein phosphopeptide	Casein	Anticariogenic

(continued)

Table 5.4 (continued)

Brand name (Manufacturer)	Type of product	Peptide	Precursor protein	Bioactivity
Tekkotsu Inryou (Suntory, Japan)	Soft drink	Casein phosphopeptide	Casein	Minerals absorption
Vivinal Alpha (BDI, Netherlands)	α-lactalbumin-rich hydrolysate	Whey protein derived peptides	Whey proteins	Aids relaxation

Staphylococcus. Arruda et al. (2012) isolated four peptides by the hydrolysis of casein with proteases from the root of *Jacaratia corumbensis*. All of the isolated peptides showed an antimicrobial effect on *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The MIC of peptides was 50 mg/ml for all microorganisms except for *S. aureus* (MIC = 40 mg/ml).

5.7.3 Opioid Agonists and Antagonists Peptides

Opioid receptors are cellular protein receptors situated mainly in the nervous system that can interact with endogenous ligands and with exogenous opioids, such as opioid peptides (exorphins). Opioid peptides bind with specific receptors on the target cell and may cause either agonistic or antagonistic activity. Exorphins can be obtained from the hydrolysis of milk proteins of different species, such as bovine, human, ovine, and buffalo, among others. The first and most studied opioid peptide from food proteins is β-casomorphin, a peptide derived from β-casein (Pihlanto-Leppälä 2001). β-casomorphins have many physiological effects, which include improvement of learning, psychomotor development, memory, hypoglycemic, antihypertensive, and anxiolytic effects. Studies carried out by Zhang et al. (2012b) showed that bovine β-casomorphin-7, the most studied β-casomorphin, attenuated renal interstitial fibrosis caused by type 1 diabetes, by regulation of epithelial–mesenchymal transition of renal tubular epithelial in rats fed with this peptide. Likewise, Sakaguchi et al. (2006) studied the effect of the μ-opioid receptor agonist, β-casomorphin-5, on short-term memory and non-spatial long-term memory in mice. These results showed that a systemic administration of low doses of β-casomorphin to mice improved their memory and lowered the disturbance of learning. Other studies conducted by Taira et al. (1990) demonstrated that the administration of bovine β-casomorphin-7 to neonatal rats increased the quiet state of sleep and decreased active sleep without respiratory depression. Other milk caseins have also been shown to contain opioid peptides within their structure. α-casein exorphin, a peptide contained in α_{s1}-casein, was found to exhibit opioid properties in vitro. This exorphin binds the δ-opioid receptors with an antagonist behavior (Teschemacher et al. 1997). Another peptide from α_{s1}-casein f(91–100)

has been shown to have anxiolytic-like stress-relieving properties in animal and human studies. This peptide, from α_{s1}-casein, is currently used as a commercial ingredient for confectionery products and drinks (Korhonen and Pihlanto 2006). Casoxin C, a peptide isolated from the tryptic hydrolysis of bovine k-casein, showed agonist behavior for C3a receptors. This peptide showed ileum-contracting effects as well as phagocyte-stimulating activities (Takahashi et al. 1997). Finally, opioid peptides from whey proteins have also been studied due to their physiological effects on the cardiovascular system. Nurmien et al. (2000) subcutaneously administrated α-lactorphin, a peptide derived from α-lactalbumin to hypertensive rats. Nurmien's research group found that low doses of α-lactorphin induced reductions in systolic and diastolic blood pressure by 23 mmHg and 17 mmHg, respectively. Studies conducted by Sipola et al. (2002) showed that β-lactorphin, a peptide derived from β-lactoglobulin enhanced endothelium-independent relaxation in adult rats, showing similar results to those from α-lactorphin to decrease blood pressure.

5.7.4 Antihypertensive Peptides

Angiotensin-I-converting enzyme (ACE) inhibitors have shown effectiveness in controlling hypertension and in the treatment of congestive cardiac failure (O'Keefe et al. 2001). Some milk peptides have been studied due to their ACE-inhibitory activity. Casokinins, derived from α_{s1}- and β-caseins, and peptides from α-lactalbumin, and β-lactoglobulin identified as lactokinins (Table 5.5), are well-known as ACE inhibitors (Erdmann et al. 2008; Pihlanto-Leppälä 2001). β-casomorphin, β-lactorphin, and α-lactorphin (from β-casein, β-lactoglobulin, and α-lactalbumin, respectively), typically recognized as opioid peptides, have also shown a moderate ACE-inhibitory activity. Nevertheless, the quest for new ACE-inhibitor peptides continues. Abubakar et al. (1998) isolated a tripeptide from β-lactoglobulin (β-lactosin A), as well as a tetrapeptide, (β-lactosin B) from β-lactoglobulin both with interesting ACE inhibiting activity (Murakami et al. 2004). ACE-inhibitor peptides may be obtained, not only by chemical or fermentative hydrolysis in vitro but also during the manufacture of fermented dairy products, such as cheese, yogurt, and fermented milks among others, by the action of microorganisms (Fitzgerald and Murray 2006). Cheeses have been shown to be a good source of peptides with high ACE-inhibitory activity. Gómez-Ruiz et al. (2004) isolated two peptides from Manchego cheese, α_{s1}-casein f(102–109), and α_{s2}-casein f(205–208), with ACE-inhibiting activity. Smacchi and Gobbetti (1998) studied the Italian cheese, Crescenza, from which they isolated a tetradecapeptide from β-casein f(58–72) with strong ACE-inhibiting properties. Gouda cheese also contains two peptides with ACE-inhibitory activity, from α_{s1}-casein f(1–9), and from β-casein f(60–68) (Saito et al. 2000). As a general conclusion, it may be said that milk and whey fermented products normally contain some degree of ACE-inhibitory peptides produced by microbial hydrolysis of caseins during product manufacture (Fitzgerald and Murray 2006).

Table 5.5 ACE-inhibitory peptides from milk proteins

Precursor protein	IC_{50} value μM	Peptide sequence	Enzyme
β-casein	5	Ile-Pro-Pro	*L. helveticus* proteinase
β-casein	9	Val-Pro-Pro	*L. helveticus* proteinase
β-lactoglobulin	43	Ala-Leu-Pro-Met-His-Ile-Arg	Trypsin
α-lactalbumin	77	Trp-Leu-Ala-His-Lys	Trypsin
β-lactoglobulin	141	Ile-Pro-Ala	Proteinase K
α-lactalbumin	327	Val-Gly-Ile-Asn-Tyr-Trp-Leu-Ala-His-Lys	Trypsin
α-lactalbumin	409	Tyr-Gly-Leu	Pepsin, trypsin, chymotrypsin
β-lactoglobulin	521	Ala-Leu-Pro-Met-His	Pepsin, trypsin,
β-lactoglobulin	556	Leu-Ala-Met-Ala	Trypsin
β-lactoglobulin	635	Leu-Asp-Ala-Gln-Ser-Ala-Pro-Leu-Arg	Trypsin
β-lactoglobulin	788	Cys-Met-Glu-Asn-Ser-Ala	Pepsin, trypsin, chymotrypsin
β-lactoglobulin	928	Ala-Leu-Pro-Met	Chemically synthesized
β-lactoglobulin	946	Val-Leu-Asp-Thr-Asp-Tyr-Lys	Pepsin, trypsin, chymotrypsin
β-lactoglobulin	1029	Val-Phe-Lys	Trypsin

IC_{50} value: Peptide concentration needed to inhibit 50 % of the ACE activity

5.7.5 Antioxidant Peptides

Antioxidant peptides are typically short-chain peptides easily absorbed by the body. As in other bioactive peptides, it is the amino acid-residue properties that define antioxidant capacity. In fact, peptides with a Pro-His-His sequence have shown the highest antioxidant activity among all peptides (Erdmann et al. 2008). Boldogh et al. (2003) found that a polypeptide complex rich in proline obtained from ovine colostrum (colostrinin) showed antioxidant activity on pheochromocytoma cells. These results showed that colostrinin was involved in the cell "oxidation-reduction" status, glutathione metabolism, and the modulation of reactive oxygen species; therefore, it may be useful in the treatment of neurodegenerative disorders, such as Alzheimer's, and those in which pathogenesis involves reactive oxygen species. Kullisaar et al. (2003) demonstrated that the consumption of fermented goat milk enhanced total antioxidant activity in humans. These results revealed an improvement in antiatherogenicity by prolonging the resistance to oxidation of lipoproteins, decreasing the levels of peroxidized lipoproteins, oxidized low density lipoproteins, 8-isoprostanes, and the glutathione redox reaction. Some in vitro and in vivo studies have explained the mode of action and health effects for this mechanism. You et al. (2011) showed that antioxidant peptides and the endogenous cellular defense mechanism contained in muscle cells (superoxide dismutase, glutathione peroxidase, and catalase) work together to eliminate reactive oxygen species, and protect against the oxidative stress induced by exercise. Additional studies in animals demonstrated that the intake of antioxidant peptides can reduce oxidative stress induced by

exercise. The reason for this can be that the intake of antioxidant peptides interacts with endogenous antioxidants to create cooperative networks against oxidative stress (Mizuno et al. 2008). However, antioxidant activity is not always directly due to peptide action. Bounous and Gold (1991) applied a dipeptide from serum albumin, L-Glu-Cys, to rats. An increment of glutathione levels in cardiac, hepatic, and splenic tissue, and consequently a cellular oxidative damage recovery was observed, in this case thanks to the promotion of glutathione synthesis caused by the dipeptide rather than because of its direct antioxidant activity (Pompella et al. 2003).

5.7.6 Hypocholesterolemic Peptides

Many studies have demonstrated that dietary proteins have a strong influence on serum cholesterol levels. Whey proteins and their hydrolysates have been widely studied because of their hypocholesterolemic activity. Nagaoka et al. (1992) observed a greater hypocholesterolemic effect in rats fed with whey proteins than in animals fed with casein or soy proteins; moreover, the ratio of high density lipoprotein to cholesterol was also higher in rats fed with whey proteins. Another study carried out by Nagaoka (1996) demonstrated that β-lactoglobulin tryptic hydrolysate decreased serum cholesterol levels in rats even more than soy and whey proteins. Peptides derived from β-lactoglobulin also inhibited cholesterol absorption in Caco2 cells more than whey protein, which implies a higher hypocholesterolemic activity of β-lactoglobulin peptides than that of β-lactoglobulin itself. Nagaoka et al. (2001) identified a peptide derived from the trypsin hydrolysis of β-lactoglobulin (Ile-Ile-Ala-Glu-Lys) named lactostatin by Nagaoka's research group. The hypocholesterolemic effect of lactostatin was studied in Caco-2 cells and in rats. This research group demonstrated that rats fed with lactostatin reduced their total serum cholesterol levels more than rats fed with β-sitosterol (a chemical used to reduce blood levels of cholesterol); HDL levels and atherogenic index were also higher in rats fed with the pentapeptide. A proposed mode of action suggests that peptides bind bile acids, inhibiting micellarization and therefore reducing cholesterol solubility; hence, the absorption of cholesterol in the gut is suppressed, reducing serum cholesterol levels (Erdmann et al. 2008). On the other hand, Morikawa et al. (2007) studied the hypocholesterolemic mode of action of lactostatin in HepG2 (a human liver cell line). They suggested that cholesterol degradation was due to the activation of cholesterol 7α-hydroxylase gene expression (CYP7A1) by lactostatin. CYP7A1 gene expression regulates serum cholesterol levels, since cholesterol 7α-hydroxylase is the rate-limiting enzyme in the synthesis of hepatic bile acid from cholesterol. Higurashi et al. (2007) showed that rats fed Gouda cheese fermented by *Lactobacillus helveticus* decreased their serum cholesterol levels. The hypocholesterolemic effect in Gouda cheese was associated with the presence of the decapeptide (His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln) obtained by fermentation of milk.

5.7.7 *Antiobesity and Satiety-Inducing Peptides*

It is generally accepted that high protein diets can help in the handling of obesity because of their ability to reduce energy intake, to lower fat storage, to suppress appetite, and consequently, to lose weight. Some studies have shown that milk proteins, casein, and whey proteins are a good alternative for use in weight control diets because of their antiobesity and anorectic properties. Belobrajdic et al. (2004) demonstrated that rats fed with high concentrations of whey protein reduced weight gain, fat deposition, and plasma insulin concentration better than when fed with red meat protein. However, whey and casein hydrolysates have proved to have greater effect on weigh control, insulin resistance, lipid metabolism and other risk factors associated with obesity, type 2 diabetes, and cardiovascular disease than their precursor proteins (Aoyama et al. 2000). Several studies have demonstrated that the antiobesity activity of these peptides depends on their capacity to promote cholecystokinin release. Cholecystokinin is a hormone released in the gut, the main functions of which are gastric emptying and energy balance regulation through stimulation of satiety (Reidelberger 1994). Beucher et al. (1994) found that gastric digestive hydrolysates from casein stimulated cholecystokinin release by intestinal cells in rats. Cholecystokinin secretion stimuli was associated with the presence of glycomacropeptide, a 64-amino acid residual peptide from k-casein f(106–169) released by digestive enzymes. However, studies carried out on humans demonstrated that glycomacropeptide by itself did not have a relevant effect on weight or satiety induction when volunteers consumed it for a short period of time (Gustafson et al. 2001). Currently, several commercial products containing glycomacropeptide have been released in the global market with the main purpose of overweight control by inducing satiety, though clinical effects and regulations must still be established (Korhonen and Pihlanto 2006). Some other hormones involved in weight control, insulin resistance and other risk issues, are also regulated by peptides. Studies in rats carried out by Higurashi et al. (2007) showed that the antioxidant decapeptide His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln obtained from Gouda cheese fermented by *Lactobacillus helveticus* plays a role in the production of adiponectin, a hormone whose expression inversely correlates with fat mass, and with anti-inflammatory, antidiabetic, antiatherogenic, and antihypertensive functions (Ding et al. 2012). These results suggest that the consumption of Gouda cheese may help suppress the accumulation of mesenteric adipose tissue and can prevent the development of metabolic syndrome. Similarly, Uenishi et al. (2012) demonstrated that octapeptide from β-casein isolated from a water-soluble extract of Gouda cheese, Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu f(70–77), inhibited the production of dipeptidyl-peptidase 4, an enzyme which degrades incretin hormones (insulin regulating hormones) in rats. These results suggest that this peptide may be useful as well in improving glucose tolerance in type 2 diabetes.

5.8 Conclusions

Scientific and technological advances of the past several decades have revealed a large number of old food components with new, previously unknown properties. The use of these components in the formulation and manufacture of functional foods is a task that is being gradually carried out by the food industry, and many more products are expected in the near future. The use of dairy products as a vehicle or source of functionality has been without a doubt one of the most successful approaches; therefore, a continued trend is expected, especially with regard to the development of new dairy-based functional ingredients (i.e., milk peptide-based ingredients). The segment of products with "natural functionality," as is the case of functional peptide-rich cheeses and fermented milks, is also expected to grow in the near future. Still ahead, much more research, especially human clinical trials, is required in order to solidly support the functionality of current and future functional ingredients. Legal and commercial claims issues related to the production and commercialization of functional dairy products are also a subject that requires attention and should be dealt with on a worldwide level.

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Chapter 6
Brazilian Native Fruits as a Source of Phenolic Compounds

Neuza Mariko, Aymoto Hassimotto, and Franco Maria Lajolo

6.1 Introduction

Brazil presents significant biodiversity in tropical foods which are excellent sources of macro- and micronutrients as well as bioactive compounds that promote health and reduce the risk of several chronic diseases. In addition to traditional fruits, many other fruits are present in the Brazilian diet that has not been yet industrialized. These fruits are usually consumed in nature or home processed into frozen pulps, jellies, ice cream, and sweets. The fruits are produced on a small scale using simple technology. Unfortunately, information regarding their nutritional value and phytochemicals composition is limited. Further, a lack of knowledge concerning the fruits' postharvest condition makes it difficult to obtain a standardized high-quality product at the market.

Many studies have demonstrated the potential antioxidant, anti-inflammatory, anticarcinogenic, and antiallergic health benefits of phenolic compounds (Xiao et al. 2011). Potential explanations for such properties include the ability of these molecules to modulate the expression and activity of several enzymes in cell signaling and metabolism (e.g., kinases, lipoxygenases, cyclooxygenases, and ATPases) (Tan et al. 2011; Paredes-López et al. 2010) in addition to their direct action as antioxidants (Pietta 2000; Erdman et al. 2007).

Thus, understanding the nutritional value and phytochemical composition of native fruits may aid in discovering new functional food sources and assist in reducing nutritional deficiencies of micro- and macronutrients.

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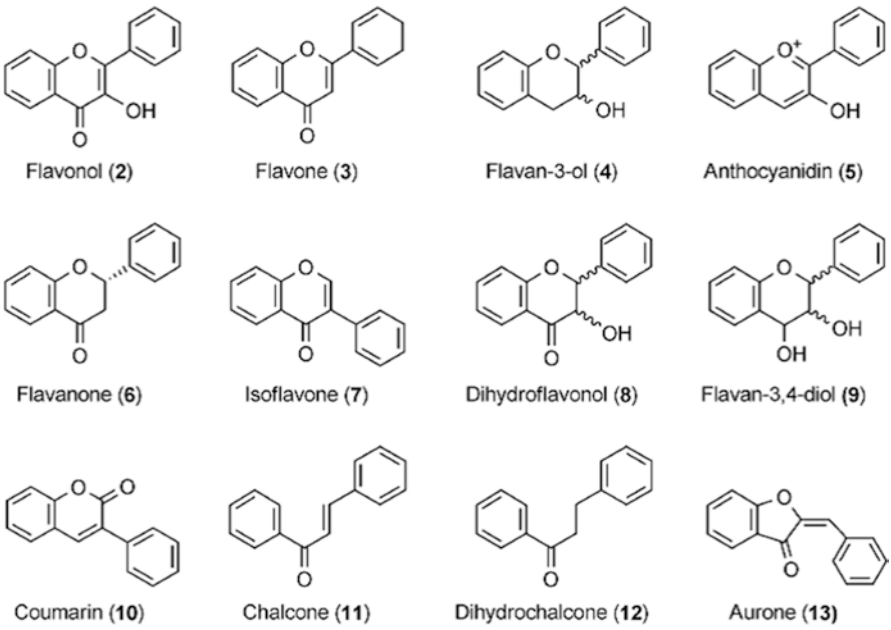

Fig. 6.1 Basic structural skeletons of phenolic and polyphenolic compounds (Crozier et al. 2009)

6.2 Main Class of Phenolic Compounds

Phenolic compounds represent the largest category of phytochemicals widely distributed in the plant kingdom. The two largest groups of phenolic compounds in the human diet are phenolic acids and flavonoids. Flavonoids are polyphenolic compounds comprising 15 carbons, with two aromatic rings connected by a three-carbon bridge; the basic skeleton can be described as C6–C3–C6. Flavonoids are concentrated mainly in the epidermis of leaves and the skin of fruits. The main subclasses of dietary flavonoids are (see Fig. 6.1) flavonols (2), flavones (3), flavan-3-ols (4), anthocyanidins (5), flavanones (6), and isoflavones (7), while those that represent lesser components of the diet include dihydroflavonols (8), flavan-3,4-diols (9), coumarins (10), chalcones (11), dihydrochalcones (12), and aurones (13). The basic flavonoid skeleton can have numerous substituents. Hydroxyl groups are usually present at the 4′-, 5′-, and 7′-positions, and the majority of flavonoids exist naturally as glycosides derivatives. Whereas both sugars and hydroxyl groups increase the water solubility of flavonoids, other substituents, such as methyl groups and isopentyl units, make flavonoids lipophilic (Crozier et al. 2009).

6.3 Brazilian Native Fruits

6.3.1 Fruits as Sources of Anthocyanins

In general, most of the native fruits of Brazil are not commercially grown and are usually collected by the local population. These fruits are typically consumed in nature or processed as frozen pulp, jelly, ice cream, or juice. The nutrition and functional potential of these fruits has been explored, and most of the fruits appear to be a good source of bioactive compounds such as flavonoids and other phenolic compounds (see Tables 6.1 and 6.2). Moreover, they exhibit good taste and nutritional value of both micro- and macronutrients.

The health benefits of anthocyanins have been described in the context of their antioxidant properties. However, recent findings have demonstrated that anthocyanins could aid in obesity control, diabetes, cardiovascular disease prevention, and the improvement of visual and brain functions. However, the physiological functionality of anthocyanins requires further elucidation (Tsuda 2012; Kaume et al. 2012).

Fruits such as açaí (*Euterpe oleracea* Mart.), which is obtained from the açaí palm tree (*Arecaceae family*), are very popular and exhibit a high caloric value and fat content. The plant is widely distributed in northern South America. The seeds account for most of the fruit size and are present in a thin edible layer. A concentrated aqueous extract is typically prepared by macerating the edible layer that has a lipid content of approximately 6 % (Del Pozo-Insfran et al. 2004). Açaí berries contain oleic acid (60 % of total fat), linoleic acid (12 % of total fat), palmitic acid (24 % of total fat), and phytosterols, including beta-sitosterol (78–91 % of total sterols) (Lubrano et al. 1994; Schauss et al. 2006). In addition, açaí is a good source of antioxidants, such as anthocyanins, which are responsible for the dark purple color of the fruit (1040 mg cyanidin 3-glucoside/L açaí pulp), and other phenolics (4–212 mg/L açaí pulp) (Del Pozo-Insfran et al. 2004). Cyanidin 3-rutinoside, cyanidin 3-diglycoside, and cyanidin 3-glucoside are the major anthocyanins found in açaí berries (Jensen et al. 2008). A complete review of açaí berries and pulp was performed by Ulbricht et al. (2012), including evidence of the açaí consumption and potential biological activity, toxicology, and side effects. Several potential biological activities have been attributed to açaí, such as antioxidant (Jensen et al. 2008; Guerra et al. 2011), anti-inflammatory (Xie et al. 2011a, b, 2012), proapoptotic and antiproliferative activities against HL-60 leukemia cancer cells (Del Pozo-Insfran et al. 2006), and hypocholesterolemic activity (De Souza et al. 2012). According to Ulbricht et al. (2012), in Belém, a major port and gateway into the Brazilian Amazon, an enormous açaí fruit market called "Feira do Açaí" houses 70–120 vendors selling over 200,000 kg of açaí fruit daily during the dry season.

Another palm tree fruit is jussara (*Euterpes edulis*). Like açaí fruit, jussara is round and has a purple pulp covering a hard seed. Despite its wide distribution in Brazil, jussara is much less commonly consumed than açaí (De Brito et al. 2007). Jussara is also rich in anthocyanin (Table 6.1), mainly cyanidin derivatives, and

Table 6.1 Anthocyanins content (milligrams per 100 g of sample) of Brazilian native fruits

Fruits	Family	Scientific name	Cyanidin	Petunidin	Pelargonidin	Peonidin	Delphinidin
Acerola[a]	Myrtaceae	*Malpighia emarginata*	181–359	nd	80–169	nd	nd
Camu-camu[b]	Myrtaceae	*Myrciaria dubia* Mc. Vaugh	306	nd	nd	nd	nd
Jambolão[c]	Myrtaceae	*Syzygium cumini*	8.8 ± 0.4	68 ± 1.1	nd	nd	95.6 ± 0.4
Jambo rosa[d]	Myrtaceae	*Syzygium jambos* L.	0.5 ± 0.0	nd	nd	nd	nd
Jabuticaba peel[d]	Myrtaceae	*Myrciaria jaboticaba*	229 ± 6	nd	nd	nd	nd
Jabuticaba flesh[d]	Myrtaceae	*Myrciaria jaboticaba*	0.1 ± 0.0	nd	nd	nd	nd
Jussara[a]	Arecaeace	*Euterpe edulis*	2943	nd	13	nd	nd
Guajiru[a]	Chrysobalanaceae	*Chrysobalanus icaco*	nd	726	nd	25	207
Wild mulberry[d]	Moraceae	*Morus nigra* L.	131–256	2.7–3.6	nd	nd	nd

Adapted from: [a]De Brito et al. (2007) (DW); [b]De Souza Schmidt Goncalves et al. (2010) (DW); [c]Faria et al. (2011) (FW); [d]Arabbi et al. (2004) (FW), Hassimotto et al. (2007) (FW)

Table 6.2 Flavonoids (flavan-3-ol and flavonol) and total ellagic acid contents of Brazilian native fruits

			Flavan-3-ol		Flavonol		
Fruits	Family	Scientific name	Catechin	Epicatechin	Quercetin	Kaempferol	Total EA
Graviola[a]	Annonaceae	*Annona muricata* L.	15.9±0.3	15±1	nd	nd	nd
Tucumã[a]	Arecaceae	*Astrocaryum aculeatum*	79.5	nd	2.96±0.05	nd	nd
Cambuci[a]	Myrtaceae	*Campomanesia phaea* Berg.	nd	nd	21.6±0.3	0.4±0.1	240±15
Araçá-boi[a]	Myrtaceae	*Eugenia stipitata* Mc. Vaugh	nd	nd	14.4±0.2	2.5±0.1	nd
Araçá[a]	Myrtaceae	*Psidium guineensis* Sw.	nd	nd	40±2	0.7±0.1	262±12
Buriti[a]	Arecaceae	*Mauritia flexuosa*	nd	nd	0.6±0.1	nd	nd
Abiu[a]	Sapotaceae	*Pouteria caimito*	nd	nd	nd	nd	nd
Maná-cubiu[a]	Solanaceae	*Solanum sessiliflorum*	nd	nd	nd	nd	nd
Murici[b]	Malpighiaceae	*Byrsonima verbascifolia* Rich	nd	nd	nd	nd	nd
Gabiroba[b]	Myrtaceae	*Campomanesia cambessedeana* Berg	79.1±0.9	nd	nd	nd	nd
Camu-camu[a]	Myrtaceae	*Myrciaria dubia* Mc. Vaugh	nd	nd	42±4	2.1±0.1	490±20
Jambo rosa[c]	Myrtaceae	*Syzygium jambos* L.	0.2±0.0	nd	0.7±0.0	nd	nd
Jabuticada peel[c]	Myrtaceae	*Myrciaria jaboticaba*	16.0±1.4	nd	nd	nd	2250±130
Jabuticana flesh[c]	Myrtaceae	*Myrciaria jaboticaba*	0.9±0.0	nd	nd	nd	460±20
Wild mulberry[d]	Moraceae	*Morus nigra*	nd	nd	14–15	nd	nd
Bacuri[a]	Arecaceae	*Scheelea phalerata*	nd	nd	nd	nd	nd
Cupuaçu[a]	Sterculiaceae	*Theobroma grandiflorum*	nd	nd	nd	nd	nd
Uxi[a]	Humiriaceae	*Endopleura uchi*	nd	nd	nd	nd	nd
Granadilla[a]	Passifloraceae	*Passiflora ligularis* Juss	nd	nd	nd	nd	nd
Sweet passion fruit[a]	Passifloraceae	*Passiflora alata* Curtis	nd	nd	nd	nd	nd

EA ellagic acid; values expressed as milligrams per 100 g of sample

Adapted from: [a]De Souza Schmidt Goncalves et al. (2010) (DW); [b]Malta et al. (2012) (DW); [c]Arabbi et al. (2004) (FW), [d]Hassimotto et al. (2007) (FW)

smaller amount of pelargonidin. The major cyanidin derivatives are cyanidin 3-glucoside and cyanidin 3-rutinoside (De Brito et al. 2007).

Acerola (*Malpighia emarginata* DC.) and camu-camu (*Myrciaria dubia* Mc. Vaugh) are also native fruits grown in the northern part of South America and are known to be a good source of vitamin C. Acerola is a small, round fruit with bright red skin and orange-red pulp. The ascorbic acid content ranges from 695 to 4827 mg/100 g (Mezadri et al. 2006), and its bioavailability is similar to pure ascorbic acid (Uchida et al. 2011). The variability in vitamin C content may be related to the ripening stage, in which a green fruit exhibits higher vitamin C content than a ripened fruit (Nogueira et al. 2002). Further, vitamin C is associated with an increase in ascorbate oxidase activity during the fruit ripening (Butt 1980). Solar radiation, which increases photosynthesis, increases the level of sugars in the tissue and, consequently, the ascorbic acid content because ascorbic acid is synthesized from the hexoses (Mezadri et al. 2006). In addition to ascorbic acid, acerola contains bioactive compounds such as carotenoids and polyphenols. Acerola was found to contain the anthocyanins cyanidin 3-*O*-rhamnoside and pelargonidin 3-*O*-rhamnoside in different amounts according to variety (De Brito et al. 2007; Hanamura et al. 2005) and quercetin 3-*O*-rhamnoside (Hanamura et al. 2005).

Camu-camu possesses significant amounts of anthocyanins (306 mg/100 g of DW), mainly cyanidin derivatives, which are responsible for the red-to-purple color of the peel in the ripe fruit. According to Zanatta et al. (2005), cyanidin 3-glucoside is the major anthocyanin in camu-camu fruits, followed by delphinidin 3-glucoside. Variation in the content of anthocyanins is observed between 337 and 606 mg/100 g (DW) in camu-camu cultivated in two different regions in Brazil (Zanatta et al. 2005). In addition, camu-camu exhibits high total ellagic acid content (Table 6.2), higher than fruits such as cambuci and araça. In addition, camu-camu fruits are a good source of vitamin C, which varies in amount from 2010 ± 65 mg/100 g (red stage) to 2280 ± 34 mg/100 g FW (green stage) (Chirinos et al. 2010). However, other studies have reported low levels of vitamin C in camu-camu fruit (397 mg/100 g FW) (Genovese et al. 2008).

Jabuticada is another Brazilian native fruit completely adapted to commercial plantation that exhibits a deep purple peel and white flesh with a sweet taste. No anthocyanins have been detected in the flesh, but anthocyanins concentrated in the peel have been identified as cyanidin glycosides (Table 6.1). In addition to the high content of anthocyanins in the peel, jabuticaba is a good source of ellagic acid derivatives. The seed exhibits the highest ellagic acid content (40.2 ± 0.6 g/kg DW), followed by the peel (22 ± 1 g/kg DW) and flesh (4.6 ± 0.2 g/kg DW) (Abe et al. 2012). In general, the fruit is consumed in nature. Unfortunately, although the peel is the part of the fruit richest in phenolic compounds, the flesh is the edible part. Some manufactured products, such as jelly or liquor, are deep purple in color because the peel was added during processing.

Guajiru has been used in traditional medicine, but its consumption as a food is limited. Petunidin is the main anthocyanidin present in the fruit, followed by delphinidin and peonidin (minor components). Notably, most of the anthocyanins in guajiru appear acylated (approximately 88 % of the total anthocyanins). Researchers

have shown that anthocyanins with acylating substituents are more stable during processing and storage than unacylated anthocyanins. Unacylated anthocyanins are sensitive to pH changes and exhibit increased heat and light sensitivity. For this reason, acylated anthocyanins are suitable not only in foods with a low pH, but also in neutral and slightly alkaline products (Bakowska-Barczak 2005), providing better color quality in processed foods. The improved stabilization has been attributed to the stacking of the acyl groups with the pyrylium ring of the flavylium cation, thereby reducing the susceptibility of nucleophilic attack of water and the subsequent formation of a pseudobase or a chalcone (intramolecular copigmentation) (Bakowska-Barczak 2005). Full color stabilization is better achieved when the anthocyanins bear aromatic rings compared with aliphatic rings, and more stable complexes are formed when the aromatic acids are substituted in ring B of the flavylium cation rather than in ring A (Giusti and Wrolstad 2003). Red cabbage and purple potato anthocyanins typically contain diacylated cinnamic acid moieties, which can simultaneously stack on both faces of the anthocyanin chromophore in a sandwich-type complex and thus offer greater color stability, whereas black carrots contain only monoacylated moieties that can protect only one face of the pyrylium ring (Del Pozo-Insfran et al. 2004).

6.3.2 *Fruit Sources of Other Classes of Flavonoids and Phenolic Acids*

Catechins are present in many fruits and derivatives, especially grapes and wine (up to 300 mg/L), but the main sources are green tea and chocolate. Catechins can be found free or esterified with gallic acid, which is most commonly observed in tea leaves (*Camellia sinensis*) and grapes (Crozier et al. 2009). Among the native fruits from Brazil listed in Table 6.2, catechin and epicatechin are detected only in tucumã, gabiroba, and graviola, ranging from 15 to 79 mg/100 g of sample (DW). Gabiroba is a typical fruit from the Brazilian cerrado. It exhibits a round shape and citrus fragrance, and its color ranges from a dark to light green and/or yellow color; it is considered a good source of vitamin C (234 mg/100 g FW) (Vallilo et al. 2006).

As mentioned previously, the seeds and peels of jabuticaba are the best source of ellagitannins (ET) and could be recovered by the waste industry. The health benefits of particular ellagitannins and ellagic acid have not been completely elucidated, but published review of ellagitannins and ellagic acid describes the anti-inflammatory, antimicrobial, prebiotic, antioxidant, and estrogenic and/or antiestrogenic activity of these compounds (Landete 2011). Ellagitannins are hydrolyzable tannins, a class of polyphenols with a structure that consists of esters of hexahydroxydiphenoyl groups with a polyol core (usually glucose or quinic acid) and that often contains galloyl groups. ET monomers can be further oxidized in plants and form dimers, trimers, and tetramers with molecular weights of up to 4000 Da (Clifford and Scalbert 2000). ET detection and quantification is based on the hydrolysis of these

compounds, which yields free ellagic acid (EA). The presence of ET in common foodstuffs is very limited; only a few fruits and nuts contain ET. ETs are found in pomegranates (*Punica granatum*), hazelnuts (*Corylus avellana*), persimmons (*Diospyros kaki*), oak-aged wine and berries, such as raspberries (*Rubus idaeus*), strawberries, and blackberries (Clifford and Scalbert 2000; Mullen et al. 2003; Cerdá et al. 2005; Koponen et al. 2007). Koponen et al. (2007) reported an average total EA content of 260–311 mg/100 g (FW) in berries of the Rosaceae family (cloudberry, raspberry, rosehip, and strawberry).

In addition to native fruits rich in polyphenols, it is important to mention a popular infusion consumed mainly in southern Brazil that is made with the leaves of the plant *Ilex paraguariensis*, or yerba maté. This plant originates in the subtropical region of South America, and it is present in southern Brazil, northern Argentina, Paraguay, and Uruguay. Yerba maté leaves are used to prepare different beverages, such as chimarrão (green dried leaves brewed with hot water in a vessel called a cuia), tererê (green dried leaves brewed with cold water in the same vessel), and maté tea (roasted leaves brewed with hot water or used to produce soft drinks) (Bastos et al. 2007a). Maté beverages are rich in polyphenolic compounds such as caffeine (approximately 8 % in the dry base); polyphenols, represented mainly by phenolic acids (65 %) and flavanols (25 %); and triterpenoid saponins, which are partially responsible for the taste of the beverage, foaming, and a choleretic effect (Bracesco et al. 2011). The main phenolic compounds identified in green yerba maté include caffeic acid, quinic acid, caffeoyl glucose, caffeoylquinic acid, feruloylquinic acid, dicaffeoylquinic acid, and rutin (Table 6.3). After the roasting process, two new

Table 6.3 ESI-MS/MS fragments of compounds identified in extracts of green yerba maté and roasted yerba maté (*Ilex paraguariensis*)

	ESI-MS ions (*m*/*z*)	
Compound	$[M-H]^-$ (*m*/*z*)	MS/MS (*m*/*z*)
Caffeic acid	179	135, 179
Quinic acid	191	85, 93, 111, 127, 173
Catechin/epicatechin	289	109, 125, 179, 203, 205, 245
Caffeoylshikimic acid	335	135, 161, 179
Caffeoyl glucose	341	119, 179
Caffeoylquinic acid	353	135, 173, 179, 191
Feruloylquinic acid	367	173, 191, 193
Epicatechin gallate	441	135, 169, 289
Methyl epicatechin gallate	455	375, 407
Epigallocatechin gallate	457	169, 305, 331
3 Methyl epigallocatechin gallate	471	407, 441
Dicaffeoylshikimic acid	497	161, 179, 335
Dicaffeoylquinic acid	515	173, 179, 191, 35
Rutin	609	301

From: Bastos et al. (2007b)

compounds are formed, caffeoylshikimic acid and dicaffeoylshikimic acid. These compounds contain one molecule of water less than the analogous caffeoyl- and dicaffeoylquinic acids (Bastos et al. 2007b). Among the biological properties attributed to *Ilex paraguariensis*, the choleretic effect, intestinal propulsion (Gorzalczany et al. 2001), and vasodilatory effects (Stein et al. 2005) have been described in rats, as well as the inhibition of glycation in vitro (Lunceford and Gugliucci 2005).

6.4 Bioavailability of Phenolic Compounds

The use of bioactive compounds from vegetables and fruits in functional foods presents certain barriers, mainly due to the lack of understanding of the mechanism of action, tissue target, and bioavailability, thereby causing difficulty in establishing nutritional recommendations and proper use of the food sources.

Currently, few databases of phenolic compounds are available due to the large number of structures and multiple factors that may influence their contents in foods. A flavonoids composition table of selected foods is available in the USDA database (U.S. Department of Agriculture 2012). In Brazil, a bioactive compounds database has been produced by TBCA-USP (Tabela Brasileira de Composição de Alimentos) (Universidade de São Paulo 1998). These data are important for calculating daily intake of phenolic compounds and correlating them with the incidence of certain diseases in epidemiological studies.

In Brazil, the estimated total flavonoid intake ranges from 60 to 106 mg/day, where oranges represent the major flavonoid dietary source (Table 6.4), corresponding to over 70 % of the total flavonoid intake, followed by lettuce, corresponding to 8–12 % (Arabbi et al. 2004). For American and Latino Americans adults, the daily intake of total flavonoids is estimated to be 189.7 mg/day (mainly flavan-3-ol) and 313.26 mg/day (mainly procyanidin), respectively (Chun et al. 2007; Zamora-Ros et al. 2010). In addition, the daily intake of anthocyanins is estimated to be 82 mg/day and 12.5 mg/day in Finland and the US, respectively (Wu et al. 2006).

Table 6.4 Flavonoid content (milligrams per 100 g of FW) of oranges (edible portion) (Citrus sinensis (L.) Osbeck), expressed as aglycon

Sample	Quercetin	Sinensetin	Naringenin	Hesperetin	Total
Pera orange, pulp	0.9 ± 0.0	nd	17.0 ± 0.8	16.9 ± 1.9	34.8
Lima orange, pulp	0.8 ± 0.0	nd	28.6 ± 0.2	14.9 ± 0.9	44.3
Pera orange, skin	4.1 ± 0.0	19.6 ± 0.4	37.4 ± 1.5	109.4 ± 5.1	170.5
Lima orange, skin	3.6 ± 0.1	17.0 ± 1.2	25.1 ± 1.1	79.7 ± 0.3	125.4

Nd = Not detected
From Arabbi et al. (2004)

In general, it is accepted that the absorption of some, but not all, components of dietary flavonoids into the circulatory system occurs in the small intestine. There are two generally accepted routes of flavonoid absorption: (1) flavonoids could be absorbed in a glycosylated form with the involvement of the active sodium-dependent glucose transporter found in the epithelium of the small intestine and subsequently hydrolyzed by cytosolic glycosidase; or (2) they could be absorbed in the aglycone form by passive diffusion as result of the action of lactase phloridzin hydrolase (LPH) in the brush-border of the small intestine epithelial cells (Manach et al. 2005). Flavonoids may also be metabolized by colonic microflora, releasing the aglycone and later aromatic acids.

An extensive review by McGhie and Walton (2007) reports low bioavailability of anthocyanins, with recovery in the plasma and urine usually less than 1 % of the total intake from an anthocyanin food source; the remaining 60–90 % is not detected in any tissue. Thus, the metabolic fate of dietary anthocyanins has not been fully clarified in humans.

In our research, we have observed that anthocyanins are apparently absorbed and further detected in the plasma in an intact glycosylated form or in a glucuronidate or methylated form, reaching the circulatory system in 15 min–2 h (Hassimotto et al. 2008a; McGhie and Walton 2007). Anthocyanins are then eliminated in the first 6–8 h after oral intake (Hassimotto et al. 2008b; Cao et al. 2001; Felgines et al. 2005).

In a recent human study in our laboratory, 400 mL of blackberry juice containing 400 mg/50 kg body weight of cyanidin derivatives were consumed by six subjects, after which plasma and urine were collected over 4 h and 6 h periods, respectively (Fig. 6.2). As expected, the absorption of anthocyanins was variable by subject due to the individual differences in absorption efficiency. After intake, cyanidin-3-*O*-glucoside, cyanidin-*O*-glucuronide, and methylated cyanidin-*O*-glucuronide were detected in the plasma in substantial levels along with trace amounts of peonidin-*O*-glucuronides and cyanidin aglycone. The plasmatic C_{max} of 0.14 ± 0.09 μmol/mL

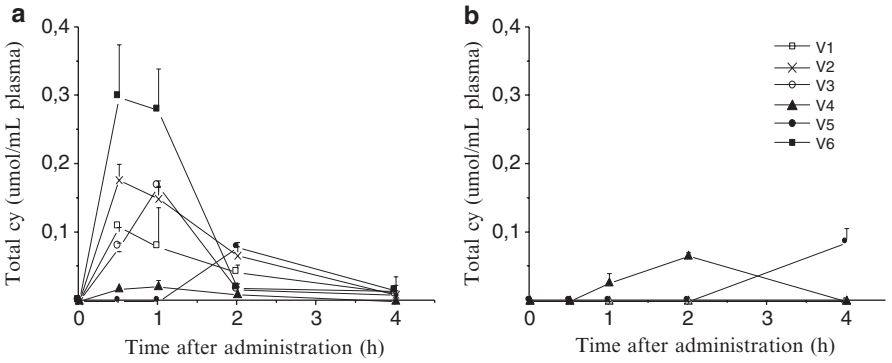

Fig. 6.2 Time course concentration of total anthocyanin in plasma of six volunteers after oral administration of blackberry juice prepared with water (**a**) and milk (**b**), containing 400 mg total cy/50 kg body weight. Total anthocyanin was expressed as cyanidin aglycone (average ± SD). From: Hassimotto et al. 2008b

was reached at a T_{max} of 15–30 min. All of the anthocyanin metabolites detected in the plasma were also recovered in urine (Fig. 6.3). When the same juice was prepared with nonfat milk, we observed a delay in C_{max}. However, no influence in the total excretion of these compounds was observed when it was compared with the same beverage prepared with water. The total anthocyanin recovered from the plasma and urine was 0.12 % of the total amount ingested.

The influence of the dietary components or food matrix on the absorption of anthocyanins is well known. Mullen et al. (2008) reported that when strawberries were eaten with cream, the C_{max} of the main metabolite, pelargonidin-*O*-glucuronide, did not statistically differ compared with when the berries were ingested without cream, but the t_{max} at 1.1 ± 0.4 h without cream was delayed to 2.4 ± 0.5 h with the addition of cream. The reduction in anthocyanin absorption was also observed when acai fruit was administered as a pulp or juice, drastically decreasing the $AUC_{(0–12\ h)}$ in 50 % when administered as juice compared when in taken as pulp (Mertens-Talcott et al. 2008); however, in this case, the total amount excreted was not evaluated. Further, the ingestion of sucrose with elderberry juice led to a reduction in the excretion of anthocyanins (0.035 % and 0.028 % of the ingested anthocyanin without and with sucrose, respectively) (Mulleder et al. 2002), supporting the finding that intestinal sugar carriers (SGLT1) may play a role in flavonoids absorption (McGhie and Walton 2007; Hassimotto et al. 2008a). In these cases, it is possible that the ingestion of sugar leads to a saturation of the glucose transporter, reducing anthocyanin absorption. Based on our results using an everted sacs model, significant inhibition of anthocyanin absorption was observed when anthocyanins were simultaneously incubated with D-glu, most likely due to competition among cyanidin 3-glucoside and the sugar for the binding site of the SGLT1. Thus, it seems

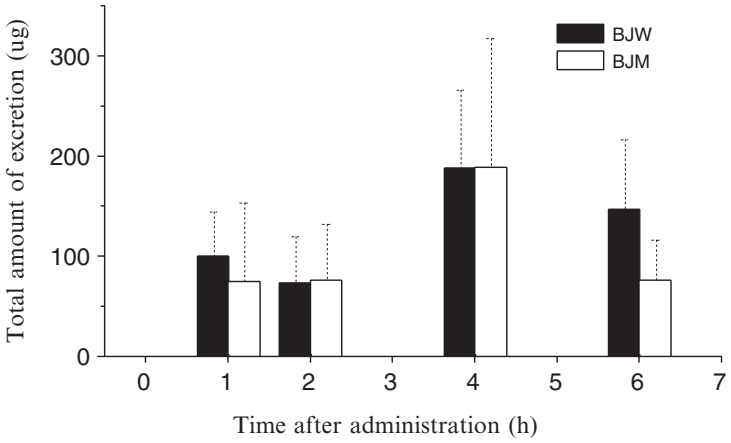

Fig. 6.3 Time course concentration of total anthocyanin in urine after single oral administration of blackberry juice prepared with water (BJW) and milk (BJM), containing 400 mg total cy/50 kg body weight, of six subjects. Total anthocyanin was expressed as cyanidin aglycone per total volume excreted (average ± SD) of six subjects. From: Hassimotto et al. 2008b

that cyanidin 3-glucoside is substrate for SGLT1. In support of this hypothesis, the removal of Na^+ from the medium inhibits the uptake of cyanidin glycosides by almost 100 % (Hassimotto et al. 2008a).

Because anthocyanins are poorly absorbed, large amounts of anthocyanins reach the colon and could be susceptible to the action of intestinal microbiota. Further, the metabolites formed by the microbiota may exhibit different biological properties as compared to the native compounds. According to some researchers, the disappearance of most of the anthocyanins may be due to degradation by intestinal microbiota to phenolic acids. The main phenolic acids identified as metabolites of anthocyanins include protocatechuic acid (PCA), vanillic and homovanillic acid, caffeic acid, and ferulic acid (Aura et al. 2005; Vitaglione et al. 2007; Hassimotto et al. 2008b; Nurmi et al. 2009).

PCA is the primary degradation product of cyanidin and is formed during in vitro fermentation by colonic microflora (Seeram et al. 2001; Aura et al. 2005) or after heat treatment (Hiemori et al. 2009). This finding is confirmed by the detection of traces of APC in the plasma and urine of humans after intake of anthocyanin sources (Kay et al. 2004; Tsuda et al. 1999). In a human intervention, after ingestion of commercial blood orange juice containing 71 mg of anthocyanins, less than 1 % of the anthocyanins and their methylated and glucuronidated forms were detected in plasma and urine. APC was the major metabolite recovered 2 h after juice consumption, accounting for approximately 72 % of cyanidin 3-glucoside intake, with 44 % detected in serum and 28 % detected in feces (Vitaglione et al. 2007).

In recent years, several studies have shown that anthocyanins have the potential to decrease the risk of developing several diseases. However, the biological activity of anthocyanins is questioned due to their low bioavailability and rapid biotransformation by the microbiota to phenolic acids. Thus, the benefits associated with a diet rich in anthocyanins may be due not only to the absorption of anthocyanins in their native structure, but to the continuous release of phenolic acids formed from their degradation by the gut microbiota.

6.5 Biological Activity of Some Native Brazilian Fruits

6.5.1 Antioxidant Properties

Many food sources of phenolic compounds exhibit a high antioxidant capacity as measured by in vitro methods. However, the biological significance of these data is not yet clear. A review of several clinical trials indicates that after ingestion of phenolic compounds through foods and beverages in a single dose, an increase in plasma antioxidant capacity (PAC) between 5 and 30 % is observed due to the absorption of these bioactive compounds (Fernandez-Panchón et al. 2008). Many studies have correlated the consumption of phenolic compounds with the increase in PAC. However, it is important to consider other antioxidant compounds that could be ingested together, such as vitamin C and vitamin E or other exogenous antioxidants.

In our work, although the increase in PAC has been correlated with the appearance of anthocyanin in plasma of the rats (Hassimotto et al. 2008a; Hassimotto and Lajolo 2011), we observed that the increase in PAC in healthy humans after blackberry juice intake was only correlated with an increase in plasma vitamin C levels and not with the polyphenol present in the fruit (Hassimotto et al. 2008b). Otherwise, in a pharmacokinetic analysis in healthy volunteers, PAC was increased up to threefold after consumption of açaí juice and pulp (Mertens-Talcott et al. 2008).

In healthy women, prolonged (7 days) consumption of Brazilian maté tea resulted in a significant reduction in plasma lipid peroxidation, decreasing TBARS levels. This finding suggests that the bioactive compounds from maté tea have reached sufficient levels in the plasma to prevent peroxidation, thereby acting primarily as antioxidants (Matsumoto et al. 2009). Despite this shift, the PAC was unchanged after 1 week of maté tea supplementation (Matsumoto et al. 2009). In most of the studies involving the evaluation of PAC, an increase in PAC after the acute intake of certain foods rich in bioactive compounds was observed, but repeated consumption of these foods either does not produce increases in PAC or the increase is not significant (Fernandez-Panchón et al. 2008).

In addition to this effect, phenolic compounds can increase antioxidant protection indirectly through modulation of antioxidant enzymes. In the same study performed by Matsumoto et al. (2009), a significant increase in leukocyte antioxidant enzyme gene expression (GPx, SOD, and CAT) was observed after 1 week of maté tea ingestion, compared to the baseline period. The authors proposed that bioactive compounds of maté tea or their metabolites mediate the upregulation of phase II enzymes in leucocytes. Phase II gene inducers are typically electrophiles, and their electrophilic interactions with nonreceptor stress-sensing proteins may lead to activation of the mitogen-activated protein kinase (MAPK) cascades. Activation of MAPK by phase II gene inducers will subsequently activate transcription factors such as leucine zipper transcription factor NRf2 and increase antioxidant response element (ARE)-dependent gene expression, including phase II enzymes and other cellular defensive enzymes (Kong et al. 2001).

A similar result was also observed after the long-term intake of anthocyanin and ellagitannin-enriched extracts from blackberries in rats in a tissue-dependent manner (Hassimotto and Lajolo 2011). Our results demonstrate an increase in GPx mRNA expression with an increase in GPx activity in the brain after intake of ellagitannin-enriched extract. The absorption and metabolism of ellagitannin are still unclear. In vitro and in vivo studies have demonstrated that ET is hydrolyzed, yielding free EA (Seeram et al. 2004), which is further metabolized by gut microflora to urolithins (Del Rio et al. 2013). Despite this understanding of the ellagitannin metabolism pathway, our results indicate that ETs are available and can promote changes in the antioxidant status of rats. Although an increase in the mRNA expression levels of the antioxidant enzymes was observed in many tissues, the enzyme activities were not consistently regulated in the same manner; we observed an increase in GPx mRNA expression in the liver after the intake of anthocyanin-enriched extract, but the GPx activity was unchanged (Hassimotto and Lajolo 2011).

In humans, the acute intake of blackberry juice promoted an increase in catalase activity in plasma (Hassimotto et al. 2008a), which was also observed after prolonged intake (Hassimotto and Lajolo 2011) in an animal model. The biological significance of these results in health is unclear, but it is possible that the increase in antioxidant defense promoted by phytochemicals could protect cells and biomolecules from oxidative damage.

The phytochemical composition of vegetables and fruits is complex, mainly because of the existence of myriad structures. Nonetheless, the mechanisms of action exhibited by phenolic acids and flavonoids are similar in many studies. In general, it seems that phenolic compounds may increase the in vivo antioxidant status directly or indirectly in the following ways: (1) reducing ROS levels, which modulates the expression levels of antioxidant enzymes regulating mRNA levels through the activation of signaling pathways (Zhou et al. 2001); (2) modulating the expression of antioxidant enzymes, inhibiting nuclear factor-*k*B (NF-*k*B) translocation from the cytosol to the nucleus (Tsoyi et al. 2008); (3) activating antioxidant response elements upstream of genes that are involved in antioxidation (Shih et al. 2007); and (4) activating specific redox-sensitive transcription factors, such as activating protein 1 (AP-1) and NF-*k*B. Both AP-1 and NF-*k*B response elements are present in the promoter regions of genes encoding CAT, GPx, Mn-SOD, and Cu,Zn-SOD (Zhou et al. 2001).

Thus, it is assumed that an increase in the antioxidant system in plasma or tissues through the action of an antioxidant or through the modulation of the activity or the expression of antioxidant enzymes could lead to a reduction in oxidative damage of macromolecules, such as lipids and DNA, thereby reducing the risk of developing physiopathological conditions.

6.5.2 Control of Postprandial Hyperglycemia by Brazilian Native Fruits

Diabetes and obesity are emerging worldwide health problems. New prevention and treatment options for both conditions could be based on strategies to dampen or inhibit nutrient absorption (Dandona et al. 2005). One strategy for the coadjuvant treatment of diabetes is to decrease the postprandial glucose level by retarding the glucose absorption through the inhibition of the carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase, present in the small intestinal brush border. Both enzymes are responsible for the breakdown of oligosaccharides and disaccharides for absorption (Rhabasa-Lhoret and Chiasson 2004: De Sales et al. 2012).

Polyphenolic extracts from a number of plants are effective inhibitors of intestinal α-amylase and α-glucosidase activity (McDougall et al. 2005). Among the phytochemicals, flavonoids exhibit the highest inhibitory activities (De Sales et al. 2012). According to Lo Piparo et al. (2008), the potency of human α-amylase inhibition is correlated with the number of hydroxyl groups on the B ring of the

flavonoid skeleton. The interaction occurs with the formation of hydrogen bonds between the hydroxyl groups in position R6 or R7 of ring A and position R4′ or R5′ of ring B of the polyphenol ligands and the catalytic residues of the binding site and formation of a conjugated π-system that stabilizes the interaction with the active site. In this way, a high inhibitory capacity is observed in flavonols and flavones groups (De Sales et al. 2012). In addition, tannins and other polyphenol groups likely inhibit α-amylase activity in situ through their ability to strongly bind to proteins and form insoluble and indigestible complexes (De Sales et al. 2012).

Among the native Brazilian fruits analyzed by De Souza Schmidt Goncalves et al. (2010), listed in Tables 6.1 and 6.2, the best α-glucosidase inhibitory activity was exhibited by phenolics from fruits that also exhibit significant inhibition of α-amylase, such as cambuci and araça. However, given that high α-amylase inhibition is undesirable, as it can result in undigested starch, the authors considered the best antidiabetic potential to be those fruit phenolics resulting in high α-glucosidase inhibition and medium-to-low α-amylase inhibition, such as buriti and araça-boi.

Polyphenol-rich extract from berries are effective at inhibiting α-amylase in vitro. According to this study, anthocyanins and ellagitannins containing extracts were not important in α-amylase inhibition, whereas extracts rich in proanthocyanidins were more effective (Grussu et al. 2011). These results were confirmed in a study by Hanamura et al. (2005), who reported that cyanidin 3-*O*-rhamnoside and pelargonidin 3-*O*-rhamnoside are not efficient at inhibiting α-amylase or α-glucosidase.

In addition, α-glucosidase inhibition induced by theaflavins, a polyphenol present in black tea, is closely associated with the presence of a free hydroxyl group at the 3′-position of theaflavin as well as the esterification of theaflavin with a monogallate group. In addition, the R-configuration at the 3′-position of theaflavin-3-O-gallate exhibits a higher inhibitory activity than the S-configuration (Matsui et al. 2007). However, α-glucosidase inhibition is correlated with anthocyanin-rich extract from several berries, and the inhibitory component of α-amylase inhibition has been identified as ellagitannin (McDougall et al. 2005).

Kim et al. (2000) assessed the efficacy of 21 phenolic compounds, including quercetin, hesperidin, luteolin, rutin, and kaempferol, toward α-glucosidase and α-amylase inhibition. The flavone luteolin aglycon was the most efficient compound in inhibiting both enzymes, whereas the glycosylates derived from kaempferol were the most efficient in α-amylase inhibition.

Thus far, we have limited our discussion of diabetes control to decreasing postprandial hyperglycemia by suppressing glucose absorption through the inhibition of the carbohydrate-hydrolyzing enzymes by polyphenol from foods. However, it is suggested that polyphenols such as flavonoids could decrease postprandial hyperglycemia by two additional mechanisms: (1) flavonoids upregulate glucose transporter 4 (GLUT 4) in adipocyte tissue and muscle, thereby increasing the uptake of glucose from blood to tissue; and (2) flavonoids inhibit the facing-facilitated glucose transporter 2 (GLUT 2) present in the apical brush border in the small intestine, inhibiting glucose uptake from the intestinal lumen.

The upregulation of GLUT 4 in adipocyte tissue and muscle was observed in induced diabetic animals treated up to 4 weeks with anthocyanin-rich food sources (bilberry extract and black soybean coat extract) or with a pure anthocyanin, cyanidin 3-glucoside (Takikawa et al. 2010; Nizamutdinova et al. 2009; Sasaki et al. 2007). These findings could explain the ameliorated postprandial hyperglycemia observed in a healthy overweight population treated with açai twice daily for 1 month (Udani et al. 2011).

Although intestinal glucose from ingested foods is transported by the apical (lumen facing) transporters SGLT1 and GLUT2 (Kwon Oran et al. 2007), emerging evidence indicates that apical or luminal GLUT2 is a major pathway of sugar absorption (Kellett and Brot-Laroche 2005). In a recent investigation in which GLUT 2 was expressed in *Xenopus laevis* oocytes, glycosylated flavonoids (rutin and isoquercitin) and some aglycones (quercetin and myricetin) have the capability to inhibit the glucose transporter by GLUT 2 (Kwon Oran et al. 2007) and therefore represent an attractive target of such potential agents.

Thus, different dietetic polyphenolic components may influence different steps of glucose metabolism, decreasing postprandial hyperglycemia in a synergistic manner and potentially representing an strategy to treat diabetes and, potentially, obesity.

6.6 Concluding Comments

Despite scientific evidence obtained from epidemiological studies, in vitro studies, animal models, and clinical trials demonstrating a relationship between the regular consumption of fruits and vegetables and a reduced risk of developing chronic disease, it is unclear which groups of foods are most promising for this use and which mechanisms are involved. Therefore, it is important to characterize the molecular type and composition of the phytochemicals that confer specific health benefits and to conduct further investigations into their bioavailability and molecular mechanisms. In this sense, Brazilian native fruits could be a good source of bioactive compounds and may introduce new food sources.

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Chapter 7
Technology and Nutrition Opportunities for Healthful Foods from Morama Beans, an Emerging Crop in Botswana

Jose C. Jackson

7.1 Introduction

The standard definition of food security adopted by the international community in 1996 is "a state when people at all times have physical and economic access to safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (FAO 1996). There is considerable debate about how to measure the different dimensions of food insecurity implicit in this definition (Barrett 2010; Bilinsky and Swindale 2007; Coates et al. 2003). As Ruel et al. (2010) note, individuals (and households) are "generally net food buyers who rely on income for their food security, spend a large proportion of household budgets on food, and have little access to other safety nets like agriculture or land to ensure food access in times of crisis." A recent household food security survey of 11 Southern African countries, including Botswana by Frayne et al. (2010) found that 75 % of poor urban household were food insecure.

In a recent report of the Global Food Security Index, conducted by the global analytics firm, Economic Intelligence Unit (EIU), Botswana was ranked 47th out of 104 other countries around the world across three categories making up food security—affordability, availability, and quality and safety (EIU 2012). The US, Denmark, Norway, and France topped the list as the most food-secure countries in the world, scoring strongly across all three categories. A combination of ample food supplies, high incomes, low spending on food relative to other outlays, and significant investment in agricultural research and development (R&D) put these countries at the top of the global index (EIU 2012).

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Within Africa itself, Botswana was ranked second, with its major food supplier, South Africa, ranked 40th worldwide and first in Africa. Botswana's strong food security situation stems from the country's purchasing power and presence of food safety net programs. In addition, factors such as the proportion of the population living under or close to US$2 (P15) per day or the global poverty line as well as the level of agricultural import tariffs also favor the country's food security status. Furthermore, low political risk, adequate infrastructure, sufficient supply, and the country's negligible reliance on food aid also accounts for Botswana's ranking (EIU 2012). However, the biggest threat to food security would be increases in the prices of food imports from South Africa and supply interruptions due to crop failures. High prices make food less affordable for consumers, and for those closest to the global poverty line, they are the most vulnerable to food insecurity (EIU 2012).

In trying to address the problems of food security however, more attention has historically been paid to cash crops such as maize, ignoring the more appropriate, climate-smart indigenous or emerging crops (Clover 2003). Current research suggests that not only do these species have important nutritional qualities, but they also contain many phytochemicals with potential health-promoting properties that can be marketed in high-value niche markets (Shelembe et al. 2012; Chingwaru et al. 2011a, b; Jackson et al. 2010). They therefore provide a unique strategic opportunity to address and support food security efforts and improve the livelihoods of the vulnerable populations.

The term emerging crops are plants whose potential are underexploited. They could contribute to food security, nutrition, health, income generation, and environmental service and also improve both the quantity and quality of useful products (Mogotsi 2009). Many emerging crops are indigenous and are therefore strongly linked to the cultural heritage of their places of origin, or of places to which they have been introduced in the distant past (Will 2008). These foods also have most or all of the following attributes. First, they have unrealized potential for contributing to human welfare, in particular to income generation for the world's poor, food security, nutrition, and reduction of micronutrient deficiencies. Second, they have a long history of being locally produced with poor documentation of their distribution, biology, and cultivation. Third, they are typically adapted to specific agro-ecological niches and marginal land, and have weak or no formal supply systems. Fourth, traditional uses and processing of these foods typically varies and they require little or no external inputs since they are collected from the wild. Fifth, indigenous foods receive little attention from research, extension services, policy, and decision makers. Finally, while they often have significant nutritional, culinary, medicinal, or other properties, they are either little known or underappreciated (Jaenicke and Hoeschle-Zeledon 2006; Will 2008).

Emerging crops may be used for many purposes and carry many advantages in relation to the conventional crops for all people in general and vulnerable groups such as the poor, women, and those with diseases, in particular. They make a huge contribution to decreasing malnutrition, enhancing food security, reducing poverty, improving ecological sustainability, conservation of natural resources, and biodiversity (Jaenicke and Hoeschle-Zeledon 2006). They are important sources of

employment and income in rural economy and can be used as inputs for other productive activities, a niche market for small agricultural producers, as well as a source of foreign exchange as through exports. They do not require costly inputs and are quite adaptable to harsh conditions, thus complementing other crops by providing an alternative in harsh regions as well as in harsh conditions such as drought. Moreover, in some cases they contain some ingredients with important health benefits that can be used in many personal uses and support local culture (Jaenicke and Hoeschle-Zeledon 2006).

This chapter presents the nutritional and technological opportunities for small and medium enterprises to add value to the morama bean, one of Botswana's important emerging crops. It also includes a discussion on future research on morama bean processing.

7.2 Morama Beans

The morama bean is an underutilized crop native to arid and semiarid regions of Botswana, Namibia, and South Africa and forms part of the diet of indigenous populations. It is also known as gemsbok bean (English); *moramaboontjie*, *elandboontjie*, *braaiboonjie* (Afrikaans); *marama*, *morama* (Tswana); *marumana* (Thonga); *tsi*, *tsin* (!Kung San); *gami* (Khoi); and *ombanui* (Herero). Morama beans may be eaten when they are still immature green beans, but most of them are eaten as mature beans when the seeds are surrounded by hard woody seed coats, reddish to brownish in color (Fig. 7.1). It has remained underutilized largely because it is found in the wild and only consumed by a small percentage of the population in the countries where it is found. However, in these areas, it is used by indigenous communities as a source of food, feed, shelter, and medicine, thus contributing to improve their quality of life. The morama has enormous potential value that needs to be exploited for the further benefit of the communities.

7.2.1 Chemical Composition

The proximate composition of the edible part of the decorticated morama bean is shown in Table 7.1. The moisture content of morama bean is very low as the dry matter content ranged from 93.4 to 98.7 % (Jackson et al. 2010). The ash content on a dry basis, which is a measure of the total mineral content in the beans, is high and varies between 2.5 and 3.7 % (Jackson et al. 2010). Holse et al. (2010) reported on the ash content of beans from South Africa compared to those from Namibia and Botswana and found that the South African morama beans have significantly higher ash content. This is likely due to a soil richer in minerals in South Africa. Morama is a good source of minerals such as calcium, iron, zinc, phosphate, potassium, and magnesium (Jackson et al. 2010), which contributes positively to the overall mineral

Fig. 7.1 Green (**a**) and Mature (**b**) Morama Bean (*Tylosema esculentum*). Picture provided by: Chingwaru W

Table 7.1 Proximate composition (g/100 g) of morama bean

Nutrient	g/100 g
Moisture	1.3–6.6
Lipid	32.0–48.2
Protein	28.8–38.4
Carbohydrate[a]	16.7–21.1
Crude fiber	4.3–4.5
Dietary fiber	18.7–26.8
Ash	2.5–3.7

[a]Obtained by difference
Source: Holse et al. (2010), Bower et al. (1988), Ketshajwang et al. (1998)

Table 7.2 Micronutrient composition of morama bean

Minerals	mg/100 g	Vitamins	mg/100 g
Potassium	757–1316	Vitamin A (Palmitate)	56[a]
Phosphorus	334–554	Vitamin B_1 (Thiamin)	0.82
Magnesium	248–374	Vitamin B_2 (Riboflavin)	0.08
Sulfur	191–236	Vitamin B_3 (Niacin)	30.65
Calcium	119–133	Vitamin B_9 (Folic acid)	1.3
Aluminum	0.5–8.2	Vitamin C (Ascorbic acid)	1.3
Zinc	3.3–3.8	Vitamin D_3 (Cholecalciferol)	2.37
Iron	1.3–3.7	Vitamin E (Tocopherol)	0.23
Sodium	0.2–2.9		
Manganese	1.5–2.6		
Copper	0.6–1.6		

[a]iu/100 g
Source: Jackson et al. (2010)

composition. The levels of minerals are similar to that of peanuts and approaching that of soybeans (USDA 2007).

Table 7.2 indicates the micronutrient composition of morama beans. It is a good source of B-vitamins (thiamine, riboflavin, nicotinic acid), vitamin E, and vitamin C, but a poor source of β-carotene. Holse et al. (2010) and Mitei et al. (2008) found that the vitamin E composition in morama beans is dominated by γ-tocopherol followed by α- and β-tocopherols. This is of particular biological relevance as vitamin E has shown potential anticarcinogenic and anti-inflammatory activities (Brigelius-Flohé 2006). The mineral and vitamin composition is important since large percentages of the population in Africa are deficient or at risk of inadequate intake of micronutrients (UNICEF 2004).

Morama bean oil is golden-yellow, with a nutty odor and a pleasant, although slightly bitter flavor and has been described as similar to almond oil in consistency and taste (Van der Maesen 2006). The content of lipids in morama bean ranges

Table 7.3 Fatty acid composition (g/100 g flour dry matter basis) of morama bean

Fatty acid	C:D	Mitei et al. (2008)	Bower et al. (1988)
Myristic	(14:0)	–	1.3 ± 0.3
Palmitic	(16:0)	12.93 ± 0.06	13.8 ± 5.0
Stearic	(18:0)	8.82 ± 0.12	9.7 ± 7.0
Arachidic	(20:0)	3.31 ± 0.03	2.8 ± 1.3
Behenic acid	(22:0)	1.03 ± 0.02	
Palmitoleic	(16:1)		1.7 ± 0.3
Oleic	(18:1)	47.27 ± 0.43	48.5 ± 8.0
Gadoleic	(20:1)	0.61 ± 0.00	
Erucic	(22:1)	2.63 ± 0.01	
Linoleic	(18:2)	23.40 ± 0.42	19.2 ± 9.5
α- and γ-Linolenic	(18:3)		2.0 ± 1.5

between 24 and 48 %, which is comparable to commercial vegetable oils such as sunflower seed (22–36 %) and rapeseed (22–49 %), slightly less than that of peanuts (45–55 %) and twice that of soybeans (17–20 %) (Belitz et al. 2004; Jackson et al. 2010). The fatty acid composition of morama beans is shown in Table 7.3 and is mainly composed of the unsaturated fatty acids such as oleic acid (43 %), linoleic acid (22 %), palmitic acid (13 %), as well as stearic, arachidic, linolenic, arachidonic, erucic, behenic, myristic, palmitoleic, and gadoleic acid in lower concentrations (Jackson et al. 2010). The fatty acid composition is similar to that of olive oil (Mitei et al. 2008).

The protein content of morama bean ranges from 29 to 39 % (Bower et al. 1988; Amarteifio and Moholo 1998; Holse et al. 2010). This is similar to or higher than other legume sources with contents between 20 and 40 % dry matter and equals that of soybeans (33–46 % dry matter) (Belitz et al. 2004). The beans consist primarily of globulins (53 %), followed by 23 % albumins, 16 % prolamins, 8 % alkali soluble glutelins, and 1 % acid soluble glutelins (Bower et al. 1988). The amino acid composition of the proteins in morama beans is shown in Table 7.4 and is largely dominated by glutamic and aspartic acid as well as tyrosine (Bower et al. 1988; Maruatona et al. 2010). All essential amino acids are present; however, the sulfur-containing amino acids methionine and cysteine are present in low amounts, which is typical of legumes. The high protein content of morama beans offers great potential for its use in improving the nutritional composition of staple cereals in Botswana like sorghum.

The carbohydrate content of morama beans has been reported by several authors to be 23 %, 24 %, and 19 %, respectively (Bower et al. 1988; Amarteifio and Moholo 1998). The content of carbohydrate is dominated by dietary fiber, which varied between 18.7 and 26.8 %, of which soluble dietary fibers are only 4 % (Holse et al. 2010). Morama bean has considerably higher levels of insoluble dietary fiber compared with peanut (9 %) and soybean (10 %) (USDA 2007). High levels of insoluble dietary fiber are associated with a low glycemic response, low serum

Table 7.4 Amino acid composition (g/100 g flour dry matter basis) of morama bean

Amino acid	Maruatona et al. (2010)	Bower et al. (1988)
Essential		
Arginine	7.3	6.3
Cysteine	0.1	0.8
Histidine	2.8	2.4
Isoleucine	4.5	4.0
Leucine	6.6	5.9
Lysine	5.7	5.5
Methionine	0.8	0.8
Phenylalanine	4.8	4.8
Threonine	3.1	3.0
Tyrosine	11.2	11.6
Tryptophan	ND	1.7
Valine	4.9	4.4
Nonessential		
Alanine	3.6	3.1
Aspartic acid	9.3	10.8
Glutamic acid	15.5	15.6
Glycine	6.3	5.7
Proline	7.8	6.9
Serine	5.6	5.3

cholesterol levels, and a decrease of colon cancer risk factors (Serrano and Goni 2004). In contrast with other legumes however, morama beans have very low starch content (Holse et al. 2010).

The morama bean seed coat and cotyledon have appreciable levels of total phenolics and antioxidant activity. The seed coat has a higher concentration of total phenolic acids and total flavonoids than the cotyledon and may have important contributions to antioxidant activity of the seed coat and may offer potential health benefits. Shelembe et al. (2012) reported that the major flavonoids in the seed coat were the flavanols methyl (epi)afzelechin-3-O-gallate (40 %) and methyl (epi) catechin-3-O-gallate (28 %), and the major phenolic acid was gallic acid (10 %). Proanthocyanidins in the extracts were predominantly prodelphinidins composed of epicatechin-3-O-gallate and epigallocatechin present as major terminal and extension units and epigallocatechin-3-O-gallate and epicatechin present as minor extension unit constituents. The polymer structure was found to be unique compared with other legumes because of the high percentage of galloylated units. Extracts showed a high DPPH free radical scavenging activity (707 μmol TE/g), protective effect against AAPH-induced human red blood cell hemolysis, and copper-catalyzed human LDL oxidation suggesting that the extracts may have potential health benefits (Shelembe et al. 2012).

7.2.2 *Morama Bean Processing Technologies*

Interest globally in the consumption of beans is on the increase due to the emerging growth potential in legume utilization for health. Even though a variety of legumes are eaten in their immature state, the greatest interest from a nutritional point of view is in the consumption of the matured dried grains (Buruchara 2006). Furthermore, it follows that the proper processing of legumes for human nutrition relates to the efficient removal of the seed coats and husks surrounding the edible cotyledons. This allows for improved digestibility and increased body utilization of the legume nutrients.

As a food product, morama beans are mostly recognized for their good taste and high nutrient levels. The traditional way of eating morama is by roasting the dry seeds in hot sand (either in a pot or directly on hot soil by an open fire) (Lima de Faria et al. 2008). Once roasted, they are eaten as a snack (in a similar way as roasted peanuts). Like peanuts they are valued for being high in calories and for satisfying immediate nutritional needs. They can also be eaten boiled like green beans, when they are fresh. The tuber (2 years or younger) can be eaten raw, boiled or baked, while older tubers are used as a source of water since it contains up to about 90 % of water by weight. The roasted bean has a delicious nutty flavor and is cooked with cornmeal to prepare porridge or is ground into a powder that is boiled in water to produce a cocoa-like beverage. More recent research has shown that morama bean can be utilized as a wide range of value-added products and ingredients including flour, butter, oil, milk, and meat analog products as well as a range of snack foods (Table 7.5).

Table 7.5 Potential processing opportunities for morama bean

Food	Description	Uses
Morama oil	Solvent extracted/mechanically pressed of whole morama beans	Cooking oil, salads, cosmetic oil
Morama butter	Milling and grinding of whole morama beans to a paste	Confectionery
Morama flour	Milling of morama beans to fine particles; may include defatting first to produce a high protein flour	Supplement to staple cereal flours
Morama texturized foods	Milled flour extruded into various shapes	Snacks, breakfast cereals, meat analogs
Morama nuts	Whole morama dry roasted	Roasted nuts whole or chopped
Morama biscuits, cookies, muffins, bread, cake	Morama and wheat flour and other ingredients baked into snack foods	Baked snack foods
Morama roast	Morama flour and other spices baked as a meat-like loaf	Meat analog
Morama ice cream	Morama powder added to mix for ice cream	Frozen snack food
Canned morama beans in tomato sauce	Whole morama beans thermally processed in a sauce	Savory cooked beans

Source: Jackson et al. (2010)

Primary processing of morama bean includes unit operations such as cleaning, cracking or dehulling and milling. The objective is to perform a clean separation of the seed coat from the rest of the grain as well as improve appearance, texture, culinary properties, and palatability. Cleaning of the beans is done by putting them on a tray or on a flat surface and the stones and other extraneous materials are picked out by hand. After cleaning, the beans are cracked or dehulled manually; the traditional practice is to use a stone; however, this has been described as one of the main bottlenecks in the processing of morama beans (Lima de Faria et al. 2008). A prototype cracker is in the design phase to crack morama beans into halves and separate kernels from the shells. The cracking is to be achieved by a shearing process while separation is by floatation and is expected to produce between 30 and 40 kg of kernels (halves and fragments) per hour (Tjiparuro 2010). The next unit operation depends on the food product that will be processed. For most products, it is a milling operation using a hammer mill to produce flour of various particle sizes that can be used for further processing.

7.2.2.1 Morama Milk

Morama milk is a creamy white water extract of morama beans that closely resembles dairy milk or soymilk in appearance and composition and was first reported by researchers in Botswana (Mpotokwane et al. 2007). It can be consumed as a refreshing and nutritious beverage similar to dairy milk or soymilk and can be used as an infant supplement providing additional protein, energy, and other nutrients to vulnerable populations where the supply of dairy milk is inadequate. It can also be an intermediate material for other applications such as yogurt.

The processing of morama milk generally involves a thermal treatment such as blanching and roasting of the beans, cracking, milling, suspending in water, boiling and filtration to obtain a milk-like phase. Blanching and roasting techniques are believed to improve the flavor by removing the bitter flavor components and aiding in the development of desirable nutty flavor components (Iwuoha and Umunnakwe 1997). A small-scale method for processing morama beans into milk reportedly involved cracking cleaned beans, then blanching in a bicarbonate of soda solution (Mpotokwane et al. 2007). The resulting beans were drained, rinsed, then ground with hot water to form a slurry that was heat processed and then filtered successively. The residue known as morama pulp was separated and the resultant liquid extract was heated, homogenized, bottled, and pasteurized before refrigeration.

Other preprocessing treatments that have been reported in the processing of morama milk included (1) soaking then water blanching, (2) soaking only, (3) water blanching, (4) roasting shelled, (5) roasting unshelled, (6) salt water blanching, and (7) defatted shelled (Mpotokwane et al. 2007). However, these were not considered acceptable as the milks produced were too watery, too dark, too bitter, or were not creamy enough (Mpotokwane et al. 2007). While the bicarbonate soda blanch pretreatment method was suitable for small-scale processing of morama milk, it does produce milk with a characteristic aftertaste. For consumers who are not accustomed to the flavor and taste, it could be described as an undesirable “bitter”

taste. Consequently, unless such flavor and taste are reduced or masked, morama milk may not be readily accepted by some consumers. Strawberry flavored morama milk has also been developed and produced a more acceptable taste that consumers are used to (Mpotokwane et al. 2007).

The physicochemical properties of morama milk were reported by Jackson et al. (2009). They indicated that morama milk has 6 % total solids compared with 10 % for soymilk and 12 % for cow's milk. The solids include protein, which is about 1.5 %, fat is 3.1 %, carbohydrates are 1.1 %, and ash is 0.2 %. Morama milk has high levels of sodium (47.9 mg/100 g) and iron (3.7 mg/100 g) compared with soy and cow's milk, but much lower calcium (6.8 mg/1000 g). The proportion of unsaturated fatty acid in morama milk is significantly higher than in soymilk and cow's milk (Jackson et al. 2009). The essential amino acids of morama milk have the same pattern as in the beans; as with other legumes, the sulfur amino acids, methionine and cysteine are limiting, 0.28 g/100 g methionine and 0.02 g/100 g cysteine (Jackson et al. 2009). Tryptophan was not detected. Of the other essential and semiessential amino acids, leucine, tyrosine, arginine, and lysine were the major amino acids in morama bean milk. Others that were also found in high levels include glutamic acid, aspartic acid, and proline. These findings are consistent with the literature on the amino acid composition of morama bean (Jackson et al. 2009).

Fermented morama milk products such as morama yogurt have been reported by Phuthego et al. (2009). Morama milk yogurt, like soymilk yogurt, provides economic and nutritional benefits, because they are likely to have higher protein levels at comparable or lower cost than regular fermented milk products (Karleskind et al. 1991). This is because legume milks are relatively easy to prepare and can serve as a low-cost protein beverage in countries where commercial dairies are not adequate. Morama milk yogurt is processed similarly to the production of yogurt from soybeans by fermenting morama milk with lactic acid bacteria (Phuthego et al. 2009).

7.2.2.2 Morama Oil

Morama oil has been described as resembling almond oil, and being suitable for domestic purposes, having a pleasant nutty flavor, albeit with a slightly bitter taste (Van der Maesen 2006). Extraction can be done using an oil press, or an organic solvent such as hexane as with other oil seeds. A higher yield of oil is obtained with solvent extraction compared to pressing but there are inherent health risks with solvent extraction if not all the solvent is ultimately evaporated off. Ketshajwang et al. (1998) reported extraction rates as high as 48.2 % using hexane extraction, while Yeboah and Moshoeshoe (2008) using a 30 kg/cm^2 hydraulic press reported yields of 20 %. Therefore, oil pressing may provide a safer and more feasible option particularly for small-scale processors. Oil pressing involves either cold pressing or warm pressing; cold pressing does not involve the use of heat on the seeds that are being pressed, while warm pressing uses heat application. More residual oil in the press cake is thus obtained from cold pressing; warm pressing gives more yield, but compromises oil quality. The oil potentially has both food- and nonfood uses, with the latter primarily being in the processing of cosmetics.

7.2.2.3 Morama High Protein Flour

The preparation of high protein morama flour involves a number of unit operations including heating, dehulling, defatting (in some cases), and milling. These operations may impact either positively or negatively on nutritional, functional, sensory, and phytochemical quality of resultant flours. Although full fat flours are deemed to be more energy dense than fully or partially defatted flours, they have lower protein contents and are prone to hydrolytic and oxidative rancidity. Therefore, defatting is required when stable high protein flours are required. Dry heating or roasting also increases the protein content and is also able to effectively inactivate heat labile trypsin inhibitors that reduce protein digestibility from 251 to 3 TUI/ml extract (Maruatona et al. 2010). In vitro digestibility was improved by 2.7 %, but the lysine content was reduced by 11.9 % as well as sulfur amino acids (Maruatona et al. 2010).

The use of dry heating processes in the preparation morama flours can also affect their functional and sensory properties. Heating of morama beans prior to decortication reduced protein solubility and emulsifying capacity of resulting defatted morama bean flours (protein contents: 53 and 56 % for unheated and heated samples; fat contents: 7 and 1.9 % for unheated and heated samples), but improved water absorption capacity significantly (Maruatona et al. 2010). Jideani et al. (2009) also found that roasting increased the water absorption capacity of full fat morama flour (protein content: 32–33 %; fat content: 36–39 %), but reported increased protein solubility and emulsifying activity upon heating. Differences in results can probably be attributed to the fact that Maruatona et al. (2010) worked with defatted flours, whereas Jideani et al. (2009) worked with full fat flours.

The potential use of morama flours to improve the nutritional quality of sorghum porridge, a staple food to millions in Africa was reported by Kayitesi (2009). Compositing sorghum porridge with morama bean flours significantly increased protein and fat contents in porridges. Lysine content of the composite porridges was 3–4 times higher than that of sorghum porridge alone. Total phenolic content and antioxidant activity of composite porridges were also significantly higher than that of sorghum porridge. Porridge composited with full fat morama flour from heated beans was found to be as acceptable as sorghum porridge, but more acceptable than that of composites using defatted flour. A bitter taste and aftertaste were perceived in composite porridges from defatted flours, whereas composite porridges from full fat morama flours were described as buttery/creamy (Kayitesi 2009).

7.2.2.4 Canned Morama Beans

Canned morama beans in tomato and vegetable sauce, a product similar to baked beans, have also been developed and reported by Phuthego et al. (2010). Beans were sorted, cracked, soaked, and then blanched in sodium bicarbonate. The blanched beans were rinsed, added to cans, and then filled with either tomato or vegetable sauce. The cans were exhausted, sealed, inverted, and then thermally processed for 45 min. The chemical composition of the canned beans indicates slight differences

in protein, fiber, and carbohydrate content between the two processed samples, which were significantly different from the raw bean. This suggests that processing changed the macronutrient content (Phuthego et al. 2010). The canned beans in tomato sauce had a higher soluble solids content and was more viscous and acidic than the vegetable sauce sample. The texture profile of the beans was not significantly different from each other, except that the beans in the vegetable sauce were slightly harder than those in the tomato sauce. This was likely due to the increased calcium content present in the sample with the vegetables. The adhesiveness, gumminess, and chewiness characteristics were also slightly higher in the tomato sauce sample (Phuthego et al. 2010).

After the canning process, the beans became darker (decreased L value) and increased in redness (increased a value); this was especially so for morama in tomato sauce, which was darker and had significantly more redness and blueness characteristics than the vegetable sauce (Phuthego et al. 2010). The microbiological safety of the two products indicated that there were no microorganisms of public health significance and therefore the products were considered safe for consumption (Phuthego et al. 2010). Although the sensory and physicochemical quality of the two products were not significantly different and were acceptable to consumers, there were slight differences in some properties. However, these did not adversely affect the final quality. These findings demonstrated that canned morama beans in tomato and vegetable sauces have high quality and are acceptable to consumers. The results provide preliminary evidence of the potential market value for canned morama beans.

7.2.3 Community Awareness, Markets, and Enterprise Development Initiatives

Despite the high nutritional quality and value that is placed on morama by communities where it is found, morama beans are still only harvested in the wild. Furthermore, there still remain a significant number of people in Botswana who are unaware of morama and its potential benefits. Currently, only small quantities of morama are sold in the villages, mostly to neighbors, or in nearby towns by villagers who temporarily act as street vendors. These are generally channeled into households for consumption and food sustainability, as morama is easy to store and can be kept for long periods (Lima de Faria et al. 2008).

A recent study was conducted about consumers' knowledge, perceptions, and utilization of morama bean in Botswana (Mahgoub et al. 2013). Results indicated that while a high percentage of families use morama beans for food, the majority were unaware of the nutritional qualities of the bean and of the possibility of processing morama into products for the market. However, consumers did indicate that they would like to see morama bean as part of some food products in the market. They also felt that increasing knowledge about the role and benefits of indigenous

foods in ensuring food security could help communities to better utilize resources around them and could play a significant role in encouraging farmers to cultivate morama on a commercial level (Mahgoub et al. 2013).

Consumer purchasing characteristics for the morama bean products developed were reported by Jackson et al. (2010). The range of prototype morama products evaluated included morama milk, a dairy substitute; full fat and defatted morama flours aimed to enhance the nutritional value of cereal staples, for example, sorghum porridge; morama oil for use in salads, cooking, and cosmetics; and roasted morama as a snack product. Consumers in Botswana were aware of the potential competitive products for the prototype morama bean products developed. These included products such as soy milk, soybean oil, sunflower oil, and high protein soy flour. They were aware of some of the health benefits associated with the competitive products; almost all of the health benefits they identified were related to the nutritional value. None of them indicated any phytochemical properties or any specific disease that they could prevent (Jackson et al. 2010).

Consumers' willingness to pay for the morama products in comparison to similar products, for example, soy milk, cereal–protein composite porridges, and roasted peanuts in the same market segment was reported by Jackson et al. (2010). Essentially, consumers in Botswana were very price sensitive. Since the products were new to most consumers, they indicated that they would be unwilling to buy them if they were more expensive than other similar products. The recommendation was that it was better for morama to establish itself first with lower prices, and then they would pay the same price particularly if there were similar benefits identified. Only very few consumers were willing to pay more and support indigenous or natural value-added products. They admitted that in some cases they were aware that more expensive products were more likely to be healthier but the key limitation for them was their disposable income (Jackson et al. 2010).

Educational programs aimed at creating and increasing awareness about the nutritional and technological opportunities available with morama bean was initiated over a 2-year period through a project called *Morama Engaged* (Chiwona-Karltun et al. 2012). Engagement and capacity building of communities, enterprises, and policy makers of the processing and economic diversification potential with morama beans was extended through community-based knowledge networks, food processing training workshops, exhibitions, trade fairs, meetings with communities, and policy makers. The initiative clearly stimulated interest in better utilization of emerging crops like morama beans, particularly in the commercialization of morama bean oil (Chiwona-Karltun et al. 2012).

7.3 Conclusions and Recommendations for Future Research

Research suggests that not only do emerging crops like morama beans have important nutritional qualities, but they also contain many phytochemicals with potential health-promoting properties that can be marketed in high-value niche

markets. The nutritional and health benefits associated with morama bean are due to the high content of essential amino acids, fatty acids, important minerals, vitamins, lignans, phenolic compounds, and the absence of allergens and cyanogenic glycosides as potential health risks (Jackson et al. 2010). There are several possible morama bean commercial applications including nutritious milk beverage and yogurt, high value oil, high protein flours, and canned beans in sauces. Morama bean is clearly an example of the many indigenous, underutilized species native to Botswana with great agricultural potential, and which can provide a unique strategic opportunity to address and support food security efforts and improve the livelihoods of vulnerable populations in Botswana. Future research should be tailored toward developing new food ingredients with novel functional properties, which, if marketed properly to food industries, could be used in food formulation that will address food security in Botswana and globally. The result will be a greater demand for value-added products and the emerging crop themselves, thus improving livelihoods for vulnerable communities.

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Chapter 8
Advancements in Obtaining and Utilizing Bovine Milk Proteins in Foods and Nutrition

Valdemiro Carlos Sgarbieri

8.1 Introduction: Physical Nature and Composition of Milk

Milk is perhaps the most functional food nature has ever made. It is a product of the mammary glands and in its natural form constitutes a liquid emulsion in which the dispersed (particulate) phase is formed of casein micelles and fat globules. Each fat globule is limited by the milk fat globule membrane (MFGM). The dispersant (liquid phase) is formed mainly of water containing a multitude of water-soluble substances such as: proteins, peptides, amino acids, phosphatides, oligosaccharides (mainly lactose), minerals, vitamins, and several growth factors.

Milk is the only food capable of promoting normal growth and health in all mammal species for variable periods, starting immediately after birth. The first secretory product of the mammary glands is called colostrum which shows a completely different composition than that of regular milk. Bovine colostrum changes composition substantially in the 72 h post-partum to reach the normal average milk composition (Table 8.1). As shown in this Table, fat, soluble (whey) proteins, minerals, and total solids decrease abruptly during the transformation of colostrum into regular milk, whereas the caseins and lactose undergo significant increase.

The most significant changes occur with immunoglobulin G (IgG), which drops from 4.5 g/dL in the colostrum to 0.05 g/dL in milk and β-lactoglobulin (β-LG) from 4.05 g/dL in colostrum to 0.4 g/dL in milk (Heng 1999).

The composition of bovine milk as well as of milk proteins can change significantly depending on factors such as age of the lactating cow, stage of lactation, feeding program of the cow, climate, season of the year, and udder condition (e.g. mammary gland infections). These factors are related to handling conditions (temperature, light, time of storage, and type of container) and microbial population.

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Table 8.1 Change in composition of bovine colostrum into bovine milk after calf delivery

Components (% w/v)	Colostrum (time after delivery)		Bovine milk
	3 h	72 h	
Fat	6.80	3.72	3.50
Protein (Total)	8.42	4.68	3.20
Soluble (whey) protein	8.50	1.60	0.50
Caseins	0.92	3.18	2.73
Lactose	2.38	4.27	4.60
Minerals (ash)	1.02	0.74	0.70
Total solids	19.62	13.41	12.0

Modified from Heng (1999)

Quality and applications of bovine milk and milk components include research and activities in three main areas: (a) herd management and biotechnology, including nutrition, selection, genetic modification, and transgenic (Jennes 1974; Brophy et al. 2003; Van Berkel et al. 2002); (b) nutrigenomic, proteomic, and metabolomic concerns (German et al. 2006; German and Watkins 2004; Bauman et al. 2006; Reinhardt and Lippolis 2006); (c) new technologies and improved analytical procedures such as membrane filtration, supercritical extraction, high pressure technology, nanotechnology, column chromatography (ion exchange, molecular exclusion, and affinity chromatographies), electrophoresis, and mass spectrometry (Rosenberg 1995; Groleau et al. 2004; Korhonen and Pihlanto 2007; Groveland-Bikker and de Kruif 2006; Argov et al. 2008).

In this chapter we will apply the above-mentioned new technologies and techniques to isolate, fractionate, and purify milk caseins, milk whey proteins, and milk fat globule membrane (MFGM) proteins.

8.2 Milk Caseins: Structure and Physicochemical Parameters

Caseins are naturally present in bovine milk as micelles which are particles varying in mass between 10^7 and 10^9 Da, approximately spherical and 50–200 nm in diameter. The micelles are formed of subunits with diameters of 10–20 nm, each containing 25–30 casein monomers corresponding to an average mass of 6×10^5 Da (Sgarbieri 1996). For practical purposes, casein is made up of three components: αs_1 (50 % of total); β (33 %), and k (15 %). Other variable quantities of γ-components also exist, which are formed by proteolytic degradation of the β-casein. The micelles are formed with a hydrophobic core that contains mainly αs_1 and β-caseins, they are stabilized by ligations with calcium ions and colloidal calcium phosphate. The k-casein, which is more hydrophilic, is distributed on the micelle surface, interacting with the liquid phase and stabilizing the whole micelle. This basic structure of the whole micelle is repeated in each substructural unit.

Table 8.2 Some physicochemical characteristics of bovine milk caseins

Protein fraction	Defatted milk (% total casein)	Isoeletric point (pI)	Sedimentation coefficient (S20)	Molecular mass (kDa)	Genetic variants[a]
αS_1-casein (αS_0, αS_2, αS_3, αS_4, αS_5)	45–55	4.1	3.99	23.6	A, B, C, D
β-casein	25–35	4.5	1.57	24.0	A_1, A_2, A_3, B, C, D
k-caseins	8–15	4.1	1.40	19.0	A, B
δ-caseins	3–7	5.8	1.55	21.0	A_1, A_2, A_3, B
δ1				20.5	A_1, A_2, A_3, B
δ2				11.8	A_1, A_2, A_3, B
δ3				11.5	A_1, A_2, A_3, B

[a]Genetic Variants—Change one or more amino acid residue in the primary sequence
Adapted from Sgarbieri (1996)

Additional physicochemical characteristics of bovine caseins are shown in Table 8.2. The αs_1-caseins are formed of 199 amino acid residues presenting a hydrophilic segment (res. 43–70) with a high phosphate content and charge density. On the other hand segments 1–42 and 80–199 show strong hydrophobic character and are rich in proline, making the formation of secondary structure (α-helix) difficult. These molecules show a strong tendency to hydrophobic endothermic interaction which suggests their important role in micelle formation.

Differently from the αs_1-caseins, the β-caseins show a strong tendency for association-dissociation, depending on protein concentration, temperature, and pH. At temperatures below 8 °C or high pH values β-casein is found as a monomer. At high temperatures and pH near neutrality, the β-caseins form polymers with a "rosary" appearance (i.e. string of beads). The β-casein A_2 is formed from 209 amino acid residues, and the amino acid sequence reveals an N-terminal segment (1–43) that is highly hydrophilic with a high charge density containing four phosphate radicals in the segment 15–19. The remaining polypeptide shows a strong hydrophobic character with a high proline content, which impedes secondary structure (α-helix) formation. Due to their structures and high content of phosphate radicals, αs_1 and β-caseins are classified as phosphoproteins with a high capacity to react and bind Ca^{2+} ions and other minerals.

Contrary to αs_1 and β-caseins, k-caseins do not react with calcium; however, in the presence of Ca^{2+} ions they react with αs_1 and β-caseins to form thermodynamically stable micelles.

In the amino acid sequence (res 1–105) of k-caseins, there is a predominance of apolar (hydrophobic) residues, whereas more polar residues predominate in the C-terminal portion of the molecule (res 106–169). The k-caseins contain two cysteine residues/mole which participate in the formation of disulfide linkages, leading to formation of a series of polymers with MM between 60 and 600 kDa. Treatment with mercaptoethanol breaks down the polymers into monomers.

The charges of k-caseins do not result from phosphate radicals which would explain the nonreactivity of k-caseins with calcium. The negative charges present in the C-terminal portion of the molecules are mainly due to the presence of carbohydrate residues. One residue corresponds to *N*-acetylgalactosamine, bound to the hydroxyl group of threonine 131 (Thr_{131}), the second carbohydrate derivative is neuraminic acid which appears as the terminal residue in the chain. All of the carbohydrate present in the k-caseins molecules is associated with the C-terminal portion, which contains 18 of the 28 serine plus threonine residues. The carbohydrate residues increase the negative charge density and the hydrophilicity of k-caseins. k-caseins constitute the substrate for chymosin, which is present in commercial rennet, which cleaves the linkage (Phe_{105}–Met_{106}) liberating the C-terminal peptide (MM 6.8 kDa) known as glycomacropeptide (GMP), which is soluble in aqueous media, whereas the N-terminal peptide (MM 12.27 kDa), which is insoluble, remains associated with the casein micelles. The high hydrophobicity of this insoluble peptide, known as ρ-k-casein, favors aggregation of the casein micelles following chymosin action.

8.2.1 Technological Functional Properties and Main Applications of Caseins

Due to their flexible structures and peculiar compositions, caseins have been used as an important ingredient in food and other industries for a long time. As nutrient caseins are much used and appreciated for their adequate amino acid composition, high digestibility, and absence of toxic components. As a food ingredient, casein can be obtained in different forms such as acid casein, enzyme coagulated casein, caseinates, and casein coprecipitated with whey proteins (total milk protein). In most cases, caseins cannot be replaced as food ingredient in certain applications. Caseins have been produced and commercialized for more than a century (Fox 1989). Global production of casein has surpassed 325.000 t/year, with New Zealand and the European Union being the major producers, whereas Japan and the United States of America are the highest consumers of caseins (O'Regan and Mulvihill 2011).

Sodium and potassium caseinates are largely used as food ingredients due to their excellent nutritional and functional properties (Lucey et al. 2000). Sodium caseinate forms stable solutions at neutral pH and jellifies when pH is decreased to its isoelectric point, which, for example, characterizes lactic products such as yogurt. Gelation of milk or milk proteins is an important stage in the manufacturing of several dairy products (Chen et al. 1999).

Sodium and potassium caseinates, at concentrations higher than 15 %, form translucent solutions of high viscosity because both exhibit good water ligation property. Such ingredients are used in the formulation of foods that require solubility, color stability, and surface activity. They function as efficient emulsifying, foaming, and thickening agents, capable of forming gels at around 17 % protein, which are thermostable at 40 °C for 15 min at pH 9.0 without coagulation (Lourenço 2000).

Calcium caseinate has been used in products such as cake mixes, breakfast cereals, soups, cheese analogs, and other dairy products enriched with calcium caseinate because of its high solubility, high water absorption, and heat stability (Linden and Lorient 2000).

Processing operations such as heat treatment (Fox 1989), high pressure (Lopez-Fandino 2006) and hydrolysis (Roman and Sgarbieri 2005a) of proteins can change their behavior regarding the functional technological properties. A more extensive and complete review of the traditional applications of caseins and caseinates in the food industry is available (O'Regan and Mulvihill 2011).

Cheese has been the main growth product within the dairy sector for many years, increasing at a rate of 2–3 % a year. Cheese, especially the minor, low volume, non-commodity varieties, is an economically valuable outlet for milk and milk protein consequently an increase in cheese production will increase the value of milk protein (Fox 2001). Cheese can be consumed directly as food, but it can also be used as sandwich filler, or it can be grated or diced and used as a condiment or as a component of cooked dishes. The main applications of cheese as an ingredient are discussed in Fox et al. (2000).

Casein from milk proteins has been produced commercially since the early twentieth century. Initially, it was used only for other industrial applications such as glues, plastics, and paper glazing, and was essentially a by-product of minor economic importance. It was only after the pioneering work done in New Zealand and Australia in the 1960s that casein was upgraded for use as food ingredient, becoming a much more valuable product. At present, casein is one of most important functional food proteins (Fox 2001).

8.3 Bovine Milk Whey Proteins

8.3.1 Composition and Physicochemical Properties

Whey proteins represent around 20 % of total bovine milk protein. The major whey proteins are β-lactoglobulin (β-LG), α-lactoalbumin (α-LA), bovine serum albumin (BSA), and the immunoglobulins (Igs) which is formed of a heavy and a light chain. In addition, other important proteins such as lactoferrin (LF), lactoperoxidase (LP), and a number of polypeptides (hormones and/or growth factors) are present in bovine milk whey.

The typical proportion (composition) and physical properties of the main bovine whey proteins are shown in Table 8.3.

Contrary to caseins, whey proteins are water soluble in a wide range of pH, in aqueous solutions. The most abundant whey protein (β-LG) presents a complex globular structure formed of 50 % antiparallel β-sheet, 15 % α-helix, 15 % randomized structure and 20 % turn structures (Rapiz et al. 1986). It presents approximately 12 genetic variants and β-LG A and B are the most highly represented.

Table 8.3 Composition and physical properties of bovine milk whey proteins

Protein components	Content (% of total)	Molecular mass (kDa)	Isoeletric pH
β-Lactoglobulin (β-LG)	48–58	18	5.4
α-Lactalbumin (α-LA)	13–19	14	4.4
Glycomacropeptide (GMP[a])	12–20	8.6	<3.8
Bovine serum albumin (BSA)	6	66	5.1
Immunoglobulins (Igs)	8–12	150	5–8
Lactoferrin (LF)	2	77	7.9
Lactoperoxidase (LP)	0.5	78	9.6

[a]Present only in "sweet whey" (released from k-casein by the action of chymosin)
Adapted from Etzel (2004), Kilara and Vaghela (2004)

The spatial configuration of β-LG A has been thoroughly elucidated (Brownlow et al. 1997). The molecule presents nine segments in an antiparallel beta sheet forming a barrel-like structure capable of holding hydrophobic molecules in its interior. This type of structure is typical of a family of proteins known as lipocalins with transport function (Lange et al. 1998).

β-LG is a thermolabile protein susceptible to various effects by heat treatments, such as loss of solubility and exposure of regions of the molecule suitable to different types of interactions with other components in complex systems (Fugate and Song 1980). Reversible modifications start to occur around 50 °C, and irreversible changes happen above 65–70 °C. At neutral pH, heating initially transforms the natural dimer into monomers following a complete loss of the globular conformation resulting in increase of volume and flexibility, in which hydrophobic groups are exposed (molten globule state). In this state, intermolecular association of β-sheet structures takes place via formation of disulfide bridges and hydrophobic interaction (Photchanachai and Kitabatake 2001).

A similar phenomenon occurs with β-LG by applying high pressures (Botelho et al. 2000; Yang et al. 2001). Monomerization seems to start with pressures in the range 100–150 mPa. Between 140 and 250 mPa, modifications occur at the secondary structure levels, but β-structures are not completely denatured until reaching pressure on the order of 330 mPa. Under this condition, the β-LG structure exhibits high flexibility and can be renatured to monomer by releasing the pressure. Pressures of 600–900 mPa promote stabilized aggregates through hydrophobic interaction and disulfide bond formation.

The second most abundant protein in bovine whey is α-lactoalbumin (α-LA). Two genetic variants (A and B) have already been identified, however, only variant B has been identified in the milk of occidental cow breeds. The variant B is formed of 123 amino acid residues and MM 14 kDa, containing in the molecule four disulfide bridges.

The most characteristic property of α-LA is the strong tendency to form associations at pH lower than its pI (pH 4.4). In natural milk pH (pH 6.6) and above, α-LA appears as a monomer in its tertiary structure. Its amino acid sequence is completely

known showing homology with egg white lysozyme of 32 %, and both proteins have a very similar tertiary structure (Browne et al. 1969; Warme et al. 1974). α-LA has an ellipsoidal form with a deep cleft dividing the molecule.

α-LA shows high affinity for Ca^{2+} and other ions such as Zn^{2+} Mn^{2+}, Cd^{2+}, $Cu^{2+,}$ and Al^{+3}. The binding constant for Ca^{2+} (kap) is 2.5×10^{-8} M (Stuart et al. 1986). Differential scanning colorimetry (DSC) has shown that α-LA undergoes reversible denaturation at 64 °C, whereas irreversible denaturation occurs when it is heated in the presence of β-LG and bovine serum albumin (BSA).

The bovine serum albumin (BSA) is another important protein that appears in bovine milk whey. This protein is not synthesized in the cow's mammary glands. It passes to the milk through the vascular system by a route similar to that used by immunoglobulins, and it is present in milk at high concentrations in cows with mastitis. BSA was crystallized from milk in 1950. It has a globular native conformation, soluble in water, and it is formed of a single polypeptide chain with 580 amino acid residues, MM 66 kDa and pI at pH 4.7–4.8 (Sgarbieri 2005).

At pH lower than the pI, alterations occur in physical and chemical properties, such as increase in intrinsic viscosity and molecular volume, with important reduction of solubility in 3 M KCl solution. A secondary structure is formed of 54 % α-helix, 40 % β-sheets alternating with three specific domains for the binding of metal ions, lipids, and nucleotides, respectively.

An important structural characteristic of BSA is the presence of one free sulfhydryl group (res 34) in the N-terminal peptide and the existence of 17 disulfide bridges in the molecule. Breaking disulfide bonds results in modification of some of its physical and chemical properties, especially ultracentrifugation sedimentation profile, immunological properties, and solubility as a function of pH. In the native state it presents high solubility in the pH range 1.5–8.0. When disulfide bonds are broken, a region of minimum solubility appears at the pH range 3.5–5.0, but solubility increases with extensive breaking of disulfide bonds. Further details of structural and physicochemical properties of milk proteins can be found in reference (Sgarbieri 2005).

8.3.2 Functional (Technological) Properties of Bovine Milk Whey Proteins, Applications

The study of the relation between protein structure and functionality is basic in the development of protein ingredients to be utilized in food formulations or fortifications.

The technological (functional) properties of food proteins can be classified in three main groups (Sgarbieri 1998; ABD El-Salam et al. 2009): (a) hydration properties (depending on protein-water interaction), such as absorption and retention of water, succulence, adhesion, and dispersibility; (b) properties depending on protein-protein interaction (precipitation, gelation, association, aggregation, cohesion, adhesion, and films formation); and (c) surface (interfacial) properties (hydrophobicity, emulsion,

superficial tension, and foaming capacity). The majority of formulated and industrialized foods belong to one of three categories: foamed, emulsified, or jellified foods (Sgarbieri 1998).

The most important functional characteristics of some milk whey proteins, such as high solubility in the entire range of pH, high dispersion capacity, water ligation, foaming capacity, gelation, and buffering power, make them highly desirable for food formulations and fortification. Milk whey proteins are highly nutritive and functional therefore, they can be applied to a variety of food products such as beverages for athletes, protein bars, infant formulas, dairy foods, meat, and other products. Whey protein concentrates (WPC) are currently used in baking products, frozen desserts, chocolates, infant formulas, humanized milks, cheese analogs, nutritional beverages, and fermented milk, among other products. Whey protein isolates (WPI) are mainly used in baking, dairy products, and confectionaries.

For further information about technological applications, functions, and benefits of bovine milk whey proteins in food products readers are referred to USEDEC (2011).

8.4 Functional Biological Properties (Bioactivities) of Bovine Milk Proteins

8.4.1 Bioactivities of Caseins

Caseins (αs, β, and k) exert their bioactivities on different systems of organisms (animal and human) mainly through various types of peptides that are liberated by proteolysis, both in natural digestion processes or controlled in vitro hydrolysis, by various enzyme systems.

In their original molecular forms caseins are important for removing calcium ions and phosphate from the mammary glands, thus avoiding pathological calcification of mammary tissues (Holt 1997). Calcium and phosphorous incorporated into caseins are used as essential nutrients for newborns and infants.

The types of bioactive peptides that have been isolated from caseins and studied to a certain extent include caseinophosphopeptides (CPPs), antihypertensive peptides (ACE inhibitors), opioids and antiopioids, glycomacropeptides, and immunomodulatory peptides (Korhonen and Philanto 2006; Miquel et al. 2006; Srinivas and Prakash 2010; Malkoski et al. 2001).

8.4.1.1 Caseinophosphopeptides (CPPs)

The term caseinophosphopeptides (CPPs) was introduced in the 1950s to describe phosphorylated peptides derived from caseins and capable of improving the calcification of children with rickets. These peptides presented high contents of

phosphoserine and were able to increase calcium balance by 39–70 % in neonate children with rickets. Later investigation showed that CPPs were able to bind mineral elements (both macro- and microelements) such as Ca, Mg, Fe, Ba, Cr, Ni, Co, and Se (Fitzgerald 1998; Scholz-Ahrens and Schrezenmeir 2000).

Subsequent studies showed that caseinophosphopeptides (CPPs) consisted of a mixture of peptides with different MM ranging from 1400 to 9600 Da. In about 50 % of identified CPPs, the sequence (SeP-SerP-SerP-glu-glu) was present and seemed to be responsible for the mineral binding property (Erba et al. 2001; Bouhallab et al. 2002). More recent research (Bouhallab et al. 2002; Miquel and Farré 2007) has shown that CPPs can positively influence the bioavailability of iron and Zn.

The presence of CPPs was observed in the ileum of experimental animals and adult humans a few hours after ingestion of milk (Takanori et al. 1995). Caseinophosphopeptides form with Ca^{2+} at alkaline pH, soluble complexes which prevent precipitation of calcium phosphate increasing the concentration of soluble calcium both in vitro and in the lumen of the small intestine (Kitts 2005).

Bovine α-S_2 caseins contain 13 phosphate groups whereas β-caseins contain only one phosphate group. Phosphate appears to be bound to caseins through monoester linkages with serine residues, creating an acidic domain that favors metal ligation (Kitts 2005).

Casein hydrolysate can be produced by various combinations of endoproteases such as trypsin, chymotrypsin, pepsin, subtilysin, and pancreatin, among others. These enzymes can be of animal, plant, or microbial origin (Zhao et al. 2007). A study was conducted with patients above 50 years of age who were supplemented with 1200 mg/day of bioactivated calcium (calcium bound to CPP) for a period of 34 months. The main observation was a significant decrease in bone fractures, resulting in a decrease in the cost of medical care for these patients (Hansen 1995). In a recent study (Garcia-Nebot et al. 2010) it was shown that use of CPPs as functional ingredients in beverages, could improve the bioavailability of iron.

The consumption of milk and milk products is associated with a reduction in dental cavity. Dental cavities are related to tooth demineralization caused by organic acids produced by bacteria during sugar fermentation. Adherence of *Streptococcus sobrinus* and *S. mutans* to the salivary film can be reduced by incorporation of CPPs. A formation of a biofilm probably occurs, which increases tooth remineralization, blocking bacteria adhesion (Schupbach et al. 1996). The activity of CPP was compared to fluoride in an animal model (Reynolds et al. 1995). It was shown that application of CPP (0.5–1 % w/v) on teeth, twice a day, was equivalent to using 500 ppm of fluoride.

In addition to improving calcium bioavailability, protecting teeth against cavities and erosion, caseinophosphapeptides (CPPs) have also shown antioxidant and immunomodulatory properties. CPPs exhibit primary and secondary antioxidant activity at the intestinal level, thus protecting against oxidative stress, and helping to maintain intestinal health (Kitts 2005).

Oral administration of CPPs to mice resulted in an increase in production of IgA by their intestinal mucosa (Otani et al. 2000). Further study by the same authors

(Otani et al. 2001) concluded that the sequence SerP-X-SerP in CPP was responsible for the immunomodulatory activity.

Caseinophosphopeptides produced by tryptic hydrolysis of sodium caseinate, followed by mineral aggregation and precipitation with ethanol were evaluated by an endotoxemia experimental model in rats. The level of interleukine 6 (IL-6) was determined in the rat plasma. A decrease in IL-6 was found as a result of oral administration of CPP, which led the author to suggest that CPP had an immunomodulatory role in reducing inflammatory processes in the treated animals (Krüger and Cândido 2009).

8.4.1.2 Antihypertensive Peptides

Caseins, and particularly milk whey peptides, liberated on proteolysis (ACE inhibitors) normally containing 3–10 amino acid residues, are capable of inhibiting the conversion of angiotensin I into angiotensin II.

Blood pressure regulation is partially dependent on the rennin-angiotensin system. In the kidney, rennin, a proteolytic enzyme, acts on the polypeptide angiotensinogen liberating angiotensin I, which is an inactive decapeptide. Angiotensin I is then converted by ACE into angiotensin II, an octapeptide which is a very strong vasoconstrictor. The enzyme ACE also acts on the hormone bradikynin, a vasodilator, converting it into inactive peptides. In addition, angiotensin II stimulates the liberation of aldosterone, a hormone produced in the adrenal glands which acts to promote retention of Na^{2+} and water, further contributing to vasoconstriction and increasing of blood pressure. Therefore, the inhibitors of ACE contribute to maintaining or lowering blood pressure by three different mechanisms: (a) inhibiting the formation of angiotensin II (a strong vasoconstrictor); (b) inhibiting the degradation of bradikynin (a vasodilator); and (c) helping to maintain the normal level of aldosterone which acts to retain sodium and water, therefore contributing to maintaining blood pressure (Pihlanto-Läppäla 2001; Silva and Malcata 2005). The enzyme ACE can be found in various tissues. ACE inhibition can influence different systems involved in blood pressure regulation as well as the immune and nervous systems (Meisel 1998).

ACE inhibitors have been found in peptides from bovine αS_1-caseins (f 23–34, 23–27, 294–299), β-caseins (f 177–183), human β-caseins (f 43–52), and k-caseins (f 63–65). These peptides are known as casokynins (Schanbacher et al. 1998).

Srinivas and Prakash (2010) isolated bioactive peptides from α-caseins, observing that they presented different bioactivities. IC_{50}, as low as 0.1 mg/mL was determined (concentration sufficient to inhibit 50 % of ACE activity). The IC_{50} value for the synthetic standard Ramipril was 0.25 mg/mL. Ramipril is a hypotensive drug that acts on the ACE enzyme system. Peptides also showed antioxidant activity with IC_{50} values of 1.25 mg/mL in comparison with a standard ascorbic acid with an IC_{50} of 0.175 mg/mL.

8.4.1.3 Opioid and Antiopioid Peptides

Opioids are liberated from all caseins and from some whey proteins. Three types of opioid receptors (δμ, μ, and δ) have been recognized in the cell membranes of the peripheral and central nervous systems. They interact with protein molecules which can bind to them (Bitri 2004).

Typical opioid peptides are the enkephalin, endorphin, and dynorphin which are peptides derived, respectively, from proopiomelanocortin, proenkephalin, and prodynorphin. These substances show similar N-terminal sequence, i.e. Tyr-Gly-Gly-Phe.

Peptides derived from milk proteins show a different N-terminal sequence, and therefore they are known as atypical opioids. However, they maintain some characteristics of typical opioids, such as the presence of either tyrosine or phenylalanine in the third and fourth positions (Meisel 1998).

Opioid peptides are obtained from β-caseins (f 60–70), β-casomorphins, and α-S_1-caseins (f 90–96), α-casomorphins.

Morphicetin is the peptide with the highest opioid potential. It belongs to the β-casomorphins group with the N-terminal sequence, Tyr-Pro-Phe-Pro. It has been shown that casomorphins can produce analgesia and increase the time of food intestinal transit they also have an antidiarrheal effect, increasing absorption of amino acids and electrolytes, stimulating secretion of insulin and somatostatin (Meisel and Schlimme 1990; Kohronen et al. 1998; Shah 2000). In addition, Drewnowiski (1992) has demonstrated that opioids can influence the preference and level of intake of certain types of foods, namely high sugar and fatty foods.

Contrary to the αS- and β-caseins, the k-caseins are reported to liberate, on hydrolysis, opioid antagonists or antiopioid peptides (Shah 2000). k-caseins can also liberate glycomacropeptide (GMP) and casoplatelins, with antithrombus activity.

Casoplatelins are peptides derived from the proteolysis of k-caseins (f_s 106–116, 106–112, 113–116) which act to inhibit platelet aggregation (Jollés et al. 1986). There are similarities between the residues 106–116 from k-caseins and the residues 400–441 from human fibrinogen. The peptide named casopiastrin was obtained from tryptic hydrolysis of k-caseins and showed antithrombotic activity by inhibiting the binding of fibrinogen peptides to platelets (Smacchi and Gobbetti 2000).

Glycomacropeptide (GMP), which results from enzymatic cleavage of k-caseins (chymosin or trypsin) MM 6.8 kDa residues 106–169, shows a singular amino acid composition. GMP does not contain aromatic amino acids, but it is rich in branched chains amino acid residues. This peculiar composition makes k-casein GMP fragment an interesting option for the elaboration of formulas to be used in patients with liver disease, phenylketonuria, and other metabolic disorders. It has also been shown that GMP can act as a prebiotic, immunomodulator, protection against dental erosion (Nejad et al. 2009) as well as stimulating the production of cholecystokinin hormone in the small intestine (Schupbach et al. 1996; Krüger and Cândido 2009). Cholecystokinin is claimed to stimulate satiety, thereby decreasing food intake.

A summary of distribution of the main caseins in bovine milk and the various types of peptides released by enzymatic hydrolysis is illustrated in Table 8.4.

Table 8.4 Active peptides released from the various caseins fractions

Caseins of bovine milk	Peptide bioactivities
αS_1-casein (10.3 g/L); αS_2-casein (2.7 g/L); β-casein (9.7 g/L)	• Caseinophosphopeptides (CPPs)
	– Increase solubility, absorption, and fixation of minerals in bone tissues and teeth
	– Decrease dental cavities and erosion
	– Participates of antioxidant and antimicrobial actions
	• Antihypertensive peptides (ACE inhibitors)
	– Convert angiotensin I in angiotensin II (a vasoconstrictor)
	– Degrade bradikyn (a vasodilator)
	– Angiotensin II stimulates production of aldosterone (which contributes to blood pression)
	• Opioids (opium-like action)
	– Act on gastrointestinal tract (GIT)
	– Decrease intestinal peristalsis
	– Decrease food mass intestinal transit and increase nutrient absorption
	– Decrease diarrhea episodes
	– Act on the nervous system as: antidepressants, analgesic property
	– Improve sleeping, memory, and learning
k-casein	• Antiopioids (opioid antagonists)
	– Act to neutralize opioids
	• Glicomacropeptide (GMP): (f 106–169), k-casein C-terminal
	– Act as a prebiotic
	– Act as immunomodulator
	– Stimulate cholecystokinin production
	• Casoplatelins: (f 106–116; 106–112; 113–116), C-terminal
	– Have antithrombotic properties

Modified from Korhonen and Pihlanto (2007), Korhonen and Philanto (2006), Hartman and Meisel (2007)

8.5 Bioactivities of Bovine Milk Whey Proteins and Peptides

Bovine whey proteins have gained much prestige (added value) in the last three or four decades due to the discovery of their multifunctional bioactivities. Important properties have been demonstrated in cell cultures, animal experiments and, in lesser proportion in clinical studies.

Most of this bioactivity has been demonstrated in whey protein concentrates (WPC), isolates (WPI), hydrolysates (WPH), and in some purified whey proteins and derived peptides. Some of the bioactivities that have been described are as follows: immune stimulation (cellular, humoral, and glutathione synthesis); anticancer

activity; antiulcer effect; anticholesterolemic effect; antivirus and antibacterial effects; antioxidant and anti-inflammatory effects; prebiotic support; antihypertensive effect and improvement of athletic performance.

8.5.1 Immunostimulation Properties

In the 1980s and 1990s, Canadian researchers were successful in demonstrating that a commercial lactoalbumin preparation fed to mice was much superior to other proteins such as commercial casein, a soy protein isolate, a wheat protein isolate and a rodent purine ration, in promoting higher content of glutathione (GSH) and immunoglobulin M (IgM) in mice spleen. Further investigations led the Canadian group to propose the hypothesis that the immunomodulatory property of bovine whey proteins depends on their primary structure (amino acids sequence) and the high content of cysteine forming disulfide bonds (-S-S-) in some of the important whey proteins, namely 17 in lactoferrin (LF), 17 in bovine serum albumin (BSA), 4 in α-lactoalbumen (α-LA), and 2 in β-lactoglobulin (β-LG) (Bounous et al. 1991, 1993; Bounous 1997).

According to these authors, the digestion of undenatured whey proteins would liberate peptides with the structure glu-$(Cys)_2$ which, during the processes of absorption and metabolism would be converted into the dipeptide glu-Cys, an ideal substrate for the biosynthesis of GSH (Bounous et al. 1991). It was also demonstrated that in vivo inhibition of GSH synthesis by butathionine sulfoximine (BSO), a specific inhibitor of the enzyme glutamylcysteine synthetase, GSH synthesis as well as the production of IgM in mice spleen were completely inhibited. Based on this series of studies, the Canadian authors developed a humanized whey protein concentrate named Immunocal®, which was patented in Canada by the Immunotec Clinical Foundation (Bounous 1997). More recently, similar immune responses have been demonstrated by different research groups in several countries, using various WPC preparations (Sgarbieri et al. 2000; Dias et al. 2006; Rutherfurd-Markwick et al. 2005).

Relatively little investigation has been done with peptides derived from the main whey proteins such as β-lactoglobulin (β-LG) and α-lactoalbumin (α-LA). In vitro models permit the study of different parameters of cell metabolism such as: proliferation and activation of lymphocytes, production of antibodies, and impact on non-specific immune response, among others. Particular interest has been shown in the study of B lymphocytes, which are responsible for the production of antibodies and T lymphocytes, which control specific immune responses to antigens, including inflammatory reactions that may lead to tissue degradation in gastrointestinal tract (GIT) diseases (Gauthier et al. 2006).

Two synthetic peptides corresponding to α-LA (f 50–51), Tyr-Gly, and (f 18–20), Tyr-Gly-Gly, enhanced protein synthesis and peripheral lymphocytes proliferation from human blood when stimulated by concanavalin A (ConA). Maximum stimulation

was reached at peptide concentration of 10^{-4} mol/L for Tyr-Gly and 10^{-8} mol/L for Tyr-Gly-Gly (Kayser and Meisel 1996).

Oral administration of lactoferrin (LF) to mice previously treated with cyclophosphamide promoted partial reconstitution of humoral and cellular responses through elevation of T and β cells number (Artym et al. 2005). Cyclophosphamide is a drug normally used in the treatment of cancer and auto-immune diseases, including multiple sclerosis (Marshall 2004).

According to Gauthier et al. (2006) there are many discrepancies among authors on the positive effect of peptides on lymphocyte proliferation. The agreement is much greater as to the effect of milk peptides on antibody production. According to these authors, many reasons may explain these discrepancies, such as difference in applied methodologies, differences in raw materials studied, as well as differences in the study models applied.

8.5.2 Anticancer Property of Whey Proteins

The anticancer property of whey proteins has been demonstrated in vitro, in various types of cells and in experimental animals (Marshall 2004; Parodi 2007). Bounous and Molson (2003) were the first investigators to demonstrate the importance of bovine milk whey proteins (WPC) in the treatment of colon cancer. These authors reported that the anticancer properties of whey proteins were due to an elevation of GSH in tissues and organs that stimulates immune defense mechanisms.

A study by McIntosh et al. (1995) showed that whey protein in rats was more efficient than soybean protein or meat protein in protecting rat intestine against tumors. It was also demonstrated that rats fed with whey proteins had a higher concentration of GSH in various organs (liver, spleen, colon, and in tumor tissues), compared to rats that were fed soy protein or meat protein.

In another experiment, McIntosh et al. (1998) used the standard American Institute of Nutrition (AIN-93) diet with 15 % total protein from the following sources: WPC protein (15 %); soy protein (15 %); soy protein (10 + 5 % LF); and soy protein (10 + 5 % β-LG). Diets containing only soy protein (15 %) or WPC (15 %) were used as controls. The diets with 5 % LF or 5 % β-LG replacing soy protein were as efficient as the 15 % WPC diet, and all three were superior to the soy protein diet (15 %) in suppressing tumor development in rat intestines, suggesting that both LF and β-LG acted as intestinal tumor suppressors.

More recently Dias et al. (2006), using the same techniques applied by the Canadian workers, tested the efficacy of a WPC produced in their own laboratory to simulate GSH synthesis in the liver and IgM in the spleen of mice (AJ strain). Efficacy of this WPC was compared to Immunocal® patented in Canada, a commercial casein and a commercial soy protein isolate. The immune response and liver GSH was similar for the Immunocal® and WPC which were superior to soybean isolate and casein diets.

Among the isolated whey proteins, lactoferrin (LF) has received the most attention in anticancer studies. Sekine et al. (1997) fed male F344 rats diets supplemented with 0.2 or 2.0 % LF, following administration of azoximethane (AOM). After 36 weeks the incidence of adenocarcinomas on the colon was 15.25 and 56 % for animals receiving 2 % LF, 0.2 % LF compared to the control diet.

Another study (Tsuda et al. 1998) showed that diet with 2 % LF hydrolysate (LFH) or 0.1 % lactoferricin (LFcin), N-terminal peptide derived from LF by the action of pepsin, significantly reduced the incidence and number of AOM-induced intestinal tumors in rats. Anticancer action of dietary LF has been reported in single studies at a number of other sites (Tsuda et al. 2002) including tongue, liver, bladder, lung, and others. As a multifunctional protein, LF seems to act through various mechanisms in tumor suppression and inhibition.

Alpha-lactoalbumin (α-LA) is another highly functional milk whey protein. Bovine α-LA shows 76 % amino acid sequence homology with human α-LA (hLA). It was recently shown that bovine α-LA, like human α-LA, under slightly acidic pH loses some bound Ca^{2+}, acquiring a flexible structure which, in the presence of a monounsaturated long chain fatty acid (e.g. oleic acid) forms a complex capable of killing cancer cells by apoptosis. Swedish investigators named the human α-LA-oleic acid complex, HAMLET (human α-lactoalbumin made lethal to tumors cells), and by analogy attributed the similar name BAMLET for the complex formed between bovine α-LA and oleic acid (Gustafsson et al. 2005; Permyakov et al. 2004).

8.5.3 Antiulcerative Property of Whey Proteins

The ability of a whey protein concentrate (WPC) to inhibit gastric mucosa ulcerative lesions caused by oral administration of absolute ethanol was investigated in a rat model (Rosaneli et al. 2002). Acute administration (single doses) of WPC resulted in 41 % inhibition of the ulcerative lesion index (ULI) and 73 % inhibition with repeated doses. In a 10-day subchronic treatment study, the inhibition was 64 %, which was relative to saline treatment (negative control). In vivo alkylation of sulfhydryl compounds by subcutaneous injection of N-ethylmaleimide essentially eliminated the WPC protection. Intraperitoneal injection of butathionine sulfoximine (BSO), which inhibits glutathione synthesis, reduced WPC protection to 35 % and 52 % for single and double doses, respectively.

In another work (Rosaneli et al. 2004) the protective effect of WPC against stomach ulcerative lesions was studied, following subcutaneous injection of indomethacin in adult Wistar rats (300–400 g body weight). The best protection for repeated and single doses and for a 10-day subchronic treatment was 50.1 % and 44 %, respectively.

Matsumoto et al. (2001) investigated the effect of the major proteins in cow milk on gastric mucosa injuries in rat ulcer models. They found that α-lactoalbumin (α-LA) has marked preventive effects against gastric mucosa injuries and that prostaglandin (PG) synthesis may contribute to these effects.

Ushida et al. (2003) investigated the effects of α-LA on several defense mechanisms of gastric mucosa by evaluating gastric PGE_2 content, gastric mucin content, gastric luminal pH, gastric fluid volume, and gastric emptying in naive rats. The authors concluded that α-LA enhances both PG-dependent and PG-independent gastric defense mechanisms in naive rats.

A more recent investigation (Mezzaroba et al. 2006) isolated α-LA and β-LG from a WPC using anion exchange and molecular exclusion chromatography. Both preparations were used to evaluate stomach mucosa protection against absolute ethanol. It was demonstrated that the purified β-LG showed no protection to gastric mucosa, whereas α-LA and two fractions of its enzyme hydrolysate (F1 < 1 kDa) and (F2 > 1 kDa) protected the rat mucosa from lesions in the range of 32 % and 33 % for both hydrolysates fractions and 49 % for the intact α-LA preparation, with no statistical difference between α-LA and the hydrolysate fractions.

8.5.4 Anti-Hypercholesterolemic Property

Sautier et al. (1983) maintained rats on 23 % protein from one of the following sources: a whey protein concentrate, a commercial casein, a soy protein isolate, and a sunflower protein isolate. The authors found that the casein diet produced the highest level of blood cholesterol, while the WPC and soy protein diets were equivalent and produced the lowest level of blood cholesterol. Liver cholesterol was significantly lower in the rats fed the WPC diet.

Another study (Jacobucci et al. 2001) compared diets with 20 % protein from a WPC, a commercial casein, or a soy protein isolate which were added to the standard AIN-76A diet, modified by the addition of 6 % coconut fat plus 1 % cholesterol, to make the diet hypercholesterolemic to rats. The feeding period was for 45 days. The main result was that both the soy protein isolate (SPI) and the WPC diets were effective in maintaining the initial cholesterolemia, whereas the casein diet exhibited a hypercholesterolemic effect in both blood and liver.

Nagaoka et al. (2001) reported the hypocholesterolemic action of a peptide derived from β-lactoglobulin (β-LG). The β-LG tryptic peptide (f 71–75), Ile-Ile-Ala-Asp-Lys, lactostatin, presented stronger hypocholesterolemic action in animal studies than did the drug β-sitosterol. The mechanism of action seems to be related to a decrease of cholesterol micelles solubility, inhibiting cholesterol reabsorption. Absorption of cholesterol by caco-2 human cells dropped 40 % when treated with 1 mg/mL lactostatin, compared with the same treatment with a tryptic hydrolysate of casein.

The peptide β-lactotensin (β-LG, f 146–149) presented hypocholesterolemic activity in mice, 90 min after intraperitoneal administration (30 mg/kgbw) or orally, in doses of 100 mg/kg body weight (Yamauchi and Ohinata 2003). The intraperitoneal administration of β-lactotensin was more efficient in reducing total serum cholesterol with a 23 % reduction, compared to 14 % by oral administration.

These observations suggest that both lactostatin and β-lactotensin, peptides derived from β-LG, show potential to be used as nutraceuticals or in functional food development, aiming at controlling human cholesterolemia.

8.5.5 Antivirus and Antibacterial Properties

Whey proteins in various preparation forms (WPC, WPI, WPH), and some isolated proteins have presented antiviral and antibacterial properties.

Oxidative stress, a known activator of HIV replication in vitro, has a potential role as a cofactor in HIV-disease progression. Glutathione (GSH), a tripeptide and major intracellular antioxidant accounts for over 90 % of the intracellular nonprotein thiols. One mechanism of action of GSH is through removal of intracellular H_2O_2 by providing substrate for GSH peroxidase, the major H_2O_2 removing enzyme.

In a previous citation (Bounous 1997) we have seen that Immunocal®, a proprietary WPC preparation was efficient in stimulating the synthesis of GSH in various rat tissues.

A prospective double blind clinical trial with 30 patients (35 male, 5 female; mean age 42 ± 9.8 years) with stable HIV infection were randomized to a supplemental diet with a daily dose of 45 g of whey proteins, either Protectamin (Fresenius Kabi, Bad Hamburg, Germany) or Immunocal® (Immunotec, Vandreuil, Canada) for 2 weeks. At the end of 2 weeks of oral supplementation with whey proteins, plasma GSH levels increased 45 % ($P = 0.004$) in the Protectamin group and 24.5 % ($P = 0.43$) in the Immunocal® group, which did not reach statistical significance with the placebo control. It was concluded that in GSH-deficient patients with advanced HIV-infection, plasma GSH level was increased in a short-term oral supplementation with whey proteins (Micke et al. 2001).

A more recent investigation (Moreno et al. 2005) performed a prospective double-blind clinical trial in which 18 HIV-infected children (1.98–6.36 years), under antiretroviral therapy, were supplemented during 4 months with a WPC produced in the author's laboratory. Erythrocyte glutathione concentration, T lymphocyte counts (CD^+_4 and CD^+_8) and occurrence of associated coinfections was evaluated. A significant median increase ($P = 0.018$) in erythrocyte GSH was measured in the whey protein supplemented group; the TCD^+_4/CD^+_8 lymphocyte ratio showed a nonsignificant increase. The WPC group presented a lower occurrence of associated coinfections, compared with placebo group. The conclusion was that WPC supplementation can stimulate erythrocyte GSH synthesis, and possibly decrease the occurrence of coinfections.

The antibacterial properties of enzyme hydrolysates of bovine lactoferrin (LF) were examined to determine whether active peptides were produced from this protein (Tomita et al. 1991). Hydrolysates prepared by cleavage of LF with porcine pepsin or acid protease from *Penicillium duponti* showed strong activity against *Escherichia coli*, whereas hydrolysates from trypsin, papain, or other neutral proteases were much less active. Low molecular weight peptides generated by porcine

pepsin cleavage of LF showed a broad-spectrum antibacterial activity, inhibiting the growth of a number of gram-negative and gram-positive species, including strains that were resistant to native LF. The antibacterial potency of the hydrolysate was at least eightfold greater than that of undigested LF, with all strains tested.

Lactoferrin is a multifunctional protein that has been studied extensively over the last decades. It is best known for its ability to bind iron, which led to the discovery of its antibacterial activity. In addition, LF has shown antifungal and antiparasitic activity towards a broad spectrum of species (Jensen and Hancock 2009).

Studies have demonstrated that most of ingested LF goes through the stomach and small intestine undegraded, reaching the large intestine where it can chelate free iron, which is indispensable to replication and growth of pathogenic bacteria (Jensen and Hancock 2009; Troost et al. 2001). Interestingly, *Lactobacilii*, known as probiotic bacteria, can utilize iron bound to LF therefore, LF can function as a bacteriostatic for pathogenic bacteria and as a prebiotic for *Lactobacilii*.

LF also plays an important bioactivity as an anti-inflammatory and antioxidant. Rogan et al. (2004) have shown a decrease in LF activity in patients with cystic fibrosis (CF). Levels of LF in *Pseudomonas aeruginosa*-positive sputum samples showed significantly higher cathepsin activity and reduced ability to inhibit formation of biofilm, compared with *P. aeruginosa*-negative sputum samples. It was shown that the cleavage of LF by cathepsin results in loss of both its microbiocidal and antifilm activity which is important for the pathogenesis of chronic *P. aeruginosa* lung infection in patients with CF.

Lactoferrin antiviral activity has also been tested against hepatitis C virus (HCV) in cultured human hepatocytes (Ikeda et al. 1998) and human clinical trials (Tanaka et al. 1999).

Ikeda et al. (1998) demonstrated that LF effectively prevented HCV infection in a susceptible cell line (PH5–CH8) hepatocytes. They showed that anti-HCV activity of bovine LF was due to interaction of LF with HCV, not to interaction of LF with cells. Their findings suggested that LF could be a candidate for an anti-HCV reagent that would be well-tolerated and effective in the treatment of patients with chronic hepatitis C.

In another publication (Tanaka et al. 1999) the hypothesis that LF inhibits HCV viremia in patients with chronic hepatitis C was tested. Eleven patients with chronic hepatitis C received 8-week treatment with bovine LF (1.8 or 3.6 g/day). At the end of the treatments, a decrease in serum alanine transaminase and HCV-RNA concentrations was apparent in three, (75 %) of the four patients, with low pretreatment serum concentration of HCV-RNA. However, seven patients with high pretreatment concentration showed no significant changes in these indices. This suggests that LF is one potential candidate as an anti-HCV reagent that may be effective for the treatment of patients with chronic hepatitis.

Another important minor protein present in bovine whey is lactoperoxidase (LP). LP is found in the mammary, salivary, and lachrymal glands and their products, such as milk, saliva, and tears (Wolfson and Sumner 1993). In addition to antimicrobial action, LP also acts on the degradation of some carcinogens and protects animal

cells against peroxidative damage. The lactoperoxidase system consists of three components: LP, thiocyanate, and H_2O_2.

Lactoperoxidase is the most heat-resistant enzyme present in milk. It is only partially inactivated by pasteurization at 74 °C (15–20 seg), retaining sufficient residual activity to catalyze the reactions between thyocyanate and H_2O_2, generating the anion hypothyocyanate ($OSCN^-$) capable of causing damage to cell membrane and killing bacteria.

Lactoperoxidase (LP) has also been used as a conserving agent, to extend shelf-life and quality of milk as well as other dairy and cosmetic products. Savci et al. (2002) described a system containing SCN^-: H_2O_2 (thiocyanite: hydrogen peroxide) at levels of 10:10 and 20:20 ppm added to raw cow, sheep, and goat milks. The samples were stored at 20 or 35 ± 1 °C. With increasing concentrations of SCN^-:H_2O_2, the content of bacteria and titrable acidities of the milk samples increased very slowly and their storage time was prolonged. On the contrary, pH and coagulation times decreased slowly with increasing concentrations of SCN^-:H_2O_2. The longest storage time was obtained at 20 °C and 20:20 ppm SCN^-:H_2O_2.

Two other whey proteins containing bactericidal domains encrypted in their primary structures are α-lactoalbumin (α-LA) and β-lactoglobulin (β-LG). According to Pellegrini et al. (1999), proteolytic digestion of α-LA by pepsin, trypsin, and chymotrypsin yielded three polypeptide fragments with bactericidal properties. Two fragments were obtained from tryptic digestion. One was a pentapeptide (res 1–5) and the other (res 17–31)-S-S-(109–114) was composed of two polypeptide chains, held together by a disulfide bridge. Fragmentation of α-LA by chymotrypsin yielded (res 61–68)-S-S-(75–80), as well as two peptides held together by a disulfide bridge. The amino acid sequences of these peptides were identified. The peptides were synthesized and found to exert antimicrobial activities. Digestion of α-LA by pepsin yielded several polypeptide fragments without antibacterial activity, against all bacteria strains tested.

Further investigation (Pellegrini et al. 2001) by the same group submitted β-LG to digestion by trypsin, obtaining four peptide fragments with bactericidal activity. The peptides (fs 15–20, 25–40, 78–83, and 92–100) were isolated. The amino acid sequences were determined and then synthesized. These peptides showed bactericidal properties in relation to various strains of bacteria. The authors suggested a possible antimicrobial function of β-LG after its partial digestion by pancreatic endopeptidases.

8.5.6 Antihypertensive Peptides from Bovine Whey Proteins

Researchers have identified and characterized a large number of peptides liberated from food proteins, under natural digestion or controlled in vitro proteolysis (Ariyoshi 1993). These peptides are capable of inhibiting the angiotensin converting enzyme (ACE), as we have already discussed for caseins in this chapter. By inhibiting the

Table 8.5 Individual whey protein bioactivities

Whey proteins	Bioactivity
β-Lactoglobulin (3.4 g/L)	Allergenicity (2–4 % newborn)
	Antihypertensive property (ACE Inh.)
	Antitumor action
α-Lactalbumin (1.3 g/L)	Lactose synthesis (mammary GLs)
	Antiulceration (gastric mucosa)
	Antihypertensive peptides (ACE Inh.)
	Rich in tryptophan (neurotransmitter)
Bovine serum albumin (0.4 g/L)	Lymphocyte stimulation/IGs production
	Antihypertensive peptides (ACE Inh.)
Immunoglobulins (0.8 g/L)	Primary in specific immune defense (newborn)
	Humoral immune defense (antigens, toxins)
Lactoferrin (0.1 g/L)	Prebiotic, bacteriostatic, bactericidal, antiviral, antioxidant, anti-inflammatory, antitumoral, immunostimulant
Lactoperoxidase (0.15 g/L)	Antioxidant, bactericidal, conservation

Modified from Korhonen and Pihlanto (2007), Korhonen and Philanto (2006)

angiotensin converting enzyme (ACE), these peptides can control, to a certain extent, the increase of blood pressure and contribute to the avoidance of hypertension.

A great deal of research has been done in isolating and characterizing a large number of ACE inhibitors from bovine milk whey proteins, as can be found in several publications (Pihlanto 2004). Table 8.5 summarizes some of the studied bioactivities of individual whey proteins (Korhonen and Pihlanto 2007; Korhonen and Philanto 2006).

A significant number of bioactivities described for caseins and whey proteins has already been applied in preparations of different functional foods and ingredients for commercial purposes, in many countries such as the United States of America, Canada, various European countries and Japan, as exemplified in Table 8.6, modified from (Hartman and Meisel 2007; Korhonen 2009).

8.6 Bovine Milk Fat Globule Membrane (MFGM) Proteins

Reinhardt and Lippolis (2006) applied modern genomic and proteomic techniques to identify proteins in an MFGM preparation. They detected 120 different proteins associated with this material, of which 71 % were proteins associated with the MFGM structure, while the remaining were cytoplasmatic or secretory proteins. Only 15 of these membrane proteins had been previously identified in rat or human milk.

Some of the proteins which have already been extracted from bovine MFGM and studied to some extent are listed in Table 8.7, along with their claim of benefits to human health.

Table 8.6 Commercially available milk-derived functional foods and ingredients

Bioactive peptides	Health claim	Product type	Brand name	Manufacturer
β and k-CASs (VPP; IPP)[a]	↓ Blood pressure	Sour milk	Calpis	Calpis Co; Japan
β and k-CASs (VPP; IPP)[a]	↓ Blood pressure	Fermented milk	Evolus	Valio, Finland
Whey peptide	↓ Blood pressure	WPI	Biozate	Davisco, USA
CAS (12 AA Res)	↓ Blood pressure	Ingredient	C12 Peption	DMV, The Netherlands
CAS (12 AA Res)	↓ Blood pressure	Drink	CAS DP·Peptio	Kanebo, Japan
α-CAS peptides (f 1–6; f 1–7; f 1–9)	↓ Blood pressure	Fermented low-fat hard cheese	Festive	MTI Agrifood Res., Finland
Milk-derived Peptides	↓ Blood pressure	Margarine	Evolusdouble effect	Valio, Finland
CPP	↑ Mineral absorption	Ingredient	Copolac	Arla Foods, Denmark
CPP	↑ Mineral absorption	Drink	Tekkotsu Inryou	Suntory, Japan
CPP	↑ Mineral absorption	Drink	Kotsu Kotsu alcium	Asahi, Japan
CPP	↑ Mineral absorption	Ingredient	CE90CPP	DMV, The Netherlands
CPPs	Anticarciogenic	Chewing gum	Recaldent	Cadbury Enterprises
CAS peptide	Athletic performance, muscle recovery	Ingredient	Peptopro	DMV, The Netherlands
Milk-derived peptide	Energy, sleep quality	Ingredient	Cys Peptide	DMV, The Netherlands
Whey-derived peptide	Aids relaxation, sleep	Ingredient	Vivinal Alpha	BDI*, The Netherlands
αS_1-CAS (f 91–100)	Reduces stress	Milk drink confectionary	GSPHP ProDiet F-200	Ingredia, France
GMP-kCAS (f 106–169)	Anticarcinogenic, antithrombotic, antimicrobial	WPI (Chymosin)	BioPURE·GMP	Davisco, USA
Glutamine Rich Peptide	Immunomodulatory	Dry milk, protein hydrolysate	Gln Peptide	DMV, The Netherlands

[a]V = valine, P = proline, I = isoleucine

Modified from Hartman and Meisel (2007), Korhonen (2009)

Table 8.7 Main MFGM proteins and their potential bioactivities

Protein	Bioactivities
FABP–FA binding protein: MM 13 kDa, pI (5–5.5)	• Transport FA; modulate lipid metabolism
	• Inhibits cellular growth
	• Anticancer action
BTN–Butyrophilin: MM 66–67 kDa, pI 5.32	• Belongs to the IGs family
	• Suppressed experimental autoimmune encephalomyelitis (EAE) in C57B2/6 mice
Xanthine oxidase/redutase: (XO/XDH) MM 146–300 kDa, pI 7.8	• Purines metabolism
	• Oxi-reduction reactions
	• Bactericidal action
	• Anti-inflammatory action
MUC1–Mucin-1: MM 160–200, pI < 4.5	• Protection against pathogens (bacteria/virus) in the GIT
BRCA1/BRCA2–MM 210 kDa	• Supressors of breast and ovarian cancer
ADPH–Adipophilin: MM 52 kDa, pI 7.5–7.8	• Absorption and transport of FA/TG
PAS VI/VII–Lactadherins: MM 47 kDa, pI 6–6.6	• Protect GIT against virus
	• Protect against neuritis
	• Stimulate synapse formation (CNS)
Inhibitors	• Inhibit glucuronidase (large intestine)
	• Inhibit *Helicobacter pylori* (upper GIT)

GIT = gastrointestinal tract, FA/TG = fatty acid/triglyceride ratio
Modified from Deuvettinck et al. (2008)

Fatty acid binding protein (FABP) has been described as capable of inhibiting in vitro proliferation and growth of colon cancer cells at extremely low concentration (Spitsberg 2005). In addition this protein is also claimed to modulate lipid metabolism.

Two MFGM proteins, $BRCA_1$ and $BRCA_2$ are present in both bovine and human milk and are claimed to suppress the expression of oncogenes for breast cancer (Spitsberg 2005). Both proteins are involved in DNA repair processes, although $BRCA_2$ is also involved in cytokines regulation (Deuvettinck et al. 2008; Vissak et al. 2002; Daniels et al. 2004).

Another MFGM protein with anticancer properties is an inhibitor of the enzyme β-glucuronidase (Spitsberg 2005). This enzyme is produced by bacteria in the large intestine and acts to degrade liver excretion products, such as glucuronides, which release tumor producing compounds. Therefore, the β-glucuronidase inhibitor acts as a protector against large intestine tumors.

Butyrophilin (BTN) is a complex protein that runs across the fat globule membrane and is formed of different functional peptide domains. The N-terminal domain is external to the membrane, while the C-terminal is encrypted between the external and internal layer of the MFGM. It has been reported (Guggenmos et al. 2004) that BTN can modulate the action of T cells against myelin oligodendrocyte glycoprotein (MOG), in experimental autoimmune encephalomyelitis (EAE) in animals,

which is related to human multiple sclerosis. This BTN property has been explained by the presence in its extracellular domain of a sequence homologous to IgV, which was conserved in the myelin oligodendrocyte glycoprotein (MOG), making possible a cross reaction with the peptide sequence 76–80 in the MOG.

Mana et al. (2004) demonstrated in mice C57BL/6 that treatment with BTN before or after immunization with MOG prevents or eliminates the manifestation of experimental animal encephalomyelitis (EAE), suggesting that consumption of dairy products enriched with MFGM protein may modulate the MOG pathogenic response in a positive way.

Some MFGM proteins exhibit antibacterial and antiviral properties. The antimicrobial action of the xanthine oxidase-reductase system (XO/XDH) in the gastrointestinal tract has been revised recently (Harrison 2004). XO/XDH is expressed in different epithelial cells of the gastrointestinal tract (GIT) and its action is related to elimination of the reactive oxygen species (ROS), superoxide, and hydrogen peroxide in the GIT.

A purified XO/XDH system inhibited the growth of the bacteria *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella entiriditis*, through formation of H_2O_2 or stimulation of the lactoperoxidase system (Harrison 2006).

Defatted MFGM material showed similar potential as gastric mucins to inhibit the bacteria *Helicobacter pylori*. This result suggested that MFGM, similar to mucins, is important in inhibiting *H. pylori*, which colonizes the stomach and upper small intestine and is claimed to be responsible for diseases such as chronic gastritis type B, peptic ulcer, and stomach cancer (Atherton 2006; Fox and Wang 2007).

Lactadherins (PAS VI/VII) were originally characterized by their exclusive association with the MFGM, nevertheless, it is known today that these proteins can be expressed in other tissues. Bovine lactadherins (PAS VI/VII) represent glycosylation variants of the same protein (4099 AA res.), molecular mass 47 kDa and 52 kDa, respectively (Rasmussen 2009). It is a multifunctional protein capable of elimination of apoptotic cells in situations like macrophage phagocytosis, inflammation, autoimmune disease, splenomegaly, falciformis anemia, Alzheimer disease, neovascularization, and angiogenesis (Hanayama et al. 2006).

In spite of a relatively large number of publications about MFGM proteins, investigation on their biological functions and their effect on human health is still in its infancy.

8.7 Isolation Fractionation and Purification of Milk Proteins

The production of milk proteins in different forms and degrees of purification has been under improvement during the entire twentieth century, and is still under development (Tunick 2008). The use of membrane technology for ultrafiltration of whey was reported for the first time in 1971, and the use of recombinant chymosin in cheese processing, only in 1990 (Marianki 1995).

According to Rosenberg (1995) membrane filtration technology was in the past, and is still nowadays, of extreme importance in the dairy industry, and for research in the area of milk proteins. There are four types of membrane applicable to research and commercial processes: (a) microfiltration (MF), pore size 0.2–2 μm, particle retention of MM > 200 kDa; (b) ultrafiltration (UF) pores of ~0.01 μm, retention of MM 1–200 kDa, pressure < 1000 kPa; (c) nanofiltrations (NF), MM in the range of 300–100 Da, separate ions based on diffusion and charge; and (d) reverse osmosis, retains MM ~100 Da under pressure 5–10× > UF.

Commercial production of milk proteins can be done by three different processes (Zinsly et al. 2001; Roman and Sgarbieri 2005b), as illustrated in Fig. 8.1. The operation starts with whole milk which is submitted to defatting and pasteurization, producing milk fat (cream), and defatted pasteurized milk.

Process 1 is used for the production of caseinates, precipitating the caseins in the isoelectric pH (pH 4.6). The isoelectric caseins are insoluble and nonfunctional. Solubilization is accomplished by neutralization (pH ~7) with sodium, potassium, or calcium hydroxide, resulting in Na, K, or Ca caseinates, which have good solubility and functionality. These caseinates are important ingredients in food industries. The remaining liquid, which constitutes the acid whey, can now be submitted to membrane microfiltration (MF), ultrafiltration (UF), and diafiltration (DF) to obtain a concentrate containing practically all whey proteins, which are then dehydrated by spray drying to obtain whey protein concentrate (WPC).

Process 2 is employed in cheese manufacturing where the milk is treated with commercial rennet containing the enzyme chymosin which produces the casein coagulum used as raw material for cheese manufacture. The liquid is called "sweet whey" to differentiate it from the acid whey generated in process 1. Chymosin acts on k-casein (Phe_{105}–Met_{106}) liberating the C-terminal polypeptide known as glycomacropeptide (GMP), which consists of the water soluble remained in the whey fraction.

The "sweet whey" undergoes the same series of membrane filtration and dehydration to be transformed into a WPC, with different composition and properties than the acid whey-derived WPC.

Finally, process 3 starts with a microfiltration (0.2 μm ceramic membrane) to fractionate the defatted milk into a retentate, which after UF and dehydration will be transformed into a casein micelles concentrate (CMC) that can be used directly as an ingredient, or it can be used as a raw material for obtaining different casein fractions in their purified forms. The remaining liquid (permeate) can be seen as a "natural whey" because it is a product of simple MF, without chemical or enzymatic treatment. The same series of membrane filtration and dehydration is applied to obtain a WPI with more than 95 % purity. Process 3 is still under adaptation for scaling up to be applied in commercial operations.

Because different casein fractions present diverse functional properties, both technological and physiological, it is of great importance to develop methods to obtain these casein fractions separated and purified. The fluxogram presented in Fig. 8.2, for isolation and partial purifications of the main casein fractions, has been modified from Rosenberg (1995) which offers such a possibility because β-caseins

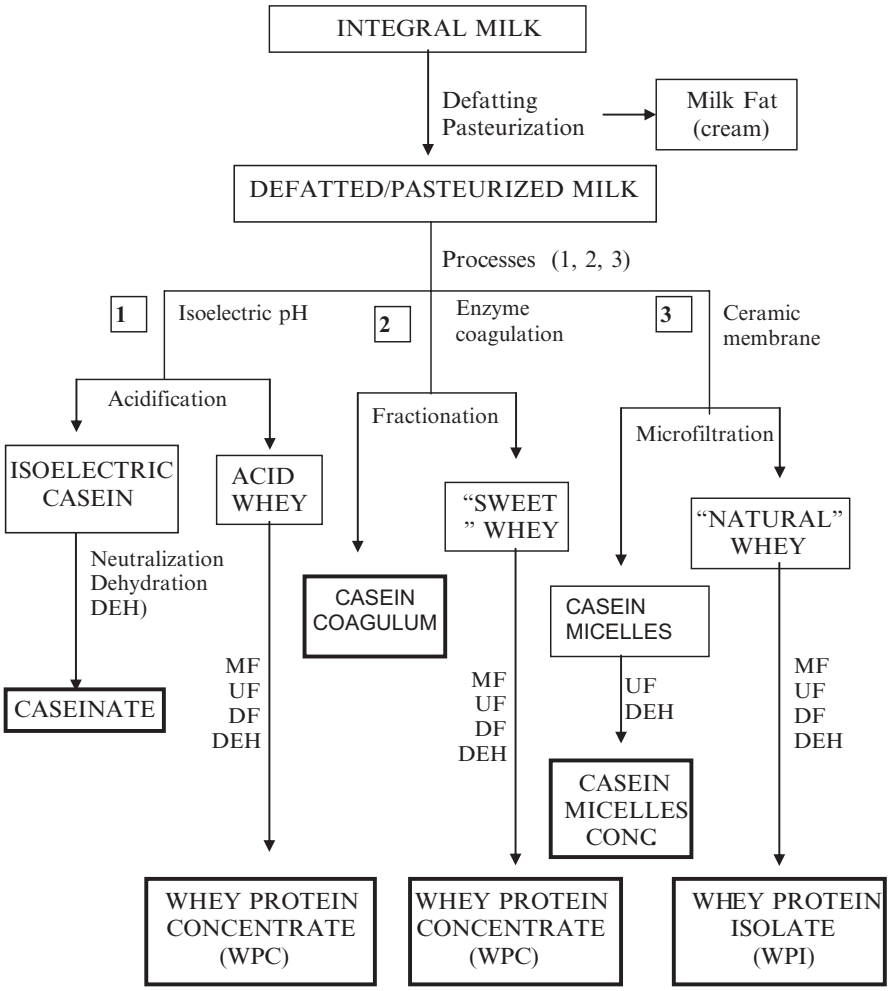

Fig. 8.1 Industrial processes for production of caseins and whey proteins concentrates: MF = microfiltration; UF = ultrafiltration; DF = diafiltration; DEH = dehydration. Adapted from Zinsly et al. (2001), Roman and Sgarbieri (2005b)

dissociate from the micelles at low temperatures ($T < 5$ °C), and separate from the casein micelles (CM) retentate as a permeate that can be transformed into a purified β-casein preparation (approximately 95 % purity) after UF and dehydration. On the other hand, the retentate, which contains the αs- and k-caseins, can be resolved into k-caseins and αs-caseins by adequate combination of MF, UF, and chromatographic techniques.

This fractionation and purification is of utmost importance because k- and β-caseins predominate in human milk and can be used in developing humanized infant formulas or recombination of these casein fractions in adequate proportions

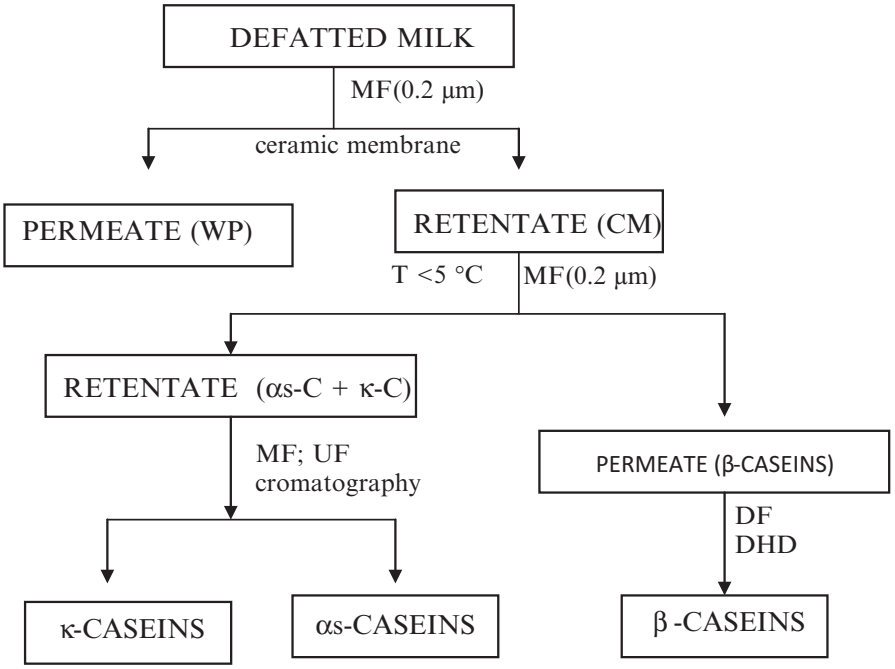

Fig. 8.2 Fractionation and partial purification of main caseins. Modification from Rosenberg (1995)

to develop novel products with improved properties. Also, the purified fractions can be used advantageously to isolate bioactive peptides after proteolysis by adequate enzyme system.

A possible process to obtain purified whey protein is shown in Fig. 8.3, which presents modified fluxograms based on (Rosenberg 1995; Maubois and Olivier 1992). The process starts with natural whey which is treated with appropriate concentration of Ca^{2+}, at 55 °C, 8 min, then submitted to MF (0.2 μm membrane) to clarify the whey, eliminating as a retentate the remaining P-lipides. The clarified whey can be used directly for purifying LF and LP by cation exchange chromatography (CEC), or it can be concentrated (MF/DF) to a clarified whey concentrate.

The whey clarified concentrate is then submitted to another cycle of MF (0.2 μm, pH 4.2, $T = 55$ °C) to separate β-LG as permeate, which after concentration (DF) and dehydration permits obtaining purified β-LG.

The retentate, containing (α-LA, BSA plus Igs) can be resolved into their components, by judicious application of UF and chromatographic techniques, at pH near neutrality.

Two minor proteins present in low concentration in bovine whey are lactoferrin (LF) and lactoperoxidase (LP). Isolation of these two proteins in purified form has not been an easy task because they have practically the same MM (~78 kDa) and isoelectric point (pI) in alkaline pH: LF (pH 8.0) and LP (pH 9.6). Due to these characteristics, they cannot easily be separated by membrane filtration or chromatography.

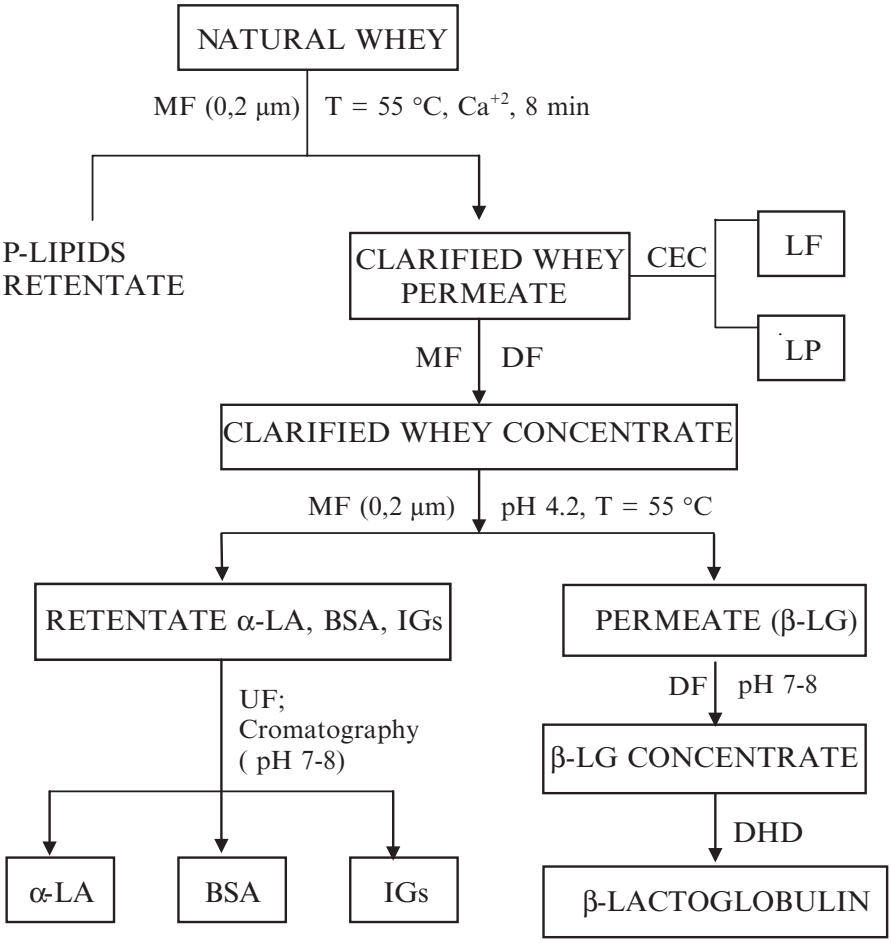

Fig. 8.3 Fractionation and purification diagram of the main bovine whey protein. Modified from Rosenberg (1995), Maubois and Olivier (1992). *CEC* cation exchange chromatography, *LF* lactoferrin, *LP* lactoperoxidase, *α-LA* lactalbumin, *BSA* bovine serum albumin, *IGs* immunoglobulins

A commercial process to obtain LF which uses a combination of chromatographic and membrane ultrafiltration procedures to isolate and purify LF at quantities around 40 t/year was reported (Tamura 2004). The process starts with bovine whey which is introduced in a CM-Sephadex column, equilibrated with 0.05 M Tris/HCl buffer to adsorb LF and LP, among other whey proteins. The column is then washed with water to elute nonadsorbed materials, and then treated with a citrate buffer containing 0.3 M NaCl, to elute proteins other than LF. The adsorbed LF is then eluted from the column by treatment with a citrate buffer containing 1 M NaCl.

The eluted LF is then submitted to UF for demineralization, following the cycles of UF/DF for concentration. Finally, the LF concentrate is dehydrated by freeze-drying,

resulting in an LF preparation with 96% purity. According to Tamura (2004), this commercial production has been under operation since 1989.

A more sophisticated chromatographic system to purify, the same operation in both LF and LP has been recently proposed by Anderson and Mattiasson (2006). The system uses the Simulating Moving Bed (SMB), applied to cation exchange chromatography. The pilot unity used (C920 Calgon Carbon Corporation, Pittsburg, USA) works with 20 columns, which were packed with "Streamline SP" resin. The key unity component is a central valve coordinating the flux in and out of the columns.

This chromatographic technique involves: (a) conditioning of the resin with 0.02 M sodium phosphate buffer, pH 6.5, containing 0.12 M NaCl, to adjust the columns pH; (b) loading the columns with the WPC solution (42% protein); (c) washing the columns with the 0.02 M sodium phosphate buffer, pH 6.5, containing 0.12 M NaCl; (d) elution of LP with 0.02 M sodium phosphate buffer, pH 6.5, containing 0.42 M NaCl; and (e) elution of LF with the 0.02 M sodium phosphate buffer, pH 6.5, containing 1.25 M NaCl.

At the end of the operation cycles, the columns are regenerated by treatment with 1 M NaOH solution and re-equilibrated (conditioned) with the sodium phosphate buffer, pH 6.5, 0.12 M NaCl, prior to initiating a new cycle.

Once scaled up to commercial operation, this method could probably be time-saving and economically feasible for obtaining both LP and LF in the same equipment with a high degree of purity.

8.8 Need for Further Investigation

In spite of the enormous advancements achieved in bovine milk science and technology, recent publications have described the complexity of milk as a functional food and raw material for industries. Genomic and metabolic studies (German et al. 2006) are underway in attempting to explain the mechanisms of metabolic production and functions of bovine milk components. Dietary (feeding) management of milking cows has been demonstrated to be a relatively easy and efficient way of modifying milk production and composition (Lanna 2000).

Genetic markers and genetic modifications have been studied aiming at modifying the proportions of important milk proteins, such as k-caseins, β-caseins, and lactoferrin (Sabikhi 2007). Important advances in membrane technologies and other techniques have permitted the separation of milk components (Sgarbieri 2012), offering the opportunity to specifically mix separate components whose action complement each other to achieve a net nutritional or technological result that is greater than that provided by each separate component.

The advantage of reaching such higher order functions represents future opportunities for nutritional scientists and technologists to develop novel foods and ingredients with improved nutritional and technological functionalities.

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Chapter 9
Chocolate and Cocoa Products as a Source of Health and Wellness

Suzana Caetano da Silva Lannes

9.1 Introduction

The early history of cocoa and chocolate began thousands of years before the Spanish conquest of Mexico and Central America. The origin of processed chocolate seems to have begun in southern Mexico, around 4000 years ago (Coe and Coe 1996). The native population of Central America originally consumed chocolate in the form of a spicy and foamy drink. When Spanish explorers came to Mexico, they found cocoa plantations and discovered a spicy, bitter red drink, called Xocoatl. Aztecs used the drink during tribal ceremonies worshipping their gods.

The basic Aztec method of preparing chocolate (which, like cocoa, was called *cacahuatl* in early sources) was similar to that prevalent among the Maya, although the Aztecs consumed the beverage in a cool drink, while the Mayans preferred to drink it hot (Coe and Coe 1996). Chocolate preparation was probably introduced in the Americas and Europe from Spain during the sixteenth century. Chocolate was prepared by roasting the seed in a crock before grinding it with stones. Then, hot water, spices, and honey could be added, with shaking to introduce a foamy texture to the drink. All aspects of the process involved in modern chocolate manufacture were already in place by the turn of the nineteenth or twentieth century (Coe and Coe 1996; Beckett 2009). In 1850, *The Lancet* announced the creation of a health commission for the analysis of foods, which analyzed samples of chocolate and found starch grains from potatoes or from two tropical plants, *Canna giganta* and arrowroot, as the results from French analyses. This investigation inspired the British Food and Drug Act of 1860, and the Adulteration of Food Act of 1872.

Cocoa originated from the Amazon River resulting in Criollo, which reached Central America and southern Mexico, cultivated by the Aztecs and Mayans, with

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large white or pale violet seeds, with rich and complex aroma and flavor, deep gentle taste, and requiring only short fermentation and roasting to get the flavors. Forastero, which spread below the Amazon basin and toward the Guianas, with violet or dark seeds, sometimes almost black, and requiring a long period of fermentation (Coe and Coe 1996; Afoakwa 2010; Beckett 2000).

Forastero can be mixed with Criollo cocoa, which, because of its bitter and pure taste, improves palatability. The Trinitario variety, which originated in Trinidad, is a hybrid of Criollo and Forastero. These seeds require an average fermentation time to release peak flavors, but a short fermentation can create other acceptable flavors, and thus the roasting time can also be either short or medium. The flavor is almost complex in Forastero as that of the Criollo. *Nacional* (Arriba) has distinctive floral and spicy flavor notes. It is cultivated mainly in western South America, especially in Ecuador (Coe and Coe 1996; Afoakwa 2010; Beckett 2000).

There is no agreed upon definition of *fine* cocoa type, or cocoa flavor, except that it is necessary to pay a much higher price, and it has distinct characteristics of aroma and color. The manufacture of bitter chocolate uses *fine* cocoa type, referring to flavor and color. The varieties of cocoa include Criollo, Trinitario, Arriba, or *Nacional*. However, none of the cocoas of these varieties are classified as *fine* or *aromatic*. If the seeds have no characteristic flavor due to poor fermentation or drying, they are marketed as ordinary cocoa (Afoakwa 2010; Beckett 2009).

9.2 Cocoa and Chocolate Processing

In aspects of cocoa quality, food safety is of great importance, and it is well defined in the legislation of each country. The agro-industrial processing of cocoa is microbial and must ensure quality for human consumption. One risk may be salmonella, which is eliminated in the process of roasting. The guarantee of good storage, avoiding growth of fungi production of mycotoxin, is key to food safety standards.

Steps for processing seeds occur in cocoa farms. The dried seeds are treated in cocoa processing industries (Fig. 9.1). Specific lots of beans and blending after cleaning, breaking, and winnowing result in cocoa liquors. Alkalizing, or Dutching, is the treatment of cocoa nibs with an alkali solution, modifying the color and flavor of the cocoa powder or liquor. Subsequently, the roasting process has the aim of reducing water content and further developing flavor. Cocoa liquor can either be stored in tanks to await pressing, or it can be shipped and used by chocolate manufacturers for additional processing into chocolate. In the pressing stage, cocoa liquor is shared through pressing in two fractions, i.e., cocoa butter and cocoa cake. Cocoa butter, however, suffers from a deodorization process (Beckett 2009; Afoakwa 2010).

Chocolate is a mixture of cocoa liquor (or cocoa mass), cocoa butter, sugar and emulsifier (PGPR-polyglycerol polyricinoleate, soy lecithin), and milk in various forms (for milk chocolate). Chocolate can be mainly categorized as dark, milk, and white. The main constituents of these chocolates are carbohydrates (63.5 %, 56.9 %, 58.3 %), lipids (28.0 %, 30.7 %, 30.9 %) and proteins (5.0 %, 7.7 %, 8.0 %) found in dark, milk, and white chocolates, respectively (Afoakwa et al. 2007).

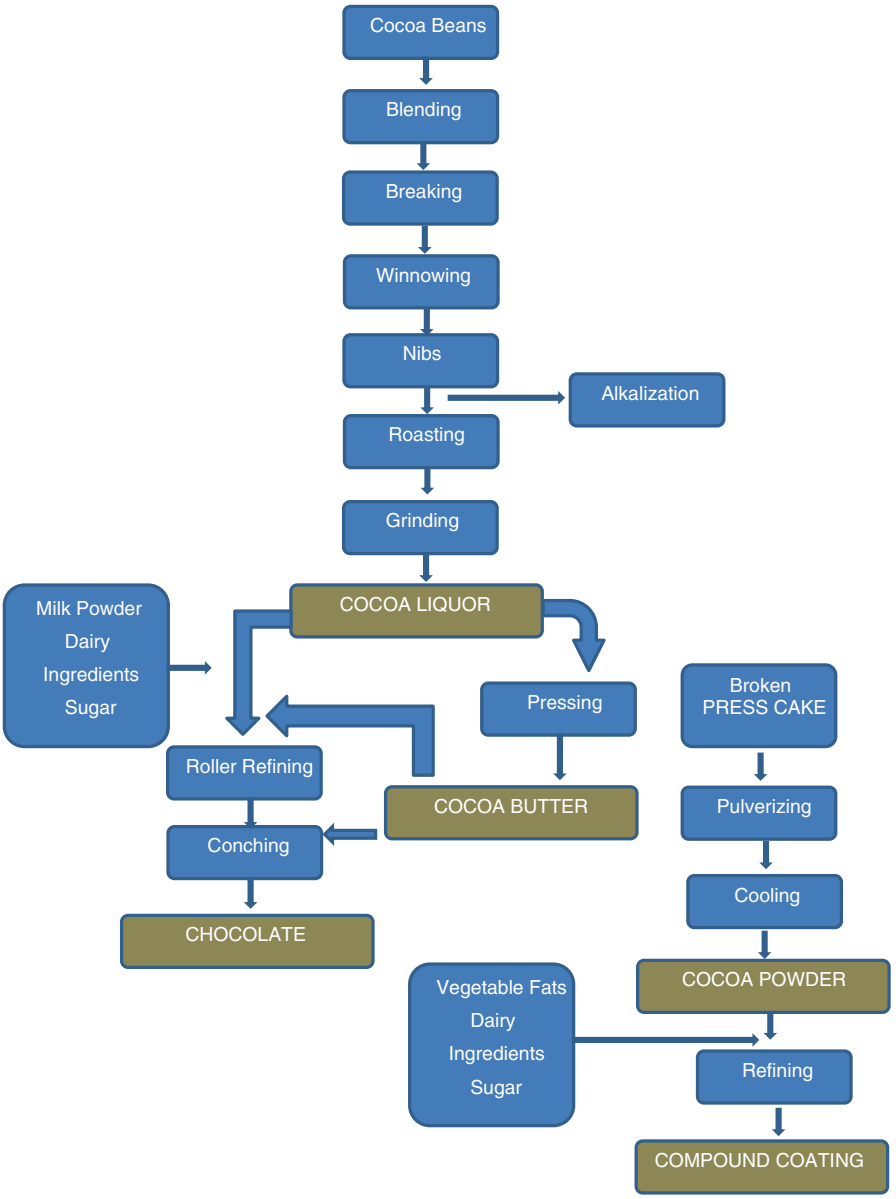

Fig. 9.1 Cocoa beans processing and chocolate production (*Source*: ADM)

Chocolate processing can be summarized as follows:

- Refining: This step is necessary to reduce and homogenize the particle size of chocolate solids ingredients. Otherwise, a feeling of grittiness will be felt in the mouth (Beckett 2000).

- Conching: This mixing step involves the volatilization of acids, short chain fatty acids, aldehydes, and water, and also develops the smooth texture characteristic of chocolate. Volatilization of the compounds develops the chocolate flavor. Solid particles such as sugar, cocoa, and milk powder are coated with fat, dissociated by friction, and become rounded. Coating the particles with fat promotes the smooth texture desired for chocolate. Time/temperature conditions during conching can reasonably vary for each type of chocolate being processed. For milk chocolate, for example, 10 ± 16 h/49 ± 52 °C can be used; for dark chocolate, higher temperatures, e.g. 70 °C up to 82 °C may be used (Prawira and Barringer 2009; Wollgast and Anklam 2000).
- Tempering: A process in which the fat phase of chocolate is conditioned by heat and mechanical means to acquire desired stable polymorphic form, in a good number, size, and at the appropriate time. Well-tempered chocolate is formed by the maximum number of small crystals in crystalline form, well distributed in the mass. Tempering involves precrystallization of a small proportion of triglycerides, with crystals forming nuclei (1–3 % total) for the remaining lipid to set in the correct form. This process can be broken into four steps: melting to completion (at 50 °C), cooling to the point of crystallization (at 32 °C), crystallization (at 27 °C) and conversion of any unstable crystals (at 29–31 °C) (Afoakwa 2010).

9.3 Some Health Aspects of Cocoa and Chocolate

Functional foods can be mentioned as foods or dietary components that may provide a health benefit beyond basic nutrition. The development of functional products presents several critical points, among them a demonstration of their bioefficacy. The safety of chocolate consumption is another critical point, since when consumed in high quantities it may have some toxicity, among other concerns. It is important to tailor the product to the food matrix, as well as diet and to suit the needs of each person. Food formulators face challenges in developing foods and ingredients to meet consumer demands. Thus, it is necessary to ensure that the finished products taste good and have other acceptable properties and that the ingredients used in these products function well under processing conditions and have appropriate shelf stability.

Bioactive compounds have health promoting activities by being able to exemplify antioxidant capacity: vitamins A, C, and E; carotenoids; and phenolic compounds (flavonoids, phenolic acids, tannins). Enhancing bioavailability by mixing phytochemicals is the newest formulation direction.

A fully grown cocoa bean contains more than 200 substances that promote good health. One group of these beneficial substances is cocoa flavanols. Scientific research has shown that these powerful antioxidants may have a positive effect on both body and mind (Belščak et al. 2009; Castell et al. 2012; Lipp 2012).

Description of the microbiology of cocoa processing and biochemical compounds of cocoa and chocolate is important, as is the composition of chocolate sources and related plant components. Chocolate metabolism and its activities in culture and a great approach to bioavailability are described by Elwers et al. 2009 and Lipp 2012, who point to the significant effects of these bioactives on the human gut microbiota; and some points to take in consideration to understand cocoa actions are discussed by Grassi et al. (2012), Lee et al. (2008), McShea et al. (2008), and Visioli et al. (2009).

Chocolate contains a number of bioactive molecules that can be of value to many aspects of health. Nevertheless, it depends on the cocoa sources, chocolate manufacture, and the physical and biochemical aspects of the chocolate components. Also, clinical studies need to be placed in the proper perspective to serve correctly for comparisons between studies (Cruz et al. 2015; Elwers et al. 2009; Ioannone et al. 2015; Jolic et al. 2011; McShea et al. 2008; Wollgast and Anklam 2000).

Chocolate can be categorized as a functional food that recognizes and generates interesting physiological effects, likely to promote or maintain health, and therefore chocolate can be considered as "medi-food," which exalts its nutritional functions and its therapeutic abilities (Lipp 2012).

It is a common misconception that every dark chocolate contains high levels of polyphenols. However, there is no relationship between the darkness of chocolate and polyphenol content, and not every dark chocolate contains high polyphenols. Healthy chocolate is polyphenol-rich chocolate, and there is nothing about the color of the chocolate that will inform consumers about flavonol content (Selmi et al. 2008; Watson et al. 2012).

The consumption of cocoa and chocolate contributes to human nutrition, supplying lipids, sugars, minerals (potassium, magnesium, copper, and iron) and antioxidants, especially polyphenols, including flavonoids such as catechin, epicatechin, and procyanidins. As a chemical group, polyphenols range from simple structures to polymeric phenolic molecules. Some basic structures of polyphenols occur in a variety of fruits, vegetables, nuts, seeds, and in some manufactured products. Polyphenols have become an intense focus of research interest because of their health benefits (Afoakwa et al. 2007; Dreosti 2000; Wollgast and Anklam 2000). Teas and red wine are also well known to possess this characteristic.

The antioxidant activity of chocolate seems superior even when compared with traditionally healthy fruits. For example, compared with *açaí* (*Euterpe oleracea*), which is currently recognized as a food very rich in natural antioxidants, dark chocolate and processed cocoa powder have approximately 20 and 75 % of the antioxidant activity of cocoa per gram, based on measurements of oxygen radicals absorbance capacity (ORAC) tests (McShea et al. 2008). A comparison of the antioxidant activity of different foods mentioned ORAC values of antioxidant foods per 100 g: unprocessed cocoa powder (26,000), *açaí* berry (18,500), dark chocolate (13,120), prunes (5770), raisins (2830), blueberries (2400), blackberries (2036), strawberries (1540), raw spinach (1260), and red grapes (739) (USDA 2013).

Bioactive content, physical and sensory properties of milk, semisweet and dark chocolates enriched with concentrated (1 and 3 %), and freeze-dried (1 %) red rasp-

berry leaf (Rubus idaeus L.) extract were examined by Belscak-Cvitanovic et al. (2012). Polyphenolic content (total phenols, flavan-3-ols, and proanthocyanidins), as well as antioxidant capacity of enriched chocolate was increased by the incorporation of 3 % concentrated raspberry leaf extract, while 1 % concentrated or freeze-dried extract showed an insignificant trend in comparison to plain chocolate.

White chocolates differ from milk and dark chocolates due to the absence of cocoa liquor, and therefore no antioxidants content, which results in decreased product shelf life. Dark chocolates are manufactured with higher amounts of cocoa liquor, which results in a greater amount of antioxidants than in other products made with cocoa (Beckett 2009; Afoakwa et al. 2007).

The polyphenols content (mg phenols/serving size) in some chocolate products can consist of: dark chocolate (40 g) = 951; milk chocolate (40 g) = 394; chocolate milk (240 mL) = 34; homemade chocolate milk (240 mL) = 81; hot cocoa mixes (180 mL) = 45; homemade hot cocoa (180 mL) = 211 (Visioli et al. 2009).

Aspects such as cocoa origin, microbiology of cocoa fermentation, cocoa constituents, aroma compounds, and cocoa and chocolate processing can contribute to some clinical approaches. Cocoa has a cardioprotective effect (improving endothelial function and decreasing platelet aggregation and blood pressure), regulating mood and brain disorders, anti-inflammatory effects (flavonoids), and reducing certain states of autoimmunity and hypersensitivity, antitumoral effects (more conclusive studies are needed) (Castell et al. 2012). It has been stated that chocolate could be recommended in the treatment of diabetes mellitus, cancer prevention, neurocognitive functioning problems, hypertension, coronary heart disease, vascular tone cognitive improvement, and beneficial brain effects (Watson et al. 2012).

Control of food cravings has been made possible by certain activities of cocoa compounds, and some suggestions about this have been offered, in that the chemical family of unsaturated *N*-acylethanolamines might activate cannabinoid receptors in humans or increase endocannabinoid levels, resulting in heightened sensitivity and euphoria. In other words, cocoa compounds have the ability to imitate the nervous system effects of cannabis when combined with caffeine, which is also present in cocoa and chocolate. However, its possible activity in controlling food cravings may be more related to the sugar contained in its composition than with the characteristic properties of cocoa to activate cannabinoid receptors, or increase the level of anandamide which is in the chemical family of *N*-acylethanolamines (Di Tomaso et al. 1996).

The main healthy chemicals in chocolate are epicatechin and its esters. These chemicals could be responsible in part for the beneficial vascular effects that are observed after the consumption of flavanol-rich chocolate (Al-Dujaili et al. 2012).

Polyphenols are products of the secondary metabolism of plants derived from glucose, which are also responsible for the astringent taste of cocoa. Comprised of about 10–20 % (dry weight) of cocoa cotyledons, cocoa polyphenols are stored in the pigment cells of cotyledons. They consist of anthocyanins, shown by the purple color of the seeds, as well as colorless compounds such as catechin, epicatechin, and their dimers, trimers, tetramers, and higher oligomers called procyanidins. Epicatechin is a more bioavailable antioxidant in cocoa, but it tends to polymerize under the influence of oxygen (McShea et al. 2008; Wollgast and Anklam 2000; Beckett 2009).

The group of methylxanthines contributes to the bitter taste of chocolate, along with compounds formed during roasting; serves as a quality parameter, such as indicating the presence of cocoa (cocoa is the only American plant that has theobromine as a principal alkaloid); has effects on pharmacological nervous, cardiovascular, gastrointestinal, respiratory, and renal systems; and, in high doses methylxanthines can cause excitement, headache, and insomnia (Castell et al. 2012).

Polyphenols and alkaloids, approximately 14–20 % of the seed weight, are central to the aroma characteristic of chocolate. Three groups of polyphenols can be differentiated: catechin or flavan-3-ols (~37 %), anthocyanins (~4 %) and proaanthocyanidins (~58 %). The (-)-epicatechin (primary catechin) is greater than 35 % of total polyphenols and 34.65–43.27 mg/g of defatted Criollo and Forastero cacao seed. Less abundant is the (+)-catechin. The inclination to form complex compounds with proteins, polysaccharides, and alkaloids enhances the complexity of cocoa products (Belščak et al. 2009).

Polyphenols present in cocoa and cocoa products can be classified into three main groups: flavonoids (37 %), anthocyanins (4 %) and proanthocyanidins (58 %). Cocoa beans before the fermentation process contain 120–180 g/kg of phenolic compounds with epicatechins, which is quantitatively the major component. Catechins, traces of gallocatechins, epigallocatechin, epicatechin-3-gallate, and many procyanidins, as well as small amounts of quercetin, quercetin glycosides, naringenin, luteolin, apegenina, clovamida, and phenolic acids such as caffeic, ferulic, gallic acid, and p-coumaric have also been found in cocoa beans. The tendency of these compounds to form complexes with proteins, polysaccharides, and alkaloids increases the complexity of cocoa. It should be noted that cocoa is not only rich in polyphenols, but also in methylxanthines corresponding to 3.2 % of the composition of defatted and without sugars chocolate. The main methylxanthine present in cocoa is theobromine (3.7 % in a defatted sample) and caffeine (0.2 %) (Belščak et al. 2009; Genovese and Lannes 2009).

Elwers et al. (2009) noted differences between some cocoa types (Upper Amazon Forastero, Lower Amazon Forastero, Nacional, Criollo—varying fermentation stages—and Trinitario from different origins). They mentioned the influence of genetics and site-specific features on phenolics. Criollo samples were found with no content of anthocyanins, and with greater amounts of caffeic acid aspartate than for other samples. No genetically determined differences in the amounts of total polyphenols and (-)-epicatechin were denoted. Soil fertilization was indicated as leading to cocoa seed with significantly smaller amounts of total polyphenols, flavan-3-ols and anthocyanins, and larger quantities of caffeic acid aspartate than those from unfertilized locations. Catechins diminution was mentioned during fermentation and drying of Criollo, which may be responsible for the mild flavor of Criollo chocolates. Caffeic acid aspartate turned out to be highly resistant to degradation.

Fat-soluble polyphenols in Forastero cocoa (dried fat-free fresh) form are 15–20 %, which falls to approximately 5 % after fermentation, denoting poor fermentation. Criollo cocoa beans have approximately 2/3 of the content of polyphenols of Forastero seeds, and anthocyanins have not been found. Sugar and amino

acids in reaction with polyphenols contribute to flavor and color of cocoa beans, and alkaloids contribute to bitterness (Afoakwa 2010).

During fermentation, protein breakdown occurs partly by hydrolysis to peptides and amino acids and partly by conversion to insoluble forms by the actions of polyphenols. Polyphenol oxidase promotes oxidative browning to give the characteristic chocolate brown color of well-fermented Forastero beans (Afoakwa 2010; Beckett 2009).

Epidemiological and clinical studies have pointed out that supplementing a diet rich in flavonoids contained in cocoa and chocolate may exert suppressive effects on LDL oxidation and development of atherosclerosis associated with implications due to its interference in many pathophysiological mechanisms. Some of the benefits identified include antioxidant properties, lowering of blood pressure via the induction of nitric oxide (NO)- dependent on vasodilatation, enhancement of endothelial function, increased insulin sensitivity, decrease of activation and platelet function, as well as modulation of immune function and inflammation (Ding et al. 2006; Grassi et al. 2010, 2012; Djoussé et al. 2011; Castell et al. 2012).

Cocoa polyphenols play an important role in cardiovascular protection and the function of the autonomic nervous system, with a lower incidence of atherosclerotic lesions in the aorta through the consumption of cocoa (polyphenols), and preservation of parasympathetic nerves (Akita et al. 2008).

The benefits and effects of polyphenols present in chocolate have been mentioned as anticarcinogenic, anti-atherogenic, anti-ulcer, antithrombotic, anti-inflammatory, antiallergic, immune modulators, antimicrobials, vasodilators, and analgesic. These effects have been attributed to different properties of polyphenols, including antioxidant benefits, chelation of divalent cations, and inhibition of the activity of enzymes. Such antioxidant effects are characterized by the ability of polyphenolics in stabilizing free radicals through the resonance of electrons in the structure (Wollgast and Anklam 2000; Watson et al. 2012).

Polyphenol-rich chocolate may potentially be used as a nutraceutical medication to help treat diabetes, stroke, and vascular dementia; thus, future studies should provide information on polyphenol content and flavanol plasma levels achieved. A number of mechanisms have been suggested to explain chocolate's positive effects for diabetes mellitus, not only on insulin sensitivity and vascular endothelial function, but also its metabolic (fat and carbohydrate), antihypertensive, antithrombotic, and anti-inflammatory effects (Al-Dujaili et al. 2012).

Chocolate has been reported to affect the release of serotonin and phenylethylamine when consumed, producing aphrodisiac effects and mood elevation, which may be of great importance to understand the critical factors involved in this process and the potential beneficial effects for consumers. Chocolate is rich in cocoa polyphenols, especially catechins (flavan-3-ols) and procyanidins, which are stored in the cotyledon of the pigment cells and cocoa leaves, and, depending on the content of anthocyanins, pigmentation of the seeds ranges from red to white (Afoakwa 2010).

Because chocolate consumption has sometimes been recommended for reducing the risk of cardiovascular disease due to high levels of stearic acid and flavonoids, an examination of more than 100 articles studying the subject suggested that cocoa and chocolate may exert beneficial effects on cardiovascular risks due to the lowering of blood pressure, platelet and anti-inflammatory functions, increased HDL, and decreased oxidation of LDL. Also, a broad screening suggests that stearic acid is actually cholesterol-neutral. However, epidemiological studies of serum and dietary stearic acid are inconclusive due to methodological limitations. However, most studies suggest that flavonoid content of chocolate can reduce the risk of mortality from a cardiovascular event. Meta-analysis indicates that intake of flavonoids may reduce mortality risk of coronary heart disease. In conclusion, several laboratory experiments and randomized clinical studies suggest that stearic acid may be neutral, due to the protection of flavonoids against mortality from coronary heart disease. The priority now is to conduct larger randomized studies to definitively investigate the impact of chocolate consumption in the long term by looking specifically at cardiovascular events (Ding et al. 2006).

It has been accepted that some types of cancer, cardiovascular and cerebrovascular diseases, as well as diabetes and rheumatic diseases, are caused or accelerated by oxidative stress, and that eating certain foods that contain antioxidants can slow or prevent the appearance of these pathologies. Chocolate flavonoids have also gained notoriety primarily for their benefits related to heart health. Several studies have been conducted in this direction, to try to prove the benefits of daily consumption of dark chocolate, which is richer in flavonoids. In addition to its beneficial actions for the heart, the flavonoids present in cacao bean have also been attributed to anticarcinogenic, anti-inflammatory, antibacterial, antioxidant, and antiallergic properties, as well as being able to modulate or reduce platelet activation, and helping to maintain the cardiovascular system (Ohno et al. 2009; Selmi et al. 2008; Sies 2010).

Regular intake of dark chocolate can cause beneficial effects on cardiovascular disease by lowering blood pressure, insulin resistance, triacilglycerols, vascular reactivity, endothelial dysfunction, oxidative stress, markers of inflammation, and antiplatelet activity. Each of these physiological events can be observed in preeclampsia, providing strong support for future research on the effects of the ingestion dark chocolate in early gestation for reduced risk of preeclampsia (Saftlas et al. 2010).

Chocolate can also possibly cause loss of appetite. Eating dark chocolate before meals corresponds to consumption of 15 % fewer calories during meals than eating milk chocolate. Eating dark chocolate also helps reduce the desire to eat sweet, salty, or fatty foods (Berry 2009).

9.4 Chocolate Processing and Bioactive Compounds Preservation

Based on increasing scientific evidence, which points to the benefits of a diet rich in polyphenols, it is of great interest to maintain the level of cocoa polyphenols in chocolate. However, some polyphenols present in cocoa beans are actually destroyed

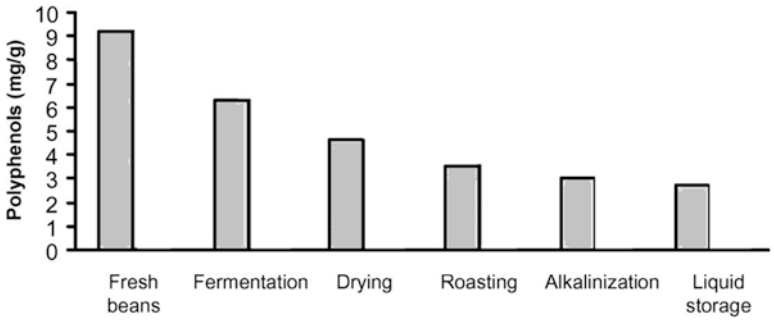

Fig. 9.2 Chocolate making process—polyphenols lost during process (based on Visioli et al. 2009)

during processing (Visioli et al. 2009). Different studies show that all of the steps involved in the processing of cocoa and chocolate decrease polyphenol content (Beckett 2000, 2009; Ioannone et al. 2015; McShea et al. 2008; Dreosti 2000; Wollgast and Anklam 2000; Andres-Lacueva et al. 2008; Visioli et al. 2009).

Reduction of cocoa polyphenol content can occur during the following events (Fig. 9.2):

- Fermentation: Polyphenols decrease is due to the action of enzymes present in cocoa fruit and exposure of seeds to oxygen;
- Drying: Reduction of enzymatic action (reduced water activity) can occur; seeds are exposed to sun, while still in contact with oxygen;
- Roasting: Loss of phenolic compounds occurs by heating seeds;
- Alkalization: (not a required step) Aimed at increasing solubility of cocoa products by neutralizing acetic acid.

Due to the dynamics of the conching step, which consists of continuous agitation of the chocolate mass at high temperatures (above 70 °C) during 8–24 h, in the presence of oxygen, polyphenol content can be reduced. Reduction in the amount of polyphenol in chocolate mass can be perceived during this step (Beckett 2000).

The Barry Callebaut Company developed a special process (ACTICOA) which maintains the health properties of cocoa in chocolate. A 7.6 g portion of dark chocolate contains a minimum amount of 168 mg flavanols, which is required to achieve antioxidant effects; 21 g of milk chocolate provides the minimum amount of 168 mg flavanols; while a minimum portion of 2.1 g of cocoa powder is sufficient to reach the minimum amount of 168 mg flavanols (Barry Callebaut 2012).

The alkalization process has been proven to affect polyphenols, methylxanthines, amino acids, and volatile compounds in commercial cocoa powder (Li et al. 2012). Total polyphenols content decreases with the increasing degree of alkalization. Under different degrees of alkalization, the content of some flavor compounds is significantly changed. The polyphenols in cocoa cause interaction with aroma precursors as well as aroma compounds. Amino acids analysis indicated that

polyphenol binding is preferred on hydrophobic amino acids. The addition of glucose in the alkalizing solution restrained the loss of acetic acid and fructose during alkalization.

Alkalization, especially a heavy degree of alkalization, greatly alters the components in cocoa powder, which leads to the unique flavor and color of the end products.

The loss of cocoa phenolics and their antioxidant activity varies with the degree of the technological process. The process of roasting and alkalizing seed has greater influence on individual and total phenolics as well as antioxidants. Research shows that the roasting process resulted in 14 % loss of total phenolic content, and alkalization 64 %; catechin increased during cocoa processing; caffeic acid and its derivatives showed greater stability in technological processes (Jolic et al. 2011).

Cocoa and chocolate products are rich in flavanol monomers, oligomers, and polymers (procyanidins). Epicatechin comprises the largest fraction of total chocolate flavonoids, with the remainder being catechin and procyanidins. It has been shown that dark chocolate can contain higher levels of total flavanols (93.5–651.1 mg of epicatechin equiv./100 g of product) than milk or white chocolate (40.6 mg and 0.0 mg of epicatechin equiv./100 g, respectively). The amount and integrity of procyanidins often suffer in the manufacturing of chocolate, mainly due to oxidation and alkalization. If the flavanol content of chocolate is accepted to be a key determinant of health benefits, then continued monitoring of flavanol levels in commercially available chocolate products may be essential for consumer assurance (Langer et al. 2011).

Sarriá et al. (2015) suggested that flavanols appeared to be responsible for the increase in HDL-cholesterol, while insoluble dietary fiber and theobromine in cocoa product rich in dietary fiber were associated with the hypoglycemic and anti-inflammatory effects observed.

9.5 Fats in Chocolate

Physical and chemical properties of fats are based on their composition. The properties of any specific fat molecule (triacilglycerol) depend on the particular fatty acids that constitute it. Long chains also yield more energy per molecule when metabolized. The physical properties of fat materials is relevant from scientific, technological, and medical perspectives, and they are governed by a complex confluence of the various structural levels in a fat material beginning with triacilglycerol molecules (Marangoni et al. 2012).

Dietary fats and closely related are quite all polyunsaturated fats. As dietary oils and fats, structured modified fats have received attention, as they are used extensively to ensure safety and wellness. Oils and fats represent a major dietary constituent. Modified fats can be obtained by rearrangement or structural alteration of the fatty acids or triacylglicerols in original oils, to generate a new oil or plastic fats. These

processes can be a mixture, fractionation, interesterification and/or hydrogenation, producing fats with desirable functional properties (Puligundla et al. 2012; Lannes and Ignácio 2013).

Functional lipids can be broadly described as lipids that provide specific health benefits when consumed and/or that impact a specific functionality of a food product. The desired functionality may be a physical or chemical property. Other terms that can be used to describe functional lipids include lipids with physiological function, nutritional lipids, and medical and pharmaceutical lipids. Natural fats and oils contain highly unsaturated fatty acids at the sn-2 position and saturated or monounsaturated fatty acids at the sn-1,3 positions of the triacylglycerol molecule. Positional distribution of fatty acids in triacylglycerol fats and oils is not ideal for human nutrition, and modification of the triacylglycerol structure has been applied to improve its physicochemical and nutritional values, leading to structured lipids (functional fats) that have been used in the formulation of nutritional foods.

Brazil nut oil can be used to represent this category of lipids, with the presence of palmitic, stearic, oleic (*n*-9), linoleic (*n*-6), lauric, myristic, erucic, and cis 4,7,10,13,16,19 docosahexaenoic fatty acids. It can be used in partial fat replacement, and it contains phytosterols and tocopherols. It can decrease levels of LDL cholesterol, reduce the risk of cardiovascular diseases and certain types of cancer. It can also be added in chocolate product formulations (Gonçalves 2011; Santos et al. 2013).

Addition of omega 3 fatty acid in chocolate products can help in cardioprotective effects, since it is a natural anti-inflammatory (Gonçalves 2011; Muggli 2006; Jacobsen et al. 2008).

In an effort to provide alternatives to trans and saturated fats, scientists have been modifying the physical properties of oils to be similar to those fats. In this way, many food products requiring a specific texture and rheology can be made with these novel oil-based materials without causing significant changes to final product quality. Some of these fats can be substituted for cocoa butter, for example. Emerging technologies have included catalytic transfer hydrogenation (selective hydrogenation with lower trans fatty acids formation); genetically modified oilseed varieties (generally with saturated fatty acids, i.e. palmitic and stearic acids, and oleic acid at varying levels); structured lipids (developed for specific purposes such as reducing caloric value and fats available for metabolism); and biotechnological modifications applying position-specific and acyl-group specific modifications by lipases (Puligundla et al. 2012; Lannes and Ignácio 2013).

This emerging technology of oil-based materials (oil gels, or *oleogels*) is the focus of many scientific investigations geared toward helping to decrease the incidence of obesity and cardiovascular disease. The major approach to form these materials is to incorporate specific molecules (polymers, amphiphiles, waxes) into the oil components that will alter the physical properties of the oil. Fluidity will decrease and the rheological properties will be similar to those of fats (Marangoni and Garti 2013).

9.6 Cocoa Butter

Chocolate confectionery comprises all confectionery products that contain one or more ingredients derived from cocoa beans. Chocolate confectionery fats are characterized by sufficient solid fat at 20 °C to make the product be snapped together with a steep melting profile between 30 and 35 °C such that the product melts cleanly and completely in the mouth to a liquid fat or oil (Timms 2003).

In the case of chocolate and some creams, certain characteristics like sensory, mouthfeel, texture, and flavor are a function of the nano- and meso-scale structure, structural failure, flow characteristics, and melting behavior of an underlying elastoplastic fat crystal network within foods (Marangoni et al. 2012).

Some factors can affect the composition of cocoa butter, such as the cocoa cultivation area, including soil characteristics, altitude, latitude, growing conditions, local temperature, rainfall, solar radiation, seed genetics, and maturation period. Variation in some of these factors can increase or decrease the class of fatty acids in cocoa seeds. As a result of these variations, cocoa butter may have different hardness, and consequently different melting points, which designate the classifications of cocoa butter.

Fermentation of cocoa seeds is very important, because fermentation develops in the seeds' essential oils that give cocoa its characteristic flavor, and reduces natural bitterness, releases theobromine, kills the radicle (small root), thus preventing seed germination, and removes some moisture from the seeds, thus increasing fat content.

Cocoa butter contains on average 20–30 % stearic acid (C 18:0). Stearic acid has a unique position within triacylglycerol, showing a neutral cholesterolemic effect, unlike other saturated fatty acids. Cocoa butter contains large amounts of saturated fatty acids in triacylglycerol in positions that are less easily absorbed and so do not help those at risk for atherosclerosis. Cocoa butter constains a significant amount of sterols, of which sitosterol (56.2–73.6 %) is very important. Also are found stigmasterol (18.8–33.9 %), campesterol (6.0–9.0 %), cholesterol (0.6–1.0 %) and tocopherols (natural oxidizers). These are beta and gamma tocopherols in their natural form, being extracted during the roasting of seeds. The amount of tocopherols is between 158 and 256 mg/g, increasing resistance to oxidation, rancidity, and not requiring the addition of antioxidants (Timms 2003; Beckett 2009; Afoakwa 2010).

Cocoa butter, which amounts to 25–36 % in finished chocolate, is responsible for the smooth texture, contractibility, flavor release, and gloss of the product. The fat phase is the only continuous phase in chocolate, thus being responsible for melting behavior and the dispersion of all other constituents. A careful tempering of the chocolate is necessary in order to obtain the fine crystals in the correct form (β-modification).

Fat crystals observed in milk chocolate are more uniform in size than those in dark or plain chocolate. Milk chocolate fat crystals are between 0.5 and 1 μm long. All of the fat crystals are between 3.0 and 5.0 nm thick, and all of them form V polymorphs, which is the acceptable form for chocolate.

Cupuassu [*Theobroma grandiflorum* (Willd ex Spreng) Schum, Sterculiaceae] fat can be used to replace cocoa butter and could be of interest to the confectionery industry, due to its properties and interactions with cocoa butter. Cupuassu fat shows polymorphic behavior like cocoa butter (β form) and thus needs to be tempered like cocoa butter. Cupuassu seeds comprise about 16 % of dry weight, and content of fat in the seed is about 60 %. In general, fat content is similar to cocoa butter (*T. cacao* L.). At some test temperatures, cocoa butter has a higher solid fat content than cupuassu fat. This suggests that cupuassu fat would be useful in chocolate manufacture as a softer filling fat compatible with cocoa butter. For fatty acid composition, palmitic acid in cupuassu fat is present in a much smaller amount (7.8 %) than in cocoa butter (26.1 %); stearic acid is about the same, while oleic acid is higher in cupuassu. The amount of arachidic acid (20:0) is high and can be notable in cupuassu fat. Triacilglycerol composition reflects the fatty acid composition, but gives more useful information. Although cupuassu has a higher SOS content than cocoa butter, its contents of POP and POS are much lower, reflecting its low level of palmitic acid. Total SOS-type triglycerides, i.e. POP + POS + SOS + SOA, is 57 % in cupuassu and 83 % in cocoa butter, thus explaining the melting profiles. Crystallization velocity of fats is linked with the time of process, and then the price of the final product, and it can be related to fat behavior and quality. Cocoa butter has lower minimum temperature (22.7 °C/24.6 °C) and higher minimum time (37 min/21 min), lower maximum temperature (26.6 °C/27.1 °C) and higher maximum time (63 min/35 min) than cupuassu (Lannes 2003).

9.7 Maillard Reaction

The most important factors in the formation of cocoa flavor are cocoa bean variety, fermentation and drying, alkalization and roasting. During fermentation, enzymatic reactions play a principal role in the formation of cocoa flavor precursors. Peptides and amino acids are generated by proteolytic enzymatic breakdown of proteins. Sugar from the pulp is split into glucose and fructose. The peptides and amino acids and reducing sugars are precursors for the formation of the volatile flavor components formed by Maillard reactions during the later stages of processing. Enzymes are also responsible for the conversion of flavonoids into tannins, leading to a decrease in astringency of the cocoa and changing the original purple color of the fresh beans into the typical brown color of cocoa. Most of the various compounds found in the flavor of cocoa are generated by Maillard reactions. Aldehydes and pyrazines in particular are considered to be important for the character of cocoa flavor (Beckett 2000, 2009; Afoakwa 2010). Antioxidant compounds in cocoa-derived products represented by Maillard reaction products are formed in food matrices containing reducing sugars and proteins as a consequence of high temperature processing, which can be formed during chocolate conching. These are mainly focussed on volatile compound evolution, and can take into account other Maillard reaction products such as melanoidin and their contribution to the antioxidant properties (Di Mattia et al. 2014).

9.8 Nutritive Chocolate Products

In 1914, during World War I, it was decided to end the expansion of chocolate industries. Export product restrictions were put into place. Chocolate tablets became a component of emergency food provided to American soldiers in service, but this product was not suitable for that use. In 1934, Captain Paul P. Logan developed a formula based on chocolate containing cocoa, sugar, milk powder, cocoa butter, vanillin, oats, and vitamin B1, which then was named "Feed D." The Hershey Company developed a new formulation for World War II, which contained other nutritious ingredients and about 600 cal. The ingredients were chocolate, sugar, dry milk, cacao fat, oat flour, and flavoring.

More recently, consumers wanting products with "no added sugar/sugar-free" or "no preservatives, additives or artificial flavoring" has led to innovation focused on these issues and brought a wealth of new chocolate choices to the market. Cocoa and chocolate have been characterized for years by their potential health benefits, but more recently have been scientifically studied (Al-Dujaili et al. 2012; Castell et al. 2012; Ding et al. 2006; Djoussé et al. 2011; Ioannone et al. 2015).

Cupuassu powder, prepared from *Theobroma grandiflorum* fermented seeds, is a very promising cocoa powder substitute. In Brazil, some formulations of *cupuassu chocolate* have already been produced. These formulations are composed of cupuassu fermented seed, cupuassu fat, and other normal chocolate ingredients. In order to know the potential health benefits of cupuassu powder, a comparison was made between cocoa and cupuassu powders and chocolates in relation to the content of total phenolic compounds, flavonoids, and DPPH scavenging capacity of methanolic extracts. The findings showed that although cupuassu powder may represent a better flavonoid source than the cocoa powder, the total polyphenol content and in vitro antiradical activity of cocoa is much higher (Genovese and Lannes 2009).

Many chocolate products have been developed to include more functional ingredients than polyphenols. Recipes for all technology and specifications, such as texture, water activity, no sugar added, organic, gluten-free, fair trade, bake and/or freeze stable products have been adapted. Murici (*Byrsonima verbascifolia*, Rich) is a Brazilian fruit found in abundance in the highlands of the southeast, in the Cerrado of Mato Grosso and Goiás and the coast of Brazil's north and northeast. Rich in calcium and vitamin C, murici can be consumed raw or in various forms such as in fillings for chocolates (Amaral and Lannes 2013).

Chocolate spread can be categorized as a chocolate formulation that does not solidify at ambient temperature, and may contain cocoa solids, vegetable fats and oils, milk (optional), sugar, flavor, nuts, and honey, and which presents a creamy consistency. Spreads are generally consumed at ambient temperature. Partially hydrogenated oils may be used to improve oxidative stability and required consistency. One example of this kind of product is Nutella® spread, which in its earliest form was created in the 1940s by Pietro Ferrero. At the time, cocoa was in short supply due to World War II rationing, so hazelnuts were used to replace some of the chocolate (Nutella USA 2013).

Fig. 9.3 Cupuassu fruit and its products (*Source*: Author)

Chocolate *fondue* is another spread variation; it can be defined as a formulation of chocolate consistency solid or semi-solid at room temperature, which is molten for consumption and can contain dark, milk, or white chocolate, as well as other ingredients that do not decharacterize the product. Formulations of chocolate fondue were developed by aggregating nutritional value by adding functional ingredients like oat extract, Brazil nut oil, and Omega 3 fatty acid (Gonçalves 2011).

Cocoa butter has been replaced in some chocolate formulations by β-glucan-rich hydrocolloid (C-trim30), providing health-enhancing benefits to consumers, and lending low-fat and low-calorie characteristics to chocolate (Lee et al. 2008) (Fig. 9.3).

One suggestion for nutritional chocolate formulation is to consider the following points: (1) The type of cocoa used influences the chocolate; (2) Products with high levels of polyphenols and procyanidins should contain at least 70 % cocoa; (3) A controlled alkalization process must be used; (4) A hot pressing system should not be used; (5) Care must be taken when roasting; (6) Formulation should contain at least 70 % cocoa; (7) Product should contain cocoa butter instead of other fats or vegetable oils; (8) Low glycemic sweeteners should be used (not refined sugar).

It is necessary to remember that the development of functional products presents several critical points, including a demonstration of their bioefficacy; safety of consumption is another critical point, and it is important to tailor the product to the food matrix, as well as diet and to suit the needs of each consumer.

9.9 Consumer and Innovation Trends in Chocolate

Chocolate, in the form of candy, gum and cookies, are considered as confectionery products. Confectionary products consists of a set of foods that are not only enjoyable, but also functional in their use. Despite difficult market conditions, there have been high levels of innovation in the chocolate category, which has evolved in line with consumers' complex and dynamic needs. This section outlines the most important consumer and product trends impacting the chocolate category globally. New product opportunities are spurred by consumer demand for more unique and novel product flavors. Nearly three-quarters of consumers claim to experiment by trying new foods all or most of the time. Chocolate products can capitalize on consumers' willingness to experiment through new product development. Many

consumers demonstrate a desire to return to a time when life was simpler and less demanding, and also when there was a perceived credibility of manufacturers (Datamonitor 2012; Innova 2015).

Some prospects and challenges for the future are frequently discussed by both researchers and consumers, including functional and nutritional aspects of foods. Consumers are stimulated to discuss with the food industry the best way to promote wellness and health. They are more knowledgeable than ever about their food products (or at least think they are). Where claims once flourished, transparency and credibility are now issues facing many food producers, both from consumers and governments. Social media provides the perfect platform for consumers to discuss their opinions. In global activities for ethical products, not only is health high on the list of consumers' desires, but the environment is also of great concern. Sustainability matters, and consumers demand that food producers incorporate this ideal. Therefore, terms such as sustainability, fair trade, recyclability, environment-friendly, and animal-friendly are commonly used (Cargill Webinar 2012).

Traditional, innovative and trendy fillings, suitable for cereal products (breakfast cereal, biscuits, cakes, pastries, chocolate) have been proposed, including fruity: strawberry, pear, raspberry, apricot; chocolate: dark chocolate plus coconut, milk chocolate; creamy: vanilla, caramel; innovative: lemon-curd, kiwi-cactus, grapefruit-hibiscus; savory: tomato, olive. Recipes must be adapted for all technologies and specifications, including texture, water activity, no sugar added, organic, gluten-free, fair trade, and bake and/or freeze stable.

The percentage of product launches tracked with a specific active health claim has stagnated since 2009. Natural ingredients carry functional claims in chocolate products, i.e., sugar-free chocolate covered with wolfberries, and organic chocolate with cranberry and mandarin. Categories including ingredients for aging well and wellness can be exemplified by chocolates with collagen, fruits, fiber and vitamins; chocolate with protein; low calorie chocolate; chocolate with added probiotics (e.g. *Lactobacillus helveticus*, *Bifidobacterium longum*); and chocolate with added phytochemicals (Innova Database 2012). Snacks for special occasions, good fats, good carbs, more protein, adding fruits, chewy and crunchy textures are also requested (Innova Database 2015).

9.10 Conclusions

Research should continue to explore the mechanisms of action and metabolism of flavonoids absorption/metabolite/breakdown products in the body, as well the effectiveness of cocoa flavonoids levels during the shelf life of products and during the production process. Also, the stability of flavonoids with respect to interactions with other components of the food matrix, points to new and promising applications, and can assist in increasing consumption of these phytonutrients by the general population. The development of products with added nutritional value by the use of natural products such as "superfruits" must continue, because the market has shown good acceptation of these foods.

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Chapter 10
Advances on the Production and Application of Peptides for Promoting Human Health and Food Security

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10.1 Introduction

Since ancient times, mankind has believed that nature equipped foods with health-promoting and therapeutic properties (Labuza 1994) as indicated by the use of natural products and edible substances for medicinal purposes (Ji et al. 2009; Winterhalter and Straubinger 2000). For instance, the oldest medical text known, written around 2600 BC, details the therapeutic and chemo-prophylactic use of about 1000 plants and plant products including *Papaver somniferum* (poppy juice), *Glycyrrhiza glabra* (licorice), and *Cedrus* spp., among others (Newman et al. 2000). While the concept of mining so-called "medicinal plants" for remedies to ailments has its origins in millennia past, the formal recognition of empirical research into the derivation of health-promoting benefits from "mainstream" food and food products can be traced to the end of the twentieth century, a development fostered by advances in food processing technologies (Hardy 2000).

Biologically active peptides have been defined as specific protein fragments possessing properties beneficial to the function or condition of humans and thus able to improve their health (Kitts and Weiler 2003). Bioactive peptides enzymatically derived from food protein sources have been credited with various health-promoting functions including antihypertensive, antioxidant, immunomodulatory, anti-inflammatory, hypocholesterolemic, hypolipidemic, anticancer, and opioid properties. As opinions on global health crises steadily lean toward the economic argument

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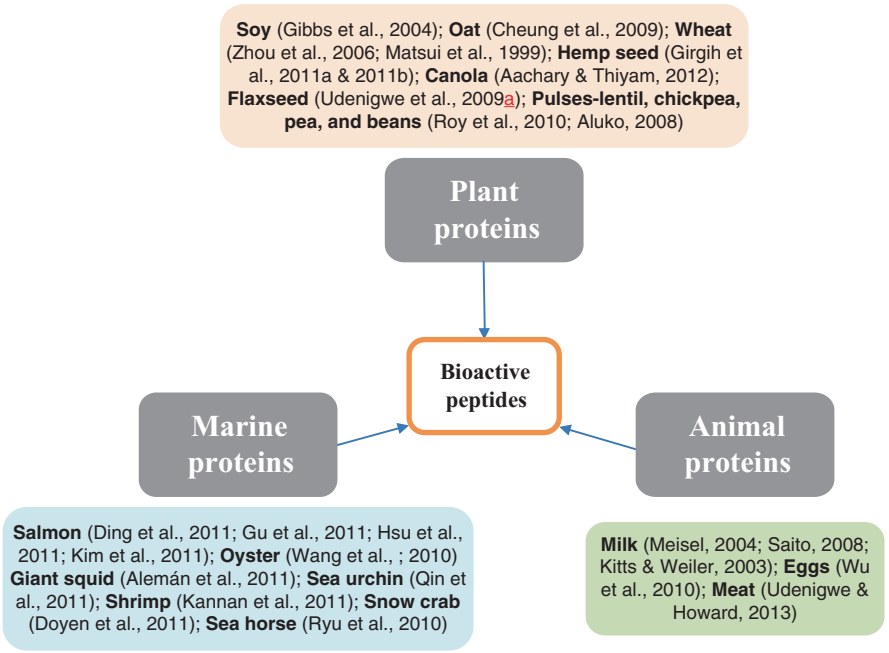

Fig. 10.1 Multiple food protein sources of bioactive peptides

for disease prevention rather than treatment, functional proteins and peptides are expected to play a crucial role in reducing the global epidemic of chronic diseases, which are estimated to account for millions of untimely deaths every year (Mine et al. 2010), especially with rising healthcare costs (Catlin et al. 2008; Congress of the United States 2007; Orszag and Ellis 2007), adverse reactions associated with chemically synthesized drugs and public perception of "natural" as safe (Slusarenko et al. 2008). As investigations into the health-promoting properties of various bioactive peptides and functional proteins continue, it is expected that fundamental problems encountered in peptide research such as their gastrointestinal stability, bioavailability, and safety will receive more attention.

10.2 Sources of Bioactive Peptides

Over the years, investigators have derived bioactive peptides from a variety of food protein sources including plants, marine and animal proteins as shown in Fig. 10.1 (Udenigwe and Aluko 2012). While a majority of research efforts have focused on animal proteins, especially milk proteins (Meisel 2004), attention has been increasingly directed towards bioactive peptides from marine sources in recent years. Recently, marine protein sources such as fish (Ding et al. 2011; Gu et al.

2011; Hsu et al. 2011; Kim et al. 2011), oyster (Wang et al. 2010), macroalgae, giant squid (Alemán et al. 2011), sea urchin (Qin et al. 2011), shrimp (Kannan et al. 2011), snow crab (Doyen et al. 2011), and sea horse (Ryu et al. 2010), have proven successful in the search for novel bioactive peptides. The decision to select a food protein as a potential bioactive peptide source is thought to rest on the presence of abundant underutilized proteins or protein-rich industry by-products, and/or the presence of proteins containing specific peptide sequences or amino acids of known pharmacological property (Udenigwe and Aluko 2012). The need to ensure that underutilized food proteins are exploited as sources of bioactive peptides is important, given the concerns that using food otherwise intended for wholly nutritional purposes could affect food availability and food security.

10.3 Production of Bioactive Peptides

The discovery of bioactive peptides has spawned profound interest in methods for isolating, analyzing, purifying, identifying, and quantifying these health-promoting molecules for use in physiological, biochemical, clinical, and pharmacological studies (Perez-Espitia et al. 2012). The methods for producing bioactive peptides of interest or isolating them from proteins are: (a) chemical synthesis; (b) enzymatic hydrolysis of proteins; and (c) peptide production by means of recombinant DNA technology.

10.3.1 Chemical Synthesis

The production of bioactive peptides by chemical synthesis involves peptide bond formation between various amino acid residues that yields specific protein fragments (Andreu and Rivas 2002). Since amino acids have various reactive groups, the chemical synthesis of peptides is carefully and tightly controlled to minimize side reactions that could reduce the efficiency of the reaction. To ensure that the synthesis of peptides proceeds with minimal side reactions, the N- and C-terminals of amino acids are protected using special chemical groups such as tert-butoxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), benzyl- and *tert*-butyl-based groups (Albericio et al. 2005; Spengler et al. 2006). The choice of specific protecting groups for the C-terminus depends on the chemical synthesis method—liquid or solid phase synthesis (Perez-Espitia et al. 2012). Chemical reagents are then employed to activate the carboxylic acid group of the relevant amino acid, thus facilitating its donation of the R-CO- acyl group to the amino group of the second amino acid residue for peptide bond formation. Deprotection follows the coupling of the two amino acids (Albericio et al. 2005), essentially involving the removal of specific protecting groups from the newly added amino acid to facilitate the linkage of the incoming amino acid to the growing peptide chain.

10.3.2 Enzymatic Production of Bioactive Peptides

Enzyme-derived bioactive peptides and protein hydrolysates have in most cases shown greater bioactivity than their parent proteins, thus suggesting that enzymatic hydrolysis of peptide bonds is crucial for releasing potent peptides from the primary structures of proteins (Udenigwe and Aluko 2012; Meisel 2004). Of the three methods for peptide production, enzymatic hydrolysis is the most common method for obtaining low molecular weight bioactive peptides and is deemed cost-effective, efficient, and safe (Ryan et al. 2011; Xin et al. 2010). Several bioactive peptides lie inactive and encrypted within food proteins and become activated following their release by enzymatic proteolysis (Meisel 2004; Bongers and Heimer 1994). The duration of hydrolysis, extent of protein denaturation, degree of hydrolysis, ionic strength, pH of the reaction environment, temperature, presence or absence of inhibitory substances, and enzyme–substrate ratios receive critical attention during the enzymatic production of bioactive peptides since they have a direct impact on the type and functional properties of the resulting bioactive peptides (Udenigwe and Aluko 2012; Kilara and Chandan 2011). For instance, it has been shown that high hydrostatic pressure of up to 400 MPa resulted in an improvement in the hydrolysis of proteins and in obtaining potent bioactive peptides from ovalbumin (Quirós et al. 2007). Common food-grade proteases such as pepsin, trypsin, papain, or thermolysin are usually employed during in vitro procedures to liberate bioactive peptides; and choice of protease is a critical consideration, as proteases differ in their active site specificity, catalytic mechanism, as well as temperature and pH optima (Udenigwe et al. 2009a).

The bioactivities of cryptic peptides in proteins can also be released by fermentation, during gastrointestinal digestion, or by means of food processing (Hartmann and Meisel 2007; Meisel 2004). While many food proteins have been the subject of several studies on bioactive peptides, milk has been widely studied as a source of the peptides (Korhonen and Pihlanto 2006). Although studies have been conducted to simulate gastrointestinal digestion of food proteins (Majumder and Wu 2009), the in vivo production of bioactive peptides from milk is still not completely elucidated (Regazzo 2010). Prior to the cleavage of milk proteins by intestinal enzymes, they are first degraded in the stomach by the action of hydrochloric acid and pepsin in the gastric juice (Svedberg et al. 1985). This degradation prepares the proteins for the subsequent action of trypsin and chymotrypsin present in the pancreas, which ensure that a majority of bioactive peptides is released in the small intestinal part of the gastrointestinal tract (Regazzo 2010). Moreover, microbial enzymes have the distinction of being useful for the liberation of bioactive peptides either in vivo or as a starter during milk processing (Regazzo 2010). The microbial enzymes of the gut flora are only able to interact with proteins that arrive in the large intestine intact or partially degraded (Möller et al. 2008). Since these microbial enzymes differ from gastrointestinal enzymes in their active site specificity, their resulting peptide profiles may be markedly different from those liberated by intestinal digestive

enzymes (Regazzo 2010). Furthermore, bioactive peptides derived from milk proteins by the action of microbial enzymes may subsequently be acted upon by gastrointestinal enzymes, resulting in the release of significantly different bioactive peptides (Möller et al. 2008).

10.3.3 Synthesis of Bioactive Peptides Through Recombinant DNA Technology

The use of cloning and gene expression techniques in microorganisms enables the production of large amounts of a recombinant peptide or several recombinant peptides at the same time (Perez-Espitia et al. 2012). This method has been most extensively studied in *Escherichia coli* hosts; although it is the most recent method of peptide synthesis, it represents the most cost-effective strategy for producing peptides on a large scale (Li 2011). Using this technology, a number of bioactive peptides, originally identified from food proteins, have been produced in prokaryotic and transgenic plant expression systems. For instance, the rice expression system has been used to produce up to 2 mg of hypocholesterolemic pentapeptide IIAEK per gram dry seeds; high yields of several other bioactive peptides have also been produced using *E. coli*, *Chlamydomonas reinhardtii,* and soybean expression systems (González-Ortega et al. 2015). Prior to expression, protease cleavage sites are often inserted between the tandem peptide sequences. Appropriate proteases can then be used to release the expressed bioactive peptides followed by purification by reverse-phase, ion-exchange, size-exclusion, or affinity chromatography, depending on the structural properties of the expressed peptides (González-Ortega et al. 2015). Compared to the other production methods, the recombinant technology approach appears to hold strong promise (for increasing yields) in translating purified peptides into functional food products for human health promotion.

10.4 Bioactivity of Food Protein-Derived Peptides

As shown in Table 10.1, food protein-derived bioactive peptides have been found to lower elevated blood pressure, take part in mineral transport, modulate the immune system, suppress the growth of cancer cells, possess antimicrobial, CaM-inhibitory, anti-inflammatory, antioxidant, and hypolipidemic properties, and contribute to the sensory properties and nutritive value of foods, among other beneficial functions (Dziuba and Dziuba 2010; Udenigwe and Aluko 2012). In this section, a brief highlight of the major health-promoting properties of bioactive peptides is presented, with a focus on the molecular mechanisms of their functions.

Table 10.1 Some bioactive properties and mechanisms of bioactive peptides derived from food proteins

Bioactivity	Mechanism	References
Antihypertensive	Renin inhibition	Girgih et al. (2011a), Udenigwe et al. (2009b)
	ACE inhibition	Udenigwe and Aluko (2010), Yamaguchi et al. (2009), Hartmann and Meisel (2007), Li et al. (2004), Sato et al. (2002), Nakamura et al. (1995), Ferreira et al. (1970)
Antioxidant	Free radical scavenging/capture	Girgih et al. (2011b), Peng et al. (2009), Udenigwe et al. (2009a), Mendis et al. (2005)
	Inhibition of ROS-induced lipid, protein, and DNA oxidation	Chen et al. (1995), Girgih et al. (2011b)
	Metal ion chelation	Chen et al. (1996), Girgih et al. (2011b)
Immunomodulatory and anti-inflammatory	Reduced secretion of TNF-α and IL-6	Ndiaye et al. (2012), Hernández-Ledesma et al. (2009a), de Mejia and Dia (2009)
	Suppression of (1) NF-kB transactivation, (2) COX-2 expression, (3) NO and PGE-2 production (4) iNOS secretion	de Mejia and Dia (2009)
Anticancer	Apoptosis induction	Hernández-Ledesma et al. (2009b), Silva-Sánchez et al. (2008)
	Arrest of cell cycle progression at the G1/S phase and G2/M phase	Hernández-Ledesma et al. (2009b), Kim et al. (2000)
Hypolipidemic	Suppression of histone H3 Lys14 acetylation and concomitant reduction of HMG-CoA reductase secretion	Udenigwe and Aluko (2012)
	Upregulation of LDL receptors in liver cells	Lovati et al. (2000)
	Degradation of the essential LDL component, Apo B-100	Mochizuki et al. (2009)
CaM-inhibitory	Inhibition of phosphodiesterases	You et al. (2010), Kizawa (1997), Kizawa et al. (1995)
	Inhibition of nitric oxide synthases	Omoni and Aluko (2006a, b)
	Inhibition of protein kinases	Li and Aluko (2005)

10.4.1 Antihypertensive Bioactive Peptides

Hypertension is not only a major risk factor for cardiac, cerebrovascular, and other vascular diseases (Lawes et al. 2008), it is also considered a leading cause of mortality worldwide (WHO 2009). Antihypertensive drugs currently on the market include ACE inhibitors, calcium channel antagonists, endothelin antagonists, diuretics, renin inhibitors, as well as α- and β-blockers (Yamamoto 2010). Long term use of some of these drugs in the management and control of elevated blood pressure is not without adverse consequences (Coulter and Edwards 1987; Webb et al. 1986; Sesoko and Kaneko 1985), thus promoting the use of food-derived bioactive peptides for lowering blood pressure during hypertension (Udenigwe et al. 2009a; Li et al. 2004; Sato et al. 2002).

The renin-angiotensin system (RAS) occurs in the circulatory system, brain, lung, kidney, and aorta (Matsui and Tanaka 2010), and has proven to be an important target for antihypertensive drugs, since it plays a vital role in the control of blood pressure, myocardial remodeling, fluid and electrolyte balance, in addition to contributing to important physiological processes in the liver and heart (Acharya et al. 2003; Turner and Hooper 2002). Renin, one of two key enzymes in the RAS pathway, catalyzes the hydrolysis of the Leu10-Val11 peptide bond of angiotensinogen, a liver-derived serum globulin, generating the decapeptide, angiotensin I [AT-I] (Acharya et al. 2003). AT-I is then converted by the dipeptidyl carboxypeptidase ACE to the potent vasopressor angiotensin II (AT-II) following the hydrolytic cleavage from the AT-I decapeptide of the dipeptide His-Leu (Acharya et al. 2003). ACE also affects blood pressure by degrading the vasodilator, bradykinin (Yang et al. 1970). Potent antihypertensive agents should therefore be able to inhibit renin and/or ACE activities with minimal or no side effects to the body. Various in vitro studies have copiously demonstrated the inhibition of ACE and renin activities by food-derived peptide fractions obtained via enzymatic hydrolysis (Girgih et al. 2011a; Udenigwe and Aluko 2010; Udenigwe et al. 2009a). Matsui and others (2003) have reported that a single oral administration of the dipeptide Val-Tyr from sardine led to prolonged blood pressure decrease in 11-weeks-old Tsukuba-Hypertensive Mouse—a result replicated in other studies where other bioactive peptide fractions were administered to spontaneously hypertensive rats subjects (Girgih et al. 2011a; Yamamoto et al. 1999; Nakamura et al. 1995) and human subjects (Turpeinen et al. 2012).

10.4.2 Antioxidant Peptides

Although oxygen is essential for life, everyday aerobic metabolic processes result in the production of reactive oxygen species (ROS) and other free radicals including hydrogen peroxide, nitric oxide (NO), hydroxyl radical (·OH), and peroxynitrite ($ONOO^-$), which are neutralized by the body's in-built antioxidant defense mechanisms (Urso and Clarkson 2003; Uttara et al. 2009). Excessive production of free

radical and ROS, which may also come from dietary sources and mitogen-activated immune cells, may overwhelm normal physiological antioxidant defense systems, resulting in the disruption of intracellular redox homeostasis. This represents the onset of cellular oxidative stress, cellular and organismal aging, normal or neoplastic cell migration, proliferation, senescence, or death (Storz 2011), as well as the oxidative degradation of biomolecules (Pacher et al. 2007; Ames et al. 1993; Ames 1983). This process contributes to the pathogenesis of various human neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Uttara et al. 2009). Antioxidants present in various foods including cruciferous vegetables, fruits, and legumes such as soy (Sikora et al. 2008; Sun et al. 2002; Chen et al. 1998) supplement endogenous enzymatic (glutathione peroxidase, superoxide dismutase, catalase) and nonenzymatic (glutathione, flavonoids, ascorbate, ubiquinone) antioxidant defense systems (Fang et al. 2002), and are thought to reduce the risk of developing chronic neurodegenerative diseases. Food-derived bioactive proteins and peptides have also been found to possess antioxidant properties as demonstrated by standard in vitro antioxidant assays such as free radical scavenging assays (Girgih et al. 2011b; Peng et al. 2009; Udenigwe et al. 2009b; Mendis et al. 2005), inhibition of ROS-induced lipid, protein, and DNA oxidation (Girgih et al. 2011b; Chen et al. 1995), and metal ion chelation (Girgih et al. 2011b; Chen et al. 1996), as well as inhibition of the ubiquitous oxidative enzyme group, semicarbazide-sensitive amine oxidase that catalyzes the conversion, by oxidative deamination, of primary amines to corresponding aldehydes, NH_3 and H_2O (Udenigwe et al. 2009b). As with other bioactive peptides, differences in hydrolysis temperature, degree of hydrolysis, hydrolyzing enzymes, peptide size, hydrophobicity, amino acid composition, and general peptide preparation conditions affect the type of peptides generated and their antioxidant potential (Young and Mine 2010; Pihlanto 2006).

10.4.3 Immunomodulatory and Anti-inflammatory Bioactive Peptides

By suppressing nuclear factor (NF)-kB activation and consequently reducing the secretion of tumor necrosis factor (TNF)-α, interleukin (IL)-6, cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), NO and prostaglandin (PG)E-2, many food-derived bioactive peptides exert potent immunomodulatory and anti-inflammatory functions (Ndiaye et al. 2012; Hernández-Ledesma et al. 2009a; de Mejia and Dia 2009). Pro-inflammatory cytokines mediate the immune response by facilitating the arrival of immune cells at injury sites and stimulating cells to produce inflammatory molecules including cytokines, thus resulting in an exponential increase in immune response (Young and Mine 2010). Takahashi et al. (1994) reported that oryzatensin, a novel bioactive peptide isolated following the digestion of rice albumin with trypsin-enhanced phagocytosis in human polymorphonuclear leukocytes and improved the production of superoxide anion by human peripheral leukocytes. Pancreatic and tryptic

digests of phosphorylated bovine milk glycoprotein kappa-casein have been shown to significantly suppress the proliferation of murine spleen lymphocytes and leporine Peyer's patch cells (Otani and Hata 1995). Other food proteins sources from which immunomodulatory and anti-inflammatory peptides have been derived include soy (de Mejia and Dia 2009), chum salmon (Yang et al. 2010), pea (Ndiaye et al. 2012), wheat (Horiguchi et al. 2005), whey (Gauthier et al. 2006), and eggs (Mine and Kovacs-Nolan 2006). In addition to peptides, anti-inflammatory proteins have also been isolated from eggs, milk, soy, fruits, and other food protein sources (Young and Mine 2010). Hen egg lysozyme has been shown to reduce the expression of pro-inflammatory cytokines TNF-α, IL-6, interferon (IFN)-γ, IL-8, and IL-17, and heighten the secretion of anti-inflammatory IL-4 and transforming growth factor (TGF)-β in a model of chemical colitis (Lee et al. 2009). In in vitro studies, egg white protein ovotransferrin also showed immunomodulating effects in the chicken macrophage cell line HD11 and in heterophils (Xie et al. 2002) and is also to known to suppress the proliferation of murine spleen lymphocytes (Otani and Odashima 1987).

10.4.4 Calmodulin (CaM) Inhibitory Bioactive Peptides

CaM is a 16.7 kDa ubiquitous calcium-binding messenger protein involved in cell division and proliferation, neurotransmission, vasodilation, smooth muscle contraction, and many other roles in the translation of intracellular messages (Cho et al. 1998; Rasmussen and Means 1987; Veigl et al. 1984; Klee and Vanaman 1982). It plays a vital role in the regulation of a wide variety of important intracellular enzymes such as ATPase, CaM-dependent cyclic nucleotide phosphodiesterase 1, protein kinase II, endothelial and neuronal NOS, phospholipase A_2 and adenylate cyclase, which place CaM in a prime position with regard to the modulation of key physiological processes (Stevens 1983; Itano et al. 1980). In spite of its obvious importance in the normal functioning of the body, overexpression of CaM may result in physiological processes that may trigger the progression of chronic diseases such as Alzheimer's, cancer, and cardiac hypertrophy (Obata et al. 2005; O'Day and Myre 2004; Hait and Lazo 1986). Thus, bioactive inhibitors which bind to and inactivate CaM can be used for the prevention or treatment of diseases arising from or triggered by the increased activity of enzymes regulated by CaM (Martínez-Luis et al. 2007). Since reports demonstrated that food-derived peptides from the hydrolysis of α-casein with pepsin inhibited the activity of CaM-PDE (Kizawa et al. 1995, 1996), other works have shown the potential for using bioactive peptides in the prevention and management of diseases positively correlated with increased activities of CaM-dependent enzymes. For instance, cationic peptide fractions from pea inhibited CaM-dependent protein kinase II (CaMKII), the enzyme that catalyzes the phosphorylation of critically important cellular proteins (Li and Aluko 2005; Soderling and Stull 2001), while bioactive peptides derived from flaxseed protein were found to inhibit the activities of CaM-dependent endothelial NOS and neuronal

NOS (Omoni and Aluko 2006a, b). Isolation of cationic peptide fractions from egg white lysozyme with antioxidant and CaM-inhibitory dual functionalities highlighted the potential for use of such bioactive peptides in the treatment of multiple diseases (You et al. 2010).

10.4.5 Anticancer Peptides

Throughout the world, cancer is a major cause of death. Mortality from cancer is predicted to be on an upward trend with each passing year, and the disease is estimated to claim about 13.1 million lives worldwide in 2030 (IARC 2010). Although the molecular bases of cancer development and progression have not been completely elucidated, a considerable mass of scientific evidence suggests that diet plays a role in cancer development (Milner 2004). It is therefore possible that by modifying the diet, cancer development could be influenced. Moreover, the possibility of using bioactive peptides as anticancer agents has been the focus of many research efforts (Wang et al. 2008; Hernández-Ledesma et al. 2009b). Anticancer bioactive peptides have been isolated from soy (Kim et al. 2000) and milk (Damiens et al. 1999) proteins, while a number of egg proteins including lysozyme (Kovacs-Nolan et al. 2005; Sava et al. 1989), cystatin (Verdot et al. 1999), avidin (Gasparri et al. 1999) and ovomucin (Oguro et al. 2001) have shown demonstrable anticancer properties. One of the mechanisms by which anticancer peptides are thought to halt cancer progression is by inducing apoptosis in cancerous cells (Hernández-Ledesma et al. 2009b; Silva-Sánchez et al. 2008). The multifunctional peptide, lunasin, first isolated from soy and now known to be present in many other plants including wheat, barley, and amaranth (Hernández-Ledesma et al. 2009b) has been demonstrated to suppress carcinogen-mediated transformation of mouse fibroblast C3H10T1/2 cells (Galvez et al. 2001) as well as the oncogene-directed transformation of mammalian cells (Jeong et al. 2003). It was suggested that lunasin selectively induces apoptosis in cells undergoing transformation such as C3H cells transfected with the adenovirus early region 1A (E1A) gene, a known oncogene (Routes et al. 2000) by disrupting histone acetylation; E1A-transfected cells not pretreated with lunasin did not show the characteristic morphology associated with apoptosis induction (Galvez et al. 2001). Hernández-Ledesma and others (2009b) have also demonstrated the capacity of food-derived bioactive peptides to initiate cell cycle arrest and induce apoptosis by inhibiting the activity of histone acetyl transferase. Enzymatic hydrolysates from different soy varieties have also been shown to reduce the viability of L1210 leukemia cell lines, but not as much as lunasin (Wang et al. 2008). In spite of lunasin's promising anticancer activity, its large molecular size raises concerns about its absorption, bioavailability, and use as an orally administered health-promoting bioactive peptide, especially in light of the report of a lunasin absorption rate of only 4.5 % in human subjects (Dia et al. 2009). Interestingly, efficient absorption of lunasin from rye has also been

reported (Jeong et al. 2009), thus underlining the need for more studies to expand current knowledge of the absorption kinetics of lunasin, increase our understanding of the differential absorption potential of lunasin from different food sources, as well as pave the way for the isolation of other novel (anticancer) bioactive peptides.

10.4.6 Hypolipidemic and Hypocholesterolemic Bioactive Peptides

Two peptide-based hypocholesterolemic products are known to have been developed and commercialized. The products, Lunasin^{XP} and LunaSoy™ were produced from the soybean-derived polypeptide, lunasin. The mechanism by which lunasin exerts its cholesterol-lowering effect begins with the suppression of the acetylation of Lys14 residue on histone H3, thereby slowing the secretion of HMG-CoA reductase and reducing the rate of cholesterol biosynthesis (Udenigwe and Aluko 2012). The soy 7S globular storage protein β-conglycinin has also received attention for its involvement in the lowering of cholesterol levels; the protein's $\alpha+\alpha'$ subunit significantly upregulated LDL receptor expression in cultured liver cells, resulting in increased LDL uptake and degradation (Lovati et al. 1998, 2000). Results suggesting hypotriglyceridemic functions have also been obtained with bacterial protease-hydrolyzed β-conglycinin fractions. β-conglycinin enzyme-derived peptides also lowered the accumulation of apolipoprotein B-100 (Apo B-100) in Hep G2 cells due in part to the upregulation of the expression of LDL receptors (Mochizuki et al. 2009). Degradation of Apo B-100 impairs the biosynthesis of very low density lipoprotein. Additionally, various studies have reported the hypocholesterolemic effects of different legume-based diets such as reduction in total LDL-cholesterol among (a) Chinese women following soy consumption (Zhang et al. 2003); (b) in chickpea-fed rats (Yang et al. 2007); and (c) in adult men and women on a chickpea-supplemented diet (Pittaway et al. 2006, 2007). It has been suggested that such cholesterol and lipid-lowering effects are possibly due to a combination of factors such as the binding of the saponins and fiber to bile acids resulting in the excretion of greater quantities of bile salts in the feces, the consequent production of more bile from cholesterol by the liver, and a reduction in total body cholesterol (Jiang et al. 2010; Howard and Udenigwe 2013). The insoluble high molecular weight hydrophobic amino acids-rich peptide fractions present in soy protein hydrolysates have also been partly credited with the capacity of enzyme-derived hydrolysates to bind bile acids, as is the case with high molecular weight trypsin-derived hydrolysates from buckwheat (Kayashita et al. 1997). Although traversing the intestinal epithelium to reach the blood circulation and exerting their salutary hypolipidemic effects may pose a formidable challenge to bioactive peptides, they are still important contributors to cholesterol homeostasis given their facilitation of bile acids and exogenous cholesterol expulsion via feces (Howard and Udenigwe 2013).

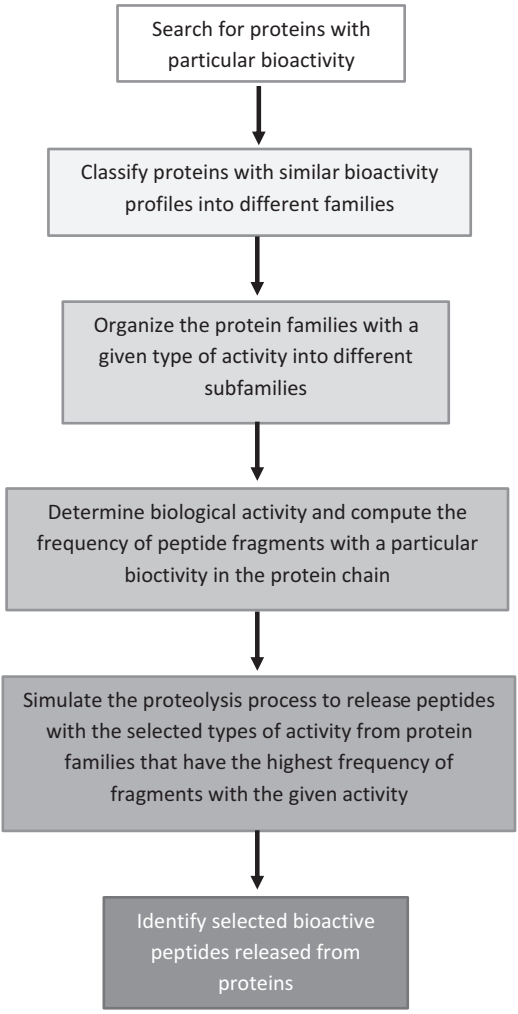

Fig. 10.2 Computer-aided simulation of food protein digestion for the derivation of bioactive peptides (Dziuba and Dziuba 2010)

10.5 Bioinformatics and the Discovery of Bioactive Peptides

At the turn of the millennium, computer-based strategies were proposed for use in the search for novel food-derived bioactive peptides. This approach employs computational tools in assessing food proteins as precursors of bioactive peptides prior to wet lab experiments (Udenigwe 2014). As shown in Fig. 10.2, bioinformatics tools can help in the prediction of secondary structure of proteins and searching for homologies between proteins in order to identify their functions as well as classifying proteins into families with similar biological activity profiles (Dziuba and Dziuba 2010). This approach can potentially contribute to determining if potential

hydrolysates from a selected protein source would be bioactive, predicting possible functional, biological, sensory, and physicochemical properties of peptides, and resolving how to deal with possible allergenic, toxic, or other unwanted properties of peptides (Minkiewicz et al. 2008). The "*in silico*" approach uses a number of parameters such as the protein's potential biological activity profile, the frequency of occurrence of bioactive motifs in its sequence, its potential activity, and the integrated biological activity coefficient in evaluating food proteins as precursors of bioactive peptides (Minkiewicz et al. 2008; Dziuba and Iwaniak 2006). A myriad of computer-aided tools and databases are available for the analysis and simulation of enzymatic hydrolysis of proteins and polypeptides with the purpose of revealing bioactive peptides encrypted in their sequences.

10.6 Bioavailability of Bioactive Peptides

In order to exert a specific physiological effect, bioactive peptides must remain active and intact from the time they are ingested until their intestinal absorption into the blood circulation, a property known as bioavailability (Vermeirssen et al. 2004). For instance, an effective and highly bioavailable ACE-inhibitory peptide would be one that remains intact during gastrointestinal digestion, resisting the proteolytic action of human proteases (Segura-Campos et al. 2011) and passing through a series of barriers that could potentially inactivate it (Yamamoto et al. 1999) to reach the cardiovascular system where it would essentially exert its antihypertensive effect (Vermeirssen et al. 2004). Limitations in bioavailability, enhanced in part by the enzymatic digestion of bioactive peptides to inactive residues, are often linked to the wide gap between in vitro peptide bioactivity and in vivo physiological and pharmacological effects (Vermeirssen et al. 2004). Consideration of the physiology and mechanics of peptide (and protein) digestion and absorption highlights the enormous challenge in the form of the gastrointestinal tract and peptide-bond hydrolyzing peptidases that bioactive peptides must surmount in order to reach the circulation intact. While the gastrointestinal tract proteases primarily function in hydrolyzing dietary proteins into small subunits, the peptidases are known for their wide substrate specificity and can be considered as an important barrier against bioactive peptide absorption (Woodley 1994; Pauletti et al. 1996). To put this in perspective, it is thought that an ingested protein could encounter at least 40 different enzymes as it passes through the small intestine (Woodley 1982). A logical deduction from the foregoing is that short length bioactive peptides would have a higher propensity of being absorbed intact into the blood than longer oligopeptides, as the former would hardly need to undergo further hydrolysis. This inference is supported by studies showing that while most peptides with more than three amino acid residues tend to be hydrolyzed by extracellular enzymes in the brush border membrane of the intestinal epithelial tissue, di- and tripeptides can be absorbed intact and hydrolyzed afterwards (Segura-Campos et al. 2011). The size of peptides

therefore exerts an enormous influence on their absorption across cell membranes and bioavailability in target tissues (Udenigwe and Aluko 2012).

Dietary protein digestion begins in the stomach where the action of pepsins under acidic conditions results in the production of polypeptides which are further hydrolyzed by a battery of enzymes in the lumen of the small intestine including carboxypeptidase A and B, α-chymotrypsin, trypsin, elastase, and pancreatic proteases to smaller oligopeptides and some single amino acid residues. Oligopeptides usually undergo a second hydrolysis by peptidases in the intestinal villi resulting in the production of dipeptides, tripeptides, and free amino acids (Segura-Campos et al. 2011). The latter hydrolysis must be taken into consideration when designing dietary bioactive peptides intended for passage through the enterocytes without enzymatic degradation. In fact, the lumen and brush border membrane of the intestinal epithelium are known to pose the most formidable threat to therapeutic proteins and bioactive peptides, as they contain gram quantities of pancreatic proteases, proteases from mucosal cells, as well as peptidases with broad specificities for both proteins and peptides (Segura-Campos et al. 2011). As a strategy for ensuring that peptide sequences are shielded from enzymatic degradation, scaling the hurdles posed by the digestive enzymes is essential to the bioavailability of bioactive peptides. Moreover, the amino acid composition of bioactive peptides has also been shown to have a considerable influence on their bioavailability. Particularly, proline and hydroxyproline-containing peptides are more likely to resist the action of proteolytic enzymes (Segura-Campos et al. 2011), as demonstrated for peptides obtained from casein and gelatin with an impressive capacity to withstand cleavage by digestive peptidases (Fitzgerald and Meisel 2000). Finally, inter-individual variations in peptide transport can possibly influence peptide bioavailability. Differences in the human intestinal di- and tripeptide transporter hPEPT1, a proton-dependent peptide-specific transporter (Yang et al. 1999), in the form of single nucleotide polymorphisms (SNPs), have been recorded in different genomic DNA sample collections (Gerloff 2004). While a survey of 44 ethnically diverse individuals revealed nine nonsynonymous SNPs in hPEPT1, functional characterization of the variants revealed a significant difference in peptide transport in only one protein (Zhang et al. 2004). Nevertheless, the possible impact of inter-individual differences on transmembrane transporters and peptide transport should receive prime research attention in future investigations (Brandsch et al. 2008).

10.7 Safety and Regulation of Bioactive Peptides

The safety of food protein-derived bioactive peptides has hardly come into question given that normal metabolic processes would ordinarily convert food proteins into peptides (Wang and de Mejia 2005) and that only food-grade enzymes and processes are used in the production of such functional peptides (Udenigwe and Aluko 2012; Mao et al. 2007). Nakamura et al. (2005) found no evidence of organ toxicity in male and female canine and murine models following oral administration of the

tripeptides Val-Pro-Pro and Ile-Pro-Pro to a maximum dose of 16 mg/kg/day, while Ponstein-Simarro et al. (2009) have shown that a dose 141-fold higher than the anticipated maximal intake of the milk protein hydrolysate Tensguard™ containing Ile-Pro-Pro had no mutagenic or clastogenic effect in rats. Similarly, a study of the impact in murine models showed that tripeptides did not adversely affect the survival, post-implantation, birth weight, development, or sex ratio of the embryo, or impair the sexual maturation, viability or reproductive capability and fertility of the F1 generation (Kurosaki et al. 2005). In spite of the general perception of bioactive peptides as safe, it is imperative to ensure that processing techniques employed in their production do not impair their quality and safety (Udenigwe and Aluko 2012). Additionally, it is still necessary to consider and evaluate the safety and toxicity of the individual food proteins (Phelan et al. 2009) on a case by case basis. This may seem overly cautious, since it could be argued that it is unnecessary to conduct toxicity tests of bioactive peptides obtained from regular and usually edible food proteins (Schaafsma 2009). However, it must be remembered that a particular bioactive peptide preparation contains an aggregate amount of specific amino acids that differs markedly from the concentration of the same amino acids normally found in a regular human diet (Schaafsma 2009).

Regulatory guidelines on the use and administration of bioactive peptides as foods and/or food supplements differ from region to region. Japan pioneered efforts to regulate the use of functional foods including bioactive peptides with the introduction in 1991 of the Food for Specific Health Use [FOSHU] licensing system (Arai 2002; Halsted 2003; Yamagushi 2005). Although bioactive peptides have been extensively investigated in Europe, the Commission of European Communities has neither an authorized specific single set of health claims for food-derived bioactive peptides and proteins (Walther and Sieber 2011) nor a regulatory framework specifically for "functional foods" or "nutraceuticals" in the European Union's General Food Law Regulation (Coppens et al. 2006). Instead, under the food law (Regulations EC No: 178/2002) for which guidelines are numerous and depend on the nature of the food, the European Food Safety Authority (EFSA) is responsible for ensuring safety by carrying out risk assessments of food products in an independent, objective, and transparent manner; conveying risk assessment findings to the European Commission, tracing and recalling harmful foods and food products; and authorizing the use of certain food products after favorable risk assessments. Under the food law, the EFSA prohibits the use of any food substance or ingredient found to be harmful. If a dietary supplement or food component is thought to be potentially injurious to health, but some uncertainty still persists after risk assessment studies, the substance is placed on a list for a maximum period of 4 years during which time further scientific evidence, favorable or otherwise, about its safety is expected to be obtained (Coppens et al. 2006).

In the United States, legislation passed in 1938 [Food Drug and Cosmetic Act] and 1990 (Nutrition Labeling and Education Act) invariably covered regulation of health-related claims for foods and food components, but it was not until 1994 that the use of more specific language to regulate particular effects on the body's "structure and function" or on "well-being" following the consumption of a nutrient

or dietary ingredient was introduced in the Dietary Supplement Health and Education Act, DSHEA (Gilani et al. 2008; Halsted 2003). Under the 1994 act, the term "dietary supplement" refers to "a product (other than tobacco) that is intended to supplement the diet and that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, a herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract or combinations of these ingredients," is legally classified as food and not drug, should be labeled as a dietary supplement, and should not be the sole component of a food or diet (Gilani et al. 2008). The Center for Food Safety and Applied Nutrition of the US Food and Drug Administration is responsible for implementing the provisions of the DSHEA and of the more recent Food and Drug Administration Modernization Act (Halsted 2003). The first steps towards regulation of the administration and use of bioactive peptides in Canada were taken in 1998 when Health Canada put forward statutory definitions for the discussion and categorization of "nutraceuticals" and "functional foods" (Lee 2006). Bioactive peptides could be sold in the form of capsules, pills, pastilles, tablets, and similar forms as natural health products or as components of functional foods. The Natural Health Products (NHPs) Regulation of the Foods and Drugs Act sets out the conditions for the use of certain dietary supplements (nutraceuticals) as NHPs (Gilani et al. 2008). For functional foods to which food-derived bioactive peptides have been added for "fortification," regulations for their use are contained in Division 28 of the 1999 Food and Drug Regulations (Gilani et al. 2008).

10.8 Contributions to Food Security

At the World Food Summit in 1996, food security was defined as existing "when all people at all times have physical and economic access to sufficient, safe, nutritious food that meets their dietary needs and food preferences for an active and healthy life." To be food-secure, a household must have enough resources to consistently obtain sufficient quantities of nutritious food while making appropriate and prudent use of the food available to it. While there may be many food-secure households around the world, on a global scale, food security is still a growing concern. With the world's population projected to reach eight billion by 2030, the same year that global demand for food will grow by 50 % in comparison to current levels (FAO 2006), the need for smarter and more efficient production methods, storage, distribution, and uses of food and food products has never been more urgent. Recently, advances in food science research have seen the emergence of new strategies and technologies aimed at obtaining health-promoting bioactive peptides from proteins derived from byproducts of the agri-food industry. Although the muscles of animals contain high amounts of collagen, which make them a less attractive option for direct human consumption due to their toughness (Lepetit 2008), their utilization as sources of bioactive peptides is well documented (Ryan et al. 2011; Saiga et al. 2008;

Vercruysse et al. 2005). ACE-inhibitory peptides have been derived from industrial waste frame protein of Alaskan pollack (Je et al. 2004), from waste yellowfin sole frame protein (Jung et al. 2006) and from chicken collagen hydrolysate (Saiga et al. 2008). Valorization of these "waste" food proteins by incorporating them into the human food system through bioactive peptides may enhance their economic value and perhaps reduce waste disposal and associated carbon footprints (Ryan et al. 2011; Udenigwe and Howard 2013). This alternative protein source will contribute to food security because the need for primarily food proteins (e.g. milk proteins) for this purpose will be reduced, and enhance the burgeoning pool of food-derived agents for health promotion.

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Chapter 11
IUFoST Distance-Assisted Training Program

Daryl Lund and Donald Mercer

11.1 Development of the Website for the IUFoST Distance-Assisted Training (DAT) Program

From the outset, the DAT Program was designed for food industry personnel working primarily in developing economies. Food industry employees in these circumstances seldom have training in processing food, personal hygiene, or food safety. Consequently, the intent was to develop a set of modules based on the principles of food processing which, when presented through a mentor, will provide workers with a fundamental understanding of food processing and food safety. The DAT Program is conducted through a mentor/participant relationship. This is to facilitate educational experience when the participant does not have access to the Internet. The program relies on the mentor downloading and printing the course manual and materials and physically handing it to the participant. The procedure is described in more detail later in this chapter.

11.2 Access to the Basic Information

In this section of the chapter, we examine the website to demonstrate how the modules can be accessed. In subsequent sections, the application of the modules/courses to mentors and participants is described in detail. The starting point is the IUFoST website at www.iufost.org. The screen will appear as below. Then click "Education and Training" (Fig. 11.1).

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Fig. 11.1 Accessing Education and Training information on the IUFoST website

Once in this section, click on the link to "Distance Education" in the drop-down menu. Here, three sections that describe the work of the Distance Education Work Group of IUFoST can be found: (1) Institutions that offer food science and technology courses for academic credit using distance education technology; (2) Distance-Assisted Training programs for professional continuing education; and (3) Resources for use in teaching food science and technology. Information on the DE courses for academic credit is gathered through a survey conducted by IUFoST every 5 years. Information on resources for teaching food science and technology is gleaned from knowledge of books, modules, animations, etc., which are readily available without cost on the Internet. In this chapter, we are interested in what IUFoST has to offer in the Distance-Assisted Training Program (Fig. 11.2).

About one-third of the way through the description is a paragraph entitled:

"*Method of Delivery*"

Each course has a course instructor with a specified time frame for completing the course. The course instructor serves as a resource for the course mentor. The training materials are designed to be delivered through a local mentor who has identified local participants. The mentor and the participant both must register here.

Fig. 11.2 Accessing the Distance Education information

By clicking on the active link here, you are directed to the following screen (Fig. 11.3):

Here you can activate an account as a Mentor, Participant, or Instructor. Completed information is not shared outside the system. The information is registered with the General Secretary of IUFoST and available to course instructors.

Once the mentor and participant have registered, the next step is to log in to the e-learning site. The log-in is found by clicking on the e-learning box on the left side of the screen, as shown below (Fig. 11.4).

Clicking on e-learning will take you to this screen. Note that it requires that you log-in (Fig. 11.5).

Your log-in name and password are obtained from the IUFoST General Secretary. There is an active link to request a password. Once you are in this site, you can then register for specific courses as a mentor or a participant. There is also a very thorough description of the DAT Program.

When you have logged in, the following screen appears (Fig. 11.6):

Here, you can manage your courses as a mentor and see the materials for all courses to determine what you would like to present to your participant. There

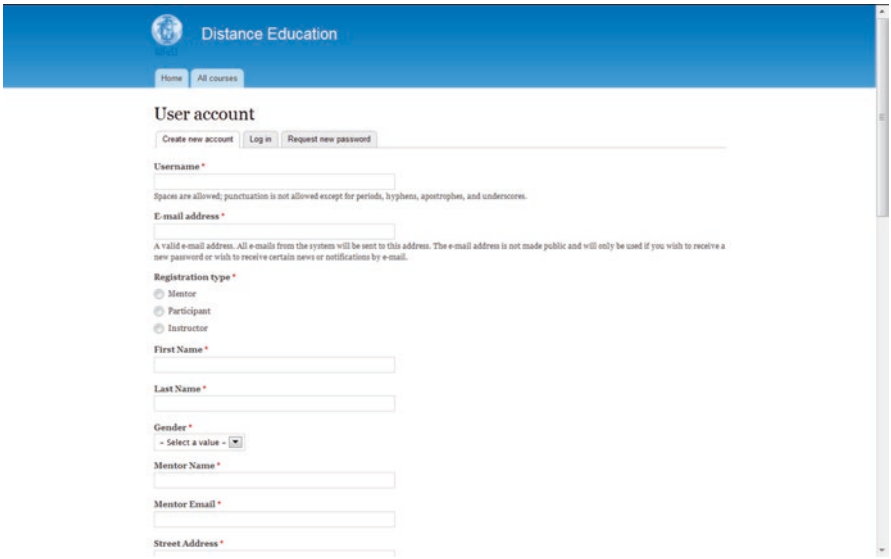


Fig. 11.3 Setting up a user account including password for accessing the information

Fig. 11.4 Accessing the e-learning information

is also an extensive Question and Answer section for mentors. When you click on "All Courses," the next screen appears. Note that each course has an acronym in its title for easy reference, and the course is described in some detail. For example, in the first course listed, IUFDEFCF: Food Chilling and Freezing, the brief

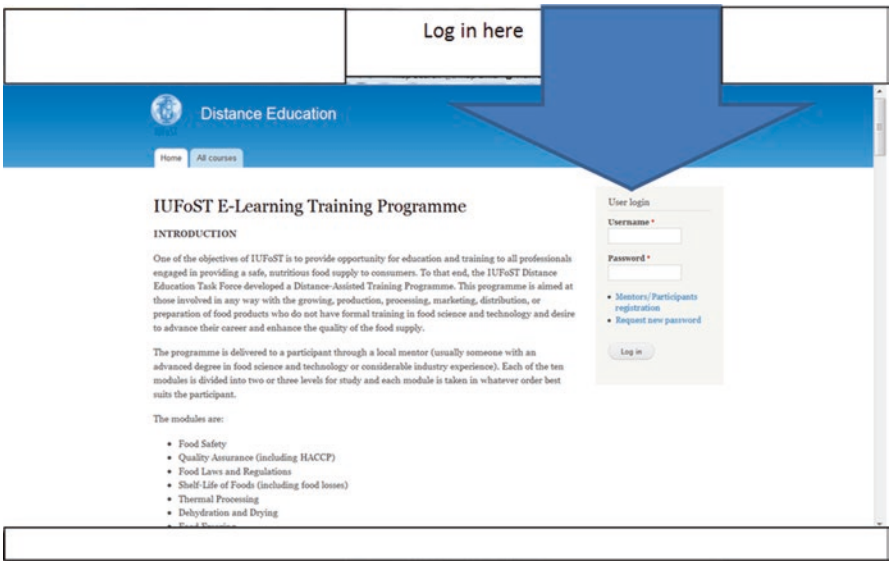


Fig. 11.5 Logging in to access the courses

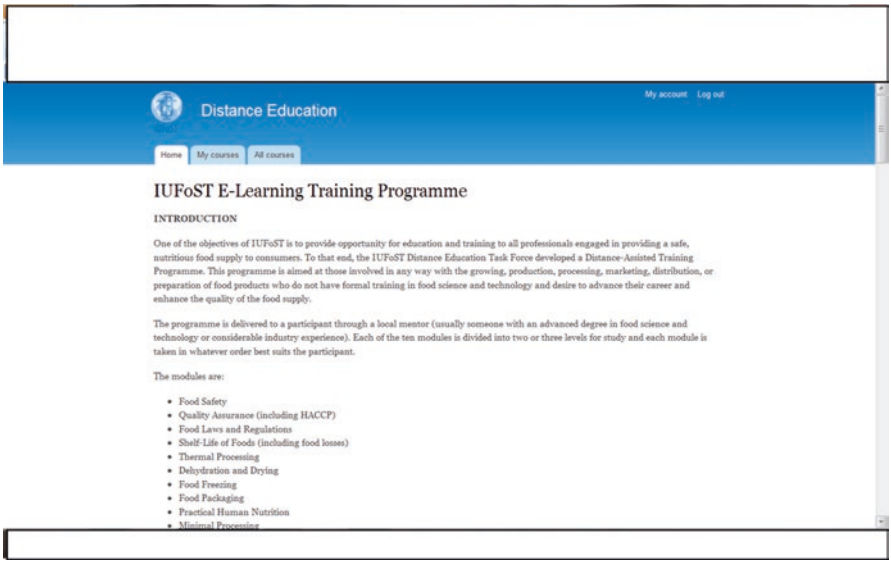


Fig. 11.6 Description of the e-learning materials

description points out that the course is divided into four sections. Furthermore, the title is hyperlinked to a description and picture of the person who developed the course.

Clicking on the heading "My Courses" will give you access to the courses to which a mentor has direct access. The screen appears as follows. For each course

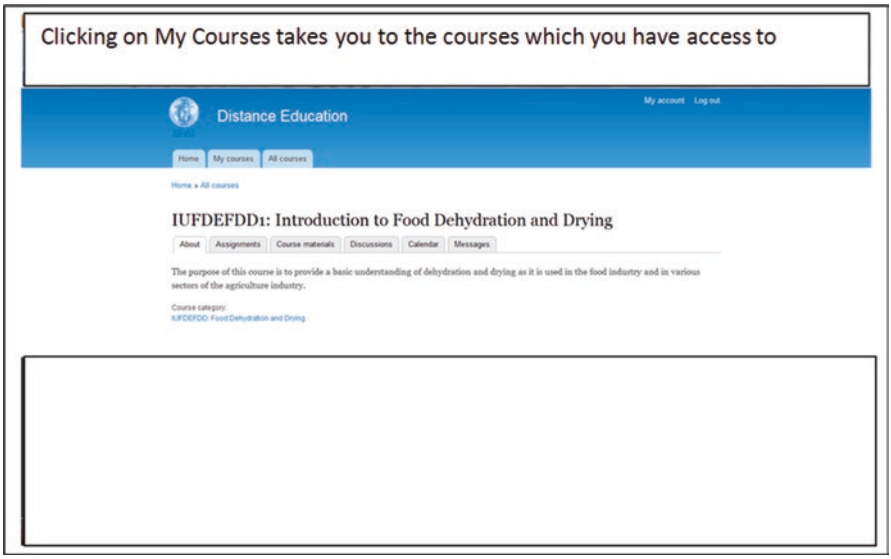


Fig. 11.7 Accessing My Courses to which a person has access

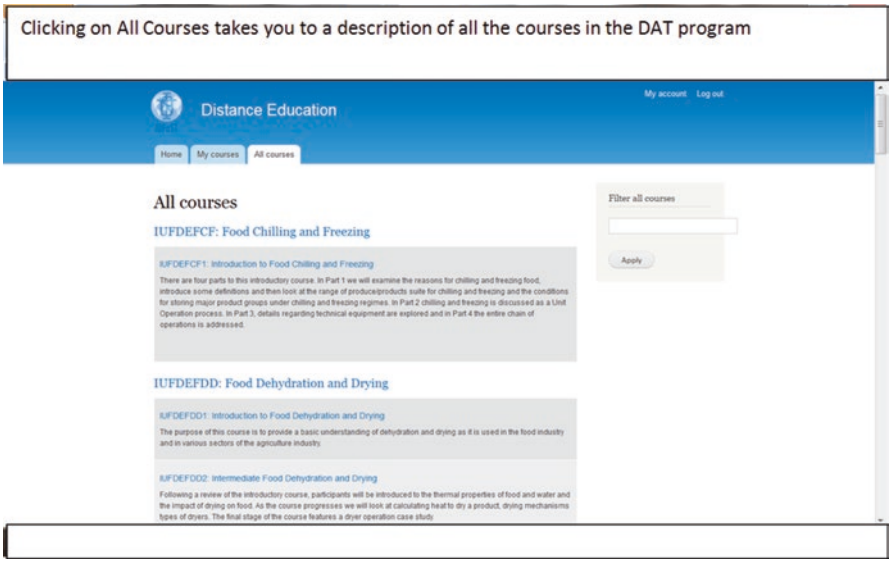


Fig. 11.8 Accessing all courses in the distance-assisted training program

there are tabs for "About," "Assignments," "Course Materials," "Discussions," "Calendar," and "Messages." Using "Messages" allows the mentor direct access to the overall course instructor and for the course instructor to all the mentors (Figs. 11.7 and 11.8).

In summary, the website has been developed for ease of navigation and to provide all of the information that a mentor needs to deliver a course to a participant. We require that each mentor and participant register with the website so we can track use and get feedback on the utility of the site. The next section of this chapter describes the actual use of the course materials as presented by course developer, Professor Donald Mercer.

11.3 Testing the Concept

The concept of offering distance-assisted training courses to food industry workers in sub-Saharan Africa was essentially untested at the time the training modules were under development. Degree level courses in food science were well established through distance education offerings at numerous universities worldwide. However, there were no courses being offered to workers employed within the food industry who had completed, or only partially completed, high school. In-house training was considered to be limited and restricted by the resources available to individual companies to provide it.

There was no easily accessible source of instructional material that would provide a uniform background of training material geared to the educational background and actual needs of individuals who were already employed in the food industry. What was missing was a series of courses designed to explain the basic concepts and principles of food processing to those employed in the industry. There were few, if any, opportunities for learning why particular things were done, and an overall lack of explanations as to cause and effect relationships when processing various foods.

The course module topics were identified based on an in-depth understanding of food processing by numerous subject matter experts from around the world. The majority of these individuals were Fellows of the International Academy of Food Science and Technology, with diverse experiences and equally diverse expertise. There was absolutely no doubt as to the relevance of the topics—the problem was how to deliver the material to those who needed it most.

It was decided to use the "Food Dehydration and Drying" module to test the concept of delivering training at the grass roots level.

11.4 Assessing the Target Audience

Since its inception, the target audience for the entire Distance-Assisted Training Initiative was defined as workers employed in the food industry with minimal formal education. Development of a course is illustrated by considering the thought process that went into the Food Dehydration and Drying courses.

Given the regions of the world in need of such training, with such a broad spectrum of indigenous crops and varied preferences in food processing, it was essential to emphasize the basic concepts that could be applied to all activities

within the area of food dehydration and drying. It was reasoned that the training manual could provide the essentials and outline the basic principles, while local mentors could assist in bridging any gaps between what was in the manual and what was being observed in the participants' particular processing activities.

Based on this approach, it was possible to identify a set of essential skills that would be required of a food industry worker involved in food dehydration and drying, regardless of the particular product being dried. These skills would vary depending upon the level of expertise of the worker. It was also considered that all participants taking the training should develop a common level of understanding of food drying in general before moving on to more advanced topics. Such an approach would address concerns regarding the entry level skills of the participant and create a reasonably uniform background for additional training within the Food Dehydration and Drying Module.

A major consideration was that attention be directed to Competency Based Education and Training (CBET), where the objectives are to learn skills and how to apply them. This is in contrast to the typical university degree program which utilizes a Knowledge Based Education and Training (KBET) approach, where emphasis is routinely placed on the uptake of knowledge from textbook sources. The CBET approach was consistent with the fact that course participants were expected to be process operators and those working on the factory floor.

The target audience also had a number of other unique needs. Due to family commitments and other factors, many potential participants were unable to relocate to larger centers to access formal training at colleges or universities; they had to maintain their employment to support financial obligations to their immediate and extended families and fulfill other related obligations. Availability of time was another consideration. Course offerings within a structured or rigid framework was not a viable option to fit in with the life style patterns of those who needed the training the most.

It was also important to understand why individuals might be seeking training. One major reason was so that they could develop the necessary skills to advance within their place of employment and enhance their potential earning power. There also seemed to be an underlying desire to learn. Opportunities to learn new skills did not appear to be as plentiful as they might be to younger persons without the responsibilities of families. In the case of the Distance-Assisted Training Initiative, training opportunities literally came to those who were interested in pursuing them.

11.5 Establishing the Level of Instruction

The Food Dehydration and Drying module was designed with three distinct sections. An Introductory Level would provide participants with the background information deemed necessary to understantd the basics of drying and develop some of the essential basic skills required in any food drying activity. At the Intermediate Level, actual drying scenarios would allow participants to see how an understanding of the

principles of drying could be applied to specific situatsions. Finally, an Advanced Level course would delve more deeply into the actual operation of a food dryer and examine how to troubleshoot dryer problems.

The following is a summary of the key subject matter areas covered in each of the three Food Dehydration and Drying Courses:

Introduction to Food Dehydration and Drying

Getting Started

Learning Objectives
Definition of Drying and Historical Development
Reasons for Drying Foods
Methods of Water Removal (Traditional and Nontraditional)
Factors Influencing Drying (Product and Equipment Attributes)
Effects of Drying on Products

Organizing Information

The Unit Operation Approach
Process Flow Diagrams
Tables and Graphs
Organization in Problem Solving
Practice Problems

Dimensional Analysis Approach to Problem Solving

Conversion Factors
Dimensional Analysis
Sample Calculations
Practice Problems (with answers)

Wet and Dry Basis Moistures

Definitions of Wet and Dry Basis Moistures
Wet and Dry Basis Moisture Conversions
Methods of Moisture Determination
Case Studies
Practice Problems (with answers)

Sources of Information

Intermediate Course in Food Dehydration and Drying

Getting Started

Learning Objectives
Review of Introductory Course
Practice Review Problems (with answers)

Thermal Properties of Food Materials

Thermal Properties of Water
Sensible Heat and Latent Heat
Thermal Properties of Foods
Heat Transfer Mechanisms
Impact on Food Drying
Case Study #1
Practice Problems (with answers)

Calculating Heat to Dry a Product

Data Organization and Preliminary Calculations
Theoretical Values versus Production Values
Role of Time in Drying
Case Study #2
Practice Problems (with answers)

Drying Mechanisms

Heat Transfer Mechanisms (revisited)
Stages of Drying
Critical Moisture Content
Calculation of Water Removal Rates

Drying Curves

What Are Drying Curves?
Case Study #3
Caveats in Scale-Up
Practice Problems (with answers)

Types of Dryers

Direct and Indirect Heating
Batch and Continuous Dryers
Airflow in Dryers
Types of Dryers

Dryer Operation: Case Study

Case Study #4
Sources of Information

Sources of Information

Advanced Course in Food Dehydration and Drying

Getting Started

Learning Objectives
Previous Course Material
Review: Mango Drying Case Study

Process Control in a Drying Operation

Basics of Process Control
Moisture Sampling
Case Study #1: Moisture Testing
Process Control Mechanisms

Psychrometrics

Definitions and Associated Terminology
Sample Problems and Calculations
Practice Problems (with answers)
Case Study #2: Applications of Psychrometry to Drying
Case Study #3: Drying Feasibility Study
General Comments

Troubleshooting

Drying Problems
General Comments

Summary Comments

Things to Keep in Mind
Sources of Information

11.6 Preparing Instructional Material

Throughout the entire Dehydration and Drying module, it was important to maintain the proper perspective on both the level of complexity of the material, and its relevance to the target audience. Previous experience had shown inherent problems in using common North American examples of commercially available dried food products when providing instruction to various groups around the world. In many classrooms, using the conversion of grapes to raisins may be appropriate to demonstrate how drying can convert a product into an entirely different form. However, in Equatorial Guinea, this example was totally irrelevant, since raisins were not available there and were completely unfamiliar to the local residents. This emphasized the need to employ local products as examples whenever possible for the sake of clarity in understanding the concepts being illustrated.

For this reason, a variety of common examples typical of those found throughout tropical and subtropical regions were used. For case studies, apples and mangoes were considered as being appropriate. In the Advanced course, where psychrometrics played an important role, it was not necessary to specify a particular product being dried. In these examples, emphasis was placed on the air itself rather than on the material being dried. In sample problems peas, beans, carrots, papaya, mangoes, apples, and tomatoes were routinely cited. Occasionally, guava, artichokes, cherries, beets, parsley, and other examples were used. However, less common fruits, vegetables, and herbs were not used in major case study examples and were only included in sets of multiple examples where a lack of familiarity with particular items would not reduce the level of understanding of a particular topic.

Preparing the actual instructional material to be included was a relatively straight-forward process once the degree of proficiency was identified for each training level. At the Introductory level, the key was to familiarize course participants with the concepts of drying in general and to make them aware of the role of drying in food processing. It was also felt that developing basic organizational skills that would be used in later problem solving should be included. Calculations of wet basis and dry basis moisture contents provided a link to the Intermediate level course while introducing participants to essential skills necessary for understanding how much water was present in a product before and after drying. "Unit operations" was included as a means of understanding the importance of individual steps in a more complex food process. In order to introduce the handling and interpretation of data, exercises involving the plotting of temperatures versus time were included in the instructional material. It should be noted that a module on "Numeracy" was also developed so those with only minimal mathematical skills could hone their skills.

Completion of the Intermediate course was felt to be the level to which most participants should be trained. This would provide them with the essential skills to be fully functional in a food drying environment as process operators or assistants. Participants would have acquired an understanding of the reasons for drying and the basic concepts in the introductory course. The intermediate course would build on this and examine the thermal properties of food, which have an impact on their processing, as well as looking at the mechanisms of drying. Drying curves were included to show the changes in moisture during a drying process, plus there is a description of various types of dryers for different applications. A chapter on calculating the amount of heat required to dry a product links theoretical considerations to practical applications.

The advance level course was designed to provide opportunities to those who wanted to go beyond the skill set provided by completing the Introductory and Intermediate levels of training. Basic drying process control, psychometrics, and troubleshooting are essential for anyone who has the responsibility for making adjustments to a drying process, but are not required by those performing routine activities within a food drying facility.

11.7 Presentation and Delivery of the Course Material

One of the first decisions to be made after developing the course material was how to present it to participants. Having a hard-copy version of a course manual was felt to be important, since the manual would serve as a future reference source. A manual was then prepared for each of the three levels. Care was taken to allow accessibility to each of the pages in the manual so that they did not appear intimidating to anyone. Individual steps were shown for all calculations, with notes explaining why various things were being done. The material itself was arranged in chapters that corresponded to a specific topic such as "Unit Operations" or "Dimensional Analysis" in the Introductory Course. Initial draft copies of each of the three manuals were reviewed by Fellows of the International Academy of Food Science and Technology for content and applicability.

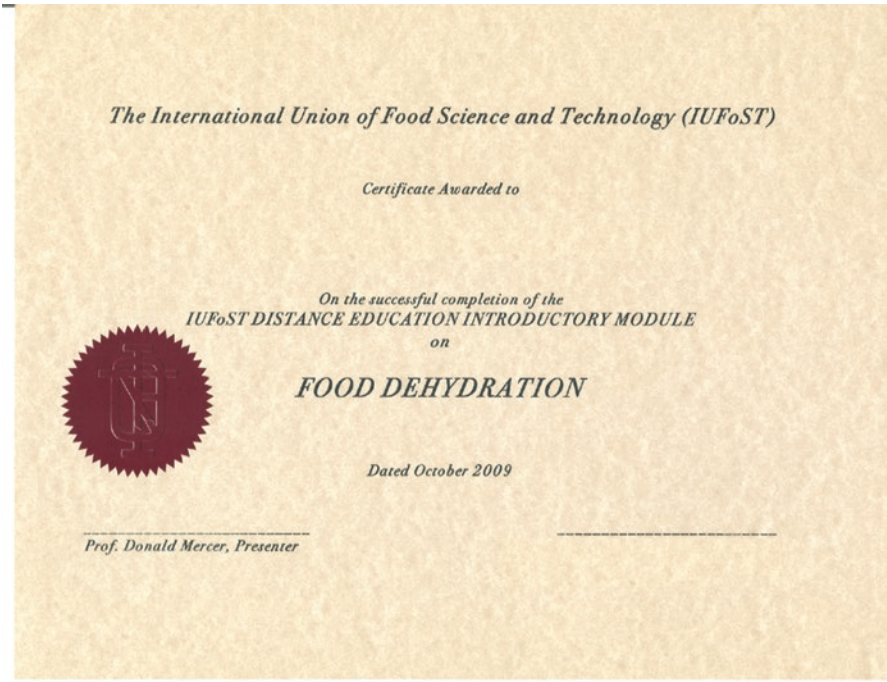
Distributing course material to participants in widespread geographical locations was initially not recognized as being a challenge. It was felt that all material would be distributed electronically to the course mentors, rather than printing hard copies and sending them from a central site. Mentors would then print hard copies of the material for each participant. Unfortunately, the level of Internet connectivity in some areas was not adequate to permit such an approach. Initially, this problem was solved by posting the material for each course manual in smaller sections on a secure section of the IUFoST website where mentors were able to download the chapters more readily. However, with the development of the improved website, this problem no longer exists.

As it now stands, course mentors are given access to the restricted material on the IUFoST website. They then print the manuals for the participants with whom they are working. In order to offset these and other out-of-pocket expenses incurred by the mentors, a small honorarium is provided on the basis of the number of participants being supervised.

11.8 Assessing and Recognizing Levels of Competency

Courses offered through the IUFoST Distance-Assisted Training Initiative are not officially recognized by universities or colleges. They are designed solely for the purpose of providing a means for food industry workers to obtain training that can assist them in advancing within their chosen area of employment. However, it is necessary to have some indication of the uptake of knowledge and the development of skills from this training.

For each of the training courses within the Food Dehydration and Drying subject module, a set of assignments was prepared. Typically, there are four or five assignments per course. Each assignment covers a specific subject area which may be contained within a single chapter of the course manual, or within two smaller chapters of the manual in some cases.


The International Union of Food Science and Technology (IUFoST)

Certificate Awarded to

On the successful completion of the
IUFoST DISTANCE EDUCATION INTRODUCTORY MODULE
on

FOOD DEHYDRATION

Dated October 2009

Prof. Donald Mercer, Presenter

Fig. 11.9 Sample of a certificate provided to those completing a course

Assignments are designed to assess the competencies that have been acquired. As an example, one of the first things to be accomplished in the Introductory Course is to have participants visit local markets or grocery stores and prepare a list of foods that are available in both fresh and dried forms. Briefly describing the differences and discussing them with their mentors helps to gauge their level of awareness in this regard. Other assignments build on the preceding ones with the addition of calculations and introduction of more difficult concepts. These assignments have been prepared by the module developer and, as pointed out above, are available on the IUFoST website for mentors. A set of suggested or potential answers is also provided as a marking aid. Once an assignment has been marked, the mentor will forward the mark to IUFoST and/or the module developer. A mark of 50 % on each assignment is considered as being a passing mark.

Those participants who successfully complete a course deserve some form of recognition for their efforts. In addition, documentation is necessary as a means of indicating to their employer that they have taken a training course. Since there is no official affiliation with any academic institutions, IUFoST has designed a certificate stating that the individual has successfully completed a certain training course. The certificate, as shown in Fig. 11.9, is signed by the Module Developer and the Mentor before being presented to the participant. In at least one of the pilot offerings, the certificates were presented by a company official in a small ceremony to recognize the individual.

11.9 Initial Pilot Offering of the "Introduction to Dehydration and Drying" Module

In June 2009, an initial pilot offering of the "Introduction to Food Dehydration and Drying" module took place, with eight participants and seven mentors. The participants represented five countries: Ethiopia, Kenya, Mauritius, Nigeria, and South Africa. Although the training courses were aimed at food industry workers with a high school or partially completed high school education, the academic qualifications of the participants in the pilot offering were extremely diverse. Seven mentors were able to secure eight participants whose formal education ranged from ninth grade education to two participants with degrees from universities. Although this was initially thought to present a potential challenge in keeping the participants engaged, it proved not to be a problem since the subject of food dehydration was new to all of the participants.

11.10 Mentor and Participant Evaluations and Feedback

Following completion of the final assignment in the module, an evaluation form was e-mailed to each mentor for completion. In addition, an evaluation form was sent to each mentor to distribute to his or her participant(s). The evaluation form was created with the assistance of "A Distance Education Consortium" (ADEC) located at the University of Nebraska.

The average responses to each of the questions by the mentors and the participants are summarized below. All scores were based on a five-point scale.

From the responses of the mentors, it was clear that:

- the instructor had provided sufficient information to the mentors (average 4.7)
- the instructions were clear and informative (average 4.3)
- generally, the mentors would participate again (average 4.6)

Regarding the presentation of the module itself, the mentors:

- were generally satisfied that the time allotted for each assignment was sufficient (average 3.4)
- felt that their time commitment was just about right (average 2.7)
- thought that they were reasonably involved with the participants whom they were mentoring (average 3.0)
- felt that the participants appreciated the course (average 4.4)
- agreed that the difficulty of the questions was appropriate (average 3.0)
- believed that the questions addressed relevant issues (average 4.3)
- considered that there were about the right number of questions (average 3.3)

In the open comment section, the mentors thought access to the website could be improved, but did not recommend major changes to the module itself. In summary,

the mentors gave high marks to the module leader, the module and questions themselves, and the opportunity to participate in the test.

The participants were extremely complimentary on the exercise itself. They felt:

- the information they were provided was excellent (average 4.4)
- the instructions were clear and informative (average 4.3)
- they would be willing to participate in future offerings (average 4.4)
- the information they received would be useful to them (average 3.4)
- the time allotted to each assignment was sufficient (average 3.0)
- the level of the assignments was about right (average 3.1)

The participants appreciated their mentor's involvement (average 4.6) and generally had follow-up discussions with their mentor (average 4.3).

From the comments, the participants were very appreciative of the module, their mentors, and the opportunity it gave them to improve their education. All felt that it would assist them in doing their job better.

11.11 Expanding the Audience

The original target audience for the Distance Education Initiative was food industry workers in sub-Saharan Africa. This was based on United Nations Millennium Development Goals and needs identified within the South African Development Community (SADC). With little or no modifications, the course material could easily be transferred to other regions and provide training support on a global basis.

Now that the courses have been successfully tested in sub-Saharan Africa, they can be offered on a worldwide basis. IUFoST has been fortunate in obtaining offers from a number of organizations to translate material into French, Spanish, and Portuguese to further enhance their uptake.

11.12 Additional Support Material

As mentioned previously, not all participants in the courses have a solid background in mathematics. In order to assist them, a "Numeracy Guide" has been prepared and is available on the IUFoST website to the mentors, who can then pass this information on to those requiring it.

As additional resources are developed, the IUFoST website will begin to include articles of specific interest to those attempting to set up their own cottage-scale food processing operations, such as the preparation of jams and jellies, or dried fruits and vegetables.

The work of IUFoST is an ongoing evolving process and welcomes input from anyone who might be able to provide additional support material. Please contact the IUFoST Secretariat for indications of interest and to learn more (IUFoST Secretariat secretariat@iufost.org).

Chapter 12
Food Science and Technology Undergraduate and Graduate Curricula in North America

Rickey Y. Yada, Charity Parr-Vasquez, and Brian C. Bryksa

12.1 Introduction

Like many programs around the world, food science/technology curricula in North America, at both the undergraduate and graduate levels, have evolved from commodity focused courses (e.g., dairy, horticulture) to programs based on the core disciplines of food science/technology (i.e., food microbiology, chemistry, engineering/processing, food safety/law). More recently, various programs recognize the strong relationships among food, nutrition and health, as well as the importance of other disciplines (e.g., business and culinary arts). Current curricula are focusing on soft skills (i.e., communication, team work, problem solving), as well as the use of social media technologies (e.g., YouTube, Instagram, and Twitter). Although course content still remains important, program development has shifted from strictly assessing content to assessing the ability to use course content to problem

Some of the information in this chapter has been previously reported in the IUFoST World of Food Science article "Food Science and Technology Undergraduate and Graduate Curricula in Canada and the United States" (http://worldfoodscience.com/content/food-science-and-technology-undergraduate-and-graduate-curricula-canada-and-united-states-0) and has been extracted from the corresponding websites.

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solve (i.e., outcome based learning). The ability to pursue degrees via distance learning and/or without research (e.g., non-thesis master's degrees, certificate programs) has arisen from developments in communication technologies, the recognition that experts may not reside at a single location and from a need for options that suit students who do not have time to undertake lab research. The advancement of new disciplines (e.g., "omics," nanoscience/technology) is also being reflected in some curricula. This chapter briefly discusses the above topics and outline some quality assurance approaches to food science education in North America as well as highlights innovative and creative food science/technology programs.

12.2 Approval Processes

12.2.1 Institute of Food Technologists (IFT)

While individual undergraduate Food Science programs in North America undergo quality assurance processes within their own institutions, many, if not all of the departments in Canada and the USA, and a select few in Mexico, have requested approval by the Institute of Food Technologists (IFT) (http://www.ift.org/Community/Students/Approved-Undergrad-Programs.aspx). IFT is an organization with the mission to advance food science and ensure a safe and sustainable food supply that contributes to the health of people globally. Originally established in 1966, IFT educational standards have evolved to their current state (see Table 1. Core Competencies in Food Science in the document http://www.ift.org/~/media/Knowledge%20Center/Learn%20Food%20Science/Become%20a%20Food%20Scientist/Resources/Guide_Approval_UndergradFoodSci.pdf) and are intended to help institutions evaluate undergraduate programs and their academic selection, guidance, and preparation of students. In addition, these standards are designed to enhance the excellence of undergraduate food science programs and offer students the highest scientific training and professional development skills. Students enrolled in IFT-approved programs are eligible to apply for IFT scholarships, and approximately 50 undergraduate programs from around the globe are currently approved by IFT.

Applications for IFT approval are adjudicated by three higher education review board (HERB) members. HERB members will evaluate applications for their description of curriculum, inclusion of core competencies in programs, evidence of course and whole program outcomes and assessment, and indications of how the results of the assessment will be used to alter the curriculum to improve the overall student experience. Outcome based measures are a core tenet of the adjudication process, where emphasis is placed on student learning as opposed to the specific knowledge being offered in a program of study.

12.2.2 International Union of Food Science and Technology (IUFoST) (http://www.iufost.org/)

In addition to IFT, the International Union of Food Science and Technology (IUFoST) is currently developing guidelines for food science undergraduate programs(http://worldfoodscience.com/content/iufost-guidelines-recognition-curricula-food-science-0). IUFoST is a country-membership organization that connects the world's food scientists and technologists to advance food science globally. In recognition of the fact that food science and technology programs are globally diverse, and catering to a range of local realities and educational practices, the intent of the proposed IUFoST guidelines is not meant to be overly prescriptive; however, undergraduate programs seeking approval should meet certain basic criteria. The Education Committee of IUFoST has recommended that programs should include fundamental, traditional science courses combined with innovative student experiences such as internships in industry/government/research laboratories. The IUFoST approach stresses that assessments should be based on outcomes both at the course and program levels. Prescribed schedules of study are not given, but rather the outcomes of such programs are emphasized.

The proposed IUFoST guidelines highlight that programs should include courses on the fundamental, hallmark disciplines of food science including food chemistry; analysis and nutrition; microbiology, safety and environment; and engineering and technology. Additional courses may include food management and business. Programs must be 4–5 years in length with the first 2 years focused on fundamental courses. Those offering a fifth year must be more advanced than fourth year courses, and include extended courses, projects, and/or research periods or industry internships. The proposed IUFoST guidelines stress the significance of the use of active teaching methods that enhance personal skills such as communication. Courses must integrate both traditional class-based study with practical and laboratory experiences. Independent projects, traditionally undertaken at the end of programs, should combine the various disciplines of food science and technology. Universities seeking recognition and approval by IUFoST will have to submit an application to the IUFoST Education Committee for adjudication. The Committee is currently developing the minimum specific requirements for approval; until these are established, programs will be evaluated against internationally accepted best practices. Programs will be evaluated on curriculum and course content, and program quality as measured by incoming and outgoing student assessment, faculty, facilities, and graduates.

12.3 Food Science Programs

Food science is a continually growing and evolving field, and undergraduate food science programs across Canada, the USA, and Mexico have evolved in parallel with that trend. Today, programs continue to offer "traditional" core food

science courses—food chemistry, sensory science, food engineering, and food microbiology. Additionally, unique courses and opportunities that better prepare the next generation of food science graduates for the increasingly demanding workforce are also being incorporated. More than ever, employers in the food sector require employees who are not only competent in advanced scientific techniques, but are also equipped to deal with regulatory and social issues, and who have knowledge and competencies in the "soft skills" (e.g., communications, leadership, ability to teach).

Examples of some of the current trends within food science programs at the undergraduate and graduate levels in Canada, the USA, and Mexico are given below. Note that the university programs listed are examples and are not meant to be either comprehensive and/or all-inclusive with the information extracted from the corresponding websites.

12.4 Undergraduate Programs

12.4.1 Cornell University, Ithaca, USA (http://www.cornell.edu/)

The Department of Food Science at Cornell University (http://foodscience.cornell.edu/) focuses on: food science, which combines fundamental and applied sciences courses; and food operations and management, which trains students in the principles and practices of efficient management of food processing, preservation, distribution, and marketing operations; and "food safety" (https://foodscience.cals.cornell.edu/undergraduate/degree-options-and-requirements). In addition to traditional academic experiences, Cornell offers a Summer Scholar Program where undergraduate students participate in a 10-week research program. Students are given the opportunity to conduct research with a faculty member at Cornell, North Carolina State, Purdue or Texas Tech University, participate in field trips to food companies, interact with representatives of industry, government, and academia, and develop soft skills.

12.4.2 Purdue University, Lafayette, USA (http://www.purdue.edu/)

Purdue offers two Majors in Food Science: Culinary Science and Food Science (https://ag.purdue.edu/foodsci/Pages/majorsminors.aspx). Within the Food Science Major, students have the option of completing an honors degree, which allows them to complete advanced level science and mathematics courses and conduct an honors project under the supervision of a faculty member. In cooperation with Ivy

Tech Community College, the Department of Food Science at Purdue University offers a unique program in Culinary Science. Students complete the first 2 years of the program at Ivy Tech Community College before completing their final studies at Purdue. This applied approach to learning makes students extremely valuable to companies in the food industry.

12.4.3 Tecnolólgico de Monterrey, Monterrey, Mexico (http://www.itesm.edu)

Tecnológico de Monterrey offers a degree in Food Engineering that is focused on the design, improvement, development and production of foods using the most modern information technology, engineering, biotechnology, and nanotechnology (https://serviciosva.itesm.mx/PlanesEstudio/Consultas/Planes/ConsultaPlanEstudio.aspx?form=PLANESTUDIO&contenido=caratula&modovista=default&Idioma=ING&claveprograma=IIA11&UnaCol=NO&VerReq=&VerEqui=&IdTipoArea=&Materia=). Graduates have solid knowledge in biological areas as well as skills in experimentally integrating normative aspects and sustainability. The program seeks to train students in designing products and processes that meet the needs of modern society while considering technical, economic, environmental, legal, social, political, ethical, health, and sustainability aspects. Students gain competencies in developing projects and solving problems in all stages of the food production system, and learn to communicate professionally in both English and Spanish.

12.4.4 University of Guelph, Guelph, Canada (http://www.uoguelph.ca/)

The University of Guelph offers a Major in Food Science with a co-op option (https://www.uoguelph.ca/foodscience/current-students-current-undergraduate-students/undergraduate-programs). In obtaining a Food Science degree at the University of Guelph, students will integrate the science of chemistry, microbiology, and physics with the study of food processing and development. In addition, students will also be exposed to the fields of law, health, nutrition, and security in relation to food science, processing, and development of food products and processes. During a co-op degree, students have the ability to work and receive hands-on training in industrial processes and practices such as pasteurization, fermentation, packaging, product development, and sensory evaluation with leading Canadian food companies, thereby preparing them for employment. Additionally, the University of Guelph has responded to the rapid growth in the food for health consumer oriented market by offering a minor in functional foods and nutraceuticals (http://www.

uoguelph.ca/registrar/calendars/undergraduate/current/c10/c10bsc-nans.shtml). The Department also offers a Certificate in Food Science for individuals who wish to obtain a general understanding of food science, and who have no previous formal food science training. This certificate is intended for an audience that may include production supervisors, technicians looking to upgrade qualifications, marketers seeking additional information about their products, dietitians and nutritionists, and government food inspectors. The certificate is composed of five degree-credit courses in food science delivered in a distance education format using print, DVD, online courses and online conferences, and includes virtual group work. The certificate is comprised of five core curriculum courses including Principles of Food Science, Introduction to Food Chemistry, Introduction to Food Microbiology, Introduction to Food Analysis, and Introduction to Food Processing (http://www.foodsciencecertificate.com/).

12.5 Graduate Programs

Graduate programs across North America have evolved, including a proliferation of non-thesis based and/or interdisciplinary graduate programs.

12.5.1 Non-thesis Master's Programs

12.5.1.1 University of Guelph, Guelph, Canada (http://www.uoguelph.ca/)

The University of Guelph offers a non-thesis master's degree in Food Safety and Quality Assurance (MSc FSQA) focusing on food safety monitoring and maintenance in the food industry and government. Students acquire knowledge in the following areas: fundamentals of food policy development, Canadian and international food law, applied aspects of total-quality management, food safety management systems, risk analysis, and detection and epidemiology of foodborne pathogens (https://www.uoguelph.ca/registrar/calendars/graduate/current/gradprog/fsqu.shtml). A graduate diploma is also offered in Food Safety and Quality Assurance (GDip FSQA) which is specifically intended for individuals who are currently employed in the food industry and who wish to obtain advanced knowledge of food safety and quality assurance. The GDip FSQA offers graduate-level study of theory and research as well as knowledge on the general principles of food safety and quality assurance, including microbiology and chemistry, domestic and foreign regulations, food risk analysis, and quality management (https://www.uoguelph.ca/foodscience/future-students-future-graduate-students/food-safety-andquality-assurance-program-fsqa).

12.5.1.2 Virginia Polytechnic Institute, Blacksburg, USA (http://www.vt.edu/)

The Master's of Food Safety program offered by Virginia Polytechnic Institute offers an online non-thesis degree. It is a broad based program suitable for students with interests in agricultural and life science industries, governmental agencies serving agriculture, cooperative extension and agricultural education. Students can choose a focus from a variety of areas, including: Food Safety; Biosecurity, Bioregulations, and Public Health; Education; Environmental Science; or Plant Science and Pest Management (http://www.fst.vt.edu/graduate/msonline_fs.html).

12.5.1.3 University of Illinois, Urbana, USA (http://illinois.edu/)

The Professional Science Master's degree in Food Science and Human Nutrition offered by the University of Illinois is a 16-month, non-thesis program. It combines science with management and leadership training by integrating a solid core graduate-level curriculum with business courses such as management and marketing, accounting and finance, and project management. The program consists of four key components: science, business, industry seminars, and internship. Students are given the option to obtain degrees focussed on agricultural production, bioenergy, food science and human nutrition, plant biology, and technical systems management (http://psm.illinois.edu/prospective-students).

12.6 PhD Programs

Many PhD programs in North America have traditionally been inter/multidisciplinary by nature, given the complexity and the need for a comprehensive and in-depth investigation of the given problem being examined by the candidate. This situation has been intensified with the development of new areas of research such as "omics" (proteomics, genomics, nutragenomics, nanotechnology, etc.), where the candidate often requires the advice of faculty/researcher expertise and/or courses from outside those traditionally found in food science departments. Course requirements vary from no course requirements (unless recommended by the candidate's advisor and/or advisory committee, e.g., University of Guelph (https://www.uoguelph.ca/foodscience/current-students-current-graduate-students-graduate-handbook/students-program-studies-and-research)) to required courses (e.g., University of California—Davis (https://gradstudies.ucdavis.edu/sites/default/files/files/graduate_program/degree_requirements/2003-gfsc.pdf)).

12.7 Interdisciplinary Graduate Programs

As with undergraduate programs, various innovative and creative interdisciplinary programs have recently become more prevalent and are neither the domain of a single department nor college.

12.7.1 Universidad de las Americas Puebla, Puebla, Mexico (http://www.udlap.mx/home.aspx)

The Biotechnology master's degree at the Universidad de las Americas Puebla is a four semester interdisciplinary program that provides an opportunity to acquire and apply biotechnological knowledge and techniques in food engineering applications (e.g., food preservation, manufacturing, processing) (http://www.udlap.mx/oferta-academica/conoce.aspx?cveCarrera=MBT&idioma=2). Through intensive course work as well as a research thesis, students navigate the research process from initial design of a research project, ensuring a keen understanding of the theoretical framework, to ultimate completion. The program stresses the significance of soft skills such as oral and written scientific communication.

12.7.2 Biophysics Interdepartmental Group: University of Guelph, Guelph, Canada

The Biophysics Interdepartmental Group (BIG) at the University of Guelph is a relatively new program at the institution (http://www.uoguelph.ca/biophysics/). Through master's and doctoral studies, BIG is a unique program that seeks to further our understanding of biological processes through the application of the concepts and techniques of the physical sciences. Faculty members currently involved in BIG are from several colleges (i.e., College of Physical and Engineering Science, College of Biological Science, Ontario Agricultural College and Ontario Veterinary College), and represent areas such as Biomechanical Biophysics, Computational Biophysics, Cellular Biophysics, Molecular Biophysics, Structural Biophysics, and Food Science. MSc and PhD students are granted degrees from BIG, although the majority of their research is done in the department of their advisor.

12.7.3 Dual Major PhD Program in Toxicology at Michigan State University, East Lansing, USA

In addition to traditional MSc and PhD degrees, Michigan State University offers a unique joint Food Science and Environmental Toxicology PhD program (http://cit.msu.edu/training/doctoral_program.html). This multidisciplinary dual-degree

graduate program is offered through Michigan State University's Center for Integrative Toxicology (CIT) in conjunction with food science, and offers training in basic food science coupled with rigorous teaching about how chemicals, drugs, and naturally occurring toxins can cause harm. The program offers two tracks for students, one intended for trainees with limited background in biology, and the second for students with a background in biology. The dual-major offers Michigan State University students increased flexibility and increased interaction with faculty from different disciplines.

12.7.4 Rutgers University, New Brunswick, USA (http://www.rutgers.edu/)

Rutgers University's unique University Professional Science Master's program brings together science, and business and policy (http://psm.rutgers.edu/). The Food Science Master of Business and Science degree requires that students complete a combination of 24 food science and 19 business credits (http://psm.rutgers.edu/food-science). The emphasis is on training students in advanced food science principles, exposing students to the fundamentals of business and management, and value-added professional development opportunities such as internships. The program seeks to produce future leaders and managers in the food industry.

12.8 Conclusions

Food Science curricula at both the undergraduate and graduate levels are evolving to reflect the need for greater emphasis on outcome based courses/programs, the development and encouragement of more hands-on/internship experience, as well as courses regarding "soft" skills (e.g., communication skills, team work, bilingual training). Programs and courses are also responding to recent developments in research and technology (e.g., "omics" and nanotechnology) as well as students' and employers' interests in areas not traditionally associated with food science and technology (e.g., business, culinary arts). Finally, the discipline of food science, by definition, has been inter/multidisciplinary; however, we are now experiencing the formalized creation of new creative programs involving food science departments (e.g., BIG, University of Guelph) which further expand the expertise, knowledge and infrastructure available to our food science and technology students.

Chapter 13
Food Science and Technology Curricula in Africa: Meeting Africa's New Challenges

Amanda Minnaar, John R.N. Taylor, Steven Haggblade, John David Kabasa, and Nelson K.O. Ojijo

Bending the Nutrition Transition Curve in Africa (BCC Consortium)

13.1 Introduction

Africa faces many critical challenges, chief among them raising the quality of its human capital, accelerating economic development and improving its peoples' well-being. Food science and technology (FST) in Africa has a critical role to play in contributing to increased food product manufacturing, improved worker productivity and incomes, and ensuring adequate food supplies, human nutrition, and good health.

Africa is changing rapidly, and so are its challenges. Across the continent, sustained economic growth now averages about 4 % per annum (Africa Economic Outlook 2012). At the same time, the population continues to grow at roughly 3 % per annum and is expected to double to two billion people by 2050 (FAO 2009).

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Even more startling is the continent's rapid urbanization. As a result, Africa's food consumption patterns will change dramatically over the coming decades. At the same time, evidence strongly suggests that dietary changes and accompanying lifestyle changes are driving a rapid "Nutrition Transition," leading to major health problems, such as an increase in obesity and its associated diseases. Food Science and Technology (FST) and nutrition professionals will play a key role in heading off these looming public health problems by developing nutrient-rich, sensorially appealing, low-cost foods, helping to improve people's health and ramping up food and nutritional sciences education at all levels.

This chapter strategizes how FST in Africa needs to develop to meet the challenges of the continent's rapidly changing food systems. Firstly, Africa's changing food systems will be examined with a focus on the markets and the nutritional and human health implication of these changes. Next, the role of FST professionals in this changing environment will be mapped out, examining their current and potential roles across the food sector, from the private food industry (multinationals to small, medium, and micro enterprises (SMMEs)), in public health and in shaping the polices of government at all levels. Then, the results of a snapshot survey of current university FST education and training across Africa will be examined in the context of the needs of this broad food sector. The chapter concludes with the authors' vision of a strategy for FST curricula to meet Africa's new challenges in food science and nutrition education and training.

13.2 Africa's Changing Food Systems

13.2.1 Changing Food Markets

Rapid urbanization and growing per capita incomes will trigger major changes in African food systems over the coming decades. With urban population growing at about 3.4 % per year (UN Habitat 2012), current projections suggest that Africa will become majorly urban by 2030 (Fig. 13.1). The corresponding decline in rural and farm population shares will translate into a rapidly increasing share of marketed food. Although the value added from on-farm food production will roughly triple in Africa over the next 40 years, to keep pace with the continent's doubling population and shifting tastes, marketed volumes will increase by a factor of 6 (Haggblade 2011). As a result, under most plausible future scenarios, the fastest growing segments of Africa's food systems will be in the post-farm segments of the supply chain, i.e., in food processing, distribution, and marketing.

The composition of food consumed will change rapidly as well. Urbanization and growing per capita incomes will translate into greatly increased demand for processed foods, high-value foods (e.g., dairy, meat, and fresh fruits and vegetables), packaged convenience foods and prepared foods. The agribusinesses emerging to meet this growing demand will require food scientists and technologists with

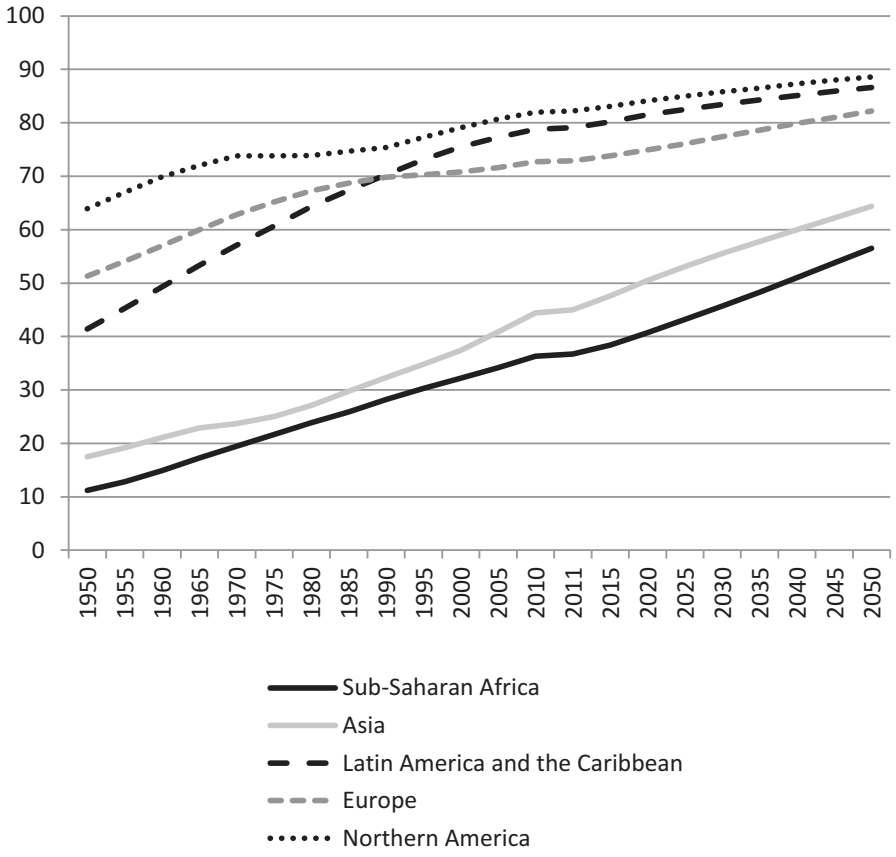

Fig. 13.1 Trends in urban share of total population, by continent. *Source*: United Nations Department of Economic and Social Affairs (2010)

expertise in modern food processing and food safety technologies. Markets for cereals, in contrast, will grow more slowly than for processed and prepared foods. Increasingly, food grain demand will become tied to the livestock sector and derivative demand for animal feeds.

Burgeoning urban demand for fresh fruits and vegetables is placing growing pressure on Africa's urban horticulture markets. Unlike processed foods and packaged dry goods, which increasingly rely on supermarket distribution channels, fresh fruit and vegetable markets remain dominated by traditional wet markets (Reardon 2007). Detailed studies from Kenya and Zambia, for example, indicate that traditional retailing through open air markets and street vendors accounts for over 90 % of all fresh produce marketed. Supermarkets, in contrast, handle less than 5 % of horticulture retailing (Tschirley et al. 2010).

Yet rapid growth in urban fruit and vegetable markets, coupled with limited investment in wholesale wet market facilities, inadequate town planning, poor zoning, traffic congestion, and often deplorable sanitation, results in high losses (in the range of 25–40 %), high prices for consumers, and low prices for farmers and traders. Despite the potential nutritional gains from growing purchases of nutrient-rich fresh fruits and vegetables, high prices and poor sanitation remain challenges.

This shifting structure of food demand holds two major implications for FST. On the supply side of Africa's growing food markets, agribusiness firms increasingly require employees with technical skills in food biochemistry, food processing technologies, packaging, food safety, storage, logistics, and distribution. On the demand side of these growing urban food markets, the widespread shift in consumers' diets holds critical implications for human health and nutrition. As a result, both public and private employers will require employees with technical training in food safety, food standards, and nutrition.

13.2.2 Nutritional Implications

All countries in sub-Saharan Africa continue to suffer from unacceptable levels of undernutrition, particularly among young children in rural areas. In sub-Saharan Africa, some 28 % of children under 5 years are moderately or severely underweight (UNICEF 2007). A major cause is unbalanced diets, which are often deficient in micronutrient-rich foods and may also have low bioavailability of essential micronutrients (FAO and ILSI 1997). Africa today has also entered the so-called "Nutrition Transition." Popkin (2003) describes the Nutrition Transition in terms of the large shifts that have occurred in diet and physical activity patterns, particularly in the last decades of the twentieth century. The diet of modern societies seems to be converging. It is characterized by high levels of saturated fats, sugar, and refined foods, and low levels of fiber. At the same time, there is a change in lifestyles, characterized by lower levels of physical activity. Associated with the change in diet and lifestyle is a shift in anthropometric factors (i.e., an increase in average stature, body composition) and changes in disease patterns.

Popkin (2003) further identifies three stages of Nutrition Transition. In Stage 1, food scarcity (and in extreme cases famine) begins to recede as income rises. During Stage 2, changes in diet and activity pattern lead to the emergence of new disease problems and increased disability. Finally, in Stage 3, behavioral change begins to reverse the negative tendencies and make possible a process of successful aging. South Africa, for example, has advanced far into Stage 2 of Nutrition Transition, from a predominantly rural population where people obtain most of their food through their own farming activities to an urban population purchasing its food from supermarkets, fast-food outlets, and street vendors. Other African countries are following closely in this transition.

The rapid pace of the Nutrition Transition means that Africa increasingly faces the double burden of simultaneously high rates of undernutrition and overnutrition

(or more strictly speaking excessive energy consumption). Across Africa, very disturbing health and disease consequences are accompanying this transition. A survey by GlaxoSmithKline in 2010 (Guardian 2010) revealed that more than 60 % of South Africans are overweight. Most worryingly, 17 % of children under 9 years are overweight. Even in Tanzania, a low-income country, a survey of equal numbers of adult women involved in farming, housework, and business found that 49 % were obese (mean BMI 30) and only 4 % were chronically energy deficient (mean BMI 17.5) (Mosha 2003). The association of noncommunicable lifestyle diseases with urbanization in Africa has been revealed by research findings by the South African Medical Research Council, which showed that 15 % of urban people in Africa have high blood pressure compared to 5 % of the rural population (Mbewu 2009). The research also revealed that today in South Africa, some 25 % of deaths result from cardiovascular disease, and across Africa the incidence is 11 %. The incidence of type 2 diabetes is also increasing rapidly in sub-Saharan Africa. The Diabetes Leadership Forum (2010) predicted that levels will double by 2040. Diabetes South (2012) estimates that in South Africa 10 % of the population has diabetes, and most are unaware of their condition. Elsewhere in Africa, diabetes is also becoming a major disease. A recent article in the Lancet revealed that in Tanzania, there has been a 3- to 7-fold increase in diabetes in the past 15 years for 6 % of urban people (Mbanya et al. 2010).

To avoid these health consequences, it is critical that consumers successfully navigate the Nutrition Transition, which is accompanied by a huge increase in the availability of and access to processed, packaged and prepared foods. This means that traditional diets rich in whole grains and indigenous fruits and vegetables lose ground to a diet based on highly refined flours, high levels of sugar and fat, and excessive amounts of salt. Figure 13.2 illustrates what happens to people's macronutrient and micronutrient intake as populations go through this Nutrition Transition. As indicated, energy-dense but empty calories, in both processed foods and beverages, contribute to growing overweight problems and associated noncommunicable disease such as diabetes, heart disease and certain forms of cancer. Africa, the last continent to urbanize, is also the last to face the public health consequences

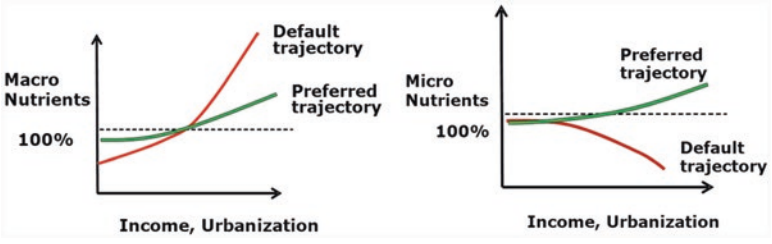

Fig. 13.2 Influence of urbanization and rising incomes on macronutrient and micronutrient intake in developing countries (BCC Consortium)

of the resulting Nutrition Transition (Popkin 2003). These trends, likewise, place new and rapidly growing demands on institutions offering scientific training in FST, nutrition, and public health.

13.3 Role of Food Science and Nutrition Professionals

13.3.1 Private Sector Food Industry

Food processing companies in Africa, in order to supply growing urban markets and to attain cost-reducing economies of scale, will increasingly need to scale up production from semi-artisanal to industrial levels. In many instances, these increases in scale imply batch sizes several orders of magnitude larger than traditional manual processing. In Southern Africa, the industrialization of sorghum beer (traditional African opaque and cloudy beers) and *mageu* (nonalcoholic fermented cereal porridge gruel) production resulted in scaling up production from 200-l batches by village women to stainless steel cookers of between 15,000 and 20,000 l (Haggblade and Holzapfel 1989). In West Africa's industrialization of *gari* (a granulated, precooked convenience food made from toasted cassava) production, emerging food processors have scaled up production from individual, episodic batches of one to two tons of processed roots to continuously operated industrial plants processing 50–60 t of roots per day (Onyekwere et al. 1989; Nweke et al. 2002).

Another uniquely African example is the development of a massive modern technology lager beer brewing industry based on sorghum, firstly in Nigeria starting 25 years ago (Ilori 1991), and in the past decade in East and Southern Africa (Mackintosh and Higgins 2004). Figure 13.3a shows the inside of a pneumatic germination vessel in sorghum maltings in Nigeria. The maltings are entirely PLC controlled. Figure 13.3b shows a lager beer brewery in Uganda. Each block of vertical fermentation tanks represents an expansion over the past decade since the introduction of sorghum-based lager beer brewing. Note that the original brewery fermenters are inside the brewery buildings and still in use. The scale of this brewing revolution in Africa can also be seen by the fact that beer production in Nigeria, based on locally grown sorghum, is currently growing at 6 % per annum and in 2011 reached 19.5 million hectoliters (Anon 2012), and is now a close second in Africa to South Africa.

In order to improve food and beverage safety, avoid spoilage and to ensure product quality at such industrial scales, the African food industry will require increasing numbers of food scientists and technologists. With industrialization of Africa's many indigenous fermented foods—such as *gari*, sorghum beer, *mageu*, *fufu* (cassava or yam porridge), *ogi* (a fermented starchy cereal-based beverage), and fermented dairy products such as amazi—increases in batch sizes will require scientific research into the biochemistry and microbiology of these fermentations in order to identify the microbial cultures driving key fermentation processes, optimal temperatures and pH, and effective protocols for controlling pathogenic microorganisms and ensuring food safety (Steinkraus 1989; Taylor and Emmambux 2008).

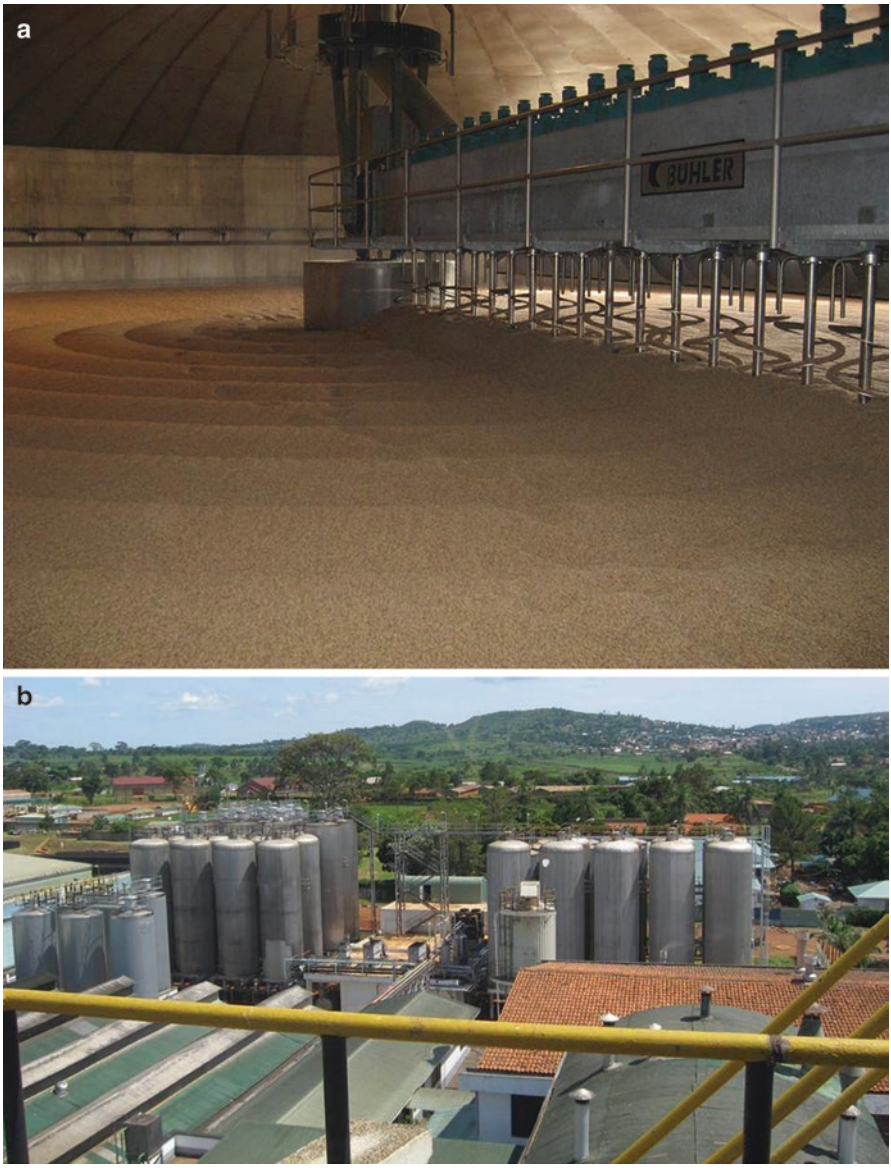

Fig. 13.3 Developments in sorghum-based lager beer brewing in Africa. (**a**) Inside a germination vessel in a Sorghum maltings in Nigeria; (**b**) Bird's-eye view of a lager brewery in Uganda

Marketing and logistics become increasingly important as supply chains lengthen. Growth in the post-farm segments of the supply chain results in a growing share of processing, packaging, distribution, and marketing in the total price of food paid by African consumers. Consequently, marketing efficiency becomes critical to efficiency of the food system. In Zambia, for example, marketing margins for maize fell

by roughly 70 % in the decade and a half following liberalization of cereal markets the mid-1990s (Jayne et al. 2010). While increasing farm productivity remains crucial for raising farm incomes, the growing scale of post-farm segments of the supply chain means that efficiency in food marketing and distribution systems will become increasingly important for moderating consumer food prices. This need translates into growing demand for employees with expertise in packaging, food storage, logistics, commodity price hedging and finance. Expanding demand for perishable products such as fresh fruits and vegetables will require people with expertise in logistics, quality and temperature control, packaging, cold storage management, sanitary controls, and monitoring of communicable bacterial diseases (Abbott 1986). Given increasingly volatile world commodity prices, market forecasting, commodity risk management, market monitoring, storage, and regional logistics platforms will all become increasingly important for Africa's food industries.

Food safety, packaging and consumer taste preferences likewise govern food marketability and food safety. As a result, private food processors will increasingly need scientific expertise in food safety, food packing and sensory evaluation techniques. To meet this need, multinational food companies operating in Africa are beginning to set up regional R&D centers where food scientists and technologists develop and adapt food products to meet local requirements. A notable example of this trend is the Nestlé Food R&D Center at Abidjan, Ivory Coast (Nestlé 2010).

Food processing SMMEs are the drivers of job creation. With increasing urbanization, small-scale production of all types of food products is expanding rapidly across Africa. An interesting trend is the development of convenience food products based on traditional local foods, such as pearl millet (Taylor et al. 2010) where three levels of value addition are taking place: simple flour products, value added flours (nutritionally enriched and flavored) and ready-to-eat (RTE) instant porridges and infant foods. Since the small and micro enterprises are usually run by persons with no technical training in food science and technology, food safety risks are high. Also, processing efficiency and product quality are often poor. These factors greatly increase the chance of business failure. Obviously, food scientists and technologists have a critical role to play in providing technology to SMMEs. However, reaching them and making a significant impact is challenging because of the high numbers of SMMEs and small number of food scientists and technologists, and the issue of the SMMEs having little or no money to pay for the services of food professionals.

Franchising is an excellent way of alleviating small enterprise technical and business failure. With urbanization and rising living standards, there is a huge growth in fast food franchises across Africa, particularly from companies based in South Africa. For example, Famous Brands, a South African fast food franchise company opened 18 restaurants in other African countries in the last quarter of 2012 (How We Made It In 2012). However, as is well known, the widely held opinion is that most fast foods from international companies are not healthy (Igumbor et al. 2012; Pretoria News 2012). Food scientists and technologists are needed, not only to improve technical efficiency, product consistency and food safety standards of fast food franchise enterprises, but importantly to substantially improve the nutritional quality of the products, while at the same time maintaining sensory quality.

Food-borne diseases (FBD) caused by pathogens (viruses, bacteria, parasites, and prions) are a great concern in Africa. Food scientists and technologists must mitigate FBD. This concern should be given priority in future FST standards, especially with regard to setting such standards for the food safety of animal products.

13.3.2 Public Health, Consumer Protection, and Consumer Education

Food and nutrition are an integral part of health. Health is not merely the absence of diseases, but rather the total well-being of an individual. Future FST activities (and education) in Africa must take cognizance of the new approaches to health. "One Health" is a relatively new approach to solving complex health related challenges that has generated significant interest and gathered momentum at global, regional, and national levels. The One Health approach seeks to promote total health by ensuring disciplinary, multisectorial, and systemic approaches to the practice of health service delivery—doing things together, learning together, and shaping the future together in this increasingly "One World, One Health and One Economy." There are multiple and varied disciplinary and professional stakeholders in promoting One Health, including FST, veterinary science, public health, wildlife health, agriculture and nursing, among many others.

Future advocacy roles for FST professionals will be geared towards measures to help curb dietary deficiencies and nutritional disorders as well as food labeling, especially genetically modified foods (GMOs), trans-fats, sugars, and other food constituents that are of health concern. FST professionals, through their national bodies affiliated with the International Union of Food Science and Technology (IUFoST), can commission independent scientific statements on contemporary and emerging issues of public interest such as GMOs, the presence of acrylamide in heat processed foods, nanostructured foods and human health, the role of antioxidants in human health, trans-fats and cardiovascular diseases, dietary fiber and health, HIV/AIDS and nutrition, and so on, to guide policy directions. Further, the African FST professional bodies affiliated with IUFoST can play an important role in moderating the practices by members so as not to profiteer at the expense of public safety. Responsible media advertising, for example, can help change people's dietary behavior.

At a more basic level, through private agencies, food professionals can provide the public with valuable advice about the nutritional composition of foods and which food products should be consumed daily or in moderation. An example worth emulating in Africa is Vegetables NZ (n.d.), based in New Zealand, which promotes increased vegetable consumption through using a variety of media and activities, even including the early childhood education setting.

Perishable foods likewise require public and private investments in sanitation, food safety and urban planning. Africa's urban wholesale markets for horticulture products often feature outmoded infrastructure and inadequate zoning that

consequently imposes heavy losses on farmers, particularly during the rainy season, as well as potential public health risks and high prices for consumers. Proactive discussions between market traders, city governments, town planners, and public health officials are required to anticipate and provide the public zoning and infrastructure necessary to facilitate continued rapid growth of the sector, particularly in Africa's rapidly growing secondary cities.

Animal health and food safety is dealt with in the related specialization area of veterinary public health. This covers, for example, a number of food science and nutrition areas, including meat hygiene, inspection and technology; milk hygiene and technology; food hygiene and food-borne diseases; food spoilage and preservation; food microbiology; fish hygiene and inspection; egg hygiene; zoonoses and emerging and reemerging zoonoses; environmental hygiene; water hygiene; and inspection of food of animal origin including meat from domestic and wild animals, fish, and honey, among others. Food scientists and technologists should therefore emphasize the need to strengthen the relationship with veterinary and other One Health disciplines. They should also contribute to the work of Codex Committees, including the Codex Committee on Food Import and Export Inspection and Certification Systems (which addresses, among other things, the issues of traceability and certification), and the Codex Committee on Milk and Milk Products.

Perhaps sometimes overlooked is the fact that agriculture and food are among the critical infrastructures susceptible to terrorism. It is advisable that FST professionals, farmers, market vendors, hoteliers, and others selling food to the public be aware of terrorism activities that could impact any portion of the food and nutrition value chain, and relevant competencies to address emerging challenges are needed today.

13.3.3 Role of Food Science and Nutrition Professionals in Shaping Public (Government) Policies in Africa

As alluded to, the future of Africa's food systems will be driven by changes in demographic profiles, structural transformation, income growth (and associated nutrition transitions) and health concerns. Africa, particularly sub-Saharan Africa, currently comprises the youngest population in the world, with 44 % of its population below the age of 15 years, the majority of which (over 60 % on average) reside in rural areas (Ashford 2007). However, by 2030, Africa will be represented by a population predominantly living in urban centers (Kessides 2005). As elsewhere, urbanization in sub-Saharan Africa is strongly associated with increased levels of chronic noncommunicable diseases such as obesity, diabetes and cardiovascular disease (Unwin and Alberti 2006).

This unfolding scenario offers immense opportunities for FST to shape the future. As an inherently inter-disciplinary field, FST is uniquely situated to stimulate value-added food processing, agribusiness and job creation; design food products for convenience to suit urban lifestyles without compromising health; manage

the food–health interface by ensuring food safety and development of nutritive and health-promoting foods; and facilitate trade in food products by ensuring compliance with agreed standards and safety regulations.

However, the potential contribution of FST to development of African countries has been limited by persistent misconceptions, especially in public policy circles. In Kenya, for instance, pioneering FST graduates were deployed in the field as home economists and extension officers under the Ministry of Agriculture. The food industry has equally been undiscerning of the capabilities of FST graduates, almost invariably engaging them at best as quality control managers, but mainly as laboratory technicians and managers, while employing chemists as production managers. In many African countries, this gross mismatch of FST skills continues, due to persistent personnel deployment traditions in the industry, nonexistent or moribund professional lobby groups, and lack of clear public perception of FST curricula (Ojijo 2005).

Food science and technology professionals, either as individuals or as a body corporate, have the onerous role to help bridge the divide between seeming public denigration and the true worth of their profession. The food industry in many African countries is still rudimentary. However, agriculture is increasingly seen as the cornerstone for economic growth of African countries, especially through innovations along the value-chains. Within government, food scientists and technologists thus have a key role to play in Ministries of Agriculture, Health, Trade and Industry, and Departments of Standards and Metrology in the general areas of food safety, food quality (including nutritional quality) and food standards. Roles in food safety could include development and enactment of food safety legislation on additives, food toxins and contaminants, microbiological and chemical safety, and GMOs. Also required are the enactment of Codex Alimentarius standards and development of African Union standards, development of harmonized regional standards, and public health inspection and certification.

Roles in food quality could include development of food product nutrition and health labeling and food fortification. Roles in food standards should include food product classes and grades, food authenticity, facilitation of food import and export (especially removal of unnecessary barriers to regional trade), and sanitary and phytosanitary agreements. As mentioned, Africa's growth is creating opportunities for food processing SMMEs, and it is imperative to help develop this key sub-sector. An important role for FST professionals in government will be development of an SMME appropriate regulatory framework for food handling, processing and distribution.

Professionals with specific expertise in food science and nutrition have a key role to play in policy dialogue with a view to bringing to the attention of governments contemporary food-health and food safety related issues. The interdisciplinary nature of FST endows its professionals with a unique inter-sectorial grasp of technical issues. Thus, FST professionals working in both public and private agencies would potentially be best placed to handle trans-boundary issues of food safety, quality, nutrition and standards that span agriculture, health, and trade and industry sectors. An interesting example in South Africa regarding how food scientists and technologists in the private sector can assist government in ensuring food safety is FLAG, the Food Legislation Advisory Group. FLAG consists of a committee of

food industry, research and academia food scientists and nutritionists, and representatives of the government Department of Health; the committee advises the government on development of food safety and labeling legislation and regulations appropriate to the rapidly changing food safety, nutrition and health environment.

Together with associated professionals (in nutrition and health), FST professionals must play catalytic roles in helping enact policies on agro-food business, nutrition, food safety, hygiene, and chronic noncommunicable diseases. For example, food scientists, nutritionists and pediatric endocrinologists can jointly help in the development of public policy to prevent childhood obesity by providing a robust voice in support of scientific facts that attribute the looming public health problem to identified dietary proclivities (Friedman and Schwartz 2008).

Food safety crises are an increasingly important issue. Numerous food crises have occurred internationally in recent years (e.g., the use of the dye Sudan Red I; the presence of acrylamide in various fried and baked foods; mislabeled or unlabeled genetically modified foods; the outbreak of variant Creutzfeldt-Jakob disease), originating in both primary agricultural production and in food manufacturing industries. Public concern about these and other events has led government agencies to implement a variety of legislative actions covering many aspects of the food chain, with expert input from food scientists and technologists.

13.4 Survey of University Food Science Curricula in Africa

13.4.1 Methodology Used for Surveying Food Science and Technology Curricula in Africa

A short questionnaire (Table 13.1) was prepared and sent to 28 Food Science or related departments in selected African universities. Seventeen responses were received from 11 countries (Southern Africa: Botswana, Namibia and Botswana; Eastern Africa: Ethiopia, Kenya, Mozambique, Rwanda and Uganda; Middle Africa: Cameroon; and Western Africa: Ghana and Nigeria). The questionnaire included questions on the following aspects: specific focus and outcomes of degree programs; important food science skills required for graduates; work experience/experiential training details for degree purposes; national and international collaborative programs and partnerships; adherence to international guidelines for FST degree programs; where graduates are employed; and major challenges facing FST educators in Africa.-

13.4.2 Curriculum Offerings

The majority of universities surveyed offer the equivalent of 4-year BSc Honors level Food Science and/or Technology degrees at the undergraduate level (Table 13.2). Some institutions, notably in Nigeria and Ethiopia, offer 5-year

Table 13.1 IUFoST Africa food science and technology education questionnaire

ITEM	ITEM (continued)
Country	Are there regional guidelines for Food Science and Technology programs?
	Yes or No
Name of Institution	Where are your students employed?
	• Tertiary education institutions
	• Research institutions
	• Private industry
	• Self-employed
Name of Department	Do you have institutional collaborators that contribute to your degree programs? (please list them)
Name of undergraduate degree program(s) and duration (e.g., BSc in Food Science or BSc in Food Science and Nutrition—3 years)	Name of postgraduate degree program(s) and duration
Is there a specific focus in terms of specialization or specific outcomes in your degree programs?	Are your postgraduate degree programs (i.e., masters and doctoral) research based, taught degrees or a combination thereof?
What skills do you regard to be important for your graduates?	List any specific food security activities in your department
Work experience/experiential training details (if applicable for degree purposes)	Are you involved in formal continuing education activities and please list?
Adherence to either IFT or IUFoST degree program guidelines (Yes/No)	What is the biggest challenge that you face as Food Science and Technology educators?
Is there a national curriculum for Food Science and Technology and if so under whose jurisdiction?	Any additional comments that you wish to make?

programs. The Technical Universities of Technology in South Africa (Tshwane University of Technology, University of Johannesburg and Cape Peninsula University of Technology) offer diploma programs at the undergraduate level and technical degrees at the postgraduate level. Only four universities surveyed (University of Pretoria, South Africa; University of Ghana, Ghana; Moi University, Kenya; and Makerere University, Uganda) offer degree programs in Nutrition or Nutrition and Food Science. The majority of universities surveyed present Masters and Doctoral degree programs. In South Africa, all MSc degrees are research based. The norm for the rest of the countries and institutions surveyed is 1 year for course work and 1 year for research-based work. Although the majority of BSc programs in Food Science and/or Technology presented at the universities surveyed adhere to either IFT (US Institute of Food Technologists) or IUFoST degree guidelines, national and in particular regional guidelines and curricula are lacking.

Food Science and Technology graduates need a comprehensive skill set (Fig. 13.4, Table 13.3). In general, degree programs include fundamental principles of FST—broad base knowledge at the undergraduate level and more specialization

Table 13.2 Degree offerings at undergraduate and postgraduate level in Food Science and related disciplines at surveyed universities in Africa

Country	Name of Institution	Undergraduate offerings	Postgraduate offerings	Work experience/ experiential training required for degree purposes	Adherence to IFT/IUFoST guidelines (Yes/No)	National or regional curricula in Food Science	Regional curricula in Food Science
Eastern Africa							
Ethiopia	Haramaya University: Dept of Food Science and Postharvest Technology	BSc in Food Technology and Process Engineering (5 years)	MSc in Postharvest Technology MSc in Food Science and Technology MSc in Food Engineering (1 year course work and 1 year research)	6 months internship	Yes	No	No
Kenya	Moi University: Dept of Consumer Science	BSc Hons Food Science and Nutrition (4 years)	None (First undergraduate class graduated in 2011)	90 working days—industrial attachment	Not exactly	No	Yes, with Tanzania and Uganda
Mozambique	Universidade Eduardo Mondlane: Chemical Engineering—Food Technology Division	BSc (Eng) in Chemical Engineering with specialization in Food Technology	MSc with collaboration of Lund and Chalmers Universities in Sweden (course work and research)	–	–	No	No
Rwanda	Kigali Institute of Science and Technology: Dept of Food Science and Technology	BSc in Food Science and Technology (4 years)	Research based MSc and PhD programs through research in the process of development	–	Yes	Not sure	Not sure

Uganda	Makerere University: Department of Food Technology & Nutrition	BSc Food Science and Technology (4 years)	MSc Food Science (2 years)	None	Yes	Yes	No
		BSc Nutrition (3 years)	MSc Applied Human Nutrition (2 years)				
			PhD Food Science (4 years)				
			PhD Nutrition (4 years)				
			Masters: course work and research				
			PhD: research based				
Western Africa							
Ghana	University of Ghana: Dept of Nutrition and Food Science	BSc Nutrition and Food Science (4 years)	M Phil (2 years)	6 weeks vacation internship	Yes	No	No
		BSc Food Science (4 years)	PhD (3–4 years)				
		BSc Nutrition (4 years)	Both degrees are a combination of taught courses and research				

(continued)

Table 13.2 (continued)

Country	Name of Institution	Undergraduate offerings	Postgraduate offerings	Work experience/ experiential training required for degree purposes	Adherence to IFT/IUFOST guidelines (Yes/No)	National or regional curricula in Food Science	Regional curricula in Food Science
Nigeria	Federal University of Agriculture, Abeokuta: Dept of Food Science and Technology	BSc Food Science and Technology (5 years)	MSc programs (2 years)—taught courses and research: Food Processing and Storage Technology; Food Quality and Assurance; Food Microbiology and Biotechnology; Food Process Engineering	6 months industrial attachment	Yes	Yes	No
			Similar programs on doctoral level (3 years—research based)				
Nigeria	University of Agriculture, Makurdi: Dept of Food Science and Technology	BSc in Food Science and Technology (5 years with O levels and 4 years with A levels)	Masters and doctoral Degrees in Food Science and Technology	6 months industrial attachment	Not sure	Yes	No
Middle Africa							
Cameroon	University of Ngaoundere: Dept of Food Science and Nutrition	None (a postgraduate school)	Professional Masters in Engineering in Agro-Food Process Engineering (3 years)	7 months over a period of 3 years of studies	No	No	No

Southern Africa							
Botswana	Botswana College of Agriculture: Dept of Food Science and Technology	BSc in Food Science and Technology (4 years) (first intake 2013)	Nonexistent at the moment	3 months industrial placement	Not sure	No	No
Namibia	University of Namibia: Dept of Food Science and Technology	BSc (Agric) (Hons) in Food Science and Technology (4 years)	None	3 months industrial attachment	No	No	No
South Africa	University of Free State: Department of Microbial, Biochemical and Food Biotechnology	BSc Food Science with either Microbiology, Biochemistry or Chemistry (3 years)	BSc Hons and BSc (Agric) Hons (1 year)	None	No	No	No
		BSc (Agric) Food Science with either Agricultural Economics. Agronomy, Animal Science, Microbiology, Biochemistry or Chemistry (4 years)	MSc and MSc (Agric) (minimum 3 years)				
			PhD (minimum 2 years)				
			Masters and Doctoral degrees—research based				
South Africa	University of Johannesburg: Dept of Food Technology	National Diploma in Food Technology (3 years) or extended	MTech (1–2 years)	1 year industrial training	No	Still follow national curriculum framework as applicable to former Technikons in South Africa	No
		National Diploma in Food Technology (4 years)	DTech (2–4 years)				
		BTech Food Technology (3 + 1 year)	Programs research based				

Table 13.2 (continued)

Country	Name of Institution	Undergraduate offerings	Postgraduate offerings	Work experience/ experiential training required for degree purposes	Adherence to IFT/IUFOST guidelines (Yes/No)	National or regional curricula in Food Science	Regional curricula in Food Science
South Africa	Cape Peninsula University of Technology: Dept of Food Technology	National Diploma in Food Technology (3 years)	MTech Food Technology (minimum of 2 years)	1 year industrial training	Yes	No	No
		BTech Food Technology (3+1 year)	Research based				
South Africa	University of Pretoria: Dept of Food Science	BSc (Agric) Food Science and Technology (4 years)	MSc (Agric) Food Science (2 years)	None	Yes	No	No
		BSc Food Science (3 years)+BSc (Hons) Food Science (1 year)	MSc Food Science (2 years)				
		BSc Nutrition and Food Science (3 years)+BSc (Hons) Nutrition and Food Science (1 year)	MSc Nutrition (2 years)				
		Nutrition and Food Science programs to be replaced in 2014 by 4 years BSc Nutrition	PhD Food Science (3 years)				
			PhD Nutrition (3 years)				
			Masters and doctoral degree programs research based				

South Africa	University of Stellenbosch: Dept of Food Science	BSc Food Science (Food Science with Biochemistry) (4 years)	MSc Food Science (minimum 1 year registration)	8 weeks minimum with at least 4 weeks at single company	Yes	No	No
		BSc Food Science (Food Science with Chemistry) (4 years)	PhD Food Science (minimum 2 years registration)				
			DSc Food Science				
			All degrees are research based				
South Africa	Tshwane University of Technology: Department of Biotechnology and Food Technology	N Dip Food Technology (3 years program extended over 4 years) B Tech Food Technology—2 years part time program	MTech and D Tech degrees are research based		Yes (IFT) but adapted to qualification structure at TUT	Still follow national curriculum framework as applicable to former Technikons in South Africa	No
South Africa	University of Venda: Department of Food Science and Technology	BSc Food Science and Technology (4 years)	MSc course work and mini dissertation (1 year)	6 months industrial training	Yes	As practised in South Africa	No
			MSc by research (1 year)				
			PhD Agriculture (Food Science and Technology) (3 years)—research based				

No information provided

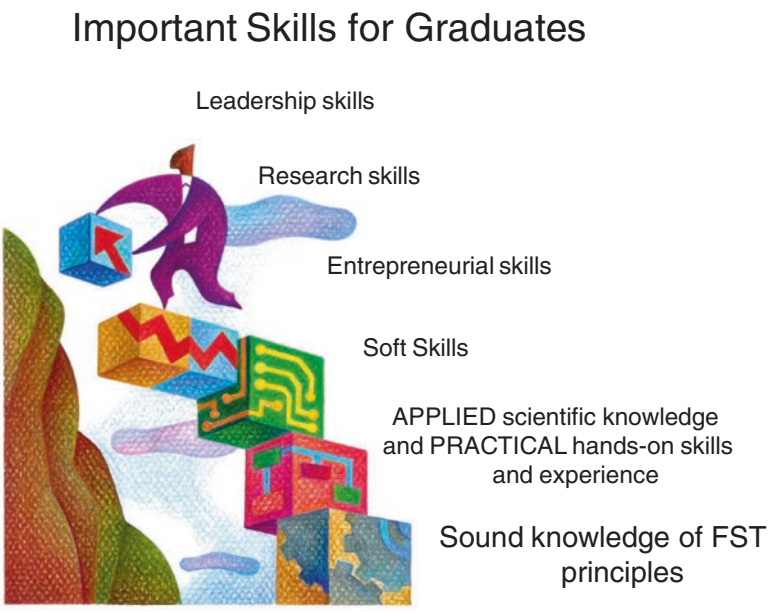

Fig. 13.4 Important skills for Food Science and Technology graduates in Africa

Table 13.3 Description of important skills for Food science and Technology graduates in Africa

Skills or competencies	Description
Sound knowledge and understanding of Food Science and Technology principles	Sound knowledge in basic food science: food chemistry and analyses, food microbiology and safety, food processing and engineering
Applied scientific knowledge and practical hands-on skills and experience	Application of basic food science (Applied food sciences—e.g., food product development, food quality assurance, sensory analyses, food research projects)
Soft skills	Computer skills, communication skills, team building, report writing skills, problem solving skills
Entrepreneurial skills	Innovative and creative thinking, willingness to take risks, self-motivated and disciplined, ability to sell ideas to others, competitiveness, ability to network
Research skills	Ability to work independently to solve problems, critical thinking and analytical skills for problem solving
Leadership skills	Take ownership and responsibility and leading by example

at the postgraduate level. Sound knowledge of food science principles (in terms of food chemistry and analyses, food engineering, food processing, food microbiology and safety) is a requirement for most programs. Another important element is that graduates need to have the ability to apply their scientific knowledge to practical situations. Practical hands-on skills and experience are regarded as important by the

majority of universities surveyed. The ability to apply knowledge and theory to practice was emphasized by many of the departments.

Most universities have a specific focus in terms of specialization or have specific outcomes for their degree programs. These are often linked to the perceived needs of their countries. In Nigeria, for example, the Federal University of Agriculture places a special emphasis through research and extension programs on the needs of local food industries in terms of local sourcing of raw materials and upgrading of traditional food processing technologies. Reduction of food wastage through the use of "appropriate" FST is also of paramount importance in their programs. At Moi University in Kenya, graduates are expected to develop healthful products to combat nutritional deficiencies and maintain good health as part of the degree program. In South Africa, BTech graduates from Tshwane University of Technology are expected to apply and integrate advanced knowledge and skills of Food Technology in the food manufacturing environment. At Haramaya University in Ethiopia, much emphasis is placed on food processing technologies and food engineering, but there is less for nutrition and post-harvest technology. At Makerere University in Uganda, graduates need to be ready and able to address challenges facing the food industry and society at large. In some countries (e.g., Botswana) one expectation of their program is that it will develop food entrepreneurs to develop a formal food industry in Botswana.

Future food science and nutrition leaders in Africa must demonstrate innovative and creative thinking skills, be able to work both independently and in teams and have strong problem solving and analytical skills. Most of the surveyed universities aim to produce well-rounded graduates equally equipped to enter private industry or to continue on to postgraduate research. Most programs include internships or experiential training in the food industry or private sector, research institutions or regulatory authorities as a degree requirement.

From Table 13.4, it is evident that the type of employment for graduates from universities surveyed differs substantially. In countries such as South Africa with a well-developed formal food industry, most of the graduates are employed by the private sector. Where the private sector is not as yet well developed, most graduates are employed by government or parastatal organizations.

13.4.3 Non-degree Training and Extension Work

Of the universities responding to the questionnaire, fewer than half present formal non-degree courses and workshops in the FST domain for persons from the wider community.

Examples of non-degree training include:

- University of Ghana: EU/Industry Council for Development—Food Safety and Nutrition Training Project. This project offers a nutrition extension and sustainable livelihood short course for the industry;

Table 13.4 Survey results of where graduates in Food Science related disciplines are employed in Africa

Country	Name of institution	Private industry	Government/NGO/Others	Research institutions	Tertiary education	Self employed
		(% of graduates employed)				
Eastern Africa						
Ethiopia	Haramaya University	20	57	10	8	2
Kenya	Moi University	40	10	20	30	
Mozambique	Universidade Eduardo Mondlane:	50	10	35	5	
Rwanda	Kigali Institute of Science and Technology:	20	20	15	5	5
Uganda	Makerere University	40	35	10	10	5
Western Africa						
Ghana	University of Ghana					5
	Undergraduates:	50	45 (incl. research institutions)			
	Postgraduates:	20	60 (incl. research institutions)			
Nigeria	Federal University of Agriculture, Abeokuta	50		10	30	10
Middle Africa						
Cameroon	University of Ngaoundere	40	7	18	25	10

Southern Africa						
Namibia	University of Namibia	80		15	5	
South Africa	University of Free State	80	5	10	5	5
South Africa	University of Johannesburg	85		5	5	5
South Africa	Cape Peninsula University	98	2			
South Africa	University of Pretoria	96		2	2	
South Africa	University of Stellenbosch	90–95		2–3	2–3	2–3
South Africa	Tshwane University of Technology	90		2.5	5	2.5
South Africa	University of Venda:	95		5	5	
Range		20–98	5–60	2–35	2–30	2–10

- University of Pretoria (South Africa): CE@UP short courses and workshops (including sensory evaluation workshops, an opaque beer brewing certificate course, and a part-time honors degree program available for people working in the industry);
- University of the Free State (South Africa): Short courses in food processing (1–4 days);
- Federal University of Agriculture (Nigeria): Part-time degree for people working in the industry and a part-time postgraduate diploma;
- Moi University (Kenya): Preparation of mature diploma students to enter their degree programs;
- Cape Peninsular University of Technology—AgriFood Technology Station (ATS) (South Africa): Mandated to assist small and medium enterprises primarily to improve their use of technology.

13.4.4 Challenges Perceived by Educators

Food Science and Technology educators in Africa face many challenges. Challenges identified in the snap survey include:

- Lack of modern food processing equipment or pilot plant facilities;
- Lack of "state-of-the-art" analytical equipment;
- Lack of technical support within institutions;
- Inadequate number of academics and support staff to run programs;
- Low student numbers and poor quality of students; many potential students see other professions to be more attractive than FST;
- Lack of sustainable partnerships in FST research or capacity development in the region.

In the majority of the universities surveyed, laboratories and pilot plant facilities are underequipped for both teaching and research purposes. In most cases, there is a lack of modern food processing equipment and "state-of-the-art" analytical equipment. This is further exacerbated by the lack of technical support within many institutions in terms of human resources, but also in terms of consumables. Some universities also appear to lack appropriate teaching and laboratory space, textbooks and access to food science research journals. In some cases, there is also an inadequate number of academic and support staff to run the programs. The quality of incoming students is another concern for some educators.

13.4.5 Core Problems and key Opportunities

Given the apparent relationship between the growing prevalence of processed foods and noncommunicable lifestyle diseases, solutions to Africa's emerging public health problems will require cross-disciplinary work linking FST, human nutrition

and public health. A core problem at present is that very few universities present BSc Nutrition/BSc Food Science and Nutrition programs. Furthermore, there is too little extension work and training (for non-degree purposes) in the area of Food Science and Nutrition.

Another core problem is that collaborative degree programs are almost completely lacking at the national, regional, and international levels. Education and research networks nationally and regionally should develop joint collaborative degree and research programs. There is also a strong need to create opportunities for food science and nutrition educators and students to interact with other each other, both nationally and regionally. Additionally, food science, food technology and nutrition educators need to engage with multinational companies operating in Africa to support local high-level FST and nutrition education and human resource development.

The African FST professional bodies, in consultation with other regional stakeholders, can help address matters of curriculum development. Graduates in FST and associated disciplines must be adequately equipped with the necessary skills and competencies that will enable them to perform their future roles. This puts a special premium on the curricula and delivery of course contents in tertiary educational centers, as well as the promotion of life-long learning. As indicated in Fig. 13.4, future skills sets that will be demanded of FST graduates include both "hard" (i.e., engineering design, product development, agribusiness and entrepreneurship, nanotechnology, food and the environment, sensory and behavioral sciences, chemometrics, nutrigenomics, biotechnology, proteomics, and metabolomics) and "soft" (i.e., community management, critical and strategic thinking, communication, and teambuilding, organizational) skills. Public policies targeting FST curricula should have the following objectives: ensuring the relevance of FST curricula to national development needs; fostering quality in the delivery of FST curricula; fostering professional probity in the practice of FST; promoting curricula reviews and guidance for such reviews; stimulating foundational or pre-tertiary interest in food sciences and the choice of FST as a profession; promoting awareness of the role and value of food in society; and fostering national accreditation systems or other means to assess skills outcomes.

There is also a strong need for concerted national and regional activities to promote FST as a profession. This will take a spirited effort of all stakeholders involved (educators, industry, and government). Postgraduate students are a special case in point; these are the young people that are on the cutting edge of new research and developments. The formal food industry needs to capitalize much more on this group than is currently the case. There should be a business incentive for graduates from industry to continue with their postgraduate studies.

Innovative approaches should be implemented to utilize scarce resources more effectively. Nationally, and even regionally, educators should consider joint purchasing, maintenance and use of expensive equipment by different stakeholders. Equipment suppliers should also be approached for an "equipment on loan" scheme, where equipment could be placed in institutions for use by the university and its students and the suppliers. This will not only improve the quality of education and training, but also expose future buyers to a supplier's equipment.

13.5 A Strategy for Addressing Africa's Food Science and Nutrition Education Challenges

Africa, the world's poorest continent, and the last continent to urbanize, is also the last continent to confront the Nutrition Transition. As a result, Africans have a chance to come out ahead of the curve, to learn critical lessons from hard experience elsewhere and to apply those lessons to bend the nutritional curve back to a healthier trajectory (Fig. 13.2).

But the Nutrition Transition appears to be accelerating in many parts of the world, with overweight, obesity and related diseases emerging in communities with lower levels of income and urbanization than historically. Increased globalization of agribusiness supply chains, improved communications and the more rapid spread of unhealthy Western-style diets may be to blame (Popkin 2003). Consequently, African nutritionists and public health officials will need to move quickly to bend the nutrition curve and moderate the public health costs of emerging overweight and noncommunicable diseases.

Africa's Bending the Curve Consortium (BCC) is a coalition of food scientists, nutritionists, and public health professionals dedicated to early, preventive action aimed at improving Africa's long-term nutritional trajectory. BCC partners have identified the four following early action priorities:

13.5.1 Advocacy

High-level executive education for government public health director generals and food industry CEOs is required to raise awareness of the early arrival and unexpected speed of the Nutrition Transition in Africa, the resulting high prospective costs to human productivity and public health systems, as well as the key lessons emerging from elsewhere about successful mitigation and prevention strategies. A strong parallel is the impact that HIV/AIDS has had on sub-Saharan Africa in terms of the magnitude and severity of health consequences, the population affected (economically productive adults), and the enormous cost of treatment versus prevention.

FST professionals must engage in championing the One Health cause and seek to become leaders in One Health approaches to sustainable health for healthy and productive humans, animals, plants and ecosystems. This new approach will require multidisciplinary research, training and community engagement so that FST professionals can contribute to the overall goal of reducing local and global health challenges and their socioeconomic impacts.

13.5.2 Food Science and Technology Curriculum Reform in African Universities

In order to educate a new generation of FST professionals capable of taking early and preemptive action in bending the curve in Africa's Nutrition Transition, curricular reform must begin now. This will demand the development of curricula that integrates food science and technology, human nutrition, and public health, as well as educational systems that facilitate internships and applied research programs linking students and university educators to the private sector food industry.

Food of animal origin is another priority that requires special attention for FST education, research, and training given the growing interface between humans and animals. In response to the demand from consumers worldwide for safe food, FST education should emphasize working together with relevant professionals to reduce food-borne and food-related risks to human health due to hazards arising from animal production.

At the postgraduate level, development and implementation of joint complementary masters and doctoral programs, nationally and regionally, will not only enhance the quality of food science and nutrition education in Africa but also promote student and academic staff mobility.

Fellows of the International Academy of Food Science and Technology (IAFoST) could assist food science and technology educators in Africa in the following ways: providing advice on design and content of curricula; spending significant time at African universities, teaching and mentoring; lobbying governments and international funding agencies; and engaging with multinational companies operating in Africa to support local high-level food science and nutrition education and HR development.

13.5.3 Food Industry Entrepreneurship

Africa's rapidly growing demand for processed convenience foods offers significant potential for the promotion of high quality convenience type indigenous foods that cater to local tastes, but which most urban food markets fail to deliver under the forces of inertia which, by default, lead to the expansion of low nutritional quality fast foods. A proactive new generation of FST professionals can contribute to the development and marketing of tasty, profitable, inexpensive nutrient-dense packaged foods in Africa, often by building on favored indigenous foods such as local grains and tubers complemented with African leafy vegetables. Coupled with agribusiness management and entrepreneurship programs, FST food laboratories, internship programs and competitive grants can translate into food entrepreneurship incubators serving private food industries as well as local consumers.

13.5.4 Fresh Fruit and Vegetable Wholesale Market Reforms

Parallel increases in urban demand for fresh fruits and vegetables offer similar prospects for raising agribusiness incomes, lowering consumer costs and improving the nutritional quality of urban diets. Early investments in urban planning, zoning, road quality, and urban horticulture market infrastructure and management systems could significantly improve the efficiency of urban fresh fruit and vegetable wholesale markets, as well as sanitation and public health. By reducing current high losses, improved horticulture markets offer prospects for raising farm incomes, significantly lowering urban consumer prices for fresh fruit and vegetables, and increasing urban consumption of nutrient-dense horticultural products.

As an important bridge to transforming agriculture as a business and an engine for economic development, food science, food technology, and nutrition professionals need to integrate more in the future with the other stakeholders in the wider agricultural innovation systems, especially in agricultural commodity value chains and the manufacturing and processing end.

In conclusion, it is necessary for FST professional associations to act strongly as formalized advocacy groups to engage with policy makers on key food science, technology, and nutrition issues based on sound scientific evidence. Professional associations should also advocate for the formation of national and regional centers of excellence, e.g., in the form of food research institutes to provide cutting-edge application of food science and nutrition to address emerging challenges associated with the new African food systems.

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Chapter 14
Yeasts from Traditional Cheeses for Potential Applications

Seda Karasu Yalçin and Z. Yesim Ozbas

14.1 Yeast Biodiversity in Traditional Cheeses

Cheese has been reported as one of the first symbols of mankind's passage to civilization and, among all dairy products, is the one that has the highest number of varieties (Kamber 2008). It has been claimed that more than 1000 cheese varieties exist today, having quite different forms, colors, odors, tastes, and structures (Stanley 1998; Kamber 2008; Jany and Barbier 2008). It has also been reported that 2000 names applied to cheese can be found in the literature (Fröhlich-Wyder 2003). It is known that the art of cheesemaking dates back to 8000–10,000 years ago originating from Middle East where the first fermented milk-based foods were made (Stanley 1998; Jany and Barbier 2008). Four basic ingredients are required to produce most cheeses: milk, rennet, salt, and microorganisms. These four ingredients are processed through different steps such as acidification, coagulation, syneresis, and ripening. It has been mentioned how fascinating it is that "such a diverse range of products can be produced" from "basically similar raw material." Interestingly, it has been reported that the composition and activity of the microflora is the least controllable of all the parameters (Jany and Barbier 2008).

The microflora of cheese may be divided into two groups: starter lactic acid bacteria and secondary microorganisms. Starter lactic acid bacteria are involved in acid production during manufacture and contribute to the ripening process. It is known

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that secondary microorganisms do not contribute to acid production, but generally play a significant role during ripening. The secondary flora are comprised of nonstarter lactic acid bacteria, which grow internally in most cheese varieties and other bacteria, yeasts, and/or molds that grow internally or externally and are usually unique to specific cheese varieties (Beresford et al. 2001). Both starter and secondary flora modify the physical and chemical properties of cheese, contributing to and reacting to environmental changes that occur during the manufacture and ripening of cheese (Jany and Barbier 2008). In some varieties, such as smear- and mold-ripened and Swiss-type cheeses, flavor development is dominated by the metabolic activity of the secondary flora (Cogan 2000).

There has been increasing interest in studying yeasts from traditional cheeses in recent years (Padilla et al. 2010; Soliman and Aly 2011). Such studies generally focus on isolating, identifying, and characterizing of the yeasts, elucidating their roles in ripening, and commercially growing them so they can be used to better control ripening and reduce the risk from spoilage microorganisms (Stanley 1998). Reports on the occurrence of yeasts in cheeses date back to the early part of this century (Ferreira and Viljoen 2003). It is known that their number can reach 10^5–10^8 cfu/g during ripening depending on the cheese type (Klein et al. 2002). Their occurrence is not unexpected because of their tolerance to low pH and moisture content, high salt concentration, and low storage temperatures (Fröhlich-Wyder 2003; Soliman and Aly 2011). They are both proteolytic and lipolytic and produce a range of volatile and peptide/amino acid flavor components (Stanley 1998). Their presence may also be attributed to assimilation and fermentation of lactose, the assimilation of organic acids, like succinic, lactic, and citric acid, and to their proteolytic and lipolytic activities (Ferreira and Viljoen 2003). Yeasts are widely dispersed in the dairy environment and appear as natural contaminants in raw milk, air, dairy utensils, brine, and smear water (Fröhlich-Wyder 2003). High numbers of yeasts are frequently observed on processing equipment, and in the air of the processing environment (Pacheco and Galindo 2010). The contribution of these surfaces to contamination, however, varies from factory to factory, depending on the sanitizing practices. It is reported that brine, one of the most important sources of contamination, contains yeast populations ranging from 10^4 up to 10^6 cfu/mL. Because of its high salt content, a specific microflora, and consequently halotolerant yeasts like *Debaryomyces hansenii* and *Yarrowia lipolytica*, are frequently encountered in brine (Viljoen et al. 2003). Other species such as *Candida versatilis*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, *Torulospora delbrueckii*, and *Trichosporan cutaneum* were also reported to be in the microflora of brine (Fröhlich-Wyder 2003). In addition, *Cryptocoocus curvatus, Geotrichum candidum, Pichia membranaefaciens, Y. lipolytica, D. hansenii*, *Clavispora lusitaniae*, *Tr. cutaneum*, *Rhodotorula mucilaginosa*, and *K. marxianus* can be isolated from raw milk (Fröhlich-Wyder 2003; Deak and Beuchat 1996).

The yeast species isolated from some traditional local cheeses around the world are represented in Table 14.1. It can be seen that *D. hansenii* and the species belonging to *Candida* are very versatile in different types of cheeses. Beresford et al. (2001) reported that *D. hansenii* was the dominant yeast and occurred in virtually

Table 14.1 Yeast species associated with different types of traditional cheeses

Cheese type	Yeast species	Reference
Cheddar	*D. hansenii, Cry. albidus, Y. lipolytica, R. minuta, Tp. delbrueckii, R. glutinis, K. marxianus*	Welthagen and Viljoen (1999)
Artisanal Portuguese ewes'	*C. zeylanoides, D. hansenii, C. intermedia, C. curvata*	Pereira-Dias et al. (2000)
Danablu	*C. famata, C. catenulata, C. lipolytica, Tr. cutaneum*	Tempel and Jakobsen (1998)
Blue veined	*C. versatilis, C. zeylanoides, D. hansenii, S. cerevisiae, Tp. delbrueckii, Cry. albidus, R. glutinis*	Viljoen et al. (2003)
Feta	*C. catenulata, Candida parapsilosis, Candida sake, Cry. laurentii, D. hansenii, Dek. anomala, G. candidum, K. lactis, R. rubra, Y. lipolytica*	Cosentino et al. (2001)
Feta	*K. lactis, D. hansenii, Dek. anomala, Dek. bruxellensis, G. candidum*	Fadda et al. (2001)
Manteca	*Tr. asahii, C. parapsilosis, R. mucilaginosa, C. inconspicua, C. rugosa, C. famata, C. zeylanoides*	Suzzi et al. (2003)
Picante	*D. hansenii, Cry. laurentii, Y. lipolytica*	Freitas et al. (1999)
Rokpol	*C. famata, C. sphaerica, C. intermedia, Geotrichum spp., S. kluyveri, C. kefyr, C. lipolytica*	Wojtatowicz et al. (2001)
Water buffalo Mozzarella	*S. cerevisiae, K. marxianus, C. butirylaaseri, Candida pararugosa, C. sorbophila, C. lusitaniae, P. cactophila, P. barkeri, P. norvegensis, P. pastoris*	Aponte et al. (2010)
Karish	*Tr. cutaneum, C. catenulata, Y. lipolytica, D. hansenii, K. lactis, G. candidum, C. zeylanoides, C. lambica, C. albicans, Cry. formans, R. glabrata, S. cerevisiae*	Soliman and Aly (2011)
Erzincan tulum	*C. lambica, C. famata, C. zeylanoides, C. kefyr, G. candidum, C. apicola, C. colliculosa, C. japonica, C. krusei, C. paludigena, C. rugosa, G. candidum, S. cerevisiae, K. lactis var. lactis, P. fermentans, Z. mellis*	Karasu-Yalcin et al. (2012b)

C: *Candida, Cry*: *Cryptococcus, D*: *Debaryomyces, Dek*: *Dekkera, G*: *Geotrichum, K*: *Kluyveromyces, P*: *Pichia, R*: *Rhodotorula, S*: *Saccharomyces, Tr*: *Trichosporan, Tp*: *Torulaspora, Z*: *Zygosaccharomyces*

all cheeses including Weinkase, Romadour, Limburger, Tilsit, Roquefort, Cabrales, Camembert, St. Nectaire, and Danish blue. According to Hansen and Jakobsen (2001), the dominant yeast species in blue-veined cheeses was *D. hansenii*. Del Bove et al. (2009) reported that *D. hansenii* was an important component in the production of Cheddar, Gouda, and buffalo mozzarella cheeses. *Y. lipolytica* and *Kluyveromyces lactis* were accepted as the next most important species in cheeses (Hansen and Jakobsen 2001). *G. candidum* was also reported as one of the most versatile species in cheeses. Pottier et al. (2008) reported that *G. candidum* was estimated to be present in about 600,000 t of cheeses produced in France. It was reported that the consumption of cheeses that contain or may contain *G. candidum*

was close to 8 kg/person/year. It was also estimated that 0.8–80 billion cells of *G. candidum* was potentially ingested per person per year.

It is known that the composition of the yeast flora of young cheese is heterogenous and depends strongly on the conditions of the cheese plant in which it has been produced. In cheese prior to brining, lactose positive species such as *K. lactis*, *K. maxianus*, and *Tp. delbrueckii* were reported to be dominant (Fröhlich-Wyder 2003).

The technology of cheese ripening also has an impact on the composition of the yeast flora (Fröhlich-Wyder 2003). It was reported that *D. hansenii*, *K. marxianus*, *S. cerevisiae*, *Zygosaccharomyces rouxii* (Tempel and Jakobsen 1998), *K. lactis*, *Y. lipolytica*, and *Candida* spp. (Viljoen et al. 2003) could be isolated from blue mold-ripened cheeses. The typical yeast flora of smear-ripened cheeses may contain mainly *D. hansenii,* but also *Y. lipolytica* and *G. candidum* (Fröhlich-Wyder 2003), as well as *Candida utilis* and *K. lactis* (Beresford et al. 2001). It was reported that *D. hansenii* is especially of importance during the production of surface-ripened cheeses such as Brick, Limburger, Port Salut, Taleggio, Tilsitter, Trappist, and Danish Danbo cheese (Fleet 2006). In the production of Gorgonzola, *S. cerevisiae* has been used as a starter culture, but it apparently also occurs spontaneously, together with other yeasts, as an integral part of the microbial population of both blue-veined cheeses and some types of soft cheeses (Fleet 2006).

Karasu-Yalcin et al. (2012b) investigated the yeast flora of Erzincan tulum cheese which is a traditional Turkish cheese ripened in goat's or sheep's skin bags. The yeast flora of Erzincan tulum cheese was reported to contain predominantly *Candida lambica*, followed by *Candida zeylanoides*, *Candida famata, Candida kefyr,* and *G. candidum*. It was mentioned that the yeast flora of Erzincan tulum cheese was unique for having predominantly *C. lambica* (Karasu-Yalcin et al. 2012b). Hayaloglu et al. (2007) reported that the packaging material used during ripening had a significant impact on the microflora of tulum cheese.

14.2 Importance of Yeasts in Cheese Ripening

Cheese curd can be defined as a complex biochemical habitat in which a variety of microorganisms coexist, anabolizing some substances and catabolizing other substances in order to live and grow. Furthermore, various types of interactions between the microorganisms of the cheese ecosystem can occur during ripening (Arfi et al. 2005). Yeasts in cheese are considered insignificant at the earlier stages of cheese production, but play a significant role in later stages, being present as natural contaminants in the curd during ripening (Welthagen and Viljoen 1999). Their presence is of major importance, as they can be beneficial or detrimental as a major component of the microflora that contribute to the ripening and flavor development (Westall and Filtenborg 1998a, b; Viljoen et al. 2003). It is known that the role of yeasts depends on the type of cheese; in some varieties they may be responsible for

spoilage, causing fruity flavors, gassiness, slime formation, softening, and discoloration, while in others they are involved in the ripening process and contribute to microbial interactions, texture changes, and biosynthesis of flavor compounds (Deak and Beuchat 1996; Suzzi et al. 2001). Since some yeasts could lead to spoilage or undesirable flavor development during the maturation stage, depending on the type of cheese, it is important to evaluate the physiological and biochemical properties, including aroma formation, lipolytic and proteolytic activities, or interaction with other microorganisms such as lactic acid bacteria and also filamentous fungi (Kumura et al. 2002; Viljoen 2006).

The main positive contribution of yeasts to the cheese ripening process was reported as the utilization of lactic acid with a consequent increase of pH values (Fröhlich-Wyder 2003; Ferreira and Viljoen 2003). This action favors bacterial growth, especially proteolytic bacteria, and initiates the second stage of cheese ripening (Deak and Beuchat 1996; Ferreira and Viljoen 2003). It was reported that in surface-ripened cheeses, the bacterial flora was primarily composed of *Brevibacterium linens, Arthrobacter* spp., *Corynebacterium* spp., and *Micrococcus* spp. The development of this bacterial surface flora has been shown to be dependent on the metabolism of lactic acid by yeasts, mainly *D. hansenii* (Petersen et al. 2002). The yeasts, by utilizing the accumulated lactic acid in cheeses, increase the pH and secrete growth factors which promote the growth of especially *B. linens*, which is essential for cheese ripening (Viljoen 2006). Yeasts also help the development of fungi in some kinds of cheeses, like blue-veined and Camembert, by gas production leading to curd openness.

Lactate metabolism is known as the most important biochemical reaction in the ripening of surface mold-ripened cheeses. Soon after manufacture, the surface of these cheeses was reported to become colonized by the secondary microorganisms (McSweeney 2004). *G. candidum* and *Penicillium camemberti* that rapidly metabolize lactate oxidatively to CO_2 and O_2, cause deacidification of the cheese surface. Deacidification leads to a pH gradient developing from the center of the cheese to its surface, and lactate diffuses from the center toward the surface of the cheese. As the pH of the surface of the cheese increases by formation of NH_3 from proteins, calcium phosphate precipitates, which results in a calcium phosphate gradient from center to surface and migration of calcium phosphate toward the surface. As a result, reduction in the concentration of calcium phosphate, together with increased pH, causes softening of the interior, which is a characteristic of mature Camembert-type cheese (McSweeney 2004).

Yeasts may also contribute to cheese ripening by assimilation or fermentation of some sugars such as glucose, galactose, and lactose. It is known that glucose is one of the sugars occurring at trace amounts in cheese. Lactose is partially hydrolyzed to glucose and galactose during cheese production, then glucose is rapidly fermented by starter lactic acid bacteria and very low amounts of glucose can be found in cheese (Ferreira and Viljoen 2003). It is known that most of the lactose in milk remains in the whey as lactose or lactate during cheese production. However, low levels of lactose (0.8–1.0 %) remain in the curd at the end of manufacture. Residual lactose is metabolized quickly to L-lactate during the early stages of ripening at a

rate largely determined by temperature and salt-in-moisture levels of curd by the action of starter bacteria. As salt levels in Cheddar and other dry-salted varieties increase rapidly on salting, starter activity is stopped very quickly at the end of manufacture. Lactose that remains unfermented by the starter is probably metabolized by the secondary flora (McSweeney 2004). According to Ferreira and Viljoen (2003), galactose in cheese has been reported as an important sugar contributing to reactions during cheese ripening. Yeast contribution to galactose metabolism was demonstrated by addition of *D. hansenii* and *Y. lipolytica* as costarters to Cheddar cheese. It was reported that no galactose was detected after 1–5 months in the yeast-inoculated samples, while 0.55 % galactose was detected even after 6 months in control samples produced without addition of adjunct starter (Ferreira and Viljoen 2003). In another study by Tempel and Jakobsen (1998), identification and technological characterization of yeasts originated from Danablu cheese were performed. It was reported that all of the yeast isolates belonging to *C. famata, Candida catenulata*, *C. lipolytica*, *Zygosaccharomyces* spp. , and *Tr. cutaneum* could utilize glucose and galactose.

Lactose fermentation of yeasts is given as an important technological characteristic directly affecting cheese flavor. It has been reported that the fermentation of lactose by yeasts influences the formation of aroma by formation of ethanol and acetaldehyde, by limiting acidification by lactic acid and thus affecting the texture of cheese, and by the formation of CO_2 (Welthagen and Viljoen 1999). It is known that yeasts belonging to genus *Kluyveromyces* have higher β-galactosidase activity than other yeasts (Petrova and Kujumdzieva 2010). It was reported that although *S. cerevisiae* cannot transport and metabolize lactose, *Kluyveromyces* spp. and certain *Candida* spp. are able to ferment lactose (Walker 1998).

In a study performed by Welthagen and Viljoen (1999), lactose concentration was determined as 2.55 % at the beginning of ripening during Cheddar cheese production, while it was detected as 0.262 % after 51 days. Besides, yeast count considerably increased between 24 and 37 days of ripening and it was concluded that decrease in lactose concentration was due to the ability of yeasts growing at low temperatures. In another study by Pereira-Dias et al. (2000), it was reported that all of the strains originated from Portuguese ewes' cheese belonging to *Candida curvata*, *Candida intermedia*, and *D. hansenii*, and 10 % of *Rhodotorula* strains were able to assimilate lactose, while none of the *C. zeylanoides* strains had this activity. In another study, *K. lactis, K. marxianus*, and *Dekkera anomala* were reported to ferment and assimilate lactose that originated from Sardinian ewes' cheese (Cosentino et al. 2001). Karasu-Yalcin et al. (2012a) investigated assimilation and fermentation of glucose, galactose, and lactose as a technological tool for yeast strains isolated from Erzincan tulum cheese. It was determined that all of the isolates assimilated glucose, while 49 % of them could ferment this sugar. Additionally, 69 % of the tested yeast strains had the ability to assimilate galactose, while galactose fermentation was positive for only 46 % of them. In this study, lactose assimilation and fermentation was detected for a few of the strains, but all of the strains belonging to *C. kefyr* and *C. famata* var. *famata* were able to assimilate and ferment lactose. It was reported that higher positive results were obtained for galactose

assimilation property when compared to lactose assimilation. In addition, two strains belonging to *K. lactis* var. *lactis* and *C. kefyr* were found to assimilate and ferment glucose, lactose, and galactose (Karasu-Yalcin et al. 2012a).

Proteolysis has been reported as one of the most complex and, in most varieties, the most important biochemical events that occur in cheeses during ripening (McSweeney 2004). Proteolysis has a direct influence on flavor through the production of short peptides and amino acids, some of which are flavored, by facilitating the release of sapid compounds from the cheese matrix and by providing free amino acids that are substrates for a series of catabolic reactions that generate many important flavor compounds. The origins of proteinases and peptidases that catalyze proteolysis in cheese were given as the coagulant, milk, starter lactic acid bacteria, nonstarter lactic acid bacteria, and secondary microorganisms (Sousa et al. 2001; McSweeney 2004). Many yeasts are known to produce proteolytic enzymes (Fröhlich-Wyder 2003). Species with high proteolytic activity were given as *K. lactis*, *Kluyveromyces fragilis*, *Candida pseudotropicalis*, *D. hansenii*, *Y. lipolytica*, and *C. catenulata* (Wyder and Puhan 1999). *Y. lipolytica*, *G. candidum*, and *C. catenulata* were reported as species with a strong extracellular proteolytic and/or peptidolytic activity. Intracellular proteinases have been detected in yeasts of the genera *Trichosporan* and *Debaryomyces* (Fröhlich-Wyder 2003). Exopeptidases that are aminopeptidases and carboxypeptidases are known to play a major role in the proteolysis of milk proteins. All intracellular enzymes would be much more significant in the cheese ripening process if released by cell lysis (Wojtatowicz et al. 2001; Fröhlich-Wyder 2003). They can play important roles in the breakdown of proteins and peptides into smaller degradation products, which contribute to the development of cheese flavor and texture (Wojtatowicz et al. 2001). The peptidolytic activity of yeasts may also play an important role in the breakdown of bitter peptides by releasing smaller peptides and amino acids. In particular, *G. candidum* is known to have this activity (Wyder and Puhan 1999). Wojtatowicz et al. (2001) reported that 90 % of the 39 *C. famata* strains originated from Rokpol cheese had low extracellular proteolytic activities, whereas higher levels of intracellular exopeptidases and peptidases were noticed for the majority of the strains. In another study, proteolytic activities of *D. hansenii, Y. lipolytica*, and *Cryptococcus laurentii* originated from Picante cheese were investigated (Freitas et al. 1999). Proteolytic and peptidolytic activities were demonstrated to be high for *Y. lipolytica*, and at much lower levels for the other tested strains (Freitas et al. 1999). Hansen and Jakobsen (2001) reported that a strain of the *S. cerevisiae* isolated from blue-veined cheese was able to degrade casein and stimulate the growth of *Penicillium roqueforti* in cheese. Proteolytic activities of 35 yeast strains isolated from Erzincan tulum cheese were investigated by Karasu-Yalcin et al. (2012a). It was reported that only four yeast strains belonging to *G. candidum*, *C. kefyr*, *C. lipolytica*, and *C. lambica* had proteolytic activities. Enzymatic activities of 69 yeast strains originated from Erzincan tulum cheese were also screened by Karasu-Yalcin et al. (2012b), and it was reported that all of the isolates had leucin arylamidase activities. In this study, a strain of *Candida japonica* was found to have both valine arylamidase and cystine arylamidase activities. Herreros et al. (2003) reported that arylamidases were impor-

tant tools in the liberation of aminoacids and development of desirable flavors in cheese. These enzymes were also reported to have a debittering effect during cheese ripening (Herreros et al. 2003).

Lipolysis results in the formation of free fatty acids, which are constituents of cheese flavor and can be precursors of flavor compounds such as methylketones, alcohols, and lactones during cheese ripening (Wit et al. 2005). Lipases and esterases of lactic acid bacteria appear to be the principle lipolytic agents in Cheddar and Dutch-type cheeses made from pasteurized milk (Collins et al. 2003). Yeasts also contribute to maturation of cheese by their lipolytic activities. Among the yeasts originated from cheeses, *Y. lipolytica* was recognized as the species having the greatest lipolytic activity (Fröhlich-Wyder 2003) and is regarded as a potential ripening agent because of this characteristic (De Freitas et al. 2009). According to De Freitas et al. (2009), addition of *K. lactis*, *Pichia fermentans*, and *Y. lipolytica* to Cantalet cheese increased lipolysis and markedly enhanced the formation of some volatile compounds. Pereira-Dias et al. (2000) reported that 98 % of the 344 yeast strains isolated from artisanal Portuguese ewes' cheese had esterase activity. Karasu-Yalcin et al. (2012a) investigated lipolytic activities of 35 yeast strains isolated from Erzincan tulum cheese and determined that 80 % of the isolates had lipolytic activities. In another study performed by Karasu-Yalcin et al. (2012b), 69 of the 121 yeast strains isolated from Erzincan tulum cheese were screened for their enzymatic activities. It was reported that 78 % of the tested isolates had esterase lipase (C8), and 7 % of them had lipase (C14) activities. The strains that had lipase (C14) activities were in the species of *G. candidum*, *Candida apicola*, and *C. lambica.*

Interactions between yeasts and other microorganisms in cheese have been reported in several studies (Jakobsen and Narvhus 1996; Viljoen 2001, 2006; Liu and Tsao 2009). The increase in pH due to lactic acid utilization encourages the growth of bacteria which not only affects flavor, but may pose a risk to public health. It is also known that yeasts assist the development of fungi in blue-veined and Camembert cheeses by gas production, leading to curd openness (Viljoen 2001; Romano et al. 2006). Yeasts also play a significant role during ripening by supporting the growth of the starter cultures. According to Viljoen (2001), a large number of yeasts present during later stages of ripening, originating as contaminants from the immediate environment, is indicative of a possible mutualistic interaction between the microflora. Yeasts may also inhibit or eliminate pathogenic bacteria or microorganisms causing spoilage in cheese (Jakobsen and Narvhus 1996). It has been indicated that *D. hansenii* inhibits the germination of *Clostridium butyricum* and *Clostridium tyrobutyricum*, possibly by depletion of lactic and acetic acids in cheese (Jakobsen and Narvhus 1996; Viljoen 2001). Some yeasts have been known to possess antagonistic property toward molds and other yeasts by producing killer toxins (Liu and Tsao 2009). In a study performed by Liu and Tsao (2009), the effects of *Williopsis saturnus* var. *saturnus* as a biopreservative against galactose fermenting spoilage yeasts were investigated in cheeses made under laboratory conditions. It was reported that this killer yeast inhibited *S. cerevisiae* and *K. marxianus* strains in cheese. It is known that there are high-, medium-, and low-risk pathogenic microorganisms in cheese. *Salmonella, Listeria monocytogenes*, and enteropatho-

genic *Escherichia coli* are categorized as high-risk threats to the cheese industry (Park et al. 2004). Inhibition effects of the yeast strains originated from cheeses on pathogenic bacteria were also reported in several studies (Georges et al. 2006, 2011; Karasu-Yalcin et al. 2012a). Georges et al. (2006) investigated the antilisterial potential of 404 food-borne yeast strains, 304 of which were isolated from smear-ripened cheeses. It was reported that only 4 % of the red smear cheese isolates clearly inhibited growth of *L. monocytogenes*. The yeast strains showing high inhibitory effect were mainly in the species of *C. intermedia* and *K. marxianus*. In another study performed by Georges et al. (2011), a total of 175 yeast strains were selected from various sources, with a focus on yeast species relevant for the production of smeared cheeses. It was reported that 14 % of the yeast strains had antilisterial activity, and a *Pichia norvegensis* strain tested was found to inhibit *L. monocytogenes* by 7 log units. Karasu-Yalcin et al. (2012a) investigated inhibition effects of 35 yeast strains originated from Erzincan tulum cheese on *Staphylococcus aureus*, *E. coli* and *L. monocytogenes*. For five of the tested strains belonging to *C. krusei*, *G. candidum*, and *P. fermentans*, weak inhibition effect on *E. coli* was observed. Inhibition effect on *S. aureus* and *L. monocytogenes* was observed for 23 % and 29 % of the isolates, respectively. In this study, some *G. candidum* strains inhibited all of the three tested pathogenic bacteria. According to Wouters et al. (2002), *G. candidum* has the ability to excrete D-3-phenyllactic acid that inhibits the growth of *L. monocytogenes.*

14.3 Potential Applications of Yeasts Originated from Traditional Cheeses Based on Biochemical Traits

Since the microflora of cheese plays a major role in cheese ripening, selection of suitable strains would enable the cheese maker to control or modify flavor development (Beresford et al. 2001). Yeasts are considered potential adjunct cultures for cheeses according to their biochemical properties, as reported in some studies (Martin et al. 2001; Bintsis and Robinson 2004; Kesenkas and Akbulut 2006a, b; De Freitas et al. 2009; Mehlomaluku 2011). The effects of addition of a cocktail of yeast species to Cantalet cheese were investigated by De Freitas et al. (2009). Three indigenous strains of *K. lactis*, *Y. lipolytica*, and *P. fermentans*, originated from raw milk Cantalet cheese, were used as adjunct starters in the production of Cantalet cheese. It was reported that the addition of yeasts to milk for the manufacture of Cantalet cheese generated complex yeast-yeast and yeast-bacteria interactions. The presence of adjunct yeasts was reported to give a better survival of lactococci, and to induce a small increase in lipolysis and a very marked enhancement in the formation of ethyl esters and branched-chain compounds (De Freitas et al. 2009). Kesenkas and Akbulut (2006a) investigated the effects of some adjunct yeast cultures on the aroma compounds of Turkish white cheese during ripening. It was reported that *Y. lipolytica* and *D. hansenii* contributed to formation of linear chain

aldehydes and methyl ketones when used as adjunct starters in white cheese. It was claimed that linear chain aldehydes, especially hexanal, were characterized by cream or caramel flavor. In another study performed by Bintsis and Robinson (2004), the influence of adjunct brine cultures on volatile compounds in Feta-type cheeses made from bovine milk was studied. *D. hansenii* and *Y. lipolytica* strains were used as adjunct starters in addition to *Lactobacillus paracasei* subsp. *paracasei*. It was reported that the addition of selected lactobacilli and yeast resulted in a richer pattern of volatiles, in particular alcohols, aldehydes, and esters, which had already been identified as essential components of Feta cheese aroma (Bintsis and Robinson 2004). The use of yeasts as adjunct starters in matured Cheddar cheese was investigated by Mehlomaluku (2011). It was reported that sensory analysis of the cheeses resulted in desired Cheddar cheese character in yeast matured cheeses compared to the control. Cheese samples inoculated with *Tp. delbrueckii* and *Dekkera bruxellensis* had favorable scores for aroma, texture/appearance, and mouthfeel (Mehlomaluku 2011). Because of their positive attributes, the use of yeast adjuncts was proposed as a way to modulate cheese flavor and to control the development of contaminating yeasts (De Freitas et al. 2009).

Several yeasts have been proposed as novel probiotic microorganisms and producers of functional ingredients (Padilla et al. 2010). Bile tolerance of microorganisms has been used as a selective criterion for potential probiotics (Gotcheva et al. 2002), as well as resistance to low pH values and antimicrobial activities. There is a developing interest in using various yeast species as probiotic organisms. Such species include *S. cerevisiae, S. cerevisiae* var *boulardii, D. hansenii, K. marxianus, Y. lipolytica, Issatchenkia orientalis, Pichia farinosa, Pichia anomala*, and *Galactomyces geotrichum* (Fleet and Balia 2006). *S. cerevisiae* var. *boulardii* has been reported to be effective in the treatment of diarrhea in adults and children infected with *Clostridium difficile*, of diarrhea in human immunodeficiency virus-infected patients, and of acute and chronic diarrhea in children and adults (Rajkowska and Kunicka-Styczyńska 2009). Several reports have focused on probiotic features of yeasts isolated from traditional foods. In a study performed by Gotcheva et al. (2002), some probiotic characteristics of *Tr. cutaneum*, *Candida rugosa*, and *C. lambica* isolated from a traditional fermented beverage were investigated. Bile salt tolerance was determined between 0.2 and 2 % oxgall and it was reported that all of the strains survived at all tested concentrations, but increase in yeast count was only observed in the media containing 0.2 and 0.3 % oxgall. Karasu-Yalcin et al. (2012a) investigated some probiotic characteristics of yeast strains isolated from Erzincan Tulum cheese. It was reported that three strains belonging to *C. lipolytica*, *C. krusei*, and *C. rugosa* gave promising results for bile salt tolerance, an important tool for evaluation of them as potential probiotics. In the same study, most of the strains were found to grow at pH 2.5 except one *Candida colliculosa* strain. It is also known that interaction of some yeasts with pathogenic bacteria, as discussed in the previous section, is also an important criterion for selection of potential probiotics.

Microorganisms isolated from natural fermentation media represent the most important source of biodiversity in the development of microbial starter cultures intended for industrial fermentations. The selection of yeast starter cultures for a

specific fermentation is generally carried out within isolates originating from the same type of fermenting substrate (Rainieri et al. 2009), as in the examples above for cheese fermentations. It is expected that in this way, the culture will be more adapted to the substrate and technologically better suited to efficiently carry out the fermentation process. Rainieri et al. (2009) noted that while this procedure promotes yeast technological performance, it limits the variety of traits that can be exploited in industrial fermentations. The selection of starters from yeasts isolated from alternative substrates was proposed as an effective method to enrich the variety of qualitative fermentation traits. It was reported that *S. cerevisiae* strains isolated from whey were able to considerably lower malic acid concentration in wine fermentations, an uncommon trait among wine starter cultures (Rainieri et al. 2009). Based on their biochemical characteristics, yeasts from fermented foods may be adapted to a wide range of food productions and/or biotechnological processes. In a study performed by Limtong et al. (2009), traditional fermented foods in Thailand were used as a source to isolate some thermotolerant yeasts for ethanol fermentation, because of the fact that the fermentation temperature of those foods was usually high in that country. It was reported that potential isolates were determined for ethanol production at 40 °C. Anvari and Khayati (2011) studied submerged yeast fermentation of cheese whey for protein production by using lactose-fermenting yeast strains isolated from cheese whey. Fermentation of cheese whey for bioethanol production is given as one of the integrated solutions for the valorization of this byproduct. The lactose-fermenting species *K. lactis*, *C. pseudotropicalis*, and *S. cerevisiae* were reported to be used for this process (Guimarães et al. 2010). Traditional cheeses may also serve as a good source for these species to be used in bioethanol production. It was reported that, for the yeast strains of a specific food, assimilation of wide or narrow ranges of carbon and nitrogen sources corresponds to the nutrients available in natural substrates (Nout 2003). Karasu-Yalcin et al. (2012c) studied carbon assimilation profiles of yeast isolates originated from Erzincan tulum cheese as a tool for identification. Carbon assimilation profiles of some isolates as obtained by API ID 32C strips are given in Table 14.2. It can be seen that all species presented specific carbon assimilation profiles, and glucose was the only substrate that was assimilated by all of the strains. Intraspecies variability in carbon assimilation was high, especially for *C. famata* and *C. lambica*. All strains of *C. colliculosa, C. kefyr, Candida lipolytica*, *Geothricum* spp., *Candida sphaerica*, and *S. cerevisiae* assimilated DL-lactate. For *C. lambica* and *C. famata* strains, DL-lactate assimilation was variable. Lactose assimilation was observed for all *C. famata*, *C. kefyr*, and *C. sphaerica* strains. Galactose assimilation was observed for most of the isolates belonging to *C. colliculosa, C. famata, C. kefyr, C. rugosa, Geotrichum spp., C. sphaerica*, and *S. cerevisiae*. It was indicated that the use of lactose, glucose, DL-lactate, and galactose could be attributed to their contribution to cheese ripening and may be used as a tool for their selection as adjunct starters. In addition, *C. colliculosa*, *C. famata*, *C. rugosa*, *C. zeylanoides*, and *Geotrichum* spp. assimilated mannitol, sorbitol, and glycerol. *Zygosaccharomyces* spp. presented a specific profile that assimilated only mannitol, glycerol, and glucose. The use of specific substrates may be important for certain biotechnological processes,

Table 14.2 Carbon assimilation profiles of the yeast strains isolated from Erzincan tulum cheese

Substrate	Yeast species	*C. colliculosa*	*C. famata*	*C. kefyr*	*C. krusei*	*C. liploytica*	*C. lambica*	*C. rugosa*	*C. zeylanoides*	*Geotrichum* spp.	*C. sphaerica*	*S. cerevisiae*	*Zygosaccharomyces* spp.
Galactose		+	+	+	−	−	−	+	−	+	+	+	−
Actidione		−	−	+	−	+	−	−	v	+	+	−	−
Saccharose		+	+	+	−	−	−	−	−	−	+	+	−
N-Acetyl-Glucosamine		−	+	−	+	+	v	−	+	−	−	−	−
DL-Lactate		+	v	+	+	+	v	−	−	+	+	+	−
L-Arabinose		−	+	−	−	−	−	−	−	−	−	−	−
Cellobiose		−	v	−	−	−	−	−	−	−	−	−	−
Raffinose		+	+	+	−	−	−	−	−	−	+	+	−
Maltose		−	+	−	−	−	−	−	−	−	+	+	−
Trehalose		−	+	−	−	−	−	−	v	−	−	−	−
2-Keto-Gluconate		+	+	−	−	−	v	−	+	−	−	−	−
α-Methyl-D-Glucoside		−	+	−	−	−	−	−	−	−	−	v	−
Mannitol		+	+	v	−	−	v	+	+	+	+	−	+
Lactose		−	+	+	−	−	−	−	−	−	+	−	−
Inositol		−	−	−	−	−	v	−	−	−	−	−	−
Sorbitol		+	+	+	−	+	v	+	+	+	+	−	−
D-Xylose		+	v	v	−	−	v	−	−	+	−	−	−
Ribose		−	−	−	−	−	−	−	−	−	−	−	−
Glycerol		+	+	v	+	+	v	+	+	+	+	−	+
Rhamnose		−	v	−	−	−	−	−	−	−	−	−	−
Palatinose		−	+	−	−	−	−	−	−	−	−	−	−

(continued)

Table 14.2 (continued)

Substrate	Yeast species	*C. colliculosa*	*C. famata*	*C. kefyr*	*C. krusei*	*C. liploytica*	*C. lambica*	*C. rugosa*	*C. zeylanoides*	*Geotrichum* spp.	*C. sphaerica*	*S. cerevisiae*	*Zygosaccharomyces* spp.
Erythritol		–	v	–	–	+	–	–	–	–	–	–	–
Melibiose		–	–	–	–	–	–	–	–	–	–	–	–
Glucuronate		–	–	–	–	–	–	–	–	–	–	–	–
Melezitose		–	+	–	–	–	–	–	–	–	+	–	–
Gluconate		–	v	–	–	v	–	–	v	–	–	–	–
Levulinate		–	–	–	–	–	–	–	–	–	–	–	–
Glucose		+	+	+	+	+	+	+	+	+	+	+	+
Sorbose		–	+	–	–	–	v	+	v	+	–	–	–
Glucosamine		–	v	–	–	–	+	–	v	–	–	–	–
Esculin		+	v	v	–	–	v	–	v	v	+	–	–

as well as for food fermentations. This raises the idea about the potential of yeast isolates to be used in the production of valuable products from alternative substrates. Some examples of these processes may be arabitol production from L-arabinose, xylitol production from xylose, and ethanol production from cellobiose. Karasu-Yalcin et al. (2012c) indicated that as another outcome of the related study, in addition to screening carbon assimilation profiles, biochemical characterization of a pool of novel endogenic yeasts was also achieved. Traditional fermented foods can be sources of microbial strains with promising features for novel products. Interest in the microflora of traditional fermented dairy products should be encouraged, and the pool of novel microbial strains from these floras should be saved for potential future applications (Wouters et al. 2002).

Traditional fermented foods, also referred to as indigenous fermented foods, are those popular products that have been known since early history and that can be prepared in the household or cottage industry using relatively simple techniques and equipment. However, upgrading traditional home-scale processes is needed so that these products can compete successfully with imported products (Nout 2003). With regard to local cheeses in Turkey, with the exception of a few varieties, most of the traditional brined cheeses have not yet been industrialized. It has been claimed that relatively little is known about the basic and microbiological characteristics of the brined cheeses native to Turkey (Hayaloglu et al. 2008). In addition, despite the fact that there is a high variety of cheeses produced in Eastern Anatolia Region, only a few of the cheeses (Gravyer cheese, Kashar cheese, Erzincan tulum cheese, Civil cheese, Saçak cheese, and Herb cheese) specific to this region have made a place for themselves in the markets. More extensive studies are needed, especially on those local cheese varieties produced in villages or rural areas, most of which are in the process of disappearing from the region (Kamber 2008). This future research will enable the discovery of distinct cheese types as sources of novel microbial strains with interesting features to be used for various applications.

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Chapter 15
Beta-Lactoglobulin Fibril Dispersions: Structural and Rheological Characteristics

Simon Loveday, M. Anandha Rao, and Harjinder Singh

15.1 Introduction

Processed foods are created as a result of mostly physical responses of proteins, mono- and polysaccharides, and lipids in aqueous media to different processing methods. Measured rheological responses occur at the macroscopic level. However, these responses are affected by changes and properties at the molecular and microscopic levels. One major challenge is to establish links between macroscopic rheological properties with changes at the molecular and microscopic levels (Rao 2007). Rheological data on a food together with data on its composition and structure or microstructure should lead to understanding the inter relationships between them (Genovese et al. 2007).

Particles with a wide range of sizes are found in foods. The size of colloidal (Brownian) particles may be considered to be between 1 nm and 10 μm (Russel et al. 1989). Examples of foods that contain colloidal particles are milk, cloudy fruit juices, and mayonnaise (an emulsion). Brownian motion promotes collisions between pairs of colloidal particles, while interparticle forces determine whether two colliding particles aggregate. Particle shape, size and size distribution, deformability, and liquid polarity may affect the structure/rheology (Tsai and Zammouri 1988).

Many foods are dispersions of solids in a liquid medium, usually an aqueous solution, or of liquid droplets in another liquid, which is called an emulsion. It is well known that the apparent viscosity of dispersions depends on the volume

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fraction of the solids. Three kinds of forces coexist to various degrees in flowing dispersions: hydrodynamic, Brownian, and colloidal forces. Hydrodynamic forces arise from the relative motion of particles to the surrounding fluid. The Brownian force is the ever-present thermal randomizing force. Colloidal forces are potential forces and are elastic in nature (Zhou et al. 2001). The relative magnitude of these forces and, therefore, the bulk rheological behavior are influenced by the particle size. For sub-nanometer-size dispersions, Brownian motion and interparticle forces quickly equilibrate. Colloidal dispersions can be defined as polyphasic or heterogeneous systems where the dispersed phase is subdivided into discrete units (particles/droplets) that are large compared to simple molecules, but small enough so that interfacial and inertial forces are significant in governing system properties (Sennet and Olivier 1965).

Here, we briefly cover recent results on the structure and rheology of fibrils made from whey protein β-lactoglobulin (β-lg). A more extensive review of the subject can be found elsewhere (Loveday et al. 2012a). When heated at a low pH ~2–3, and ~85 °C, β-lg will self-assemble into amyloid-like fibrils that are 4–10 nm wide and up to 10 μm long, and consist of β-sheets whose strands run perpendicular to the fibril axis. These long fibrils have the potential to enhance viscosity and form gels at low protein concentration due to their extreme aspect ratio. For practical application, whey protein isolate (WPI) is often used to make β-lg fibrils, as it contains up to 80 % w/w β-lg and is available at much lower cost than highly purified β-lg.

Salts are ubiquitous in food and biological systems, and we have investigated the effect of $CaCl_2$ on fibril network assembly during heating. $CaCl_2$ was chosen because it accelerates self-assembly much more effectively than NaCl (Loveday et al. 2010), and significant amounts of Ca^{2+} are present in many dairy-based food products.

WPI fibrils formed without $CaCl_2$ were long and semiflexible, and associated in large entangled networks more than 10 μm across on the TEM grid (Fig. 15.1a). Fibrils formed in the presence of 100 mM $CaCl_2$ were shorter, and were bent and twisted (Fig. 15.1b), and these are termed "wormlike" fibrils. At $CaCl_2$ concentrations between 0 and 100 mM, "long semiflexible" and "wormlike" fibril types coexist (Loveday et al. 2010). The morphology of fibrils is characterized by two length scales: persistence length, l_p, and contour length L_c. Persistence length, defined as $l_p = k/(k_B T)$, is the typical length at which thermal fluctuations begin to bend the polymer in different directions; it characterizes the flexibility or rigidity of a filament (MacKintosh 1998). The persistence length of food protein nanofibrils has been obtained from many experimental techniques (Loveday et al. 2012a), including dynamic light scattering, microscopic observation of thermal fluctuations, and transmission electron microscopy (TEM) data. The contour length, L_c, of a filament is its length at maximum extension. A filament is considered flexible when $l_p \ll L_c$, and rigid when the opposite holds ($l_p \gg L_c$); many biological filaments are in a third intermediate category; semiflexible filaments with l_p and L_c are of comparable magnitude (Storm et al. 2005). Typical values of the persistence and contour lengths of β-lg fibrils are given in Table 15.1.

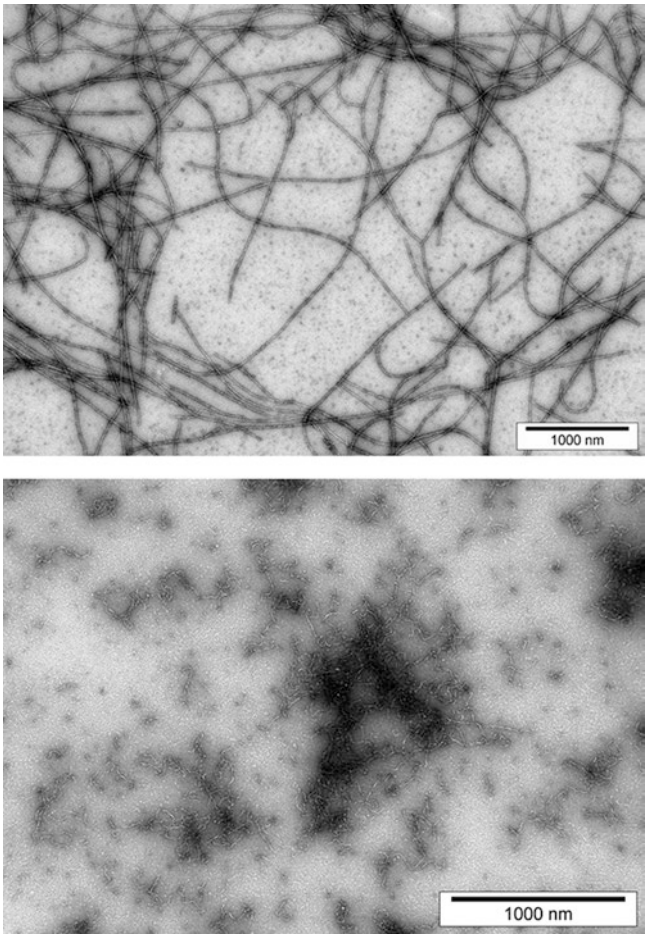

Fig. 15.1 β-lactoglobulin nanofibrils made by heating 1 % w/v protein at pH 2 and 80 °C with either (**a**) no added salts or (**b**) with 100 mM $CaCl_2$

15.2 Rheological Properties of Whey Protein Isolate Fibril Gels

On heating dispersions of β-lg at low pH and a constant temperature, the magnitude of the storage modulus (G') increases with time as the proteins self-assemble into fibrils. From the data of such a gel-cure experiment, the magnitudes of the equilibrium modulus and gel time, which reflect the morphology of the fibrils, can be determined by using the relationship (Kavanagh et al. 2000):

$$G' \approx G'_{\text{inf}} \exp\left(-B / t\right) \tag{15.1}$$

Table 15.1 Contour length (L_c) and persistence length (l_p) data for β-lactoglobulin fibrils

Study and method for measuring l_p	Concentration (% w/v)	Ionic strength	pH	Heating temperature (°C)	Heating time (h)	L_c (nm)	l_p (nm)
Aymard et al. (1999)	4	0.013	2	80	?[a]	?	600
X-ray and neutron	4	0.03	2	80	?	?	300
scattering	4	0.1	2	80	?	?	38
Mudgal et al. (2009)	1.5	<0.05	2	80	10	2500	788
TEM image analysis	4	<0.05	3.35	85	3	130	36
	8	<0.05	3.35	85	3	300	34
Loveday et al. (2010)	1	0	2	80	6	>2523	2607 ± 511
TEM image analysis	1	0.1 (NaCl)	2	80	6	>1569 (LS)[b]	4307 ± 747 (LS)
						>494 (WL)	80 ± 15 (WL)
	1	0.3 ($CaCl_2$)	2	80	6	>1170 (LS)	1846 ± 140 (LS)
						>514 (WL)	67 ± 4 (WL)

[a]Not specified
[b]Both long semi-flexible (LS) and worm-like (WL) fibrils co-existed under these conditions

where t is the time in seconds, G'_{inf} is the value of G' at infinite time, and B is the time taken for G' to reach G'_{inf}. Equation (15.1) satisfactorily reproduced both the asymptotic limit as $t \to \infty$ and the logarithmic singularity as $t \to t_{gel}$, the gelation time (Kavanagh et al. 2000). Values of both parameters, G'_{inf} and t_{gel}, are required for testing of the data against physical models, such as a percolation-based kinetic gelation model. G'_{inf} can also be obtained by extrapolating the linear portion of a G' versus 1/time plot to find the G' intercept (Gosal et al. 2004a).

The β-lg gel-cure data (Fig. 15.2) had an initial lag phase, during which G' was below 10 Pa, followed by a very rapid increase in G', then a further increase in G', but at a slowing rate. $CaCl_2$ had a marked impact on the duration of the lag phase, shortening it in a concentration-dependent manner. For 80 and 120 mM datasets there was dip in G' after long heating times, similar to that seen by Gosal et al. (2004b). They suggested that the dip could be caused by slippage due to gel shrinkage and syneresis after extended heating, or the infiltration of silicone oil between the cone and the sample.

The model in Eq. (15.1) was an excellent fit for 0 and 120 mM data, which had clear linear regions on "log(G') vs. 1/t" axes (Fig. 15.2 inset). The linear regions were less well defined with 40 and 80 mM data, and in choosing which points to

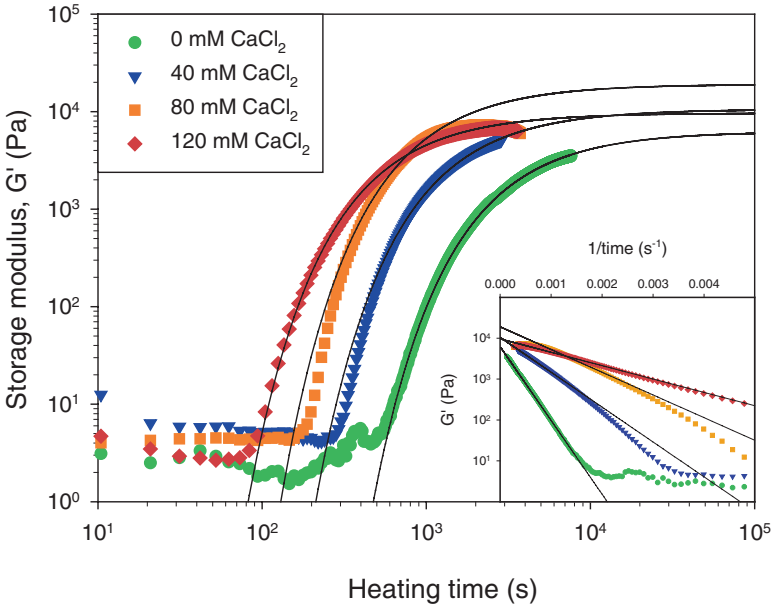

Fig. 15.2 Time course data for 10 % w/w WPI with 0–120 mM $CaCl_2$ heated at 80 °C on the rheometer. Lines show the fit of Eq. 15.1 to data; the *inset* shows the linear fitting (Eq. 15.1)

Table 15.2 Effect of $CaCl_2$ on gel times, t_{gel} (in seconds) for 10 % WPI heated at 80 °C on the rheometer

$CaCl_2$	0 mM	40 mM	80 mM	120 mM
t_{gel} when $G' > 10$ Pa	637	334	209	115
t_{gel} from manual selection	491	271	156	73
t_{gel} from Eq. (15.1)	476	212	129	82

Three different methods were used for estimating t_{gel}, as explained in the text

include in the regression, preference was given to the later points, for which G' was higher and could therefore be measured more accurately. Three different approaches were used to calculate t_{gel}: (a) choosing the time at which G' first exceeded 10 Pa, (b) manually selecting the beginning of the asymptotic increase in G', or (c) extrapolating Eq. (15.1) to the time at which it predicted $G' = 1$ Pa.

Table 15.2 shows the effect of $CaCl_2$ on t_{gel}, as calculated with these three methods. All three showed the same trend: added calcium produced a logarithmic decrease in t_{gel} with increasing $CaCl_2$ concentration. This approach has also been successfully applied to describe the effects of other mono- and divalent cations on β-lg fibril gelation (Loveday et al. 2012b).

15.3 Conclusion

Recent results on the structure and rheology of fibrils made from whey protein β-lactoglobulin (β-lg) are discussed here in brief. A more extensive review of the subject can be found elsewhere (Loveday et al. 2012a). It is shown that protein self-assembly leads to large and useful changes in rheological properties. In situ small amplitude oscillatory measurements are a useful tool that can provide information for both rheological modeling and practical purposes.

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Chapter 16
Animal Welfare and Meat Quality: Methodologies to Reduce Pre-slaughter Stress in Broiler Chicken

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16.1 Introduction

The Brazilian poultry industry is recognised as the most developed in the world, with exceptional levels of productivity. This level has been achieved by implementing quality programmes in every step of the productivity chain in recent years, particularly in genetics, nutrition, management, biosafety, good manufacturing practices, traceability, animal welfare and sustainability (UBA 2009). With these developments, Brazil has become the second largest producer and the world's largest exporter of chicken meat, with an annual production of 13,15 million tons, behind the USA of 17,97 MT and annual Brazilian exports of 4,304 MT in 2015, USA of 2,990 MT and UE-27 of 1,150 MT (USDA 2016; ABPA 2016). However, as production has increased, we have observed a concomitant increase in the rate of losses. These losses could be due to inadequate management, lack of proper technology or lack of appropriate action plans for maintaining animal welfare. In recent decades, it has increased the incidence of breast meat abnormalities such as pale-soft-and-exudative (PSE) meat. PSE meat is characterised by abnormal colour, which results from stressful conditions before slaughter. The causes of PSE meat in poultry have been well clarified over the last 30 years (Mitchell 1999; Alvarado and Sams 2002; Barbut et al. 2008; Strasburg and Chiang 2009). Simões et al. (2009a, b) reported that the incidence of pale, soft, exudative (PSE) breast

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fillets was 27.2 % in the winter season and 55.5 % in the summer season, indicating an increase in the occurrence of this problem during warmer weather.

As PSE meat occurs worldwide, several authors reported this incidence in different countries such as in Canada, 10 % (Barbut 1998), in USA, 47 % (Woelfel et al. 2002), in Italy, 10 % (Petracci et al. 2004) and in Poland, 5 % (Lesiów et al. 2007).

It is difficult to get accurate figures for the monetary losses, but some estimates suggest about $200 million a year to processors in the US broiler industry alone (Lubritz 2007; Barbut 2009). The Brazilian losses were estimated in the year of 2013, in the daily slaughter of 300,000 chickens at the facility at which this survey was conducted to be during the winter season, 81,600 broiler chickens presented PSE breast fillets, compared with 166,500 birds in the summer season. Because each broiler carcass yielded approximately 400 g of breast fillets, the calculation revealed that 32,640 kg/day of breast fillets in the winter and 66,600 kg/day of breast fillets in the summer would have been classified as PSE meat (Simões et al. 2009a, b). As determined by Oda et al. (2003), the weight loss due to the PSE content of breast fillets was approximately 1.5 %, and the loss in a single slaughter day was approximately 490 kg and 1000 kg in the winter and summer, respectively. Based on a value of US $2.94/kg for breast fillet meat, the estimated loss in economic terms was approximately US $44,117.00/month during the winter and US $88,235.00/month during the summer.

16.2 The Broiler Chicken Meat Production Chain

16.2.1 Welfare of Broiler Chickens During Production

The modern industrial production of chicken meat requires knowledge and investments in nutrition, genetics, health, the environment, animal handling and slaughter technology. Raising broiler chickens in a comfortable environment is important for their welfare and their production efficiency. Broiler chickens are raised in a variety of production systems that vary with respect to numerous factors, including the size of production (Fouad et al. 2008). An increasingly popular production system is the dark house system (DHS), which is more technologically sophisticated than the conventional yellow (CYC) and blue curtain (CBC) systems. The DHS provides comparatively better control of the aviary environment (temperature, relative humidity, gas recirculation, ventilation). Table 16.1 shows that the birds reared under the DHS presented better production performance compared with the CYC and CBC as the birds reared under the DHS showed respectively 11.4 % and 9.3 % higher average daily gain, 11.4 % and 9.3 % higher body weight and 3.8 % and finally 2.7 % lower feed conversion ratio at the end of the production cycle (46 days old). Once the broiler chickens had been acclimatised to the DHS thus in the growing phase, the broiler chickens were under less stressful conditions, resulting in a higher body comfort and improving their performance (Carvalho et al. 2015).

Table 16.1 The performance of broiler chickens reared under the dark house system (DHS), a conventional yellow curtain system (CYC) and a conventional blue curtain system (CBC)

Parameter	Treatments			*P*
	CYC	CBC	DHS	
ADFI (g)	$104.32^{a} \pm 8.63$	$106.81^{a} \pm 7.35$	$110.57^{a} \pm 4.82$	0.312
ADG (g)	$56.68^{b} \pm 0.95$	$57.79^{b} \pm 1.45$	$63.14^{a} \pm 1.40$	0.00001
BW (g)	$2{,}607^{b} \pm 44.03$	$2{,}658^{b} \pm 66.76$	$2{,}904^{a} \pm 21.47$	0.00001
FCR	$1.82^{a} \pm 0.04$	$1.80^{a} \pm 0.47$	$1.75^{b} \pm 0.33$	0.0048
V %	$96.67^{a} \pm 0.75$	$96.18^{a} \pm 0.54$	$96.88^{a} \pm 0.48$	0.143

ADFI is the average daily feed intake, ADG is the average daily gain, BW is the body weight at 46 days, FCR is the feed conversion ratio and *V* is the rearing viability

[a,b]Means followed by different superscripts in the same row differ by Tukey's test at a 5 % significance level ($P \leq 0.05$). *Source*: Carvalho et al. (2015)

16.2.2 Pre-slaughter Steps Manoeuvres

To prevent losses and ensure the quality of chicken meat and, consequently, productivity gains, the series of pre-slaughter steps must be controlled. These steps include fasting, harvesting, loading into cages on trucks, transport, reception at the commercial slaughterhouse, unloading, hanging and slaughter (Langer et al. 2010) (Fig. 16.1). The conditions during these steps greatly affect muscle glycogen stores, which are responsible for the *post-mortem* biochemical reactions that occur during the transformation from muscle to meat and, consequently, meat quality. Moreover, during the pre-slaughter steps, the chickens can suffer from stress, mistreatment and may incur injuries that cause lesions on the carcasses. These stressful challenges decrease the quality of the meat and increase the mortality rate. The main cause of losses was found to be the thermal stress experienced by the birds throughout the meat production chain (Langer et al. 2010).

16.2.3 Heat Stress

The term 'stress' is used to denote the set of reactions of an organism to physical and psychological attacks that threaten to upset the body's homeostasis (Guarnieri et al. 2004). Thermal stress is a result of interactions among temperature, relative humidity, solar radiation and wind speed (Lin et al. 2006; Simões et al. 2009a, b). Controlling these environmental conditions during pre-slaughter manoeuvres is vital to ensure thermal comfort. A typical comfortable environment for adult birds is characterised by a temperature of 21 °C and humidity of 60–70 % in which the animal's metabolic rate is minimal and homeostasis is maintained with the lowest possible energy expenditure (Macari and Furlan 2001; Simões et al. 2009a, b).

Fig. 16.1 Flowchart shows the pre-slaughter handling steps for broiler chickens, from farm to commercial slaughterhouse (Langer et al. 2010)

16.2.4 Effects of Heat Stress on Meat Quality

The birds when exposed to thermal conditions outside their comfort zone have several reactions, including depletion of muscle glycogen and this phenomenon directly affects their welfare and consequently the loss of meat quality.

The breast fillet often exhibits undesirable colour variations, which are directly related to the pH and colour values (L^*) of the meat (Fig. 16.2). These colour variations are due to changes in muscle physiology and biochemistry that lead to the formation of PSE meat. The functional properties of PSE meat are compromised by the denaturation of muscle proteins, which is promoted by rapid *post-mortem* glycolysis at reduced pH and higher carcass temperatures (Olivo et al.

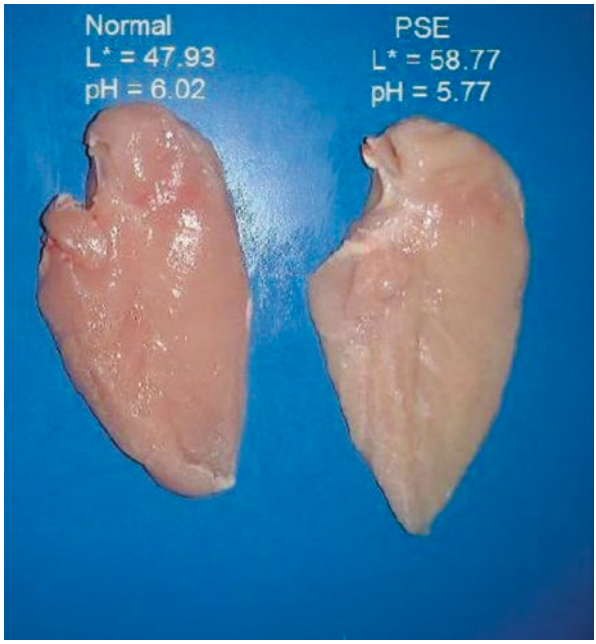

Fig. 16.2 Typical broiler breast fillet classified as PSE meat in relation to normal samples measured by the pH and *L** values, 24 h *post-mortem* (Soares 2003)

2001; Barbut et al. 2008). However, the addition of additives such as starch (Zhang and Barbut 2005), chicken collagen (Schilling et al. 2005) improved these properties although Woelfel and Sams (2001) could not find any improvement from marinating the meat. The PSE meat functionality was evaluated by examining the water retention capacity, texture profile, emulsion stability and colour of the final product. Thus, the use of isolated soy protein, sodium tripolyphosphate and cassava starch in the formulations was needed to achieve the correct properties (Kissel et al. 2009).

The conditions that lead to the development of PSE meat are likely to be the excessive release of calcium ions from the muscle sarcoplasmic reticulum, which promotes increased protease activities, thus causing fragmentation of the myofibrils in the sarcomeres and ultimately the collapse of muscle structure (Wilhelm et al. 2010). Other relevant factors that have been observed are enzyme phospholipase A_2 (PLA_2) activity, changes in lipid content (Soares et al. 2003) and genetic factors involving the ryanodine gene and the hal gene (Oda et al. 2009; Ziober et al. 2009; Paião et al. 2013). Marchi et al. (2009) suggested a genetic involvement by submitting the birds under 3 % halothane gas indicated by leg shrinkage in response to the gas and approximately 27.5 % of broilers were hal^+ and 72.5 % ($n = 248$) were hal^-.

Other authors (Cavitt et al. 2004) found 13.2 to 22.9 % hal^+ in commercial broiler lineages and Owens et al. (2000) reported a hal^+ variation from 3.5 to 10.0 % in turkey, while Wheeler et al. (1999) obtained 5.0 % hal^+, 85.0 % hal^-, and 10.0 %

halothane intermediate response in turkey. In order to prove that poultry hal gene truly plays a role in the manifestation of PSE meat, it is necessary to determine the presence of the ryanodine genotype as routinely carried out in pig PSS (Fujii et al. 1991). In pigs, PSE meat results from a set of biochemical and physiological reactions known as PSS or Pork Stress Syndrome (Fujii et al. 1991); the equivalent for birds would be ASS or Avian Stress Syndrome, although the parameters of ASS have not been established.

16.2.5 Methodologies for Avoiding Broiler Stress

16.2.5.1 Water Bath at the Farm

In order to maintain a comfortable environment for birds in transit during the summer season, it is a routine practice by some companies to submit the birds to a water shower for a few minutes just before beginning the journey with the objective to provide better animal welfare. The passive-only ventilation of standard commercial broiler transport vehicles results in low rates and heterogeneous distribution of airflow inside the container. In closed trucks, the air moves in the same direction as the vehicle; thus, the air tends to move from the rear and over the birds causing a stagnant area after the cross flow and creating a thermal core in the front (Hoxey et al. 1996; Mitchell and Kettlewell 2004). Trucks in Brazil are usually open, allowing the air to be constantly pushed from the front to the back, creating a thermal core over the rear region (Simões et al. 2009a, b; Spurio et al. 2016). Air movement in the container depends on truck speed, the pressure difference inside the truck and stock density. Under uncomfortable transport conditions, broilers can undergo unfavourable consequences, and at least two factors should be evaluated: DOA (dead on arrival) and PSE meat. As noted by Ritz et al. (2005), a DOA is a bird that died in the period between the catching manoeuvre at the farm to the moment before slaughtering. In regular transit conditions, some degree of PSE meat is unavoidable due to the thermally stressful ambient temperature that rapidly develops inside the truck. The physiological stress results in a faster post-mortem pH decline when the carcass temperature remains warm (Mitchell and Kettlewell 2004; Guarnieri et al. 2004; Simões et al. 2009a, b). This leads to denaturation of myofibrillar and sarcoplasmic proteins, compromising their functional properties and the final meat qualities (texture, flavour, juiciness) observed by consumers (Droval et al. 2012) and present in meat products (Kissel et al. 2009).

The heat stress during truck transport is one of the causes of DOA and PSE development (Simões et al. 2009a; Langer et al. 2010; Silva et al. 2011). High temperature and high relative humidity values strongly increase the heat stress during the pre-slaughter phase. DOA chickens are identified when hung on the overhead conveyor before slaughter, and the figure is used as an indicator of all deaths that

occurred while transporting the birds from the farm. Studies in the UK indicated that the deaths of birds during transport could be attributed mainly to stress and could reach a rate of 40 %, with increased mortality with increasing travel distance (Warris et al. 1990). In Brazil, the tropical climate provides chicken mortality during the summer season, and has deleterious economic consequences for poultry farmers and meat companies. Langer et al. (2010) also found that the occurrence of PSE meat increased with transport distance. Simões et al. (2009a) verified the effect of the bath at the farm after loading the truck and also the water-sprinkling during holding period at the slaughterhouse. At the rear of the truck and after the two treatments (LwoB—lot without bath and LwiB—lot with bath), there was a markedly higher temperature under the LwoB condition, causing a microenvironment at the rear region of the truck that placed heat stress on the broiler chickens. This heat build-up was the consequence of less air ventilation at the rear of the truck during transport. These results are illustrated in Figs. 16.3, 16.4 and 16.5.

Heat stress can be minimised by appropriate ventilation through the boxes on the truck. Bathing the birds on the farm with a shower of water after loading them had an important effect. The group of birds that received a shower bath (LwiB) had a lower temperature compared to the group that did not receive a bath (LwoB) (Fig. 16.3a,b). Consequently, the lack of the bath after loading was a significant risk factor for the occurrence of PSE. Moreover, this bath shower in conjunction with adequate ventilation reduced heat stress and, consequently, the occurrence of PSE meat (Fig. 16.5).

The water bath at the farm was also beneficial for reducing mortality in the summer, Silva et al. (2011) found that the DOA for LwiB chickens transported in distances of 15 km and 55 km with a 60 min holding period was 0.12 % and 0.15 %, respectively, while the LwoB chickens exhibited DOA values of 0.16 % and 0.27 %, respectively, as shown in Table 16.2. In European countries, high DOA index values have been reported, for example, Nijdam et al. (2004) reported a mortality of 0.46 % in the Netherlands, while in Italy, Petracci et al. (2006) found DOA values of 0.47 %, 0.52 % and 1.62 % for chickens, turkeys and laying hens, respectively. Thus, it is recommended to correctly apply the water bath to the chickens after loading them on the truck and immediately before transport, as illustrated in Fig. 16.1.

Recently, the performance of a designed prototype truck container provided by laterally flaps improved the microenvironment as the incidence of PSE meat was reduced. This experiment was carried out under four different conditions: regular and prototype truck, both with and without wetting loaded cages at the farm just before transporting. While there was no difference in the DOA index ($P \geq 0.05$), the prototype truck caused a reduction ($P < 0.05$) in the occurrence of PSE meat by 66.3 % and 49.6 % with and without wetting, respectively (Spurio et al. 2016) The results of this experiment clearly revealed a low-cost solution for transporting chickens that yields better animal welfare conditions and improved meat quality.

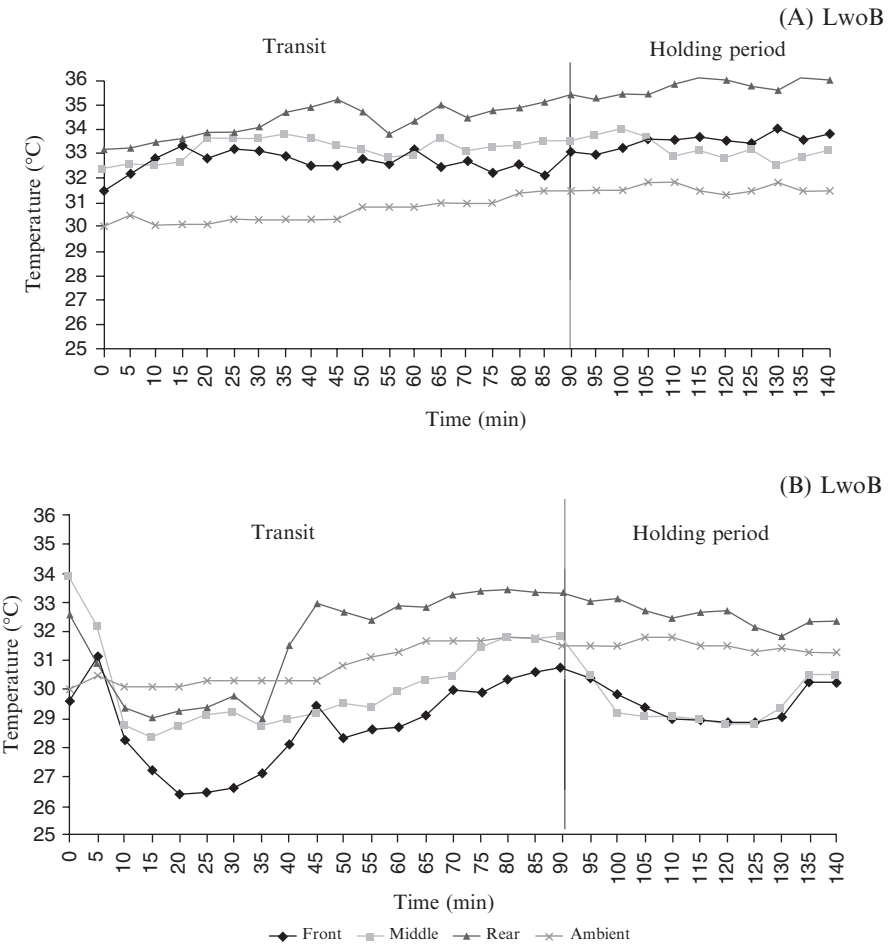

Fig. 16.3 Time versus temperature observed at the front, middle and rear regions of the vehicle for birds subjected to no bath (LwoB) (A) or bath (LwiB) (B) while on the farm, during the 55 km/90 min journey and after the holding period where the birds were left at rest at the slaughterhouse. The highest temperature value was observed at the rear region of the vehicle (34.6±0.8 °C) whilst the truck outside temperature (31.0±0.6 °C) was lower in the LwiB condition (Simões et al. 2009a)

16.2.5.2 Water-Sprinkling on Slaughterhouse

On arrival at the commercial slaughterhouse plant, the holding period for the pre-slaughter rest was essential, and it is recommended to maintain the loaded truck in a ventilated warehouse, preferably under forced ventilation system and water-sprinkling, to reduce the heat load and consequently lowering the animal stress. Guarnieri et al. (2004) found that a bath with sprayed water combined with ventilation before slaughter contributed to the recovery of normal physiology (homeostasis of calcium ions), with positive effects on biochemical processes and preventing

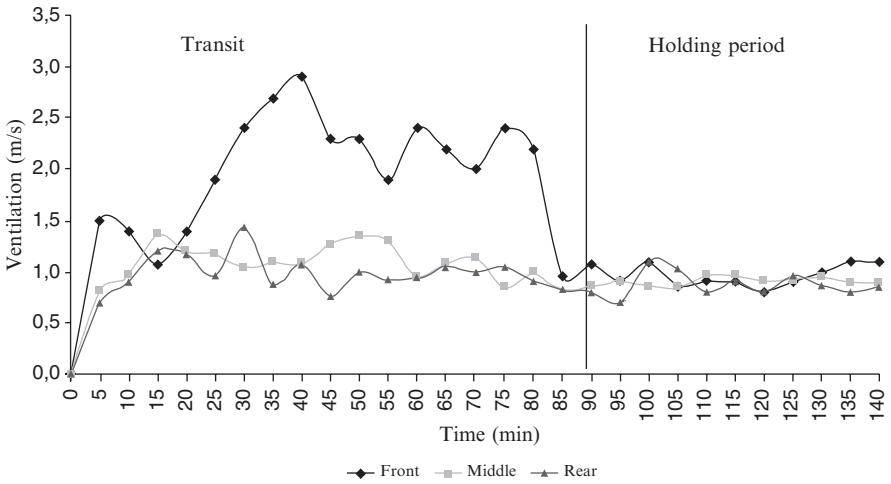

Fig. 16.4 Time versus ventilation air speed at the front, middle and rear regions of the vehicle throughout the 55 km/90 min journey and the 50 min holding period at the commercial slaughterhouse. The highest air ventilation speed was observed at the front region (Simões et al. 2009a)

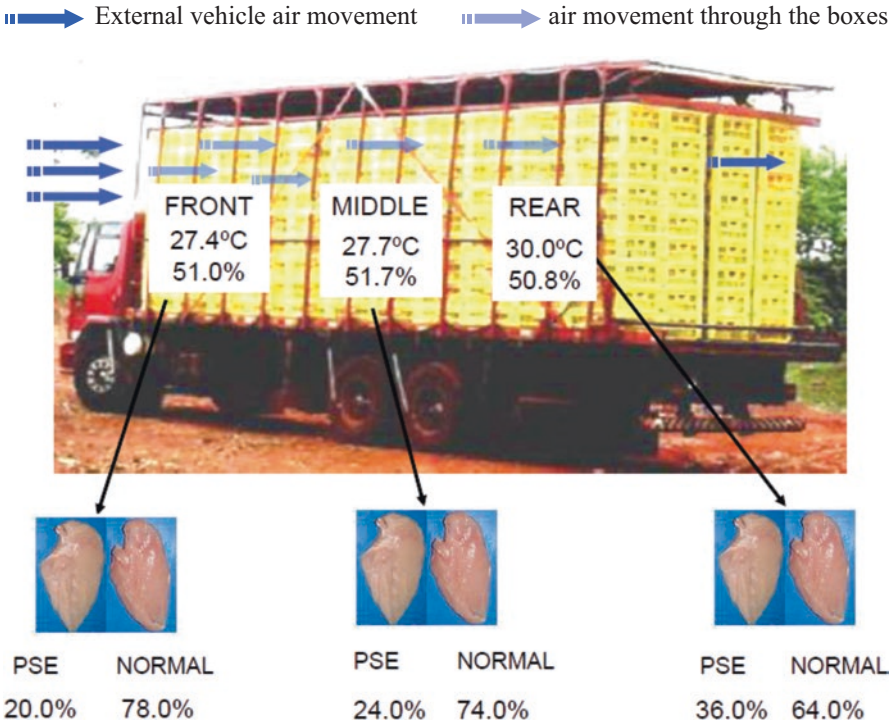

Fig. 16.5 Illustration of the air ventilation through the chicken boxes during transport. The wind speed decreases from the front to the rear of the truck. The figure also shows the occurrence of PSE and normal meat and the average temperature and relative humidity values of the front, middle and rear locations. The average distance from the farm to the slaughterhouse was 57 km (Simões et al. 2009b)

Table 16.2 Average DOA and DOA index of lots without shower (LwoB) and with shower (LwiB) of broiler chickens transported during the summer at distances of 15 and 55 km from the farm to the poultry slaughterhouse centre, with holding for 60 min

Transport distance (Km)	Lot	Total chickens[a]	DOA (*n*)	DOA (%)
15	LwoB	7,616	12	0.16
15	LwiB	7,570	09	0.12
55	LwoB	7,376	20	0.27
55	LwiB	7,760	12	0.15

n number of dead chickens, *LwoB* Broiler lot without bath, *LwiB* Broiler lot with bath
[a]Total birds in two transport trucks (Silva et al. 2011)

the development of PSE meat. Histological studies of the PSE samples from untreated group birds after 72 h *post-mortem* verified under light microscopy a shrinking of muscle cell diameter by approximately 10 % in relation to treated group with water shower (for 10 min at 25 °C) samples and an extracellular enlargement of endomysium and perimysium sheaths. Similar changes were also observed by others (Barbut et al. 2005). By electron microscopy of PSE meat, Z-lines appeared fragmented, A-bands including the M-line disappeared and a super-contraction of sarcomeres was observed, indicating that proteins were adversely affected by heat stress (Wilhelm et al. 2010).

16.2.5.3 Vitamin E Supplementation

It was also observed that supplementing chickens' rations with vitamin E effectively inhibited the formation of PSE meat promoted by physical stress contributing to the improvement of meat functional properties. However, the rapid onset of glycolysis and lactic acid formation under higher temperature apparently were the main causes for the development of PSE meat (Olivo et al. 2001). The vitamin E is a membrane associated antioxidant and inhibits the enzyme phospholipase A_2 activity by membrane stabilisation, preventing changes in the lipid content. In British Landrace pigs, supplementation of diet containing 1000 mg vitamin E/kg diet significantly decreased the occurrence of PSE carcass in PSE-prone Landrace × Large White Hal$^+$ pigs (Cheah et al. 1995). These authors suggested that vitamin E stabilised the membrane of sarcoplasmic reticulum and inhibited the PLA_2 activity, as also demonstrated in chicken by Soares et al. (2003). PLA_2 is an enzyme involved in the hydrolysis of phospholipids which produces long chain unsaturated fatty acid and lyso-derivatives (Nachbaur et al. 1972). These products could induce the uncoupling and swelling of the membrane of sarcoplasmic reticulum and mitochondria (Cheah and Cheah 1981). Therefore, vitamin E induced inactivation of PLA_2 prevented Ca^{2+} leakage into sarcoplasm and resulted in lower sarcoplasmic Ca^{2+} concentration. Lower Ca^{2+} concentration in sarcoplasm is associated with slower rate of pH decline and lower levels of protein denaturation, and thus causes increased water holding capacity (Cheah et al. 1984; Chen et al. 2010).

16.3 Final Remarks

Throughout the poultry production chain, broiler chickens face various types of stress that affect their welfare. One of the greatest causes of meat quality deffects is thermal stress. This relationship is particularly relevant for tropical countries, which have a high incidence of thermally stressful conditions in the summer that create an unhealthy environment. Thus, it is imperative to investigate in detail methods to ameliorate the conditions of broiler chickens throughout production, with the aim of promoting bird welfare and consequently improving meat quality. The water bath at the farm before transportation, water-sprinkling on slaughterhouse and vitamin E supplementation are effective methodologies for avoiding broiler chicken stress and consequently improve the meat quality.

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Chapter 17
Protein Oxidation in Meat and Meat Products. Challenges for Antioxidative Protection

Sisse Jongberg, Marianne N. Lund, and Leif H. Skibsted

17.1 Introduction

Protein oxidation in meat has, through the last decades, received increasing attention from the meat industry and meat scientists. Consumers have also noted negative effects of protein oxidation following changes in packaging practices in the meat industry in many countries. Compared to lipid oxidation, the degradation of proteins by oxidants seems to be more complex and to produce an even greater multiplicity of reaction products. While oxidation processes related to lipids, proteins, and heme proteins may individually be relatively well understood, the coupling between degradation processes in meat has not been investigated in detail. Understanding protein oxidation in biological systems, however, seems to hold the key to also understanding the connection between degradation of hydrophilic and lipophilic meat components, since certain protein oxidation products may be active at water–lipid interfaces in meat (Skibsted 2011a).

An ever-growing world population with middle class consumers showing increasing demand for high quality food products like meat and processed meats will require better and more responsible utilization of available natural resources. In most European countries fresh meat products are now commonly packed and stored in modified atmosphere packages (MAP) in order to prolong shelf life and minimize waste. The modified atmosphere contains up to 80 % oxygen, which markedly extends the shelf life by securing the product microbiologically (Asensio et al. 1988). Further, MAP prolongs shelf life through a longer period with a fresh appearance of the meat by maintaining the cherry-red color of oxymyoglobin (Ledward 1992).

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The high oxygen concentration, however, at the same time diminishes meat quality, because lipids and proteins are oxidized, resulting in reduced nutritional value, increased level of off-flavors, and decreased water holding capacity and less tender meat (Lund et al. 2011). Stress induced to the meat or meat products through phase transitions occurring during freezing and thawing enhances protein oxidation, as will heat treatment (Leygonie et al. 2012; Traore et al. 2012). Certain additives like salt, nitrite, and nitrate commonly used in meat processing also result in elevated levels of protein oxidation in products.

17.2 Protein Oxidation

17.2.1 Initiation of Protein Oxidation

Protein oxidation in meat is initiated by the same external stress factors that are responsible for lipid oxidation. In addition, certain oxidoreductase enzymes located in the meat water phase may initiate protein oxidation together with pigment oxidation, which is seen as product discoloration. Among the most important and most studied initiators of protein oxidation are transition metals, mainly Fe, Cu, and Mn liberated from metalloenzymes during heat treatment or physical treatment such as mincing. The meat pigment myoglobin will, moreover, under some conditions act as a pseudoperoxidase, generating free radicals in the meat aqueous phase and initiating protein oxidation. Ionizing radiation, which is allowed by the meat industry in some countries in order to increase food safety, will also affect lipids, proteins, and meat pigments. Exposure of light with a UV-component, such as during retail display of meat, is known to initiate meat discoloration through photooxidation of oxymyglobin to form metmyoglobin (Bertelsen and Skibsted 1987). Riboflavin, i.e., vitamin B-2, is a potent photosensitizer that is also activated by visible light. Exposure of meat to visible light, especially under low-oxygen conditions, will result in electron transfer from proteins directly to triplet-state riboflavin. Under high-oxygen atmosphere conditions, singlet oxygen is instead formed as the result of physical-quenching of triplet riboflavin, and singlet oxygen may attack both lipids and proteins (Cardoso et al. 2012). The nature of protein oxidation products formed is highly dependent on how oxidation is initiated. In general, the more reactive the formed radicals are, the less selective the initiated reactions are (Garrison 1987; Stadtman and Berlett 1988; Irwin et al. 1999; Davies and Dean 1997).

17.2.2 Protein Oxidation Products and Markers

Mechanisms behind the oxidation of amino acids and proteins in general have been reviewed in several scientific papers (Davies and Dean 1997; Garrison 1987; Davies 1987; Stadtman 1993; Dean et al. 1997; Stadtman and Berlett 1997; Hawkins and

Davies 2001; Stadtman and Levine 2003), and in a more recent monograph (Davies and Dean 1997). Hence, this section focuses on protein oxidation products known to be important in meat and meat products and how they are formed.

Reaction of radicals with proteins and peptides in the presence of oxygen gives rise to alterations of both the polypeptide backbone and of the various amino acid side chains. These oxidative changes include cleavage of peptide bonds, modification of amino acid side chains, and formation of covalent intermolecular cross-linked protein derivatives (Davies and Dean 1997; Davies et al. 1987a; Garrison 1987; Stadtman and Berlett 1997; Dean et al. 1997), as shown in Fig. 17.1. Some of the most general amino acid modifications are formation of protein hydroperoxides and protein carbonyl groups, while cross-linking most often depends on formation of disulfide and dityrosine between neighboring peptide chains (Davies et al. 1999).

Amino acids that are generally most susceptible to oxidation, are cysteine, tyrosine, tryptophan, histidine, proline, arginine, lysine, methionine, and, somewhat surprisingly, phenylalanine (Stadtman 1992), and their most common oxidation products are listed in Table 17.1.

Additionally, many amino acid side chains are susceptible to hydroperoxide formation, but as these are unstable products and not easily identified, they have not been included in Table 17.1 (Davies et al. 1999).

Susceptibility of amino acids to oxidation varies with several orders of magnitude. In cells, myeloperoxidase is responsible for the formation of HOCl, a strong oxidant equivalent to the chlorine radical, $Cl^{\bullet}$, and absolute rate constants for a number of different model compounds are available (Fig. 17.2).

From the rate constants (k_2), it can be seen that the sulfur-containing cysteine (Cys) and methionine (Met) are oxidized faster than lipids or any other substrate, and even faster than most antioxidants. In foods and beverages, the significance of HOCl is unknown, but oxidants like hydroxyl radicals (·OH) produced through

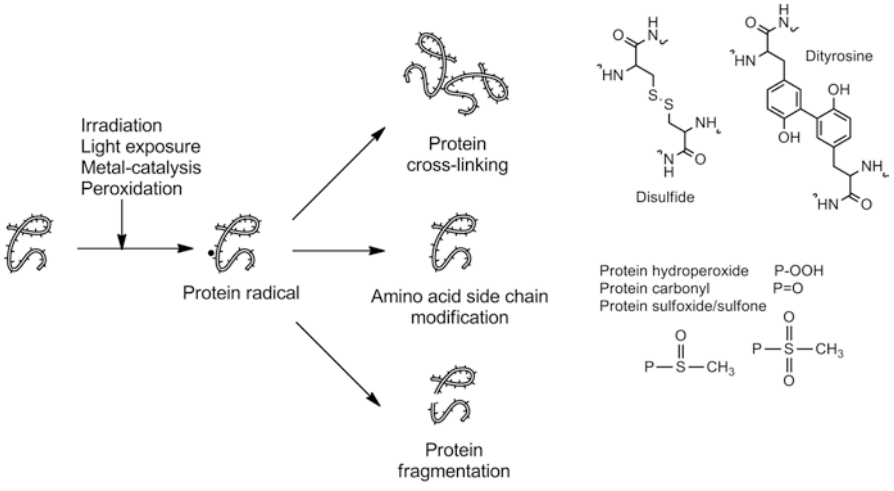

Fig. 17.1 Different consequences of oxidation of proteins (Lund et al. 2011)

Table 17.1 Amino acid oxidation products (Stadtman and Berlett 1997; Davies et al. 1999)

Amino acid	Oxidation product
Arginine	Glutamic semialdehyde[a], 5-hydroxy-2-amino-valeric acid
Cysteine	Disulfide cross-links, oxy acids
Histidine	Aspartate, asparagine, 2-oxo-histidine[a]
Lysine	3-, 4-, or 5-hydroxylysine, 2-amino-adipylsemialdehyde[a]
Methionine	Methionine sulfoxide/sulfone
Phenylalanine	*o*-, *m*-tyrosine
Proline	3- or 4-Hydroxyproline, 5-hydroxy-2-amino-valeric acid, glutamic semialdehyde[a]
Tryptophan	2-, 4-, 5-, 6-, or 7-hydroxytryptophan, kynurenine, *N*-formylkynurenine, 3-hydroxylkynurenine
Tyrosine	Dityrosine, 3,4-dihydroxyphenylalanine (DOPA)
	3-chlorotyrosine, 3,5-dichlorotyrosine
	3-nitrotyrosine, 3,5-dinitrotyrosine

[a]Carbonyl compounds

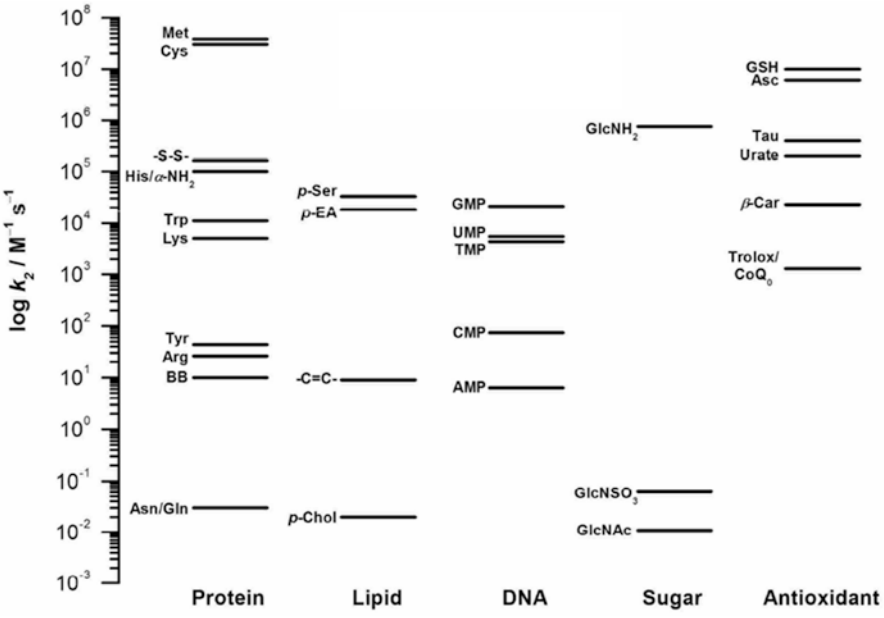

Fig. 17.2 Rate constants for reaction between HOCl and model compounds for proteins, lipid, DNA, sugar, and antioxidants (Pattison and Davies 2001, 2006)

metal-catalyzed oxidation, H_2O_2-activated heme proteins, and excited state riboflavin as a photosensitizer, play important roles and also oxidize sulfur-containing amino acids efficiently and rapidly (Lund et al. 2008; Cardoso et al. 2012). Second order rate constants for quenching of riboflavin triplet state by amino acids, peptides and proteins are shown in Table 17.2.

Table 17.2 Second order rate constants for quenching of riboflavin triplet state at 25 °C (Cardoso et al. 2012)

Amino acid, peptide, or protein	Second order rate constant (L/mol/s)	Reference
Tryptophan	1.8×10^9	Huvaere and Skibsted (2009)
Tyrosine	1.4×10^9	Cardoso et al. (2004)
Cysteine	2.2×10^7	Cardoso et al. (2004)
Methionine	6.4×10^7	Cardoso et al. (2004)
Histidine	5.2×10^7	Huvaere and Skibsted (2009)
Tyr-Gly	1.3×10^9	Cardoso et al. (2004)
Cys-Gly	6.7×10^7	Cardoso et al. (2004)
Cys-Tyr-Cys-Tyr	1.9×10^9	Cardoso et al. (2004)
β-lactoglobulin	3.6×10^8	Cardoso et al. (2004)
Bovine serum albumin (BSA)	2.3×10^8	Cardoso et al. (2004)
β-casein	4.9×10^8	Huvaere et al. (2010)
Lysozyme	3.0×10^8	Zhang and Gorner (2009)

Oxygen quenches triplet riboflavin in the biological relevant pH-region efficiently with a bimolecular rate constant close to the so-called diffusion limit. The physical quenching of triplet riboflavin results in formation of singlet oxygen and leads to so-called Type II photooxidation of lipids and proteins. However, the limited solubility of oxygen in water makes the aromatic amino acids tryptophan and tyrosine fully competitive in quenching triplet riboflavin favoring direct or Type I photooxidation with an initial formation of peptide radicals. In contrast to air-saturated solutions, anaerobic conditions in general also favor quenching of triplet-excited state riboflavin by this so-called Type I mechanism for reactive amino acids, peptides, and proteins. For the most reactive amino acids, the rate constants for quenching triplet riboflavin approach the diffusion limit, indicating rate-determining electron transfer. Notably, sulfur-containing amino acids seem to quench the singlet-excited state of riboflavin in competition with the otherwise efficient intersystem-crossing to the lower-energy triplet-state of riboflavin (Cardoso et al. 2012). Loss in fluorescence from the amino acid residue tryptophan is a convenient and often used indicator of protein oxidation together with thiol loss and detection of protein carbonyls.

17.2.3 Oxidation of Cysteine and Formation of Disulfides

The protein sulfhydryl or the protein thiol group of cysteine (RSH) is readily oxidized in meat systems. As reviewed by Nagy and Winterbourn (2010), thiol groups may form numerous oxidation products, of which the most common ones are sulfenic acid (RSOH), sulfinic acid (RSOOH), sulfonic acid (RSOOOH), or disulfide cross-links (RSSR) via non-radical oxidation reactions with hydrogen peroxide, as exemplified in reactions 17.1 and 17.2:

$$RS^{-} + H_2O_2 \rightarrow RSOH + OH^{-} \quad (17.1)$$

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (17.2)$$

Thiol oxidation products may also be generated through radical mediated reactions, and hypervalent myoglobin activated by peroxides may be involved in the oxidation of thiols to yield disulfide cross-links in meat. Protein disulfide cross-links are the only thiol-related oxidation products that have been detected in meat. Disulfide bonds are also of importance for wheat bread structure, as thiol groups in gluten become cross-linked during baking. For the whey protein β-lactoglobulin with an uneven number of thiol groups, heat-induced gel formation is due to thiol exchange reactions to yield intermolecular disulfide bonds from intramolecular disulfide bonds. Galaris et al. (1989) showed how glutathione (GSH) is oxidized to GSSG by myoglobin and hydrogen peroxide, and proposed the mechanism of Fig. 17.3 for living muscle tissue, which may also apply to proteins in meat and meat systems.

Formation of disulfide cross-links has been observed in meat model systems (e.g., Decker et al. 1993), and now also in fresh meat (Lund et al. 2007b; Kim et al. 2012; Zakrys-Waliwander et al. 2012). In fresh meat, an increasing number of disulfide cross-links correlates with meat toughness after cooking.

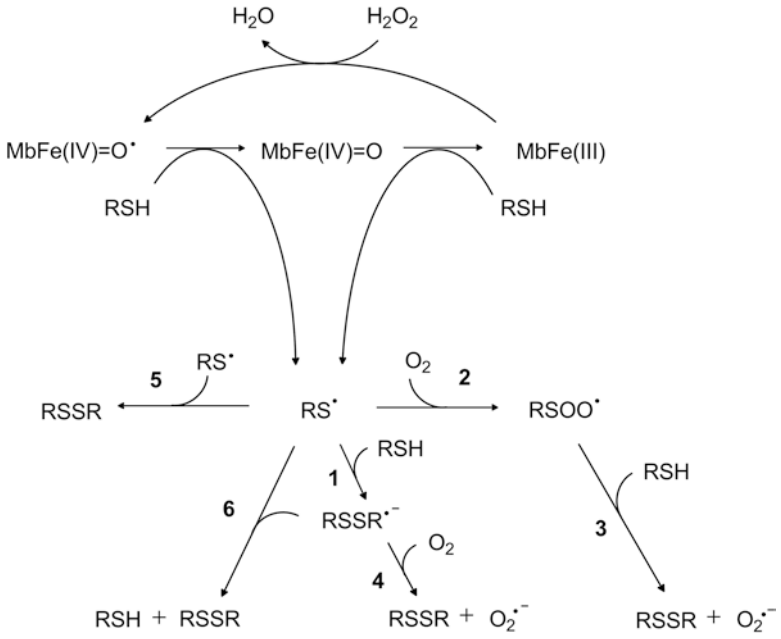

Fig. 17.3 Proposed oxidation mechanism of protein thiols by H_2O_2-activated myoglobin (modified from Galaris et al. 1989)

17.2.4 Formation of Carbonyl Compounds

Another significant marker of protein oxidation in meat is the protein carbonyls as marked by [a] in Table 17.1. Amino acid carbonyls are the most frequently used marker of overall protein oxidation for both food products and in the medical sciences. Carbonyls of amino acid side chains often seem to be the direct result of metal-catalyzed oxidation of proteins (Levine 1984; Oliver et al. 1987; Stadtman and Oliver 1991).

Carbonylation as induced by oxidative stress is an irreversible modification of protein structure. Protein carbonyls may be induced through four different pathways: (1) direct oxidation of protein side chains, such as lysine, arginine, proline and threonine (Requena et al. 2001), (2) glycation in the presence of reducing sugars through Maillard reactions (Akagawa et al. 2005), (3) oxidative cleavage of the protein backbone via the α-amidation pathway or via oxidation of the glutamyl side chain (Hawkins and Davies 2001), or (4) covalent binding of non-protein carbonyl species, such as secondary lipid oxidation products, through Michael addition (Davies and Dean 1997; Stadtman and Levine 2003). According to a recent review (Estévez 2011), direct oxidation of protein side chains are, however, the only route of carbonylation, which unambiguously has been demonstrated for meat proteins. Metal-catalyzed oxidation is accordingly considered to be the dominant pathway for protein carbonyl formation. Specific metal binding sites on proteins, moreover, seem to enhance the catalytic effect when transition metals like iron are bound in close proximity to oxidizable amino acid side chains (Stadtman and Levine 2003).

In meat, interaction with myoglobin from the water soluble protein fraction may also induce formation of protein carbonyls. Myoglobin species have thus been found to induce protein carbonylation in myofibrillar proteins, and H_2O_2-activated metmyoglobin was demonstrated to promote formation of the specific semialdehydes α-aminoadipic semialdehyde (AAS) and γ-glutamic semialdehyde (GGS) (Estévez and Heinonen 2010). Results from a meat model system have clearly demonstrated that protein carbonyls are formed to a greater extent by oxidatively activated myoglobin compared to peroxides-dependent metal catalysis by Fe(III)/H_2O_2 (Estévez and Heinonen 2010).

Concentration of protein carbonyls increases during storage of meat and meat products. Not surprisingly, high-oxygen MAP has been found to increase promotion of protein carbonyls in ground pork (Delles et al. 2011), pork loin (Ma and Xiong 2011), beef loin (Lindahl et al. 2010), beef steaks (Zakrys-Waliwander et al. 2012), and beef patties (Jongberg et al. 2011b; Lund et al. 2007a) compared to control samples packed in vacuum or other non-oxygen atmospheres.

One possible degradation pathway of protein carbonyls in meat depends on Schiff-base reactions between carbonyl groups and ε-amino groups as in lysine residues resulting in protein cross-linking (Fig. 17.4, reaction I), as suggested by Xiong (2000). Other cross-links are possible via an aldol condensation reaction between two protein carbonyls (Fig. 17.4, reaction II) (Dolz and Heidemann 1989),

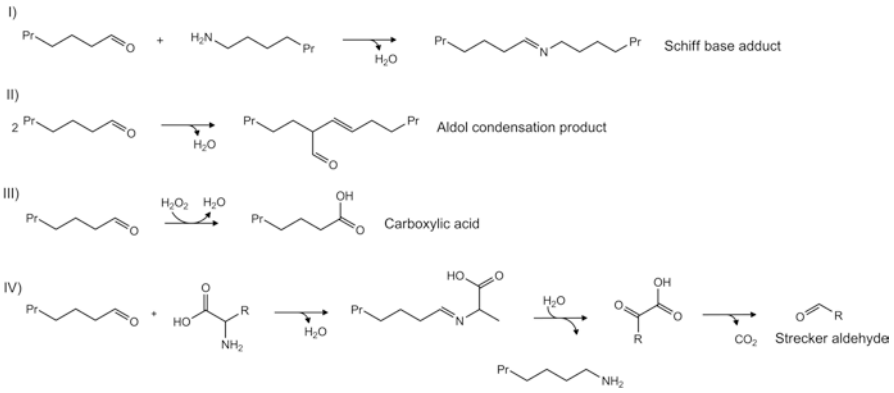

Fig. 17.4 Fate of protein carbonyls by implication in further reaction with protein amino acid residues or free amino acids (modified from Estévez 2011)

and protein carbonyls may also oxidize further forming carboxyl acids (Fig. 17.4, reaction III) (Sell et al. 2007). Schiff-base reactions with free amino acids followed by hydrolysis are known to form Strecker aldehydes (Fig. 17.4, reaction IV) (Estévez et al. 2011a).

17.2.5 Oxidation of Tryptophan

Tryptophan is readily oxidized in proteins, and tryptophan oxidation is considered as one of the early events in the cascade of oxidation reactions of meat proteins (Estévez et al. 2008). Oxidation of tryptophan may take place either on the pyrrole or on the benzene ring to generate 2-, 4-, 5-, 6-, and 7-hydroxytryptophan, but the best characterized compound after attack at the pyrrole C3 position (OH addition) followed by ring-opening is *N*-formyl-kynurenine (Fig. 17.5).

Utrera et al. (2012) recently demonstrated that tryptophan fluorescence was lost in pork patties during cooking, and that addition of avocado peel extracts as a potential source of antioxidant was unable to hinder the tryptophan loss.

17.2.6 Oxidation of Tyrosine and Formation of Dityrosine

Another cross-link observed in meat model systems is dityrosine, a compound which may be isolated following protein hydrolysis (Bhoite-Solomon et al. 1992; Lund et al. 2008). One-electron oxidation of tyrosine forms a tyrosyl phenoxyl radical, which leads to the carbon–carbon covalent bond in dityrosine (Fig. 17.6) (Davies and Dean 1997). Transfer of oxidative damage from cysteine, tryptophan,

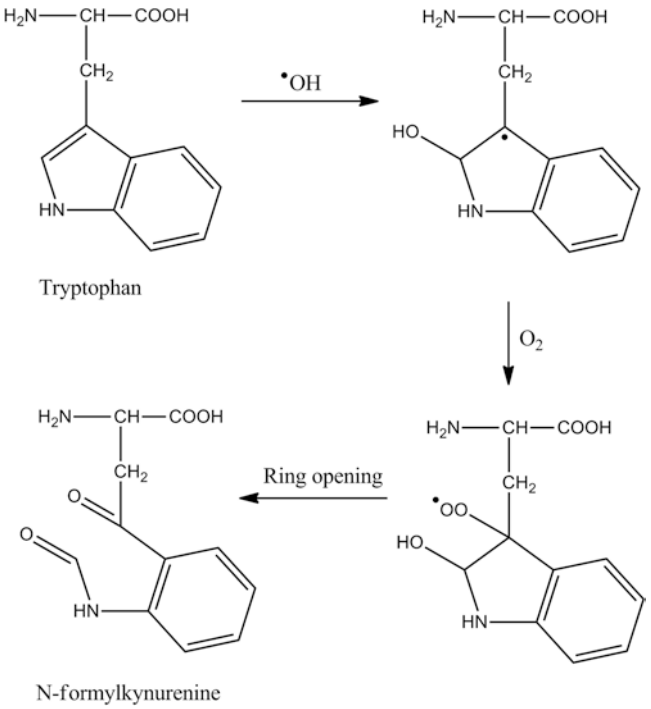

Fig. 17.5 Oxidation of tryptophan and formation of *N*-formylkynurenine

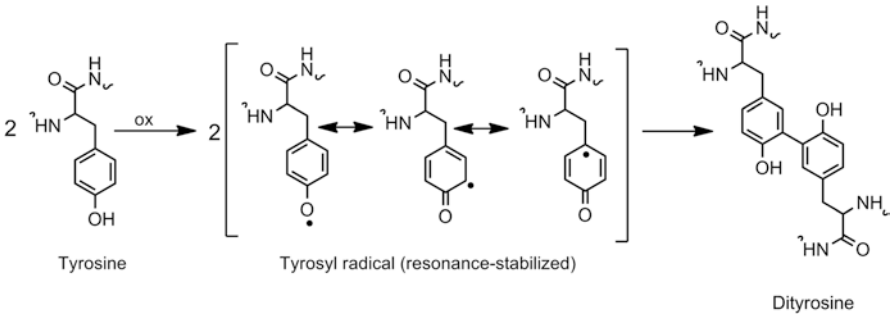

Fig. 17.6 Dityrosine formation through the mediation of resonance-stabilized tyrosyl phenoxyl radicals

and methionine to tyrosine is well characterized (Prutz et al. 1986), and it has accordingly been suggested that tyrosine residues act as the ultimate "sink" for oxidizing equivalents in proteins (Davies and Dean 1997).

The tyrosyl radical has a resonance-stabilized aromatic structure, which makes the tyrosyl radicals long-lived in some proteins such as bovine serum albumin (BSA) (Ostdal et al. 1997), and myosin (Lund et al. 2008). Apart from dityrosine, a

common oxidation product of tyrosine is 3,4-dihydroxyphenylalanine (DOPA), which has been found to be generated in the globular proteins BSA and β-lactoglobulin (Dalsgaard et al. 2011). Nevertheless, dityrosine and DOPA never seem to have been detected in meat and meat products.

17.3 Consequences of Protein Oxidation

Protein oxidation has only recently been recognized as a problem by the meat industry, but it is now being considered to be equally relevant with regard to the eating quality of meat as lipid oxidation. Oxidative degradation of meat is not only related to eating quality. In addition, the nutritional value of meat is compromised by oxidative processes, which degrade and change the structure of major nutrients, in effect making them unavailable for absorption in the digestive tract. Oxidation of proteins may also cause changes in protein functionality. Protein hydrophobicity and conformation may be affected, proteolytic enzymes may be inactivated, and protein substrates may have altered susceptibility to proteolytic enzymes (Dean et al. 1986; Wolff and Dean 1986; Davies et al. 1987b).

Figure 17.7 shows an overview of a variety of oxidative processes occurring in meat stored under oxidative conditions: (1) formation of hypervalent myoglobin species, (2) the radical-mediated chain reaction of lipid oxidation, (3) oxidative modifications of proteins induced by reaction with secondary lipid oxidation products, (4) oxidation of proteins to yield protein carbonyls, and (5) oxidation of proteins to yield protein disulfide cross-links. In the figure, the oxidation processes are divided into separate boxes; however, this is a simplification of the meat system, as all processes are assumed in one way or another to be interlocked.

A direct connection between protein cross-link formation through thiol oxidation to yield disulfides and increased toughness of meat during storage of meat in high-oxygen MAP has now been demonstrated for several meat products (Lund et al. 2007a, b; Kim et al. 2010, 2012). Some amino acids, such as methionine and cysteine, are suggested to be involved in a so-called "sacrificial protection" of other amino acids, and act accordingly as an intermolecular antioxidant scavenging reactive oxygen species (Levine et al. 1999).

Oxidatively modified meat proteins exhibit altered functionalities, as shown in Fig. 17.8. The main effects on quality traits observed for fresh meat are reduced tenderness and juiciness, whereas meat products suffer from reduced protein solubility, altered gel forming and emulsifying abilities, and changed viscosity (Xiong 2000). Altered functionalities can be utilized to manufacture meat products with certain desired properties, but for fresh meat products the impaired protein functionalities mainly damage the overall quality.

Meat tenderness is dependent on the structure of the skeletal muscle cells, and the complex processes that modify and change the myofibrillar proteins are responsible for the development of meat tenderness. Hence, protein degradation and protein oxidation are important for meat tenderness. During the maturation or aging

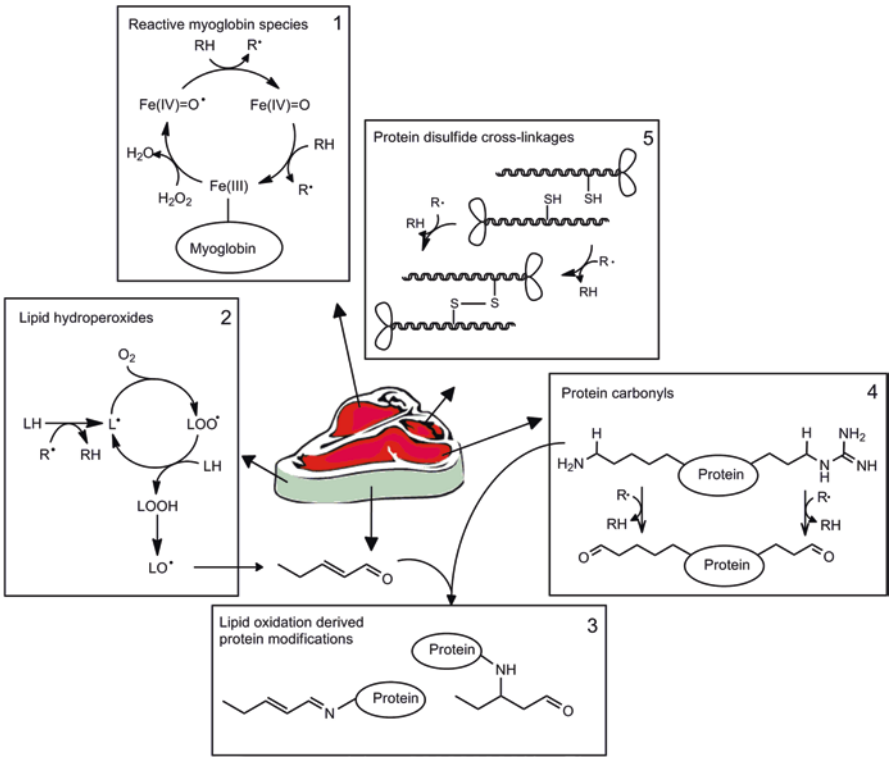

Fig. 17.7 Schematic overview of oxidation mechanisms and oxidation products in meat

process *post mortem*, the integrity of the muscle structure is compromised through protein degradation. Protein degradation in meat occurs through proteolytic digestion, and is primarily believed to be executed by the calpain system (Huff-Lonergan et al. 2010). Thus, tenderness development in fresh meat depends on the balance between proteolytic digestion and oxidatively induced protein alterations, which strengthens the protein structure by creating cross-links. However, in an oxidative environment, not only are the myofibrillar proteins affected, but also the efficiency of the calpains are modified by oxidation and may thus impact meat tenderness. Disruption of the cellular compartments *post mortem* decreases the ability of the muscle to maintain reducing conditions, which are necessary for the calpain system to function. The catalytic site of the calpains contains a cysteine and a histidine residue (Guttmann et al. 1997; Lametsch et al. 2008). Consequently, either directly or indirectly, oxidation has a large impact on the development of meat tenderness. Decreased proteolytic activity may further arise from reduced susceptibility of myofibrillar proteins to enzymatic degradation due to their oxidative modifications, which may alter the myofibrillar protein structure as an enzyme substrate (Morzel et al. 2006).

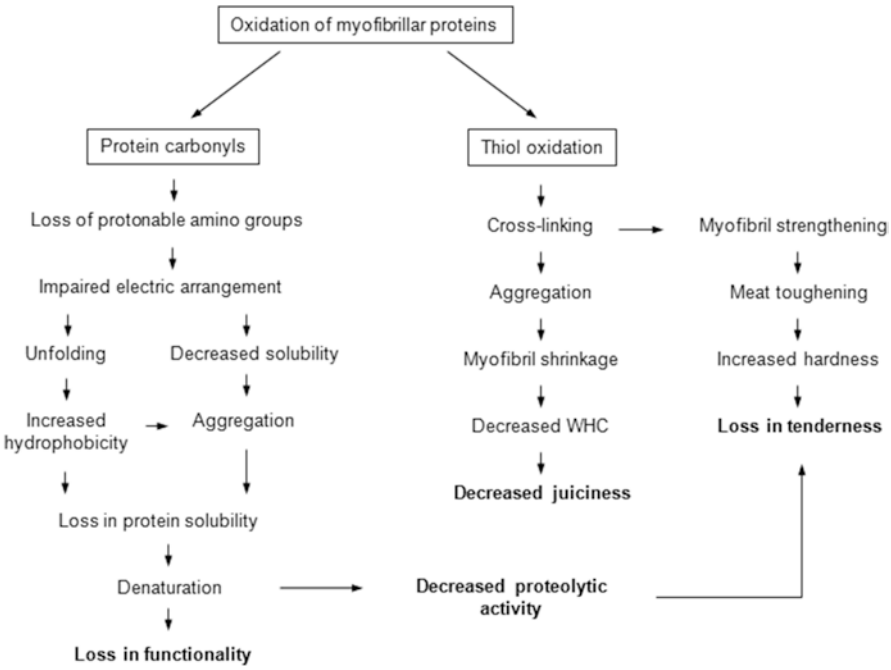

Fig. 17.8 Altered meat protein functionalities due to oxidation of myofibrillar proteins (modified from Estévez 2011)

Protein oxidation has also been suggested to be directly involved in increased drip loss of meat. The formation of protein cross-links, as described in a previous section, has been associated with decreased water holding capacity of meat. Some studies associate the formation of disulfide cross-links directly with increased drip loss (Delles et al. 2011; Lund et al. 2007a, b; Zakrys-Waliwander et al. 2012), whereas the carbonyl-amino cross-link formation has been related to the increased expulsion of water in frozen minced pork (Estévez et al. 2011b). Further, storage of beef steaks in high-oxygen MAP was found not to affect proteolytic activity, but still decreases the juiciness and tenderness of the cooked beef steaks (Kim et al. 2010), indicating that protein oxidation may exceed the constraining effects of oxidizing conditions on the proteolytic activity.

Oxidative modification of protein also seems to release a series of aldehydes and ketones, which may affect the flavor perception of meat and meat products subjected to oxidative conditions (Fuentes et al. 2010; Headlam and Davies 2002).

Meats cured with nitrite are surprisingly resistant to oxidation. Nitrite is a strong oxidant, but it is rapidly reduced by water soluble reductants in meat, forming nitrogen oxide, which serves as an antioxidant, free or coordinated to iron in myoglobin (Skibsted 2011b). Nitrite may oxidize proline in proteins forming nitrosamines, a process which, however, is inhibited by ascorbate and NADH.

17.4 Protection of Proteins by Antioxidants

Phenolic antioxidants efficiently prevent lipid derived off-flavor in meat, and extracts rich in phenolic substances from plants and herbs are recommended for use in meat products (Andersen et al. 2003; Jongberg et al. 2011a, b; Kong et al. 2010; Nissen et al. 2000; Mitsumoto et al. 2005). However, the effect of phenolic compounds on protein oxidation in meat is less clear, and the antioxidative prevention of meat toughness is only in its infancy.

17.4.1 Antioxidants and Proteins in Heterogenous Systems

The antioxidative effects of phenolic compounds are different in the protection of lipids and proteins. Often, the effect of phenolic antioxidants is more pronounced towards lipid oxidation than towards protein oxidation (Viljanen et al. 2004). A lag-phase prior to the initiation of lipid oxidation is commonly observed following addition of phenolic antioxidants to an oxidizing lipid system. Such a lag-phase indicates that the chain reaction of lipid oxidation is prevented from propagating, because the initiating radicals are scavenged or metal catalysts are chelated by the phenolic antioxidants. In contrast, inhibition of protein oxidation by phenolic antioxidants may rather be described as a retarding effect on the progression of oxidation. Protein oxidation rate is reduced by the addition of phenolic antioxidants, but oxidation is not postponed as for lipid oxidation. Figure 17.9a shows schematic diagrams of the progression over time of lipid and protein oxidation in the absence or presence of phenolic antioxidants in meat.

The difference between lipid and protein oxidation may be explained by the fact that lipid oxidation is a chain reaction mediated by free radicals in contrast to protein oxidation. Lipid radicals are highly reactive, while protein radicals are more long-lived and less reactive. Phenolic compounds will scavenge these protein radicals less efficiently compared to the more reactive lipid radicals, and the more stable protein radicals will cause the propagation of protein oxidation to proceed at a slower rate compared to lipid oxidation and at the time less affected by antioxidants.

The partitioning of oxidation substrate, antioxidants, and reactive oxygen species will influence the efficiency of antioxidants in any heterogeneous system such as meat. Phenolic antioxidants are, depending on their relative polarity, distributed in the aqueous phase or in the interface between hydrophilic and hydrophobic parts, and may be able to scavenge reactive oxygen species from transition metal catalysis or hypervalent myoglobin species before they enter the lipid phase. Proteins, on the other hand, are found in the aqueous phase or in the lipid–water interface as emulsifiers. Consequently, the fate of the oxidation initiators in the aqueous phase will depend on the relative reactivity of protein or phenols, as illustrated by the protein–liposome model system in Fig. 17.9b. In a model system, proteins have been found to stabilize catechins by scavenging hydrogen peroxide, and it was found that the

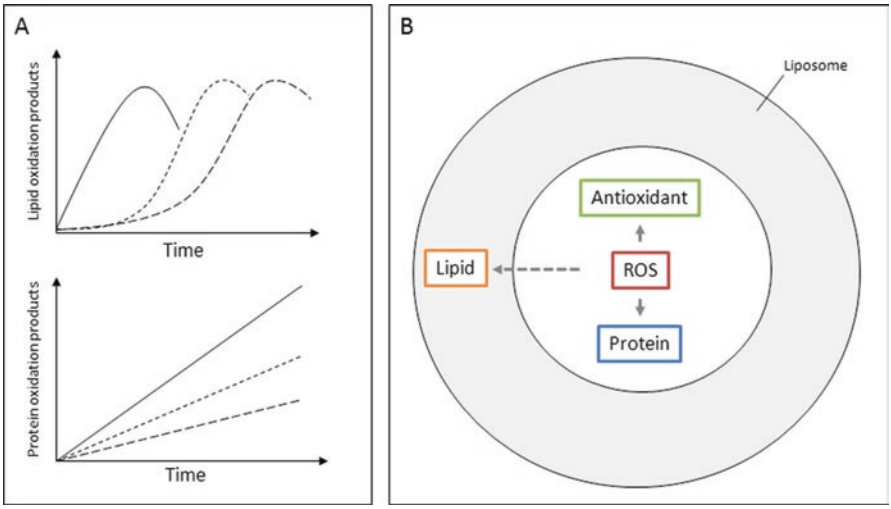

Fig. 17.9 (**a**) Proposed schematic overview of the tendency of the progression of lipid oxidation (*upper panel*) and protein oxidation (*lower panel*) in the absence (—) or in the presence of phenolic antioxidants added in two concentration levels: level 1 (·····) or level 2 (- - -). (**b**) Schematic overview of the partitioning of oxidation initiators (ROS) and oxidation substrates, proteins, lipids, and phenolic antioxidants in a biphasic system exemplified by a protein–liposome model system

protecting effect depended on the protein amino acid sequence and the accessibility of oxidizable amino acid residues to the aqueous phase (Zhou and Elias 2011). These considerations were based on observations in rather simple model systems; however, the mechanisms are still expected to be valid for more complex biological systems like meat.

Carotenoids, as another major group of antioxidants, interact with proteins. Carotenoids are lipophilic, and they seem to protect lipids against oxidation at interfaces and in membranes through regeneration of hydrophilic polyphenols (Skibsted 2012). As for proteins, carotenoids such as the more hydrophilic astaxanthin, the protein-bound carotenoid present in salmon, may protect unsaturated lipids at the expense of tyrosine in proteins, as depicted in Fig. 17.10.

17.4.2 Protection of Proteins by Natural Phenolic Antioxidants

An understanding of the relative importance of protein carbonyl formation and protein thiol loss will facilitate a rational antioxidant strategy for protection against protein oxidation in meat. Several authors have reported the antioxidative effects of natural phenolic compounds on the formation of protein carbonyls in meat, e.g., in pork patties (Rodríguez-Carpena et al. 2011a, b; Ganhão et al. 2010), chicken cuts (Rababah et al. 2004), and beef patties (Jongberg et al. 2011a, b). However, parallel

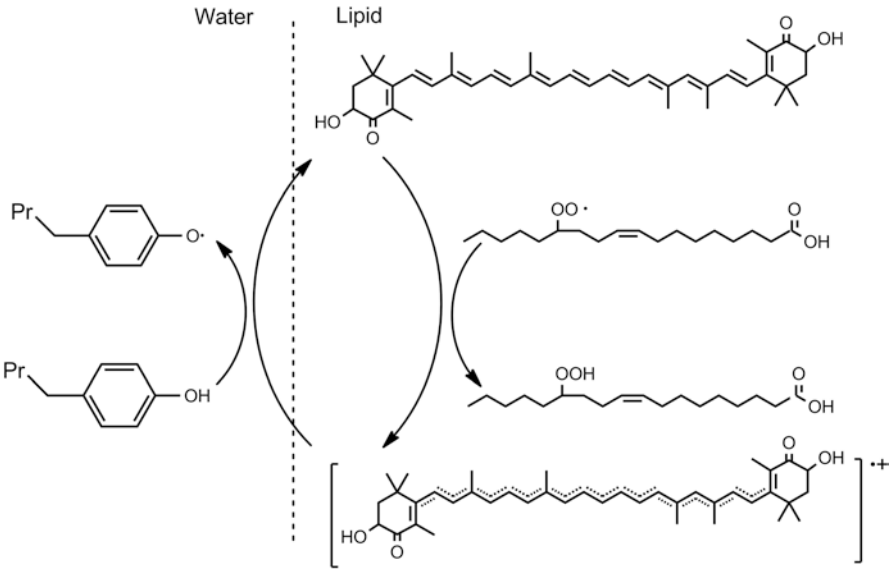

Fig. 17.10 Regeneration of astaxanthin radical cation formed in the lipid phase during oxidative stress by protein-bound tyrosine residue in the water phase

evaluations of lipid oxidation show superior antioxidative protection of lipids compared to proteins with dose-dependent antioxidative activities of rosemary or grape extracts (Lund et al. 2007a, b; Jongberg et al. 2011a, b; Viljanen et al. 2005; Estévez et al. 2005). The concentration of phenolic compounds is accordingly an important factor to consider in antioxidant strategies for proteins, together with antioxidant partition between lipid and water (Fig. 17.9).

Recent studies of the radical scavenging effects of plant extracts rich in phenols and single phenolic compounds against myosin radicals or perferrylmyoglobin radicals indicate that certain phenolic compounds are able to scavenge long-lived myosin radicals (Jongberg et al. 2012). Green tea extracts added in an amount up to 1 % (w/w) phenol to myosin were able to scavenge myosin radicals and decrease radical intensity. Addition of white grape extract or rosemary extract showed that at similar concentration levels, there was no inhibitory effect on the myosin radicals, which stresses the significance of the phenolic profile of the extract for the radical scavenging activity of plant extracts.

The radical scavenging activity of the phenolic compounds in extracts is closely linked to their ability to terminate radicals by radical–radical interactions, which regenerate the phenolic groups and enhance the antioxidative capacity of the extract. Green tea extract generated less phenoxyl radicals than extracts from rosemary or white grape, indicating that the phenolic compounds in green tea extract more efficiently scavenge phenoxyl radicals by polymerization reactions, thereby regenerating the phenolic hydroxy groups, enabling a second donation of hydrogen atoms to any oxidizing agents (Jongberg et al. 2012).

Catechin and green tea extract added at equal phenolic concentrations were found to scavenge a similar level of myosin radicals, which suggests that catechins are important active components in green tea extract. In contrast, the simple phenol 4-methyl catechol (4MC) or carnosic acid from rosemary did not provide similar radical scavenging effects, indicating that the ability of catechin to be regenerated through polymerization is important for radical scavenging and antioxidative effect.

17.4.3 Regeneration of Antioxidative Capacity by Protein–Phenol Interactions

Proteins and phenols may react to form covalent bonds. The key aspect in covalent protein–phenol interactions is the formation of quinoid structures from oxidation of phenols to quinones or semiquinone radicals (Pierpoint 1966). Due to their electrophilic nature, quinoid compounds are very reactive in the presence of nucleophilic substances, and react readily to form protein–phenol adducts. The side chains of cysteine, lysine, and tryptophan moieties in the protein chain react readily with quinoid structures due to the nucleophilic thiol groups, amino groups, and indole group, respectively (Pierpoint 1969). The reactions leading to formation of protein–phenol adducts through Michael addition between a catechol moiety and a protein-bound amino group is seen in Fig. 17.11.

Reaction mechanisms between proteins and phenols have been extensively investigated in various protein systems in combination with phenols or polyphenols. In the presence of di- or trihydroxybenzene-containing compounds, such as caffeic acid, chlorogenic acid, quinic acid, or gallic acid, Michael addition reactions have been demonstrated for several proteins, such as α-lactalbumin and lysozyme

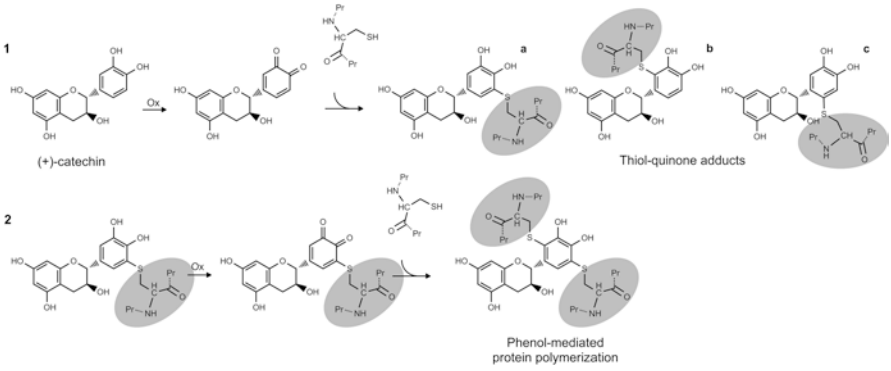

Fig. 17.11 Proposed mechanism for phenol-mediated protein polymerization in meat added plant extracts. Oxidation of (+)-catechin and subsequent reaction with protein thiols to yield thiol–quinone adducts (1), followed by a second oxidation and further adduct formation with another protein thiol to yield a di-thiol adduct (2). *Gray spheres* indicate phenol-bound protein. The scheme is modified from Jongberg et al. (2013)

(Prigent et al. 2007), bovine serum albumin (Rawel et al. 2002), whey proteins (Rawel et al. 2001), and myoglobin (Kroll and Rawel 2001). The amino acid side chains form covalent bonds with the quinone or semiquinone radicals formed by oxidation of the phenol, which in effect regenerates the hydroxyl groups (Kroll et al. 2003).

Regeneration of the hydroxyl groups after substitution of a carbon atom by a nucleophilic thiol group enables the catechol moeity to be oxidized once again and enter into a second nucleophilic attack (Fig. 17.11). Using methyl dihydrocaffeate as a model of flavonoids, Fujimoto and Masuda (2012) demonstrated that addition of thiols to catechin occurred initially in the 5′-position, then the 2′-position, and finally in the 6′-position of the flavonoid B-ring. In this sense, formation of di- or tri-thiol adducts may lead to protein polymerization mediated by oxidation of phenols (Siebert et al. 1996). Such polymerization reactions have been found to occur predominantly at higher pH levels, and may lead to the formation of dark-colored proteins of low solubility (Rawel et al. 2000). Aggregation of proteins due to protein–phenol interactions and polymerization are found to be responsible for haze formation in beer and other beverages (Siebert et al. 1996).

In meat, the formation of covalent bonds between the thiol group in the myofibrillar proteins and the phenol 4-methylcatechol (4-MC) through Michael addition has now been demonstrated (Jongberg et al. 2011a). Further, increased protein polymerization together with a concomitantly distinct loss of thiols was found in Bologna-type sausages after addition of green tea extract in contrast to control sausages and sausages added with rosemary extract, for which no protein polymerization or thiol loss were found (Jongberg et al. 2013). The difference in protein polymerization was ascribed to the different phenolic profile of the extracts. Flavonoids like green tea catechins contain multiple sites for nucleophile attack, as the B-ring has three unsubstituted carbon atoms. In contrast, phenolic acids such as carnosic acid and carnosol from rosemary contain only one unsubstituted carbon atom in the aromatic ring, and thus, are only able to bind one protein molecule (Fig. 17.12).

Formation of covalent bonds between proteins and phenols may change the physicochemical properties of the proteins due to alteration of the protein structure. Change in protein structure as a result of protein derivatization with phenolic substances has, among other changes, led to the inactivation of enzymes (Kroll et al. 2003). Also, the functional properties and protein digestibility are affected by derivatization. Studies show that tryptic digestion is restricted after derivatization, as modification of the lysine side chain interrupts the cleavage by trypsin of peptide bonds in BSA derivatized with quercetin (Rohn et al. 2004). On the contrary, other studies have shown that derivatization increases the susceptibility of enzymatic digestion, as protein unfolding increase the accessibility of the proteolytic enzymes (Rawel et al. 2000). An in vivo study on the digestibility of polyphenol-derivatized soy protein isolate by rats showed reduced net protein utilization for rats fed derivatized soy protein; amino acid distribution revealed that the amino and thiol groups, as well as tryptophan, were markedly influenced with increasing degree of derivatization (Rohn et al. 2006).

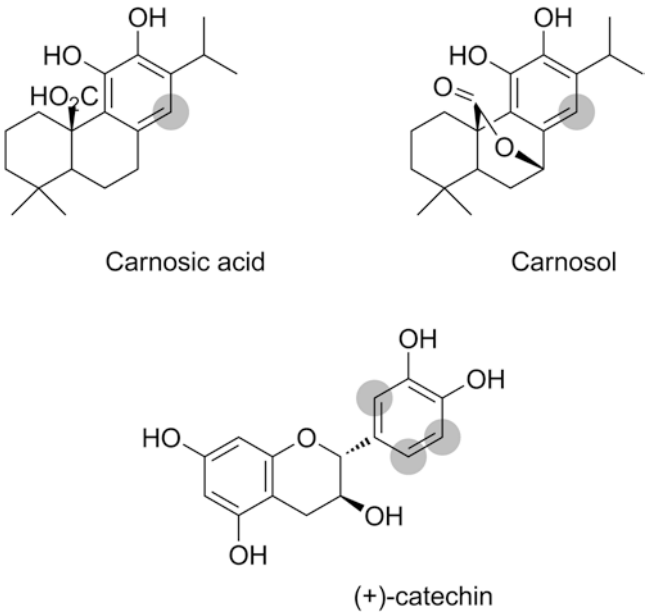

Fig. 17.12 Carnosic acid, carnosol, and catechin. *Gray spheres* indicate possible positions for substitution of a hydrogen on the carbon atom

17.4.4 Antioxidant Activity in Meat

The antioxidative capacity of protein-bound phenolic compounds has previously been evaluated in model systems. Rohn et al. (2004) found that the antioxidative capacity of protein-bound quercetin was generally inferior to the activity of non-bound quercetin, as determined by TEAC antioxidant assay (Trolox equivalent antioxidant capacity). The decrease in antioxidative activity of protein-bound quercetin was found to depend on the degree of protein derivatization with quercetin. A higher degree of derivatization resulted in better retention of antioxidant capacity compared to equivalent concentrations of non-bound quercetin. This effect was explained by better accessibility of radicals due to denaturation of the protein caused by the derivatization (Rohn et al. 2004), but may also be ascribed to the fact that an increased number of substituted carbon atoms increase the oxidability of the aromatic ring. The fact that protein-bound quercetin still exerts an antioxidant activity strongly verifies the regeneration of the phenolic moiety. Regeneration of phenolic compounds from their oxidized state is the core property of their antioxidative capacity in real food systems. The regenerated phenolic groups are able to scavenge additional radicals to form a protein-bound quinone, as exemplified for a catechol moiety in Fig. 17.11, which subsequently may take part in additional Michael addition or polymerization reactions, as recently demonstrated for epigallocatechin gallate (EGGG) cross-linking membrane proteins (Chen et al. 2011). The fact that the

protein-bound quercetin had already acted as an antioxidant when it was oxidized to the quinone may explain the decreased antioxidant capacity of protein-bound quercetin. Compared to non-bound quercetin, protein-bound quercetin will consequently contain one possible route of regeneration less than the non-bound, and will in effect be able to scavenge fewer radicals than the non-bound.

Interaction between proteins and phenols resulting in regeneration of the phenol groups and increased antioxidant capacity and activity explains the observed synergism between protein and phenols in the protection of lipids from oxidative decay. Phenols may be regenerated both through phenol polymerization and through protein–phenol interactions. Both pathways depend on the number of unsubstituted carbon atoms in the aromatic ring of the antioxidant, as multiple sites will facilitate several redox cycles of the phenol, and hence, increase the antioxidant activity of the phenol or phenol-rich plant extract. In this sense, the interplay between phenols and proteins in a multiphase system, such as meat, will influence the overall antioxidative effect, not only in the protection of lipid against oxidation but also most certainly in the preservation of protein quality.

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Chapter 18
Religious Slaughter of Animals: International Efforts to Meet This Need Responsibly

Joe M. Regenstein

The Jewish and Muslim communities have a set of dietary laws that control the food they eat. These are part of a larger set of laws that impact almost every aspect of their daily lives. The Kosher laws for Jews and the Halal laws for Muslims have been described in some detail by Regenstein et al. (2003). The focus of this chapter is on one of the most important components of these laws, i.e., how an animal is killed for food. In both communities there has been well-documented interest and concern for animal welfare, long before such concern was fashionable in the Western world. Both groups traditionally use a cut at the neck to make the animal unconscious.

The religious slaughter of animals is sometimes a challenge for the modern meat slaughter industry because the process is slower, it requires more skill on the part of slaughterhouse and the slaughter man, overall it requires more attention to details of animal handling, and it needs specialized equipment that is often expensive, especially for higher line speeds. But religious slaughter of animals also has some benefits in the modern era such as the fact that the animal is killed by someone with religious training who cares about the animal, using a razor sharp knife that is free of nicks. This process may actually be less painful than other methods if the hypothesis that endorphins are released within a calm animal at the time of slaughter with an extremely sharp knife free of nicks so that it dies in a condition that is similar to "runners high" is correct.

Yet, in recent years, with the rise of interest in animal welfare, religious slaughter of animals has become a concern in many Western countries where secular methods of slaughter are presumed to be more humane, especially when poorly done research is used to allegedly prove that hypothesis. This leads to various attempts to regulate such slaughter that are based on misleading research. New Zealand and Australia

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have for many years required an intervention prior to religious slaughter for meat destined for export. In Australia, the Jewish community is permitted to slaughter, but is doing a post-slaughter intervention for cattle although not for sheep. This is because sheep become unconscious much faster than cattle due to differences in their anatomy. Cattle have an additional set of blood vessels in the back of the neck that are not cut during religious slaughter. They normally represent less than 10 % of blood flow and after cutting the pressure drop limits blood flow through these vessels. In New Zealand, based on various newspaper reports, the local Jewish community is permitted to conduct ritual slaughter. After rejecting the advice of the government's own animal welfare committee, the agricultural minister banned domestic religious slaughter. It turned out that he had a financial interest in a halal slaughter house and worried that the Muslim export community would also ask for this right to kill without stunning. After various legal maneuvers, the situation at the time of the writing of this chapter seems to be that the Jewish community is permitted to slaughter as it always has, but on paper kosher slaughter of animals is prohibited. The next step in the legal process would be up to the government and there appears to be no interest in moving any legal action forward at this time. So the government can claim to have banned kosher slaughter but the Jewish community is able to function normally. In the meantime, Australia has become concerned with the slaughter of live animals exported to Indonesia. After providing slaughter boxes and stunners that apparently did not work properly, the Australia government is now attempting to impose a pre-slaughter intervention on the Indonesian Muslim community.

The European Union (EU), when revising their food labeling laws attempted to force punitive labeling on religiously slaughtered meat. The section of the law was eventually withdrawn. It is supposedly going to come up again as part of an EU discussion of animal welfare. The report commissioned by the EU to determine consumer interest in such labeling has just been released (June, 2015). However, that process seems to have been delayed. In the meantime, new legislation concerning animal welfare focusing on training of staff is scheduled to start on January 1, 2013, and the religious communities will need to develop appropriate compliance mechanisms although to date no actual final rules seem to have been promulgated.

In Holland, the lower house of parliament passed a bill banning un-stunned slaughter, unless it can be proven equal to secular slaughter, although no procedure for who and how such a determination might be made was provided in the legislation. The upper house of the Dutch parliament rejected the bill because it was felt to violate religious freedom. However, discussions on how to improve religious slaughter took place and led to a covenant agreement between the religious communities and the government. The requirement is that (for cattle) if an animal is not unconscious in 40 s, an alternative intervention will be used even if it requires that the animal then becomes non-kosher or non-halal.

In France, the issue of religious slaughter of animals came up in the 2012 presidential election politics. This included a widely posted statement by the prime minister of France suggesting that Islam and Judaism need to modernize their religions. Both presidential candidates who were publicly negative about religious slaughter were defeated.

In the UK, the recently adopted Red Tractor standards do not permit religious slaughter without a prior intervention. This standard was accepted by the Olympics Board and required many Muslim athletes from other countries to seek alternate sources for their meat. Furthermore, in a recent meeting of Muslim clerics representing a wide range of religious views, a collective decision has been made to not support machine slaughter of poultry, despite the fact that the largest Muslim certifying agency permitted such meat. The Muslim community has also created a new organization to serve as the sole representative of the Muslim community in dealing with the UK government, similar to the role played by Shechita-UK for the Jewish community.

A report at the time this chapter was delivered suggested that Estonia would reexamine its rules about religious slaughter and subsequently these rules were revoked to permit the religious slaughter of animals, with a focus on creating an export market. Some traditional European countries have not permitted religious slaughter, many of these instances going back to the late 1930s in sympathy with the Nazi Germany ban on religious slaughter. Ironically, although Germany retracted these laws after World War II, some of these other countries have retained these regulations such as Norway and Sweden.

In addition to the issue of how the slaughter is done, there seems to be a movement in Europe to reject meat over which a Muslim has said "Bismillah Allah Akbar" ("G-d is Great") including the Church of England. This is based on the mistaken notion that "Allah" is not G-d. But Allah is simply the Arabic word for G-d and is used in Arab speaking countries by Jews and Christians. It is also interesting that in the USA, our currency has "In G-d We Trust" and that is acceptable in a country with a stronger tradition of separation of church and state.

Why this sudden interest in religious slaughter? Obviously, the growth and focus on animal welfare is a part of the mix. This is good and is something the religious communities need to address more seriously. Religious slaughter done properly from both a religious and animal welfare point of view, as mentioned earlier, requires attention to detail, and this is slowly being addressed by the various religious communities. Also, in a very positive effort to provide guidance to the religious community and working with the religious community, the American Veterinary Association (AVMA) is working with Dr. Temple Grandin to develop a set of Humane Slaughter Documents that will include religious slaughter and which is being vetted through the religious communities. This document will hopefully become public sometime in 2015. The American Meat Institute (AMI, now the North American Meat Institute (NAMI)) also has a set of animal slaughter guidelines (also written by Dr. Grandin) that includes quantitative auditing practices. The most recent version of their document can be found on their web site (www.meat-ami.org). Pre-slaughter handling, as often emphasized by Dr. Grandin, is an important factor in presenting a calm animal for slaughter, and this is particularly important for the religious slaughter of animals. In addition, there are obviously animal activists who have pointed out some of the failings of religious slaughter. For those cases where the problems are real, they are solvable and represent less than proper practices. Groups like the North American Meat Institute, the Food Marketing

Institute (supermarkets), the National Council of Chain Restaurants, and the American Veterinary Medicine Association, along with OIE, the international animal welfare organization, are working on slaughter standards that respectfully include religious slaughter of animals. As consumers are less in touch with their food supply, and have no sense of slaughter, the ability to focus on one of the less understood forms of slaughter is attractive to groups whose agenda often extends beyond animal welfare to anti-Semitism, Islamophobia, and the end of animal agriculture. Also, as pets and their pampering becomes the normative way for people to interact with animals, all forms of slaughter are subject to attack. Efforts in Europe such as the Dialog on Religious Slaughter (Dialrel) unfortunately seemed to perpetuate the divide because of a lack of full engagement of the religious community by the scientific community and a lack of understanding by the scientists of many of the variables involved in religious slaughter, as well as a failure to appreciate the limitations of the scientific literature, as discussed in this chapter. Their various documents can be found on their website.

The question, then, is how one evaluates the science of various methods of making an animal unconscious. The vocabulary used in discussing these issues can have a significant impact on how the consumer understands the issues and how scientists frame the research.

In a subject as sensitive as religious slaughter of animals, this vocabulary can be a source of tension. Thus, calling the process "ritual" slaughter versus "religious" slaughter of animals gives it a different tone. Other members of religious communities recommend the term "traditional" slaughter, which encompasses other traditions that cause unconsciousness by a neck/throat cut such as many on-farm slaughters, but with a much wider range of acceptable practices. But this also distracts from the idea that in the case of kosher and halal slaughter, the slaughter of animals is tied to a higher religious purpose and specific rules. Specifically, the use of the term shechita in the scientific literature is clearly designed to give it a foreign, "other" context. Thus, the author prefers the term traditional religious slaughter, for both traditional kosher slaughter and traditional halal slaughter when writing about these processes in the scientific literature. For general purposes, it might be suggested to use the terms "the prophetic method of slaughter" for halal and "the Jewish religion's humane slaughter of animals" for kosher.

As may be clear from the text to this point, the author has mostly avoided the terms "stunned" and "un-stunned," which are particularly problematic. The framing of these two words is a polarization of terms. The goal in all cases is to humanely make the animal unconscious. Religious slaughter does so using a trained religious person, respectfully slaughtering the animal. Other methods of stunning could be described as "cracking the skull," "electrocuting" the animal or "putting it into a gas chamber." Those terms do not sound anywhere as nice as "carefully hand slaughtered with respect for the animals."

The author tested wording with students before and immediately after an extensive discussion of religious slaughter. Using anonymous in-class polling devices provides a rapid, relatively anonymous solicitation of opinions. The first

From the following choices, which form of slaughter do you consider most humane?

Use of a penetrating stunner going through the skull to cause unconsciousness, 29 votes

Use of a non-penetrating stunner to crack the skull to cause unconsciousness, 12 votes

Use of gases to cause unconsciousness, 42 votes

Use of an electrical current to the head to cause unconsciousness, 22 votes

Use of a sharp knife to cut the neck to cause unconsciousness, 47 votes

Fig. 18.1 Results of an in-class survey using an anonymous polling device prior to a lecture on the religious slaughter of animals

From the following choices, which form of slaughter do you consider most humane?

By smashing the animal over the head to crack its skull, 3 votes

By smashing through the skull, 14 votes

By electrocuting the animal, 12 votes

By using a gas chamber, 17 votes

By traditional hand slaughtering with respect for the animal, 109 votes.

Fig. 18.2 Another polling question before class

question used a balanced vocabulary, but framing all the methods as ways to make the animal unconscious. See Fig. 18.1:

It was interesting that gassing received the highest number of votes from among the exclusively secular slaughter methods.

In the second poll taken immediately after the first polling without showing the results and with no comments by the instructor, a number of students switched their votes to religious slaughter when a less balanced wording was used (Fig. 18.2).

Following the lecture, which was admittedly favorable to religious slaughter and covered some of the same material that is covered in this chapter, the students when presented with the neutral words still voted even more strongly than before the talk for the traditional religious slaughter (Fig. 18.3).

There is also the issue of clear definitions of technical terms. The author would propose the following: unconsciousness is when an animal generally cannot maintain posture and represents the inability to feel pain. This is the goal of the initial step of the slaughter process. Based on a careful reading of the US Humane Slaughter Act, it is clear that insensibility and unconsciousness are used to describe the same state.

From the following choices, which form of slaughter do you consider most humane?

Use of a penetrating stunner going through the skull to cause unconsciousness, 10 votes

Use of a non-penetrating stunner to crack the skull to cause unconsciousness, 7 votes

Use of gases to cause unconsciousness, 8 votes

Use of an electrical current to the head to cause unconsciousness, 4 votes

Use of a sharp knife to cut the neck to cause unconsciousness, 124 votes

Fig. 18.3 Anonymous poll after a talk on the religious slaughter of animals

At this time the animal has not lost all reflexes. The only tasks that can be done at this time are those related to noninvasively hanging and/or moving the animal. The second step in the process is waiting for the animal to lose the critical reflexes in the head, although motion of the body (tonic and clonic states) is not relevant. The loss of the reflexes can be considered as a practical determination of the loss of brain stem death. In Europe, the term insensible seems to be used most often to represent this later stage of change after slaughter. When all of the head reflexes are gone, it is then appropriate to begin further processing, i.e., invasive cutting of the animal as an ethical requirement of both religious and secular ethics.

As always, care in defining and then using all of these words consistently would prevent some of the confusion. However, it also needs to be recognized that some of the problems in presenting religious slaughter to the public is that the religious community is not always doing as good a job as they should be doing. For example, the handling of animals at the kosher plant in Postville, Iowa (which was the subject of an undercover video in 2004 by People for the Ethical Treatment of Animals which can be found on their website: www.peta.org) was unacceptable from an animal welfare point of view. Some of the handling of animals in Latin America for meat exports to Israel is beyond unacceptable in the modern era and reflects a lack of willingness to make the necessary investment in equipment to conduct a proper religious slaughter. Further, the problems in Indonesia and Turkey that have recently surfaced also suggest that work on halal slaughter still needs to be done if the religious community is to clearly demonstrate its commitment to animal welfare during the religious slaughter of animals.

On the other hand, many consumers accept traditional on-farm slaughter and hunting, both of which raise issues with how unconsciousness is obtained. These are forms of un-stunned slaughter with fewer requirements with respect to animal welfare. When the issue of the first step in the slaughter process is framed as obtaining unconsciousness, time is a variable, but not the only one. The key issue is the quality of the death. If the animal is calmly expiring and shows no signs of stress, time may be secondary. If the animal is struggling, then time is a major concern.

Now turning to the science of religious slaughter of animals, the first consideration is how the religious slaughter was conducted There are clearly many methods, e.g., upright restraint (various systems), upside down restraint (various systems),

shackling and hoisting with or without subsequent movement and casting, and various forms of traditional casting. The state of the animal at the start of such slaughters will vary greatly, yet if one looks at the literature on religious slaughter, this information is rarely provided, much less sufficient details, so one can identify and evaluate the system being studied. In two cases where the author has asked about the religious slaughter, a well-known animal welfare scientist presenting his own data has said "I do not know." Is that not a violation of science, especially the "Religion of Science"? There are clearly bad slaughter systems and one has to suspect that some research on religious slaughter has chosen a bad system to compare with a good secular system. Some of those doing the work from the religious point of view may have done just the opposite, so it is essentially impossible to generalize the work in the scientific literature. Thus, much of the literature becomes worthless when trying to conduct a critical analysis of religious slaughter's relationship to animal welfare.

Summaries of "time to death" for religious slaughter are often reported in the secondary literature as the maximum time for one animal to become unconscious, and even in the primary literature the data is not presented in such a way that one can actually determine what percentage of animals are actually beyond some reasonable cut off point for unconsciousness, e.g., 40–60 s for cattle. Please note that this author strongly supports establishing a specific time where an animal if not unconscious from the religious slaughter will have a post-slaughter intervention that puts the animal down even if this means the loss of acceptability of that animal as religiously slaughtered. The current Dutch proposal of 40 s is probably reasonable based on data collected in North America by Dr. Grandin, who suggests that good cattle slaughter will have animals that become unconscious in 17–33 s (personal communication), while improperly done religious slaughter will take longer. So the real emphasis should be placed on eliminating the improperly done slaughter that causes real animal welfare problems.

Besides the broad issue of how the slaughter was done, there are still a number of details of the actual slaughter that needs to be considered and reported in the literature, whether it be through observations in the field of actual religious slaughter, and even more importantly if the work is being done in a laboratory setting. Often in the latter case, the term "un-stunned" slaughter is used. But if the discussion is then focused on religious slaughter of animals, one has to ask if the "un-stunned" system is relevant to the religious slaughter. The most recent series of papers by Gibson et al. (2009a, b, c, d) are a clear example of this lack of congruence. The use of a 10 in. machine sharpened knife of unknown shape is totally irrelevant in comparison to the use of a razor-sharp nick-free 14 in. chalef. There are many other problems with this research, so that it is in fact far from appropriate to use these papers to generalize religious slaughter. The comments of the authors both in the papers and in public pronouncements suggest that this distinction is being blurred, presumably in some cases intentionally.

On the other hand, the animal welfare of religious slaughter needs to be improved consistent with and respectful of all religious rules. The religious community needs to vigorously take on this responsibility with help from the scientific community.

Scientists, particularly in the EU, hopefully have as their goal working with the religious community to determine how to do things better rather than focusing only on documenting what goes wrong and suggesting an end to the traditional religious slaughter of animals. Unfortunately this emphasis on documenting the problems without working on solutions seems to be too common in Europe. This contrasts with the USA where Dr. Grandin has successfully established a more pragmatic approach. Address the problems by proposing solutions. This has led to some new systems that lead to religious slaughter that Dr. Grandin deems excellent. Unfortunately, no other scientific research has been done in those plants.

Unless the best religious slaughter systems available are studied when they are working properly, science can point out one or more problems with the system being studied, but cannot be used to criticize religious slaughter as such. These studies do help to identify specific areas where improvements need to be made to a particular system and need to be used properly in that context.

Moving forward, there must be standardized methods and terminology used for evaluating and reporting all types of slaughter methods. Ironically this has not been done. Most papers do not provide enough information to even come close to permitting another scientist to duplicate the slaughter, including secular slaughter systems. Unless one works in the same plant, one has no idea what really happened.

How does one evaluate a slaughter system? Time to collapse is one concept. A good system needs to get the animal both unconscious and free of reflexes without stress, ideally in the minimal amount of time, such as the 40 s provided for in the Dutch covenant. In a good system Dr. Grandin has observed that the average for cattle is 17 s and the longest time for a good slaughter was 33 s, by her own definition. Behavioral observations should also suggest that the animal during this period is not struggling. Any animal that is not collapsed after that agreed upon time or if it is visibly stressed even if the animal becomes unacceptable for kosher or halal needs an intervention that will cause immediate unconsciousness. At least one Dr. Grandin approved plant is using this standard and routinely getting over 95 % of the cattle to collapse in about 30 s. But one should still ask: Can they do better? Has anyone done any scientific studies in these model plants to collect key baseline data as to where we are with the best religious slaughter to use for setting goals for other plants?

The impact of the actual religious slaughter needs to be separated from a number of extremely important issues that are not "religious requirements," but which confound the research results, e.g., the people, the facility, the equipment, and the non-slaughter stress of the animals need to be optimized before looking at the impact of the religious slaughter procedure. The author suggests that the literature studies do not meet the standard of sufficient information so that the experiment can be repeated or the data cleanly interpreted, which is surprising for such important questions that have taken up so much research effort and expense.

The recent papers by Gibson et al. (2009a, b, c, d) are an example of such a questionable piece of work. These papers have many serious limitations. A list of some of those concerns will be presented beyond those previously described. Dr. Grandin has put a disclaimer on her web site criticizing this work. Yet these papers are being used politically in Europe as "proof" that religious slaughter is inhumane.

The actual slaughter details (e.g., where on the neck the cut was made and how many strokes) and the restraining "pen" for holding the animals are poorly described. The custom built equipment used to restrain the animals is not shown. The head holder appears to allow the animals head to move—something that Dr. Grandin's head-holder design is specifically meant to prevent. The training of the slaughter man is not given. Like so many papers, they do not give enough details to evaluate the religious slaughter (or un-stunned slaughter as they call it). Only in the subsequent discussion is their un-stunned slaughter discussion transferred to a discussion of religious slaughter without establishing whether the research work itself is actually relevant to the religious slaughter of animals.

And they do not discuss the fact that the controlled stunning had a 28 % failure rate (2 out of 7 cuts were mis-stuns).

Why is the heart rate so high for the first paper and much lower in two of the other papers? It suggests that some animals were more stressed? This is often observed for the convulsions after slaughter regardless of method. It also seems that the normal "sticking" of the animal after non-penetrating stunning was never done and would be another important control.

They also admit in one of the papers that the halothane treatment might have had an effect on some of their results!

The papers are sloppy about how the words unconsciousness and insensibility, which seem to be used for both the same and different stages of post-slaughter states. They also created a new term and standard which they referred to as undoubted insensibility are used. And the papers also seem to reference a lot of the improper religious slaughter for the times they quote for time to insensibility. Is it insensibility or unconsciousness? The latter is the point where pain is no longer processed, so the animal welfare issues are very different after the point of unconsciousness and before insensibility. Isn't unconsciousness more critical? Words like suffering and psychological shock are used without definition or justification. And a lot of "wishy-washy" words, like "probably, likely, possibly" are used in the papers, yet the authors are publicly supporting a strong antireligious slaughter position and these papers are being widely used (e.g., in the Dutch parliamentary debates) as evidence against religious slaughter.

The issue of occlusions in the carotid arteries that block proper bleed out and the issue of blood in the lungs are issues that need to be better understood and require further research. For example, even when these occlusions occurred, according to these authors, they seemed in many cases to have had no effect. Dr. Grandin suggests with respect to blood in the lungs that what is needed is the correlation of aspiration into the trachea and the time to drop. She has also pointed out to the author that blood in the trachea is not a major concern, but if it is found in the actual lungs it is quite a concern. In a recent discussion with Dr. Grandin, she suggested based on her observations that this effect can be minimized by a cut higher up the neck, which remains to be confirmed experimentally, but which can only be as high as permitted by religious law.

Another issue that needs to be more critically addressed according to Dr. Grandin during our discussions of these issues is neck tension: The exact tension on the neck

is critical to get a cut that is clean—if it is not taut enough the cut is sloppy and if it is too taut one may get tearing ahead of the cut. Thus, this needs to be clearly specified in terms of the head position as per Dr. Grandin's work with head holders although her recent observational work in the field suggests a slightly less taut neck may be better, i.e., with the head slightly lower than the current horizontal. Can we develop a method to measure this angle routinely in research?

According to the American Meat Institute (AMI, now NAMI) standards, cattle vocalization percentages should be 5 % or less of the cattle in the crowd pen, lead up chute, and restraint device. A slightly higher vocalization percentage (5 % vs. 3 %) is acceptable for religious slaughter because the animal must be held longer in the restraint device compared to conventional slaughter. A 5 % or less vocalization score can be reasonably achieved. Animals must be completely insensible before any other slaughter procedure is performed (shackling, hoisting, cutting, etc.). If the animal does not become unconscious, it should be stunned with a captive bolt gun or other apparatus and designated as non-Kosher [non-Glatt] or non-Halal if required by the religious authorities (It should be noted that vocalization does not work with sheep.).

Clearly, there is a great deal of research that needs to be done. An outline of a possible agenda for starting the discussion of what research might be needed is presented in Appendix. The author would be happy to receive professional comments on that document.

A key hypothesis that needs further testing is that of whether the "endorphins" (opiates) release occurs in animals at the time of slaughter. The role of the sharp cut with a nick-free knife of the right size in optimizing endorphin release needs to be determined. Another need is for a way to measure the sharpness of a knife quantitatively and to develop methods to determine the absence of nicks. Since the presentation, the author has become aware that a company in New Zealand (Anago Ltd) has an instrument for this purpose and it is hoped that this instrument will be evaluated for its ability to determine knife sharpness and possibly even locate and identify nicks. It then needs to be determined if this instrument can be used for both training and validating knives. There is also a need for training of those involved in slaughter to meet this high standard for the knife, whether in the religious community or the research community. Detailed animal physiology, biochemical, and behavior measurements in a good system where during religious slaughter animals are losing the ability to support themselves in preferably 20 s or less could provide a better understanding of what is needed for good religious slaughter.

18.1 Conclusion

It is the author's personal belief that in the future good science will show that the most humane slaughter may well be religious slaughter, although this type of slaughter is slower and the persons doing the slaughter need to be highly trained and properly compensated. All research on the issue of religious slaughter (as opposed

to evaluating a particular situation) needs to be done in a system that is operating properly and provides the best possible condition for slaughter. At this time the best definition of "operating properly" in the author's opinion is the NAMI slaughter guidelines; only then can the potential and limitations of religious slaughter be properly evaluated by both the religious community and the scientific community. The author believes that someday all animals will be slaughtered religiously as the best form of slaughter. Imagine if even pigs were slaughtered that way by non-Jews and non-Muslims who have the same high level of slaughter training and concern for the welfare of the animals whose lives they are taking (However, it has been pointed out by a colleague some time ago that one actually cannot slaughter a pig using the kosher and halal methods because of the difference in the anatomy of the pig in the slaughter area.).

Supplemental Information There is a comprehensive paper at www.ift.org on the full range of kosher and halal rules; please refer to the following publications: Comprehensive Reviews, volume 2 issue 3. For online talks on kosher/halal and on animal welfare (by JMR) and animal welfare (by Temple Grandin), please refer to www.cybertower.cornell.edu. For a 2 credit distance learning course on kosher/halal refer to the Kansas State University distance learning program in food science. There is a DVD available of the movie "Temple Grandin" staring Claire Danes which depicts Temple's early life, including her overcoming the impact of being autistic and of her early work on animal welfare. This made-for-TV movie received a number of Emmy awards in 2010 including "Best Documentary." An earlier version of an essay on this same topic was published in the 2012 Proceedings of the Reciprocal Meat Conference of the American Meat Science Association.

Acknowledgement I would like to thank Dr. Temple Grandin, Professor of Animal Science at Colorado State University for many helpful conversations, although the author must take full responsibility for the views expressed in this chapter.

18.2 Appendix: Outline of Issues for a Critical Review of Religious Slaughter for Mammals

This is a suggested list of the issues that need to be addressed both by the scientific/research community, by slaughter plant management, by secular authorities, and/or by the religious communities beyond the slaughter house in trying to improve the religious slaughter of animals.

Some of the issues may not involve scientific issues currently being studied and debated, but even if not a topic of research, they are included so that (1) If the information is wrong or needs updating it can be addressed, and (2) If changes in procedures are needed, it is important to have the bigger picture in mind to hopefully minimize any unintended consequences elsewhere in the system of any proposed changes.

18.2.1 Animal Specific Information

It would be helpful to have a good set of anatomical drawings showing the various blood vessels between the heart and the brain for the key animal species being slaughtered either kosher or halal, so these can be more critically discussed and compared, e.g., cattle, sheep, water buffalo, and camel. Drawings of where on the neck the kosher or halal cut can be made also need to be made available, including the anatomy within the neck in that area.

18.2.2 Long Before Slaughter

How does one minimize stress on the farm, and during transportation and lairage at the slaughterhouse? These are covered by traditional guidelines although in recent years more emphasis is being placed on these issues.

How does one select animals that are appropriate for religious slaughter, e.g., animals that are more used to seeing humans?

How can the rejection of animals for lung adhesions or other religious defects during kosher slaughter be minimized? Can studying this in live animals (e.g., ultrasound, which preliminary results by the author with colleagues suggest is possible) improve animal health generally? Does the presence of lung adhesions actually result in slower growth of animals?

Can pre-slaughter inspections on the farm eliminate problematic animals or herds?

18.2.3 Just Prior to Slaughter

When does one remove feed and water?

How does one physically design an optimal system from lairage to slaughter, e.g., flooring, lighting, noise and glare?

How does one minimize vocalization and electric prod use? Can vibrators replace the electric prod for hard to handle animals?

Should animals be prepared ahead of time for religious slaughter, e.g., washing and shaving of the neck so the kosher slaughter men will rarely need to wait to slaughter the animal in the restraint because the neck does not meet their standard?

18.2.4 At the Time of Slaughter

Can one determine if a little stress just before slaughter is good for loss of consciousness?

Can rapid tests using glucose and lactic acid be used to measure stress during slaughter? How do you do the sampling at that time?

Are there other ways to determine unconsciousness under field conditions, i.e., for every animal in a restraining device, where loss of posture may not be evident? Are there any telemetry or medical devices that can be adapted for this purpose?

18.2.5 Religious Personnel

Exactly where on the neck should the kill be done?

How does one measure the site of cutting afterwards?

Is a more aggressive cut and/or fewer strokes better for animal welfare? How can this be monitored? (Is this possibly a role for video auditing?)

Do these differences in cutting strokes impact on the number of aneurisms?

Does the angle of the knife during the cut make a difference, i.e., cutting straight across, slightly up or slightly down? How can this be monitored and evaluated?

The Muslim community uses a unique chest stab method (Nahr) for camels that could in principle be applied to other animals, especially cattle. Is this method accepted by the Muslim community for other animals? What are the pluses and minuses of this method?

18.2.6 After Slaughter

After slaughter when the Jewish slaughter man checks that the cuts were made properly are there any special instructions needed to minimize contact with the cut skin surfaces? Should Muslim slaughter men be encouraged to do the same? Can this procedure help to decrease aneurisms?

With kosher slaughter of animals there is sometimes a second cut. Exactly how is this cut made? Under what circumstances and by whom and how might a second cut be made after the religious slaughter man if the slaughter man considers the cut insufficient? Are there different rules for kosher and halal?

How does one measure time to unconsciousness? How does one use this timing as a management tool?

How does one evaluate the quality of the transition to unconsciousness rather than just the time to unconsciousness?

At what blood pressure does an animal become “unconscious”? Is this blood pressure different in different parts of the brain? Does it vary between species and from animal to animal? Could this be used as a method to determine unconsciousness?

How in a plant does one pragmatically assure that each animal is unconscious before being hung?

How does one evaluate the many behavioral traits that might determine the “quality” of the kill?

If it becomes necessary to put down an animal that is remaining conscious longer than a specified time, what is the best way to do this from an animal welfare and worker safety point of view?

How does one check for the loss of the corneal reflex (i.e., the blinking reflex) and is this measurement the correct measurement to make for insensibility? Does it correlate with brain death? Can this or another measurement be mechanized and monitored automatically on every animal.

How in a plant does one pragmatically assure that each animal is insensible before any further cutting of the anima is done?

18.2.7 Video Auditing

How does this important technique get instituted as a management tool to assure that animals are slaughtered properly?

Where should cameras be placed in slaughter houses to optimize the ability to manage the slaughter?

Who should have access to the recordings?

18.2.8 Equipment

How should the restrainer be designed? What are the parameters that one needs to consider?

How does one measure neck tension and head movement? How does one measure the proper angle that is needed for both upright and upside down restrainers?

An upside down box needs to turn quickly and properly support the animal once upside down and while turning.

When should the head-holder be released and when should the animal be turned back upright?

How does one determine the time to unconsciousness while the animal is in the box?

With an upright SPCA box can the belly holder be better designed?

Would designing an upright pen with a double rail that actually supports the animal off the ground be beneficial?

How can a low volume upright pen be optimized for animal welfare while retaining the lowest possible price, including restrainers for large steers and bulls?

How can greater use of these pens be encouraged?

Can the ergometric design of the cutting when using a V-restrainer be improved for the slaughter man?

How long should the ideal V-restrainer be?

Is it better to cut the animal while in the middle of the restrainer or do it at the end and allow the animal to then fall onto a table?

Can the high speed moving double rail be built at a lower cost so more plants could afford them?

In general, can equipment be redesigned so that the animal can be moved away from the equipment but be given time to become unconscious and eventually insensitive without violating animal welfare considerations?

How does one deal in a systematic way with any animals that are not unconscious coming out of a restraining device?

How does one establish the ideal length and shape of the knife? Is twice the neck width and perfectly straight blade the correct requirement?

How does one measure the sharpness of the knife blade? If the new Anago knife sharpness testing instrument works, what is the minimum acceptable sharpness?

How does one measure the number and "quality" (to be defined) of this properly of a knife objectively?

Can Muslim slaughter men be trained and monitored to do this regularly?

Are all the Shochtim meeting the minimum requirement?

18.2.9 After Unconsciousness and After the Loss of Reflexes

How might one measure occlusions routinely? Should a "quantitative" measurement be developed? How can it be used as a management tool?

How does one measure where the blood is in the lung? How does one evaluate blood in the trachea versus blood in the brachea?

How does one measure the cut of the different vessels after the fact, i.e., what percent of each vein/artery was cut and where was the cut?

How does one minimize the number of occlusions?

18.2.10 Endorphin Hypothesis

Can one determine whether the endorphin hypothesis as a way for an animal to die on a high has any merit? How does one do the blood draws as the animal is losing both blood and blood pressure?

18.2.11 Beyond the Slaughterhouse

What should be the requirements for the training of slaughter men in terms of animal welfare?

Should some animal welfare licensing scheme be developed?

What type of monitoring of slaughter men is appropriate?

Should slaughterhouses be required to have a written kosher and/or halal protocol that both the secular and religious supervising body can agree on?

18.2.12 Overall Goals

What are the overall standards that one wishes to see achieved in slaughter houses doing the religious slaughter of animals? How does one get all religious slaughterhouses up to a minimal animal welfare standard?

How does one make the good plants even better?

The above outline is based on a number of articles and discussions with a number of colleagues. Khalid (2011) has an extensive literature review that is a good starting point for the comprehensive and balanced literature review that is needed moving forward. The preliminary report prepared for use in Holland (Regenstein 2011) documents in more detail some of the problems with the current literature in great detail. Dr. Grandin's website (www.grandin.com) contains a great deal of material on religious slaughter including a few papers and the disclaimer for the work in New Zealand. Stuart Rosen's (2004) critical review that was peer reviewed but was not permitted to be published as such by the Veterinary Record is an important paper. Rabbi/Dr. Levinger (Levinger 1995; Munk et al. 1976) has written two books that provide a comprehensive review of the physiology of religious slaughter, mainly kosher from a Jewish point of view, although it does not general separate the impact of the different forms of religious slaughter.

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Chapter 19
Mitigation of Acrylamide Formation in Highly Consumed Foods

Franco Pedreschi and María Salomé Mariotti

19.1 Introduction

Acrylamide (AA) (CAS number 79-06-1) (CH_2=CH–CO–NH_2), a white, odorless, toxic crystalline compound is produced mainly for the synthesis of nontoxic polyacrylamide, which is used as a flocculent in water treatment, and as a binder in pulp and paper processing. AA affects the nervous system even at low levels, causing hallucinations and drowsiness (IARC 1994). Human health effects associated with consumption of small amounts of AA over long periods of time are not known (Bent et al. 2012). AA vapors irritate the eyes and skin and cause paralysis of the cerebrospinal system (Kotsiou et al. 2011). Chronic exposure results in neurotoxicity in animals and humans, and AA has been found to be carcinogenic to laboratory animals. As a result, AA has been classified as "probably carcinogenic to humans" (Group 2A) by the International Agency for Research on Cancer (IARC 1994).

In April 2002, the University of Stockholm and the National Food Administration (NFA) in Sweden reported that rather high levels of AA could be found in normally cooked starch-rich food, compared to what had been reported earlier in other food commodities. Preliminary findings showed that French fries and potato crisps

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exhibit relatively high values of AA 424 μg/kg and 1739 μg/kg, respectively. This announcement received large attention because fried foods such as French fries are consumed daily by millions of people from multiple cultural backgrounds (Chen et al. 2012). Additionally, reports of the presence of AA in a range of fried and oven-cooked foods have caused worldwide concern because this compound has been classified as probably carcinogenic in humans with significant toxicological effects, namely neurotoxic and mutagenic (Rosén and Hellenäs 2002; Tareke et al. 2002).

AA has been demonstrated to have carcinogenic properties in animals (Smith and Oehme 1991). However, recent studies have suggested that women who eat chips or crisps appear to be at an increased risk of developing ovarian cancer. Besides, a prospective epidemiological study has found that increased dietary intake of AA is associated with increased risks of postmenopausal endometrial and ovarian cancer, particularly among nonsmokers (Hogervorst et al. 2007). Additionally, a positive association between dietary AA intake and renal cell cancer risk was observed in a prospective cohort study (Hogervorst et al. 2008). For other cancer sites such as prostate cancer (Wilson et al. 2010), gastrointestinal cancer (Hogervorst et al. 2008), bladder cancer (Mucci et al. 2003), thyroid cancer (Schouten et al. 2009), and lung cancer (Hogervorst et al. 2009), epidemiological studies have indicated that dietary AA intake would not be associated with these cancer risks. Epidemiological studies have provided valuable information regarding the associations between dietary AA intake and cancer in humans. It is suggested that more epidemiological studies are needed to substantiate this evidence (Chen et al. 2012).

Currently, a substantial body of research has been carried out worldwide to build greater understanding of AA, what the risks are for consumers and how to reduce occurrence levels. Although many effective possible ways to reduce AA content have been confirmed, the corresponding effects in sensory attributes in most of the reduction studies in fried potatoes have not yet been clearly reported (Zhang and Zhang 2007). Thermal processes are frequently used in food manufacturing to obtain safe products with a prolonged shelf life and have a strong impact on the final quality of foods. Baking, toasting, frying, roasting, and sterilization result in desired and undesired effects due to various chemical reactions, such as Maillard reaction, caramelization, with lipid oxidation being the most prominent (Capuano and Fogliano 2011).

One of the purposes of thermal processes is to improve the sensory properties of foods, their palatability and to extend the range of colors, tastes, aromas, and textures in foods produced from similar raw materials. Heating also destroys enzymes and microorganisms and lowers the water activity of the food, thereby preserving the foods. On the other hand, it is well known that some substances arising from heating processes can play a positive role on human health. Many neo-formed compounds showing antioxidant, antimicrobial, and antiallergenic effects as well as modulating activity in vitro have been detected in heated foods (Borrelli and Fogliano 2005; Lindermeier and Hofmann 2004; Van Boekel et al. 2010). The major concern arising from heating processes comes from the formation

of compounds that are not naturally present in foods, but that may develop during heating or preservation processes and that reveal harmful effects such as mutagenic, carcinogenic, and cytotoxic effects (i.e., neo-formed contaminants (NFC)). Recently, two neo-formed contaminants have gained much interest because of their high toxicological potential and their wide occurrence in foods: AA and 5-hydroxymethylfurfural (HMF). So it is a challenge to produce processed foods based on Maillard reactions with low or no HMF or AA, while maintaining their sensorial attributes intact, in order to make them attractive for the consumer (Pedreschi et al. 2011).

19.2 Acrylamide Litigation Case in California

In the USA, California laws concerning potential carcinogens are some of the strictest in the nation. California's Proposition 65 maintains a list of substances suspected of causing cancer (carcinogens) and regulates these potential cancer agents. Many products, such as paints, produced worldwide will contain labels stating that the product contains an agent known in the state of California to cause cancer. AA and the lawsuit against potato chip and French fry manufacturers fall under Proposition 65's umbrella. This carcinogen was added to the list in 1990.

In 2002, the Metzger Law Group filed the first Proposition 65 case regarding AA on behalf of the Council for Education and Research on Toxics to require fast food companies such as McDonald's and Burger King to warn consumers of the AA hazard in French fries. Eventually the California Attorney General joined the suit and the Metzger Law Group co-litigated the case with the Attorney General. After 6 years of litigation and several months of expert depositions, the case settled in 2008 when McDonald's and Burger King agreed to provide cancer hazard warnings regarding AA in their French fries, and agreed to pay civil penalties, and paid attorney's fees to the Metzger Law Group for protecting public health. As a result of this lawsuit, fast food companies in California now provide consumers such cancer hazard warnings regarding AA in French fries. The lawsuit also prompted potato chip manufacturers such as Frito Lay to improve their production process to reduce the AA content of their chips to safe levels. Additionally, thanks to a court decision in California, potato chips and French have now become a bit healthier and a bit safer for consumption. According to the Associated Press, a lawsuit filed in 2005 by the LA district attorney's office was settled requiring leading potato chip and French fry manufacturers to reduce the amount of AA in their products. Brands such as Heinz, Frito Lay, Kettle Foods, and Lance had to collectively pay $3 million dollars and reduce the amount of AA in their products as a result of the litigation.

Consumers interested in the effects of this lawsuit on their eating habits may have to wait and see what happens next. There has been some debate about what altering the process of making the potato chips and French fries in order to reduce AA will affect the taste of the products. The companies had time to work out the

kinks; as the San Francisco Chronicle reports, the lawsuit gives the companies 3 years to reduce the AA levels in their products, and they can avoid adding a Proposition 65 label to their potato chips and French fries.

In another case, in 2010, the Metzger Law Group filed a suit against ready-to-drink coffee companies to require them to give consumers cancer hazard warnings regarding AA in coffee or to reduce the AA content of their coffee products to safe levels. The Metzger Law Group is concerned that this carcinogen occurs in so many foods that we eat, and they are seriously undertaking efforts to require food companies to reduce the AA content of their food products or, if they cannot do so, to warn California consumers that this carcinogen is present in the foods that they sell.

19.3 Acrylamide Levels in Common Heat Processed Foods

It has been confirmed that a wide range of cooked food rich in carbohydrates—prepared industrially, in catering or at home at temperatures >120 °C—contain significant AA levels (μg/kg). This includes staple foods like bread, fried potatoes, and coffee as well as speciality products like potato chips, biscuits, French fries, bread, and a range of other heat-processed products (Pedreschi 2012). Browned crispy crusts in foods like French fries, potato crisps, crackers, pretzel-like snacks, cereals, and browned breads tend to have the highest levels of AA, as shown in Table 19.1.

Shortly after the announcement of the occurrence of acrylamide in foods, the necessity for monitoring and collecting data on the occurrence and extent of acrylamide in foods was recognized (Lineback et al. 2012). This led to the establishment of acrylamide monitoring databases, particularly in Europe and the USA (Lineback et al. 2005). Large databases are maintained by the US Food and Drug Administration (FDA), with analytical data for the period 2002–2006 (FDA 2006), and the European Commission (EC 2006). Considering that dietary AA is such an important food safety issue, the European Union recommended in 2007 to the Member States to perform annually the monitoring of AA in certain foodstuffs. Thus, results from 2007, 2008, and 2009 related to food AA monitoring have been recently published by the European Food Safety Agency (EFSA 2011) and are summarized in Table 19.1, showing that AA levels decreased in crackers, infant biscuits, and gingerbread over these 3 years, while AA content increased in crisp bread and instant coffee. On the other hand, some foodstuffs such as potato chips, oven fried potatoes, bread, breakfast cereals, jarred baby foods, and processed cereal based baby foods did not show significant changes in AA content during this period.

This large variability of AA levels in foods becomes important when considering dietary exposure to AA and, moreover, when evaluating possible mitigation strategies. Foods contributing the most to dietary intake will differ from country to country, according to different dietary patterns and the way in which foodstuffs are

Table 19.1 Acrylamide levels (μg/kg) of foodstuffs monitored from 2007 to 2009 (EFSA 2011)

	2007			2008			2009		
Food	*N*[b]	Mean[a] (μg/kg)	Max (μg/kg)	*N*[b]	Mean[a] (μg/kg)	Max (μg/kg)	*N*[b]	Mean[a] (μg/kg)	Max (μg/kg)
Biscuits									
Crackers	69	291–292	1526	134	203–206	1042	99	195–208	1320
Infant	97	197–204	2300	88	98–110	1200	51	88–108	430
Wafers	38	206–210	1378	49	251–254	2353	90	244–246	725
Bread									
Bread crisp	155	221–226	2430	92	229–231	1538	130	219–223	860
Bread soft	127	54–68	910	211	31–46	528	110	27–37	364
Breakfast cereals	134	130–150	1600	136	140–156	2072	153	132–142	1435
Cereal-based baby food	92	48–69	353	110	35–51	660	99	55–70	710
Coffee									
Instant	51	357	1047	58	499–502	1373	46	591–595	1470
Roasted	153	245–251	958	267	200–204	1524	172	225–231	2223
French fries	647	354–357	2668	536	281–285	2466	469	326–328	3380
Other products									
Gingerbread	357	423–425	3615	258	432–436	3307	302	376–384	4095
Muesli and porridge	48	205–210	805	19	20–41	112	92	53–82	484
Substitute coffee	61	772–775	4700	84	988	7095	34	1502–1504	4300
Potato crisps	280	574–576	4180	458	626–630	4382	388	689–693	4804
Home-cooked potato products									
Deep fried	54	344–354	1661	39	220–228	1220	49	234–241	1238
Oven baked	8	380–385	941	108	275–276	1439	72	317	1665

Update of results on the monitoring of furan levels in food. EFSA Journal 8(7): 18

[a]Values based on an upper bond scenario

[b]Number of individual samples analyzed for each food category

[c]SD: Standard deviation of the upper bond scenario (SD not available for 2009 data)

processed and prepared (Pedreschi et al. 2012). Factors such as variability of AA precursors in the raw material, differences in food composition, differences in processing parameters and other conditions could be sources of the high fluctuations in the AA content observed for the same product.

The first breakthrough in AA research was the simultaneous discovery by several groups that AA is formed from reducing sugars and asparagine in the Maillard reaction in a complex mechanism. However, achievements made for potato products cannot necessarily be transferred to cereal products due to differing process technology and limiting precursors (Claus et al. 2008). Therefore, comprehensive investigations for each food commodity were required. It is worth noting that the presence of AA in some kinds of typical foods remains poorly investigated (Bassama et al. 2012). Although bakery products together with potato derivatives are the most important sources of AA, coffee may markedly contribute to the total AA content of the diet. AA monitoring and mitigation are issues of major relevance (Rosén and Hellenäs 2002).

Furthermore, concentrations of AA present in heated foods are the result of simultaneously occurring formation and elimination mechanisms (Gökmen and Senyuva 2006). On the other hand, AA formation in starchy food heated above 120 °C only takes place when a critical moisture content in the processed material is reached, when a large amount of water has been removed from the raw material and abrupt changes in the microstructure of their original components has occurred—e.g., starch, pectin, and proteins. (Pedreschi et al. 2011).

The analytical methods for AA determination are based on (1) gas chromatography and mass spectrometry—GC-MS (Tareke et al. 2002) or (2) liquid chromatography and tandem mass spectrometry—LC-MS-MS (Rosén and Hellenäs 2002). Since Maillard reaction browning and AA formation are to some extent linked, color measurement may be used as an easily accessible and fast indicator of AA content (Pedreschi et al. 2007).

19.4 Mechanism of Acrylamide Formation

Soon after its discovery in heat processed foods, scientists reported that AA was formed principally during the Maillard reaction (Mottram et al. 2002; Stadler et al. 2002) (Fig. 19.1). This nonenzymatic browning reaction influences several aspects of food quality such as flavor, color, and aroma formation. Maillard-type reactions, in the presence of asparagine, have been shown to be the major reaction pathways of AA formation in a wide range of foods subjected to intense heating such as potato crisps, roast potatoes, breakfast cereal, bakery products, and roasted coffee (Bagdonaite et al. 2008; Becalski et al. 2004; Claeys et al. 2005; Mottram et al. 2002; Perez-Locas and Yaylayan 2008; Stadler et al. 2002; Taeymans et al. 2004; Tareke et al. 2002; Yaylayan et al. 2003; Zyzak et al. 2003).

The major mechanism of AA formation therefore involves the reaction of a carbonyl compound (preferably an a-hydroxycarbonyl) with asparagine, resulting in the corresponding N-glycosyl conjugation and the formation of a decarboxylated Schiff base (after dehydration under high temperatures) (Stadler et al. 2004; Zyzak et al. 2003). This reaction involves a cascade of reactions with different highly reactive intermediates resulting in AA formation in food (Medeiros et al. 2012).

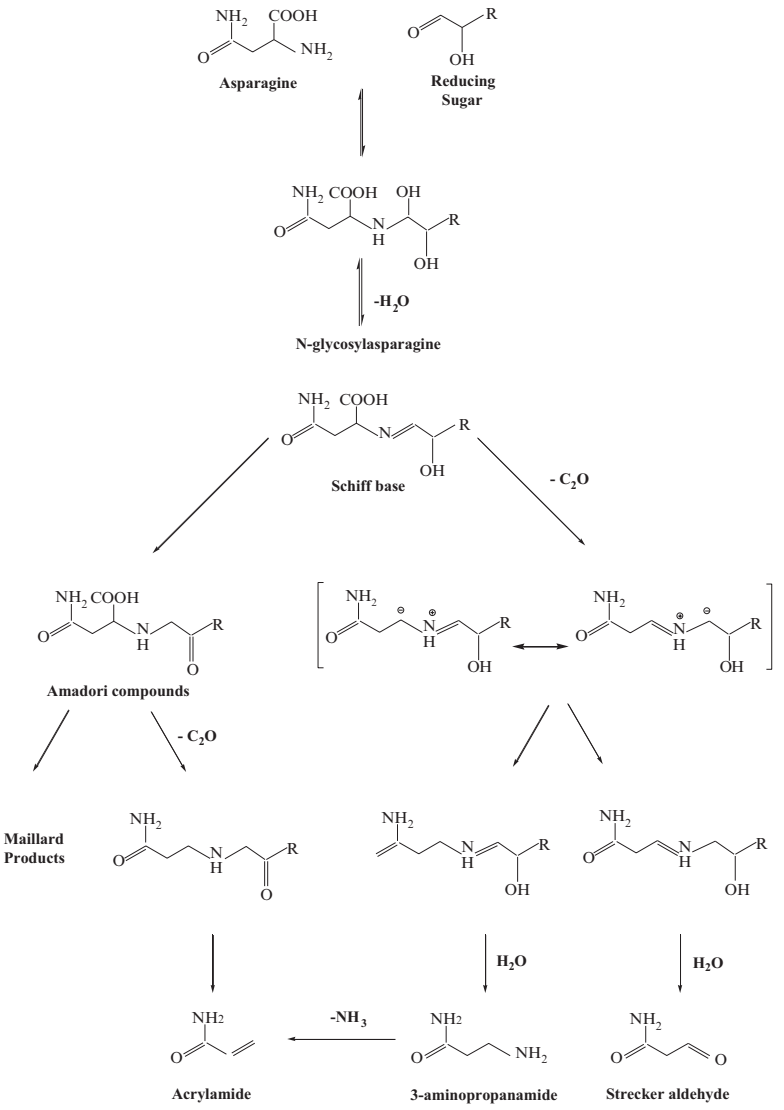

Fig. 19.1 Proposed mechanism for acrylamide formation as a side reaction of the Maillard reaction (reprinted with permission from Food Chemistry, 2012, 133, 1138–1154)

The following intermediates have been proposed (Fig. 19.1): (a) the decarboxylation of the Schiff base, which may lead after decomposition directly to AA and an imine or followed by hydrolysis to 3-aminopropamide (3-APA) and carbonyl compounds. In this respect, it should be noted that 3-APA may also occur in potatoes (Granvogl and Schieberle 2006); (b) subsequent elimination of ammonia from 3-APA can yield AA (Granvogl and Schieberle 2006); (c) alternatively, the hydrolysis of the imine which furnishes the Strecker aldehyde of asparagine (3-oxopropanamide) may also yield AA, although to a limited extent (Blank et al. 2005; Stadler and Scholz 2004). Additionally, since AA levels were high in fatty foods such as potato chips and French fries, the fatty acid oxidation product, acrolein (CH_2=CH-CHO) was noted as a possible precursor and forming AA through direct reaction with ammonia followed by oxidation to AA (Gertz and Klostermann 2002). On the other hand, the acrolein route to AA formation has been discarded since studies have confirmed that the addition of antioxidants did not affect AA formation (Vattem and Shetty 2003). Conclusively, a number of mechanistic studies have shown that Maillard reaction pathway is the most likely vehicle for AA formation.

19.5 Acrylamide Mitigation Technologies

Since AA is proven to be carcinogenic in rodents and a "*probable*" human carcinogen, with increasing evidence of positive associations with human cancers, authorities and industry promote the finding of solutions for AA formation, while no legal limits have yet been established for this contaminant in foods (Medeiros et al. 2012). However, the major challenge is to reduce AA levels in foods as much as possible while maintaining their sensorial attributes intact. Most of the proposed methods to mitigate the formation of AA seek to remove its precursors (glucose, fructose, asparagine) or to inhibit or reduce the intensity of the Maillard reaction by different process modifications (e.g., vacuum frying or conventional frying at low temperatures) (Fig. 19.2).

Post-processing exacting techniques could also eventually be implemented to decrease AA formation, but they are more unusual since they could require food structure destruction. Removing or trapping AA after it is formed with the aid of chromatography, evaporation, polymerization or reactions with other food ingredients has also been applied to food products such as coffee. For instance, the removal of AA from coffee through supercritical CO_2 extraction has been investigated. Supercritical treatment may reduce AA content by up to 79 % (with the addition of ethanol like supercritical fluid) without affecting the caffeine content of the coffee. However, to consider this method as a mitigation technology it is necessary to test its influence over the sensorial quality of the final product (Banchero et al. 2013).

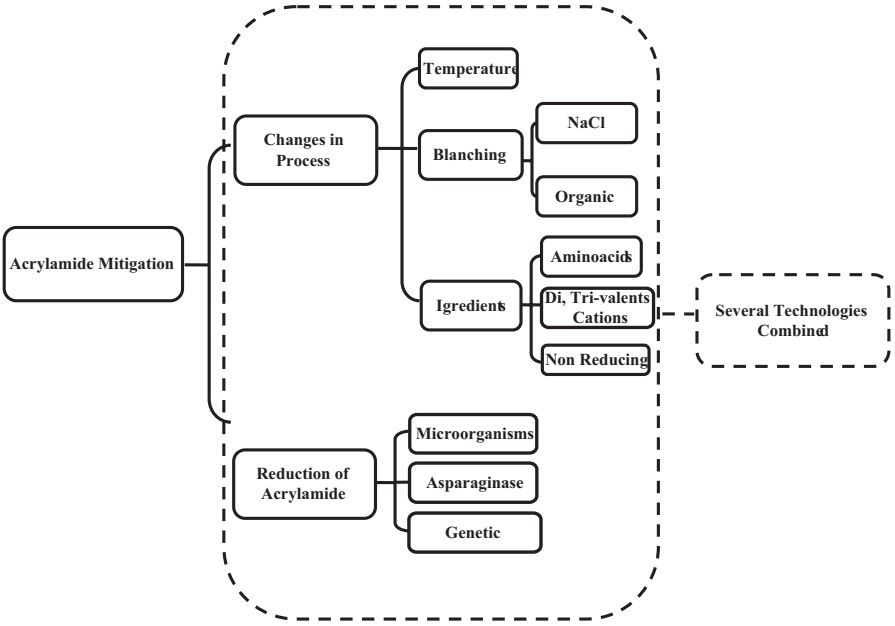

Fig. 19.2 Acrylamide mitigation techniques

19.5.1 Changes in Process Parameters

AA formation in foods may be influenced differently by several factors during heating processing, which directly or indirectly affects the occurrence and development of Maillard reaction such as temperature, heating time, browning level, water activity, and pH. The effect of temperature, heating time, surface over volume radio (SVR) and browning level on AA formation in fried potatoes was studied by some researchers. It was found that in potato shapes with low SVR, AA consistently increased while temperature and processing time increased as well. Additionally, studies on the effect of water activity on AA formation in model systems concluded that by controlling moisture, it may be possible to uncouple concurrent reactions related to Maillard reaction (De Vleeschouwer et al. 2007).

The effect of metal ions over AA formation has also been studied by several authors who concluded that the use of NaCl and $CaCl_2$ for instance could minimize AA formation during frying. These authors suggest that ionic and electronic associations between cations and asparagine suppress early-stage Maillard reactions (Lindsay and Jang 2005a). It is therefore also possible that changes in the ionic microenvironments near the potato strips contribute to the observed mitigating effects of positively charged metal ions (Mariotti et al. 2011). It has

been further discovered that AA formation in heated foods can be reduced by adding multivalent cations before cooking. The advantages of using multivalent cations in food processing are numerous, which include: (1) They generally suppress the AA reaction without causing unwanted side reactions; (2) They are active under very mild conditions of temperature and pH; (3) They are active at low concentration; and (4) Some of multivalent cations such as calcium are already widely approved for use in food processing (Corrigan 2005). Thus, the effect of metal ions over AA formation has been studied by several authors who concluded that the use of NaCl and $CaCl_2$ for instance could minimize the AA formation during frying. These authors suggest that ionic and electronic associations between cations and asparagine suppress early-stage Maillard reactions (Lindsay and Jang 2005b). The preventive effect of Ca^{2+} ions may be due to the observed inhibition of the formation of the intermediate Schiff base that leads to AA formation. Model studies showed that AA elimination, possibly via polymerization, increased in the presence of table salt (Kolek et al. 2006). Friedman and Levin (2008) found that changes of the ionic strength induced by positively charged Na^+ ions affect the rate of addition reactions of amino groups of amino acids to the double bonds of conjugated vinyl compounds such as AA (Friedman and Levin 2008). It is therefore also possible that changes in the ionic microenvironments near the potato strips contribute to the observed mitigating effects of positively charged metal ions.

Addition of free amino acids other than asparagine or from a protein rich food component to a model or food matrix are reported to strongly reduce the AA content as well in the heated products, probably by promoting competing reactions and/or by covalent binding the AA formed (Rydberg et al. 2003; Becalski et al. 2003). Several studies about the effect of some amino acids different from asparagine on AA formation/elimination kinetics have shown that addition of cysteine or lysine to model systems significantly lowered AA formation, whereas the addition of glutamine had a strong promoting effect in AA formation; interestingly, alanine shows a rather neutral effect on AA formation (Amrein et al. 2004; Jung et al. 2003; Biedermann et al. 2002; Claeys et al. 2005).

Interestingly, several results have shown that vacuum frying may be a mitigation alternative process for producing fried products with lower amounts of AA (Granda et al. 2004). Vacuum frying is an efficient method of reducing the oil content in fried snacks, maintaining product nutritional quality, and reducing oil deterioration. The unique aspect of vacuum frying is based on the fact that much lower temperatures (~120–130 °C) than those commonly used in atmospheric frying (~160–200 °C) can be applied during frying. Thus, in vacuum frying operations, food is heated under reduced pressure (<60 Torr—0.0789 atm) causing a reduction in the boiling points of the oil and the moisture in the foods. These issues make this frying technology suitable to produce fruits and vegetables with the necessary degree of dehydration without excessive darkening, scorching of the product or excessive loss of flavors and natural colors. This kind of frying also has many other advantages over atmospheric frying such as lower oil oxidation and the absence of frying vapor emission. Finally, another important issue of the use of this technology is in the area of food safety.

AA, a carcinogen found to cause cancer in laboratory rats, is present in carbohydrate-rich foods cooked at high temperatures, such as fried/baked chips, bread, etc.

Temperature and time of the processing operations used are critical factors in AA formation of the final foods. For instance, in French fries, all of the AA is accumulated in the crust (no AA presence in the core). Similarly, all of the content of AA in bread is located in the crust with no accumulation in the crumb; the amount of AA in the bread crust increases with both baking time and temperature (Brathen and Knutsen 2005). AA formation took place in the crusty part of the composite foods since the external layers of the food piece are exposed directly to very high temperatures (280–200 °C) in processing operations such as frying, baking, extrusion, and roasting, among others. The temperature of the inert part or central core of the composite foods is around 100 °C, which is far below the minimal temperatures required for AA formation (Pedreschi 2012).

19.5.2 Reduction of Precursor Levels in Raw Materials

One strategy to reduce AA content in heat-processed foods would be to reduce the precursor levels in the raw materials. In this sense, various patented pretreatments have been studied such as incorporating and/or exposing the food piece to: (1) A food grade microorganisms (yeasts, bacteria, and fungi Lindsay and Jang 2005a; Baardseth et al. 2004); (2) Asparaginase enzyme that converts free asparagine into aspartic acid (Budolfsen et al. 2008; Kim et al. 2005); (3) Another amino acid that does not form AA; (4) Saccharides and/or phenolic compounds having the ability of suppressing the formation of AA.

19.5.2.1 Acrylamide Mitigation by Blanching in Hot Water

The blanching step previous to frying in potato chip processing improves color and texture, and could in some cases reduce oil uptake by gelatinization of the surface starch (Califano and Calvelo 1987). Blanching could reduce the content of glucose and asparagine in potato slices, leading to significant lower AA formation than in unblanched potato chips (Pedreschi et al. 2004). Haase et al. (2003) reported that a reduction of sugar content by blanching could reduce the AA concentration by about 60 % according to the raw material (potato variety and field site) and the production process variables (e.g., blanching conditions and frying temperature). Glucose and asparagine determined in blanched in potato slices before frying decreased in potato slices according to time and temperature of hot water leading to significant reduction of AA formation in final potato chips and French fries. The higher the frying temperature, the higher the formation of AA in potato pieces (Pedreschi et al. 2004, 2007).

19.5.2.2 Acrylamide Mitigation by Using Microorganisms

Yeast and other microorganisms are believed to suppress the formation of AA in various high temperature heated foods by two mechanisms: (1) live yeasts and other microorganisms assimilate free sugars (especially glucose, fructose, and sucrose) that react with asparagine to produce AA under elevated temperatures conditions; (2) yeast and other microorganisms may also assimilate free asparagine, thus removing a key precursor in the formation of AA. Additionally, yeast and other microorganisms may possess the specific enzyme asparaginase, which would simply de-amidate asparagine to yield aspartic acid and ammonia, again removing a key precursor for AA formation. For instance, Lindsay and Jang (2005b) patented a method for suppressing AA formation and restoring browned color and flavor by treating an intermediate food material with a food-grade microorganism and/or a caramel coloring agent before the high temperature heating step (Lindsay and Jang 2005b). Similarly, Aziz developed a method for AA reduction in starchy foods without altering their regular cooking process parameters (temperature and time), but by using microbial cell fermentation (Aziz 2004). In this process, reducing sugar precursors of AA were metabolized by yeast and bacteria in starchy foods prior to cooking.

19.5.2.3 Acrylamide Mitigation by Using Asparaginase

Asparaginase, an enzyme that hydrolyzes asparagine to aspartic acid, is a very effective means for reducing AA formation in foods via removal of one of the precursors (asparagine) of the Maillard reaction. For instance, some researchers have prtreated potato pieces with asparaginase after blanching, and the AA levels in the resulting fried potatoes could be lowered by 60–85 % in French fries and 60 % in potato chips (Pedreschi et al. 2007; Zyzak et al. 2004; Zhang and Zhang 2007). Zyzak et al. (2004) also developed a method for AA reduction in low moisture starchy foods by using the enzyme asparaginase capable of hydrolyzing the amide group of free asparagine (Zyzak et al. 2004). This invention could be applied in batch, semi-batch, and continuous processes. This enzyme could be added to food materials in different suitable forms such as powder or solution. Furthermore, the enzyme could be added to the food material in any suitable manner such as directly (e.g., sprinkled, poured, or sprayed on the food material) or indirectly. Additionally, the enzyme could also be added to the food material at any suitable stage of the process (e.g., during the mixing of a dough or before, during or after maceration, and by soaking the piece in an enzyme solution). This invention was tested in dehydrated potato product, potato chips and French fries, finding that in all the cases the AA reduction percentage was 95. Similarly, Elder et al. also patented the use of asparaginase for diminishing AA content in foods which suffered Maillard reaction when they were processed at high temperatures (Elder et al. 2006). These

authors also remarked that for higher enzymatic AA reductions, pH control is crucial. The best performance is achieved by asparaginase at slightly acidic pH values (e.g., pH 5) and/or when the pH is slightly basic (e.g., pH 9). The use of asparaginase appears to be one of the most effective pretreatments to mitigate AA in foods processed at high temperatures. However, it is worth mentioning that this pretreatment is more expensive than others such as the using of amino acids, microorganisms and sugars.

19.5.2.4 Acrylamide Mitigation by Replacing Reducing Sugars

On the other hand, reduction of sugars is an essential precursor for AA reaction. Replacing inverted sugar and honey in the recipe with the non-reducing sugar sucrose resulted in a 20-fold decrease in AA formation in gingerbread (Amrein et al. 2004). Adding the non-reducing disaccharide trehalose (currently used in many commercial food applications) to glucose–asparagine or ascorbic acid–asparagine mixtures inhibited AA formation, presumably by suppressing the generation of intermediate carbonyl compounds such as pyruvaldehyde (Oku et al. 2005). Significantly, less AA was formed in cookies when the dough contained sucrose instead of glucose (Gökmen and Palazoglu 2008).

19.5.2.5 Acrylamide Mitigation by Using Genetic Modification

Genetic approaches for reducing the levels of AA precursors (asparagine, reducing sugars) in cereal and potato plants are active areas of research (Gerenda et al. 2007; Rommens et al. 2007). Ideally, breeding and/or suppressing genes that encode enzymes governing the biosynthesis of free asparagine may achieve a decrease in asparagine content. Additionally, selection from available varieties that contain low levels of asparagine for dietary use offers another approach to mitigate AA content. Asparagine levels in wheat grown under conditions of severe sulfate depletion were up to 30 times greater as compared to levels in wheat grown in soils with sufficient amounts of sulfate fertilizer (Muttucumaru et al. 2006). This was also reflected in observed AA levels of baked products. Levels in products prepared from high-asparagine wheat flours ranged from 2600 to 5200 μg/kg and those from wheat grown under normal conditions, from 600 to 900 μg/kg. These observations suggest the need to develop new wheat varieties with low asparagine content and that wheat should either be grown in soils with adequate amounts of sulfates or that the soil should be amended to provide adequate sulfates to the crops. AA content of breads largely depended on the wheat used to prepare the dough and is linked levels of free asparagine and crude protein (Claus et al. 2008). Nitrogen fertilization of the soil induced elevated amino acid and protein contents, resulting in increased AA levels in breads.

19.5.3 Combination of Techniques

Some combined techniques which include, for instance, blanching as the first step and then immersion in one solute could produce a synergistic effect since after the blanching step, the solute could diffuse better and faster in a heated microstructure of the raw material. For instance, blanching followed by the immersion of potato slices in 1 g/100 g NaCl solution was effective in reducing AA content in ~62 % in potato chips; however, almost half of this percentage (~27 %) could be attributed to the effect of NaCl and 35 % to the effect of the slight heating treatment during salt immersion step (25 °C for 5 min) (see Fig. 19.3). Blanching seems to make the NaCl diffusion in potato tissue easier leading to a significant AA reduction in the potato slices after frying (Pedreschi et al. 2010).

Similarly, blanching in hot water was almost as effective as asparaginase potato immersion in order to diminish AA formation in potato chips (AA reduction was 17 % of the initial AA concentration). When potato slices were blanched before asparaginase immersion, the AA content of the resultant potato chips was reduced considerably by almost 90 % (Fig. 19.4). We have demonstrated that blanching of potato slices plus asparaginase treatment is an effective combination for AA mitiga-

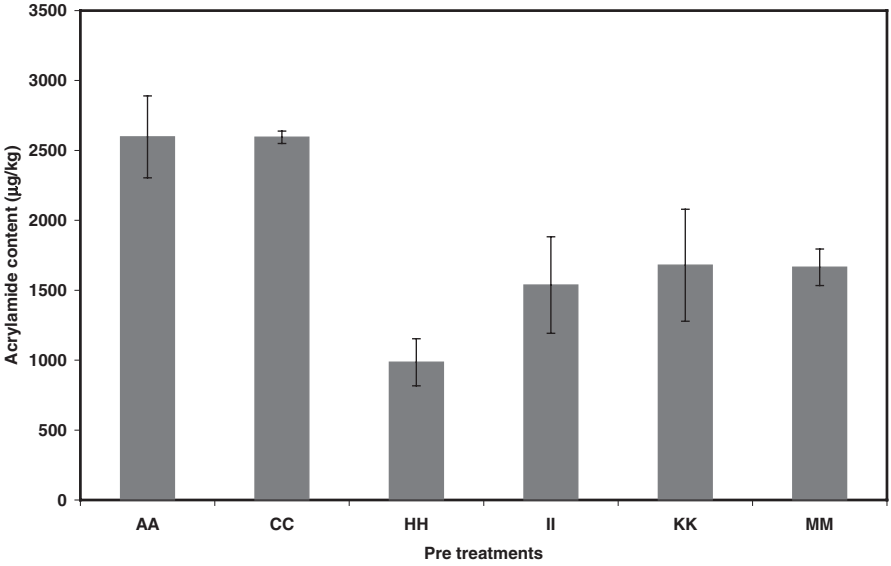

Fig. 19.3 Acrylamide content (µg/kg dry weight) of potato chips pretreated in different ways and fried at 170 °C for 5 min. AA control slices (unblanched potato slices); CC slices blanched at 90 °C for 5 min in water; HH slices blanched at 90 °C for 5 min then immersed in a 1 g/100 g NaCl solution at 25 °C for 5 min; II slices blanched at 90 °C for 5 min then immersed in a 3 g/100 g NaCl solution at 25 °C for 5 min; KK slices blanched at 90 °C for 5 min then immersed in distilled water at 25 °C for 5 min; MM slices blanched at 90 °C for 5 min in a 3 g/100 g NaCl solution

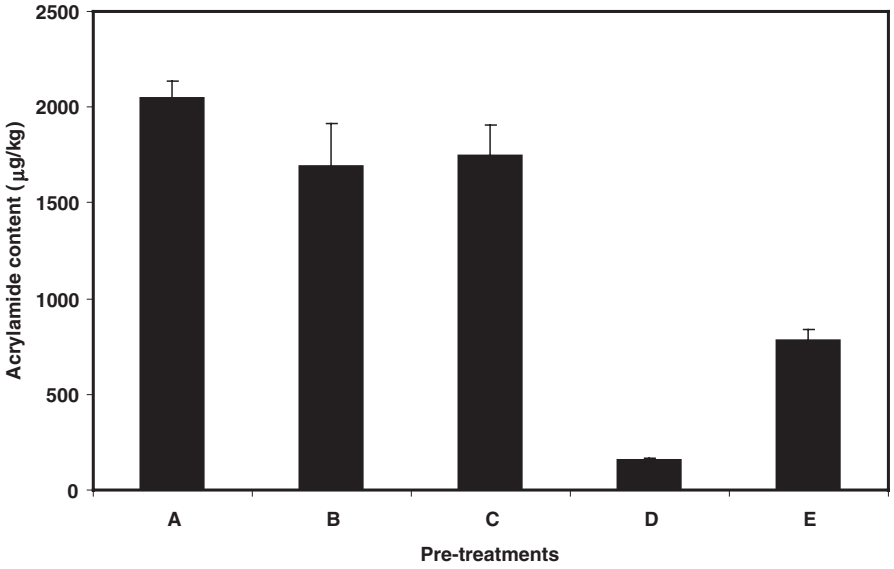

Fig. 19.4 Acrylamide content of potato slices treated with commercial asparaginase. (*A*) Raw potato slices (control I); (*B*) Blanched potato slices at 85 °C for 3.5 min; (*C*) Raw potatoes slices immersed in a 10,000 ASNU/L asparaginase solution at 50 °C for 20 min; (*D*) Blanched potato slices at 85 °C for 3.5 min then immersion in a 10,000 ASNU/L asparaginase solution at 50 °C for 20 min; (*E*) Blanched potato slices at 85 °C for 3.5 min then immersion in distilled water at 50 °C for 20 min (control II). All experiments were done in duplicate

tion during frying. It seems that blanching provokes changes in the microstructure of potato tissue, thus leading to an easier and more effective diffusion of asparaginase (Pedreschi et al. 2011).

19.6 Conclusions

The presence of AA in a range of fried and baked foods has caused worldwide concern because this compound has been classified as probably carcinogenic in humans.

The first breakthrough in AA research was the simultaneous discovery by several groups that AA is formed from reducing sugars and asparagine in the Maillard reaction. Thus, most of the methods to mitigate the formation of AA seek to: (1) remove its precursors (glucose, fructose, asparagine); (2) inhibit or reduce the intensity and (3) to combine technologies related to (1) and (2). Since this nonenzymatic browning reaction also influences several positive aspects of food quality such as flavor, color, and aroma formation, a major challenge in the food industry is to reduce AA levels in foods as much as possible while maintaining their sensorial attributes intact.

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Chapter 20
Scale-Up Issues and Cost of Manufacturing Bioactive Compounds by Supercritical Fluid Extraction and Ultrasound Assisted Extraction

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List of Abbreviations

A	Bed cross section area
CER	Constant extraction rate period
CO_2	Carbon dioxide
COL	Cost of operational labor
COM	Cost of manufacturing
CQC	Cost of quality control and research and development
CRM	Cost of raw material
CUT	Cost of utilities
CWT	Cost of waste treatment
d_b	Bed diameter
DC	Diffusion-controlled period
DFC	Direct fixed capital
d_p	Particle diameter
E	Porosity of the bed + particles
F	Feeding mass
FCI	Capital investment cost
FER	Falling extraction rate period
GRAS	"Generally recognized as safe"
H_b	Bed height
LPSE	Low-pressure solvent extraction
MAE	Microwave assisted extraction
M_{CER}	Mass transfer rate during the CER period
OEC	Overall extraction curve

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PLE	Pressurized liquid extraction
Q_{CO_2}	CO_2 mass flow rate
ρ_{CO_2}	CO_2 density
R_{CER}	Yield in the CER period
Re	Reynolds number
S/F	Solvent to feed ratio
SD	Steam distillation
SFE	Supercritical fluid extraction
t_{CER}	Duration of the CER period
t_{FER}	Duration of the FER period
TPC	Total plant cost
TPDC	Total plant direct cost
TPIC	Total plant indirect cost
t_{RES}	Solvent residence time in the extractor
UAE	Ultrasound assisted extraction
USFE	Ultrasound assisted supercritical fluid extraction
Y_{CER}	Mass ratio of solute to solvent in the bed outlet during the CER period
ν	Solvent superficial velocity

20.1 Introduction

Natural extracts containing bioactive compounds are obtained from various plant materials. The global natural product market is divided into five segments: active pharmaceutical ingredients based on a single chemical compound, herbal pharmaceutical products, dietary supplements, functional foods, and cosmetic products. These segments are not strictly separated. The market of natural products was estimated as US$ 20–40 billion per year in 2011 (Buchwald-Werner and Bischoff 2011).

There are several methods for extracting bioactive compounds, from classical technologies such as steam distillation (SD) and organic solvent extraction, to more modern techniques, such as supercritical fluid extraction (SFE), ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), pressurized liquid extraction (PLE), and the combination of these methods. The extraction method is a key component when obtaining natural products, since it is responsible for the efficient and selective recovery of the target compounds or classes of compounds responsible for extract bioactivity. When high quality plant material is processed by the appropriate extraction technique with optimized operational parameters, the resulting product is of high quality, and the process is economically feasible (Harjo et al. 2004).

SFE and UAE are considered emergent technologies because they present several advantages when compared to SD and low-pressure solvent extraction (LPSE),

especially related to toxic solvents consumption and sensory characteristics. However, these extraction methods still need scale-up study and economic assessment to determine their industrial feasibility, and to be considered among other conventional extraction technologies for industrial application.

In the past years, research on SFE and UAE has generated much experimental data on process thermodynamics and kinetics. As laboratory data is gathered, the economic evaluation of these extraction methods can be performed using simulation tools. Since each natural product is unique, it is necessary to determine the technical and economic feasibility of the processes for each raw material considering its particularities. Therefore, the objective of this chapter is to compile scale-up and economic data published in the last few years for SFE and UAE emergent extraction methods.

20.2 Emergent Extraction Methods: SFE and UAE

Every day the news spreads pessimistic numbers on the misapplication of natural resources. This information arouses the concern of both the scientific community and the general population, leading researchers to search for industrial processes that are benign to both human health and the environment. The popular belief that "everything that is natural is good" stimulates development of the industry of natural products and conventional technologies used for extraction in this field present one important drawback related to health and environmental issues: the high consumption of toxic solvents (Jiménez-González et al. 2004). New green extraction methods were developed to overcome these drawbacks, and are based on six principles (Chemat et al. 2012):

1. Innovation by selection of varieties and use of renewable plant resources;
2. Use of alternative solvents, in particular water or agro-solvents;
3. Reduction of energy consumption by energy recovery and use of innovative technologies;
4. Production of co-products instead of waste to include the bio- and agro-refining industry;
5. Reduction of unit operations and selection of safe, robust and controlled processes;
6. Aiming for a non denatured and biodegradable extract without contaminants.

The emergent extraction methods SFE and UAE fulfill most of these principles and have thus been drawing attention over the past decades. UAE has been studied in the field of natural products, especially because it decreases process time and solvent consumption. The global trend of searching for ecologically correct technologies, on the other hand, has stimulated studies on supercritical fluids, since most supercritical technologies use "generally recognized as safe" (GRAS) solvents.

However, any new technology that produces a product already in the market must be economically feasible so that it is demonstrated that it can compete with conventional technologies and be scaled-up to the industrial level.

20.2.1 The Extraction Process

From the phenomenological point of view, extraction is a mass transfer process of one or more components from one phase to another through chemical, physical, or mechanical processes. When dealing with natural products, in most cases the sample to be extracted is a solid material. The extracting solvent is usually a liquid, but it can also be a supercritical fluid.

The extraction mechanism is comprised of the following steps: (1) the solvent is transferred from the fluid phase to the solid surface and penetrates into the solid particle; (2) the soluble material is solubilized into the solvent; (3) the solution containing the solutes returns to the surface of the solid and is transferred to the bulk fluid. Each process has its particularities regarding this mechanism, which will next be presented.

20.2.2 Supercritical Fluid Extraction (SFE)

Supercritical fluids present liquid-like densities, while their viscosity is near that of normal gases and their diffusivity is about two orders of magnitude higher than that of typical liquids (Brunner 2005; Eggers and Pilz 2011). This combination of properties favors their use as solvents for the processing of natural products because they can be used to extract a wide range of compounds of interest for the food, cosmetics, and pharmaceutical industries. Because of these features, the extraction of bioactive compounds is the most widespread use of SFE (Brunner 2010; Pereira and Meireles 2010; Eggers and Pilz 2011; Herrero et al. 2010).

SFE is a physicochemical separation process based on the contact between a fixed bed of comminuted material with a solvent in its supercritical state, which removes a solute or mixture of solutes from the solid phase. It consists mainly of two steps: (1) extraction; and (2) separation of the extract from the solvent. In the extraction step the solvent flows continuously through the fixed bed of particles, solubilizing the solutes and carrying them to the next step. Separation follows, with pressure reduction, so that the solute precipitates in a separator. After separation, the solvent can be recirculated in the system. The extraction process can be conducted in multiple stages, and the depressurization step may be fractionated in multiple separators working with cascade pressures, allowing the recovery of fractions of the extract with different chemical compositions. One important advantage of the SFE process compared to the classical LPSE process is that the solute is easily recovered and the solvent can be recycled by the simple manipulation of temperature and/or pressure. SFE is also faster than LPSE.

Most applications of SFE use carbon dioxide (CO_2) as the solvent. CO_2 is a GRAS solvent, and does not leave residues either in the extract or in the exhausted raw material. It is a powerful solvent for a wide range of compounds, relatively inert, inexpensive, nonflammable, recyclable, and available at high purity. Furthermore, it is approved for food processing without declaration. Because of its low critical temperature (31.1 °C), CO_2 can be used in a wide range of temperatures, which allows benign processing of thermosensitive compounds (Clavier and Perrut 2004; Brunner 2005).

Sometimes, due to polarity limitations, modifiers (cosolvents) must be added to the CO_2 in order to increase the extractability of the target compounds. Even when that is the case, the percentage of organic solvents is reduced when compared to LPSE, and in most cases GRAS solvents, such as ethanol and water, are used as modifiers. In some situations, the moisture of the raw material itself can act as a cosolvent.

The SFE process maximizes the generation of viable waste streams because the only waste accumulated at the end of the process is the dry exhausted solid matrix (when there is no need to add modifiers), which can be incorporated into the soil or reused for many purposes, including human and animal feeding, or for nobler uses, such as a source of special starch (Braga et al. 2006), phenolic compounds (Li et al. 2008), or lignocellulosic biomass for energy production (Moreschi et al. 2004), among others.

Several factors affect the concentration of the bioactive compounds in the extract for SFE: solvent type, temperature, pressure, solvent to feed ratio (S/F), contact time, particle size, etc. To understand the kinetic behavior and how all these parameters influence the extraction process, overall extraction curves (OECs) are determined. The information obtained from the OEC is especially important for the estimate of the cycle time of the process.

The SFE process follows the kinetic behavior shown in Fig. 20.1. It can be noticed that the mass transfer rate is not constant. SFE curves usually consist of three distinct phases: constant extraction rate period (CER); falling extraction rate period (FER); and diffusion-controlled period (DC) (Brunner 1994). In the CER period the easily accessible solute that surrounds the particle is removed at an approximately constant rate. In the FER period gaps appear in the solute superficial layer that covers the solid particle; therefore, the extraction rate decreases rapidly. In the DC period the easily accessible solute layer is depleted; therefore, the extraction rate is slow and controlled by the diffusion of the solute and solute/solvent mixture.

The CER period is characterized by the following kinetic parameters: duration of the CER period (t_{CER}); mass ratio of solute to solvent in the bed outlet during the CER period (Y_{CER}); mass transfer rate during the CER period (M_{CER}); and yield in the CER period (R_{CER}). During the CER period, 50–90 % of total extract is usually recovered; therefore, it is considered the minimum duration of an SFE cycle. R_{CER} is the minimum yield expected during the CER period for the process under a certain temperature, pressure, and S/F. The duration of the FER period is denoted as t_{FER}. Usually, from an economic point of view, the most favorable cycle time is between t_{CER} and t_{FER} (Meireles 2008).

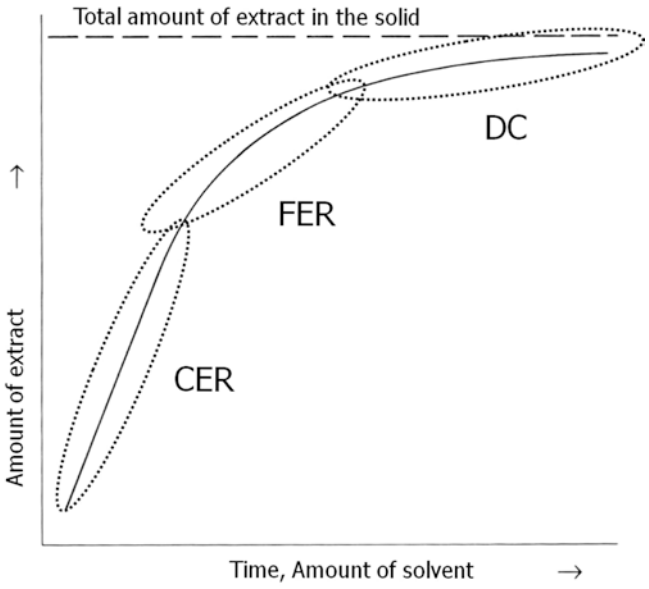

Fig. 20.1 Typical OEC of SFE process. *CER* constant extraction rate period, *FER* falling extraction rate period, *DC* diffusion-controlled period. Adapted from Brunner (1994)

20.2.3 Ultrasound Assisted Extraction (UAE)

In UAE the extraction process is improved by changes in the cell structure of the plant material caused by sound waves. Sound waves are mechanical vibrations at a frequency above the threshold of human hearing (>20 kHz). These waves travel either through the bulk of a material or on its surface. It has been suggested that the improvement of extraction by ultrasound is mainly due to the mechanical effects of acoustic cavitation, which enhances the turbulence, solvent penetration into the vegetable matrix and intracellular product release by the reduction of particle size and the disruption of cell walls (Rodrigues and Pinto 2007; Takeuchi et al. 2009; Chemat et al. 2011; Soria and Villamiel 2010; Shirsath et al. 2012; Esclapez et al. 2011; Patist and Bates 2008; Leonelli and Mason 2010; Gogate et al. 2003).

UAE technique has been systematically used in the extraction of bioactive compounds from plants. The extraction efficiency of LPSE processes can be significantly improved with intense ultrasound, achieving higher yields at shorter processing time, with reduced energy and solvent consumption, and eliminating post-treatment of waste water (Vinatoru 2001; Vilkhu et al. 2008; Wang and Weller 2006; Soria and Villamiel 2010; Esclapez et al. 2011; Chemat et al. 2011; Adam et al. 2011). The majority of material is extracted in the first 10 min of sonication (Mason et al. 1996). In addition, UAE can be carried out at low temperatures, avoiding thermal degradation of extracts and the loss of volatile components due to boiling

(Wu et al. 2001). Depending on the application, the required energy is comparable to other unit operations currently utilized in the industry, such as homogenization, milling, heat shock, etc. (Patist and Bates 2008; Paniwnyk et al. 2009). The UAE equipment is easy to install, it has high energy efficiency (around 85 %), which leads to competitive energy costs, it requires low maintenance, and the UAE technology presents strong potential for intellectual property (Patist and Bates 2008, 2011).

Many other factors govern the action of ultrasound, including design of the equipment, plant material characteristics, solvent type, solvent to feed ratio (*S*/*F*), frequency, power, pressure, temperature, and sonication time (Wang and Weller 2006; Shirsath et al. 2012; Esclapez et al. 2011; Patist and Bates 2008; Gogate et al. 2011).

Two main designs are used for UAE: indirect extraction using an ultrasonic bath, and direct extraction using an ultrasonic probe. Although baths are more often used at the laboratory scale, the direct application of ultrasound shows better results. On the other hand, direct sonication may lead to degradation of some of the bioactive compounds extracted due to intense cavitation and temperature increase. Moreover, in some cases, when soft vegetal material is employed, the *S*/*F* must be increased when using the ultrasonic probe because the raw material can dampen the transfer of ultrasonic energy (Esclapez et al. 2011; Shirsath et al. 2012; Chemat et al. 2011; Vinatoru 2001; Gogate and Pandit 2004). The extractor geometry (ratio of the diameter of the immersion transducer to extractor diameter, liquid height, position of the transducers, etc.) also affects the efficiency of the process. With an increase in the diameter of immersion transducer relative to the reactor diameter, cavitational activity increases. The extent of immersion of the transducer in direct sonication or the liquid height in indirect sonication affect the extent of reflection of the incident sound waves from the liquid surface and extractor walls. The position of the transducers in the extractors based on multiple transducer arrangement (with possibly multiple frequency operation) should be done in such a way that maximum and uniform cavitational activity is obtained (Gogate et al. 2003, 2011).

Reactors based on the ultrasonic frequency of 20–100 kHz are normally used with power ranging from 100 to 800 W; 20 kHz is usually determined as the most suitable frequency for extracting bioactive compounds from plant material (Esclapez et al. 2011; Shirsath et al. 2012).

Ultrasound-induced cavitation bubbles present hydrophobic surfaces within the extraction liquid, thereby increasing the net hydrophobic character of the extraction medium. Thus, it is possible to extract polar components into otherwise hydrophilic aqueous extraction media, reducing the need for generally undesirable hydrophobic or strongly polar extraction media. Solvent properties that affect cavitation phenomena include vapor pressure, viscosity and surface tension. Usually, lower vapor pressure, lower viscosity and higher surface tension of the liquid are preferred to maximize cavitational events (Gogate et al. 2011). Water, ethanol, methanol, hexane, and their mixture are mostly used for UAE. Ethanol, a GRAS solvent, is useful in numerous applications (Shirsath et al. 2012).

The time required for extraction will normally depend on the type of material used, structure of the cell wall, mass transfer resistance to the diffusion of solvent to the interior part of the material and penetration rate of the solvent into the plant material. Typically, UAE takes from 120 s to 1 h, which is considerably lower than the time required for the conventional LPSE processes. It is important to determine the kinetics parameters so that optimum treatment time using ultrasound can be selected (Shirsath et al. 2012). The typical OEC for UAE process is shown in Fig. 20.2. The process usually presents only two distinct phases. The first one is characterized by a rapid leaching of the solute, and the second is controlled by diffusion. The intermediate FER period observed in SFE is not evident in the UAE process.

The temperatures used for UAE usually range from 20 to 80 °C; however, 30–40 °C is most often used because this temperature range can prevent thermal degradation of sensible compounds (Esclapez et al. 2011; Shirsath et al. 2012).

Therefore, as SFE, UAE is a technique that needs study and development in order to be applied at optimal conditions. While the technology has great promise, it must be carefully developed and scaled up for each unique application (Patist and Bates 2008).

20.3 Scale-Up of SFE and UAE

The optimization of extraction processes seeks to reach the largest yield in the shortest time, with minimal energy consumption and minimal residual solvent in the product, so that an extract of good chemical and sensory quality is produced. At the

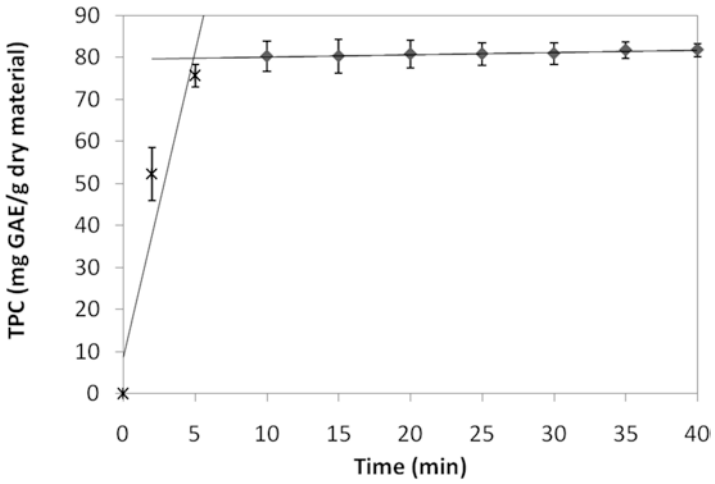

Fig. 20.2 UAE of polyphenols from jatoba (*Hymenaea courbaril* L. var *stilbocarpa*) bark. Adapted from Veggi et al. (2013)

laboratory scale, experimental determination of thermodynamic and mass transfer kinetics data is used to optimize the operational conditions of each technology and for each raw material to reach this goal.

Once the process is optimized at the laboratory scale, it is time to scale it up to pilot and industrial sizes. However, the relation between processes conducted in bench, pilot and industrial scales cannot always be simply approached or predicted. The differences observed in processes conducted in equipment of significantly different sizes must be carefully studied and evaluated to avoid mistakes when scaling-up a process. When stepping from laboratory to pilot scale extractions, the nature and intensity of some mass transfer phenomena are inevitably modified. Thus, process engineers must work to comprehend and predict the influence of each change of process characteristics that accompanies changing scales. Studying scale-up criteria is, therefore, important to establish a methodology that allows reliable prediction of the behavior of the processes at the industrial scale from laboratory data. This result is achieved by preserving some intensive extraction parameters used at the analytical scale, such as temperature and pressure, and increasing extensive parameters, such as solvent flow rate and solid feed, using predefined criteria (Martínez and Silva 2013).

20.3.1 SFE

SFE equipment used for process development can be divided into three categories (del Valle et al. 2004): (1) analytical systems, where small extraction cells are used (<25 mL) and only one depressurization step is carried out at ambient pressure; (2) screening systems, where 50–1000 mL extraction vessels are used, and to which cyclone separators can be coupled; and (3) process development units, where larger extractors are used (>1 L), separators are displayed in series, and which are operated with solvent recycling. When scaling-up a process is the goal, the objective of laboratory scale study is to provide data for the project of an industrial unit. The basic engineering to project equipment operating with supercritical fluids is available, with several studies conducted at the laboratory scale (Meireles 2003; del Valle et al. 2005; Herrero et al. 2006; Reverchon and De Marco 2006; Pereira and Meireles 2010). However, little data is available in the literature for scale-up of the SFE process.

The great challenge of studying scale-up is the choice of the criteria, that is, which parameters should be kept constant, which should vary, and how they should vary so that OECs obtained at the laboratory scale can be reproduced at the industrial scale. Effects of heat transfer, solvent distribution, solvent velocity, solvent flow rate, friction and bed geometry, among others, can be significantly different at analytical and industrial scales (Martínez and Silva 2013). The variety of parameters influencing the SFE process has led to the study of several scale-up criteria for this process. As a consequence, scale-up data of SFE found in literature are extremely divergent and inconclusive, after several criteria have been studied.

Therefore, the challenge of reproducing in the industry what was achieved in laboratory needs further study.

Scale-up criteria described in the literature include maintaining kinetic parameters constant, the development of empirical equations based on bed geometry, and the use of complex mathematical models to predict the behavior of the process when the scale is increased.

20.3.1.1 Criteria Based on Kinetic Parameters

Some of the main parameters that influence SFE are raw material characteristics, pressure, temperature, solvent to feed ratio (S/F), solvent flow rate (Q_{CO_2}) and time. These parameters influence the mass transfer rate of the process; therefore, they should be optimized when scaling-up the SFE process.

Before being used in SFE, the raw material must undergo a pretreatment step. The most common pretreatments are drying and milling. The smaller the particle diameter (d_p), the higher is the surface to volume ratio, so that there is more solute in direct contact with the solvent, which accelerates the extraction process. Moreover, the diffusive pathway of the solvent inside the particle is shorter. Therefore, when the internal mass transfer resistance is the limiting factor of the extraction process, the mass transfer rate can be increased by decreasing the particle size (Sovová et al. 1994; Roy et al. 1996; Goodarznia and Eikani 1998; He et al. 2003; del Valle et al. 2003; Fiori 2007; Fiori et al. 2008; Han et al. 2009). On the other hand, industrially, too fine particles lead to high head loss and can cause bed channeling, which are unwanted effects (Berna et al. 2000; Reverchon and Marrone 2001). Bed diameter to particle diameter ratio (d_b/d_p) between 50 and 250 is reported in the literature (Meireles 2003).

In SFE, extraction by convection is a phenomenon that exerts more influence on process kinetics, especially during the CER period. The convective mass transfer coefficients depend on solvent velocity because the degree of convection is intimately related to the movement of the solvent phase (Martínez and Silva 2013). Therefore, the solvent flow rate is directly related to the mass transfer by convection, which makes it an important parameter when mass transfer resistance is in the external film surrounding the particle. When that is the case, the extraction rate increases with increasing solvent flow rate (Povh et al. 2001; Coelho et al. 2003). This relation is valid if the equilibrium has not been reached before the solvent stream leaves the extractor (Kiriamiti et al. 2002). Moreover, very high solvent flow rates can result in mechanical drag, thus increasing the yield by the removal of some nonsoluble material (Prado et al. 2011; Martínez and Silva 2013). On the other hand, too high solvent flow rates may lead to insufficient contact time between the solvent and the solute to allow mass transfer, which leads to decrease in yield. In some extreme cases, too high solvent flow rates can lead to bed compression and consequent head loss and formation of preferential pathways (Alonso et al. 2002). Therefore, an optimum solvent flow rate must be determined for each case.

Mass transfer parameters may change when scale is increased due to heterogeneous solvent flow, due to the solute dispersion between the extractor and separator, and due to the presence of solute in the recycling stream (del Valle et al. 2005). Moreover, phenomena apparently insignificant in small scale can be important in large scale systems (Berna et al. 2000). Considering all these aspects, several scale-up criteria based on kinetic parameters have been proposed.

Eggers and Sievers (1989), as cited in del Valle et al. (2004), described a tenfold scale-up for rosehip seed, concluding that two important parameters of the scale-up were: (1) the solvent residence time in the extractor (t_{RES}); and (2) the relation between the extract concentration in the stream entering the extractor (due to drag in the solvent recycling) and the bed height (H_b).

Martínez et al. (2007) and Martínez (2005) evaluated two criteria for a 20-fold scale-up (5–300 mL): (1) keeping t_{RES} constant in the extractor (Eq. 20.1); and (2) keeping the superficial velocity (ν) of the solvent constant in the extractor (Eq. 20.2).

$$t_{RES} = \frac{\pi d_b^2 H_b \varepsilon \rho_{CO_2}}{4 Q_{CO_2}} \tag{20.1}$$

$$\nu = \frac{Q_{CO_2}}{A} \tag{20.2}$$

where d_b is the extraction bed diameter, H_b is the bed height; ε is the porosity of the bed + particles, ρ_{CO_2} is the solvent density, Q_{CO_2} is the solvent mass flow rate, and A is the bed cross section area.

When scaling-up a process from equipment 1 to equipment 2 using constant t_{RES} as the criterion, Eq. (20.1) can be written as Eq. (20.3), and if the bed porosity is kept constant, the resulting scale-up criterion is Eq. (20.4).

$$\frac{Q_{CO_2 2}}{Q_{CO_2 1}} = \left(\frac{d_{b_2}}{d_{b_1}}\right)^2 \left(\frac{H_{b_2}}{H_{b_1}}\right)\left(\frac{\varepsilon_2}{\varepsilon_1}\right) \tag{20.3}$$

$$\frac{Q_{CO_2 2}}{Q_{CO_2 1}} = \left(\frac{F_2}{F_1}\right) \tag{20.4}$$

where F is the feeding mass and the subscripts 1 and 2 refer to the different SFE equipment.

For clove, constant t_{RES} was a valid criterion; however, for long pepper and vetiver the same criterion did not apply (Fig. 20.3). Constant ν was not a valid criterion for any of the raw materials tested. The authors reported problems due to the small vessels used in the small scale experiments (5 mL), which influenced the results due to extract loss in the piping of the equipment. According to Meireles (2008), vessels no smaller than 50 mL should be used for the determination of OECs. Moreover, clove oil is a relatively simple system when compared to other vegetable extracts,

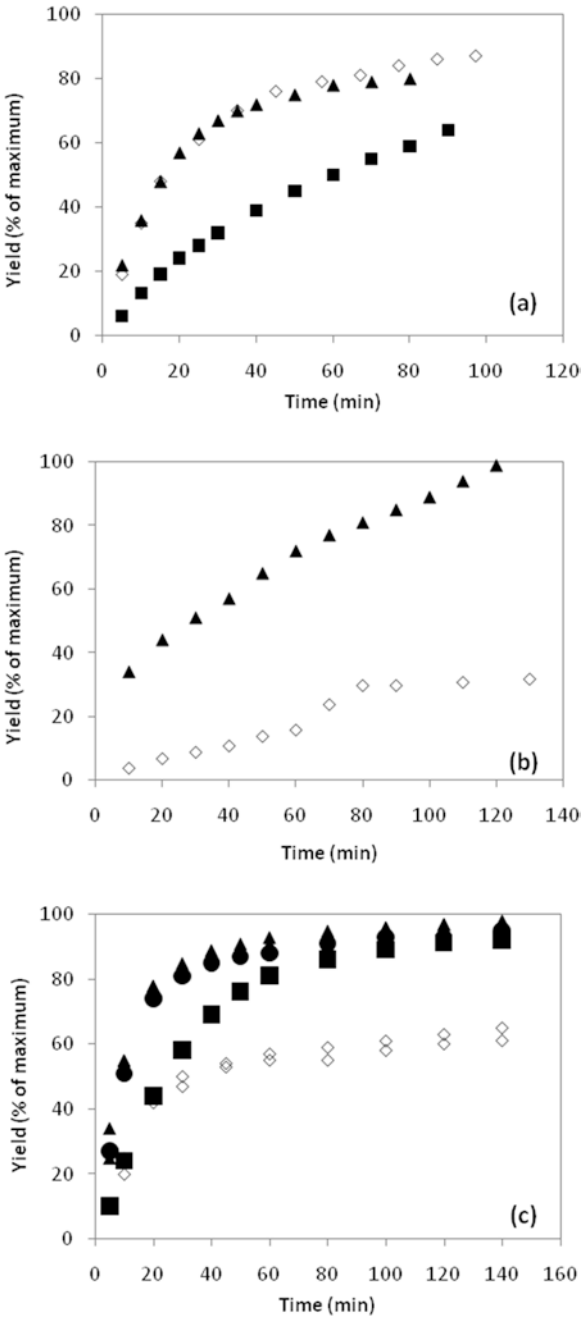

Fig. 20.3 OECs for 20-fold scale-up (5–300 mL) of SFE of clove (**a**), long pepper (**b**) and vetiver (**c**). Experiments at small scale (◇), at large scale using constant ν (■), constant t_{RES} (▲) and constant ν and t_{RES} (●) as scale-up criteria. Experimental data from Martínez (2005) and Martínez et al. (2007)

which suggests that there is a broader criterion that can be applied to clove and also to other raw materials. Therefore, it is important to validate scale-up criteria using different raw materials. Nevertheless, Martínez et al. (2007) concluded that *S*/*F* is a relevant parameter in the scale-up of SFE process, suggesting that there is a correlation between *S*/*F* and the mass transfer rate coefficient.

Casas et al. (2007, 2008) evaluated the scale-up of SFE of sunflower leaves from $F = 2$ g to $F = 180$ g. They kept the H_b/d_b ratio constant and evaluated two scale-up criteria: keeping ν + Reynolds number (*Re*) constant or keeping t_{RES} constant. For constant $\nu + Re$ the yields of analytical and pilot scales were similar, while for constant t_{RES} the yield was around 30 % higher at pilot scale. However, when 5 % water was added as a cosolvent to the CO_2, this same trend was not observed; there was no correlation between yields and ν, *Re* or t_{RES}. The addition of cosolvents modifies the extraction behavior, and there are almost no studies dealing with scale-up of SFE process for this specific case. However, in these studies, again, small vessels were used.

Perrut et al. (1997) studied the scale-up of sunflower seeds SFE from 150 mL to 1.5 L (tenfold). They studied different solvent flow rates at pilot scale, and obtained the same kinetic behavior when the Q_{CO_2}/F ratio was kept constant (5 kg/h at small scale compared to 45 kg/h at pilot scale) (Fig. 20.4). They attributed this behavior to the equilibrium between the solids and the fluid phase being the controlling step during the extraction process.

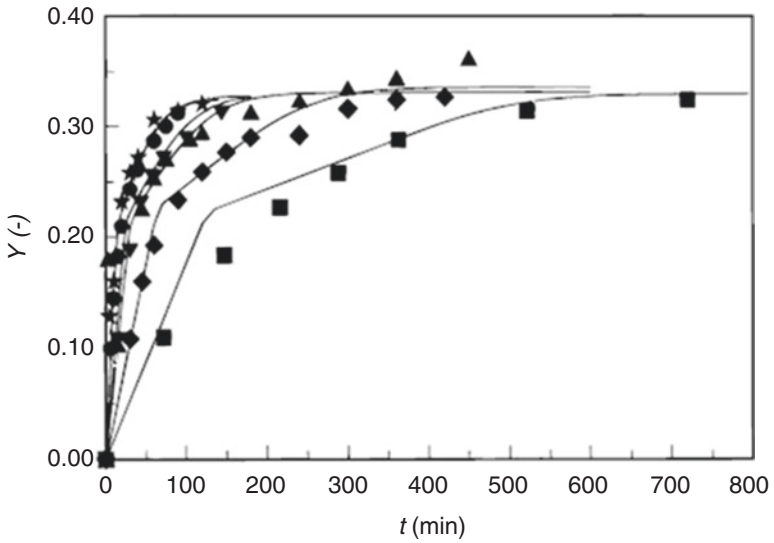

Fig. 20.4 Experimental and modeled OECs of SFE of sunflower seeds determined in a 150 mL extractor (★, $Q_{CO_2} = 5\,kg/h$) and in a 1.5 L extractor (■, $Q_{CO_2} = 5\,kg/h$; ◆, $Q_{CO_2} = 10\,kg/h$; ▲, $Q_{CO_2} = 20\,kg/h$; ▼, $Q_{CO_2} = 25\,kg/h$; ●, $Q_{CO_2} = 45\,kg/h$). Reprinted with permission from Industrial & Engineering Chemistry Research, 36, M. Perrut, J.Y. Clavier, M. Poletto, E. Reverchon, Mathematical modeling of sunflower seed extraction by supercritical CO_2, 430–435, Copyright 1997, American Chemical Society

Mezzomo et al. (2009) evaluated the scale-up from 12.4 to 88 mL based on four different mass transfer mechanisms for peach almond oil: maintaining *S*/*F* constant; maintaining Q_{CO_2}/F constant; maintaining both *S*/*F* and Q_{CO_2}/F constant; and maintaining *S*/*F*, Q_{CO_2}/F and *Re* constant (Fig. 20.5). They concluded that maintaining Q_{CO_2}/F constant was the best scale-up criterion. Aguiar et al. (2012) successfully used the Q_{CO_2}/F constant as scale-up criterion for SFE of striped weakfish wastes from 5.5 to 27.5 g (fivefold). However, again, in both studies small vessels were used.

del Valle et al. (2004) suggested that since several parameters influence the SFE process, an efficient scale-up criterion should be complex, including the influence of the interactions among these parameters. On the other hand, using a simple criterion can help to develop a scale-up method easily applicable, which would decrease time and cost employed on developing the SFE process. Therefore, LASEFI research group has recently been studying a very simple scale-up criterion, maintaining constant the solvent to feed ratio (*S*/*F*), with encouraging results (Prado et al. 2011, 2012; Prado and Meireles 2010).

When validating scale-up criteria, it is necessary to assess their applicability to different types of raw materials, since the mass transfer mechanisms may differ among species and parts of the plant used for extraction, due to the different types of cell structures where the extract is located, its bonding to the solid matrix and the solute extraction mechanism. Clove, sugarcane residue and grape seeds present completely different profiles. While the first one is a flower bud, rich in volatile oil, the second one is a by-product of the sugar/ethanol industry, containing a wax

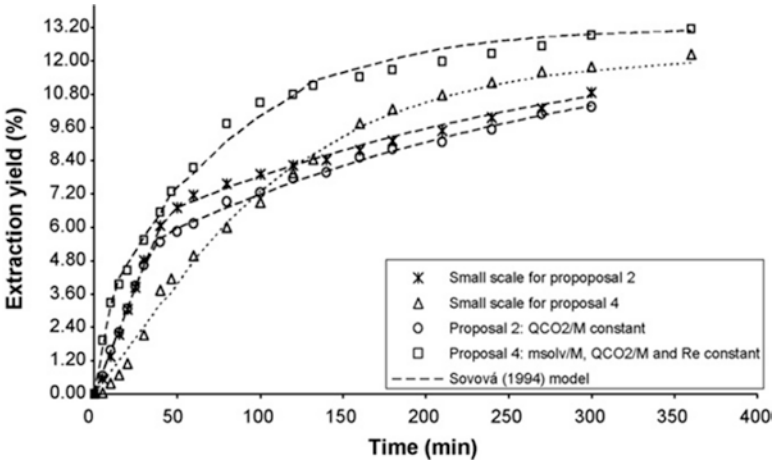

Fig. 20.5 Experimental and modeled OECs of scale-up (12.4–88 mL) of SFE of peach almond for Q_{CO_2}/F constant (proposal 2) and *S*/*F*, Q_{CO_2}/F and Reynolds constant (proposal 4) criteria. Reprinted from Journal of Supercritical Fluids, 51, N. Mezzomo, J. Martínez, S.R.S. Ferreira, Supercritical fluid extraction of peach (*Prunus persica*) almond oil: Kinetics, mathematical modeling and scale-up, 10–16, Copyright 2009, with permission from Elsevier

composed by long-chain fatty alcohols and phytosterols, and the third one, which is rich in vegetable oil, is a residue of the winery industry.

Prado et al. (2011, 2012) found that Q_{CO_2}/F constant was a valid criterion for 15-fold scale-up of SFE of clove, sugarcane residue and grape seeds, from 290 mL to 5.15 L (Figs. 20.6 and 20.7). The OECs presented similar shape and yields slightly higher in pilot scale than in laboratory scale (20 % higher for clove, 15 % higher for sugarcane residue and 6 % higher for grape seeds). The authors then evaluated the *S*/*F* criterion for clove by increasing the solvent flow rate at pilot scale while keeping the *S*/*F* constant, which proved to be valid, although some influence of the solvent superficial velocity was observed (Fig. 20.8). According to Clavier and Perrut (2004), when an extraction process is limited by solubility, keeping the *S*/*F* ratio constant is an efficient scale-up criterion for SFE. This same behavior was observed by del Valle et al. (2004) in the SFE of rosehip seeds. They observed that although the CO_2 flow rate (4–24 g/min) presented an effect on the OEC of "yield vs. time," when the OECs were plotted as "yield vs. *S*/*F*," they were overlapped, which indicates that *S*/*F* can be constant even when some other important kinetic parameters are altered.

On the other hand, Berna et al. (2000) used constant *S*/*F* as a scale-up criterion of SFE of orange peel for a 14-fold scale-up (360 mL to 5.18 L), finding lower yield at pilot scale (10–30 % lower), which they attributed to heterogeneous distribution of the particles inside the extractor, leading to the formation of preferential pathways. According to Clavier and Perrut (2004), in processes limited by diffusion, Q_{CO_2}/F as criterion presents more reliable results, as long as the contact time between the solvent and the solid sample (t_{RES}) is enough to allow mass transfer. This can be achieved by using very long extractors or using several extractors in series.

Recently, Martínez and Silva (2013) used the criterion of Q_{CO_2}/F constant for scaling-up the SFE process of *Capsicum frutescens* peppers from 290 mL to 5.15 L

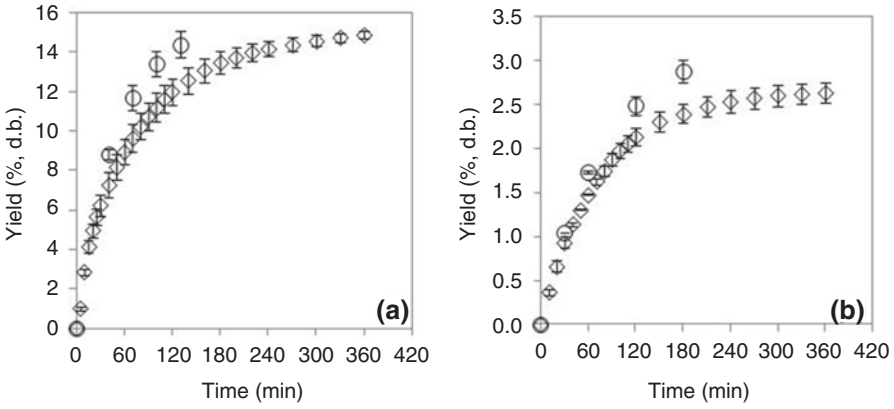

Fig. 20.6 Scale-up of SFE of clove (**a**) and sugarcane residue (**b**) from 290 mL (◇) to 5.15 L (○) using Q_{CO_2}/F constant as criterion. Reprinted from Journal of Supercritical Fluids, 56, J.M. Prado, G.H.C. Prado, M.A.A. Meireles, Scale-up study of supercritical fluid extraction process for clove and sugarcane residue, 231–237, Copyright 2011, with permission from Elsevier

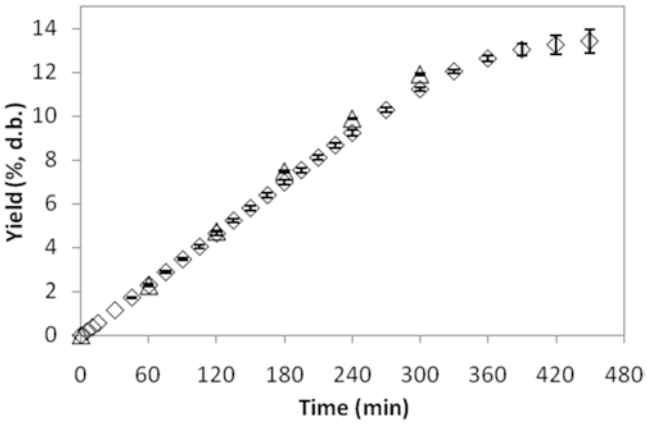

Fig. 20.7 Scale-up of SFE of grape seeds from 290 mL (◊) to 5.15 L (Δ) using Q_{CO_2}/F constant as criterion. Reprinted from Journal of Food Engineering, 109, J.M. Prado, I. Dalmolin, N.D.D. Carareto, R.C. Basso, A.J.A. Meirelles, J.V. Oliveira, E.A.C. Batista, M.A.A. Meireles, Supercritical fluid extraction of grape seed: Process scale-up, extract chemical composition and economic evaluation, 249–257, Copyright 2012, with permission from Elsevier

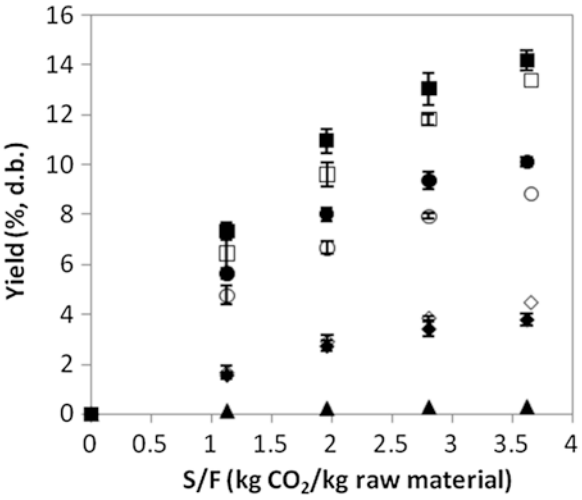

Fig. 20.8 OECs for clove SFE at pilot scale (5.15 L) for solvent flow rate of 1.2×10^{-3} kg/s (*empty symbols*) and 3.0×10^{-3} kg/s (*filled symbols*): total extract (■, □), fraction obtained in the first separator (◊, ♦), fraction obtained in the second separator (○, ●) and fraction obtained in the third separator (▲). Reprinted from Journal of Supercritical Fluids, 56, J.M. Prado, G.H.C. Prado, M.A.A. Meireles, Scale-up study of supercritical fluid extraction process for clove and sugarcane residue, 231–237, Copyright 2011, with permission from Elsevier

and did not obtain reproducible results (Fig. 20.9). The most outstanding differences appeared in the t_{CER}, which was considerably higher for the pilot scale extraction, and in the convective mass transfer coefficient, which was lower at pilot scale. The solid phase mass transfer coefficient was also higher in the pilot scale. The authors presented possible reasons for this behavior: heterogeneous packing, which may lead to the formation of preferential pathways; loss of the volatile fraction of the extract at pilot scale; positive influence of bed geometry (the H_b/d_b ratio increased from 1.7 at small scale to 4.5 at pilot scale), by decreasing the radial diffusion and increasing the contact time between the solvent and the solute; the effect of solvent flow rate, which influences the contact time of the solute and the solvent, and can promote the mechanical drag of some insoluble compounds from the solid. The authors concluded that the scale-up criterion of keeping Q_{CO_2}/F constant can be satisfactory only for a limited range.

Gathering the scale-up data from the literature, it can be noticed that they still are divergent and inconclusive. Nevertheless, the best results so far have been found for scale-up criteria of keeping S/F and/or Q_{CO_2}/F constant. These are simple criteria that can help to develop the SFE process at industrial scale, decreasing costs and time spent. However, it is important to remember that these criteria are not universal; therefore, further study and development is required in this field.

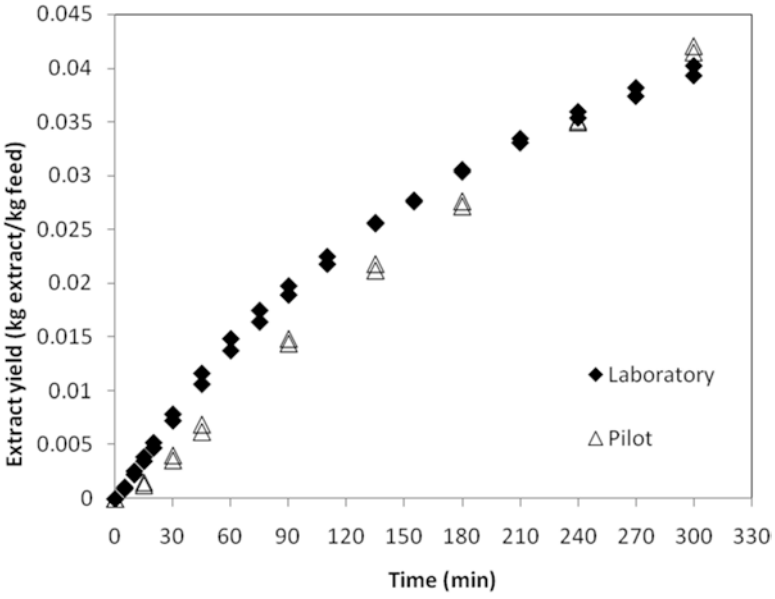

Fig. 20.9 OECs for *Capsicum frutescens* SFE at laboratory (290 mL) and pilot scales (5.15 L). Adapted from Martínez and Silva (2013)

20.3.1.2 Criteria Based on Bed Geometry

The concentration of solute in the extraction bed varies with process time and according to the bed height (Fig. 20.10). Therefore, extraction bed geometry is a crucial point for scale-up purposes. In analytical scale, the extractor shape and dimensions are usually not important. However, there are some reasons for considering the H_b/d_b ratio a fundamental aspect of scale-up in SFE. The effect of radial diffusion of the solute in the solvent phase, which can be responsible by the solvent nonuniform distribution and consequent preferential contact, can be softened when using high H_b/d_b. On the other hand, too high H_b/d_b can lead to head loss due to friction and free convection of the solute in the axial direction due to gravity effects (Martínez and Silva 2013). Therefore, industrially, H_b/d_b relations generally used are between 5 and 10 (Alonso et al. 2002; Pronyk and Mazza 2009).

Reverchon and Marrone (1997) and Braga and Meireles (2007), working with clove and turmeric, respectively, used extraction beds packed at different heights, with H_b/d_b ratios varying between 0.96 and 3.9 (clove) and 1.8 and 5.4 (turmeric), and concluded that the OEC shape (yield vs. *S*/*F*) was not altered by this relation (Figs. 20.11 and 20.12). On the other hand, Berna et al. (2000), working with SFE of orange peel, with H_b/d_b relations varying between 0.8 and 2.6, found little influence of this parameter on the kinetic behavior of the extraction (yield vs. *S*/*F*) (Fig. 20.13).

Quispe-Condori et al. (2008), Moura et al. (2005) and Carvalho et al. (2005), performing the same type of experiment, with H_b/d_b relations varying between 0.04 and 0.12, between 2.21 and 8.84, and between 2.8 and 8.4, for *Cordia verbenaceae*, fennel and rosemary, respectively, reported significant influence of H_b/d_b parameter on the SFE process (yield vs. *S*/*F*) (Figs. 20.14, 20.15 and 20.16). However, there is a limit for the influence of H_b/d_b on the process kinetics, which is related to the solvent saturation during its residence in the extractor. Small H_b/d_b values imply short solvent residence time in the extractor, which may not be enough for the phases to reach equilibrium (Alonso et al. 2002). The minimal H_b/d_b value varies for each raw material, as different classes of compounds present different solubilities in the supercritical solvent, requiring different t_{RES} for its saturation.

Considering these aspects, Moura et al. (2005) and Carvalho et al. (2005) developed two empirical equations based on bed geometry (H_b/d_b) and successfully used them to transpose the SFE process to an equipment of different bed geometry for fennel and rosemary (Eqs. 20.5 and 20.6). Equation (20.5) is used to calculate the solvent flow rate in order to reproduce the OEC in terms of yield vs. *S*/*F* (Fig. 20.17a), while Eq. (20.6) is used to calculate the solvent flow rate so that the OEC is reproduced in terms of yield vs. time, that is, the mass transfer rate is kept constant (Fig. 20.17b).

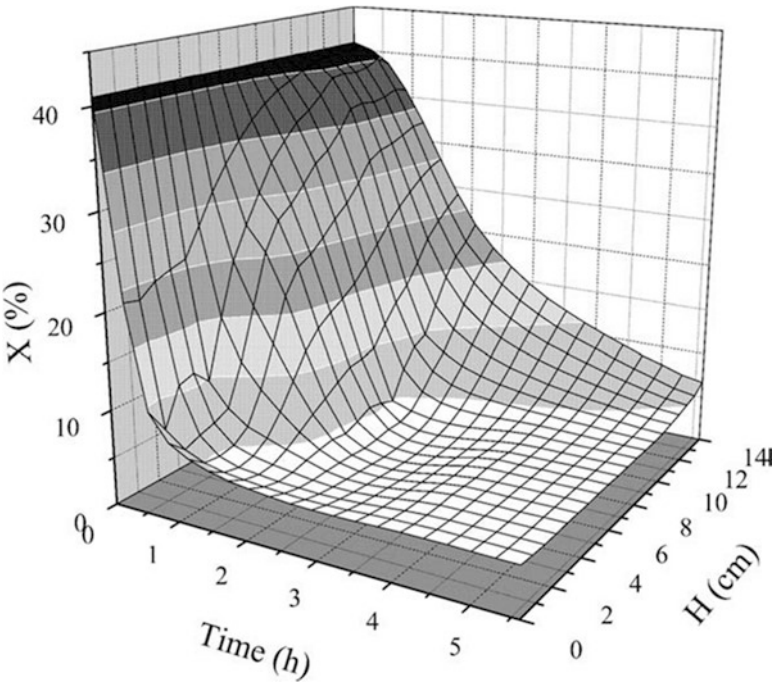

Fig. 20.10 Oil content in safflower particles as a function of distance from the bed entrance and time in SFE process. Reprinted from Journal of Food Engineering, 92, X. Han, L. Cheng, R. Zhang, J. Bi, Extraction of safflower seed oil by supercritical CO_2, 370–376, Copyright 2009, with permission from Elsevier

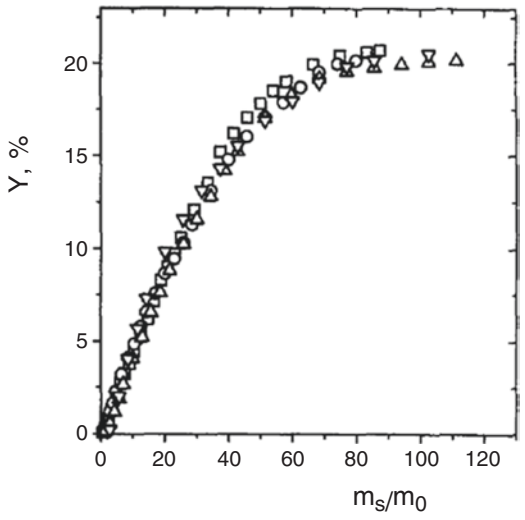

Fig. 20.11 OECs for clove SFE at different H_b/d_b ratios: 3.9 (□), 2.9 (○), 1.95 (▽) and 0.96 (△). Reprinted from Chemical Engineering Science, 52 (20), E. Reverchon, C. Marrone, Supercritical extraction of clove bud essential oil: isolation and mathematical modeling, 3421–3428, Copyright 1997, with permission from Elsevier

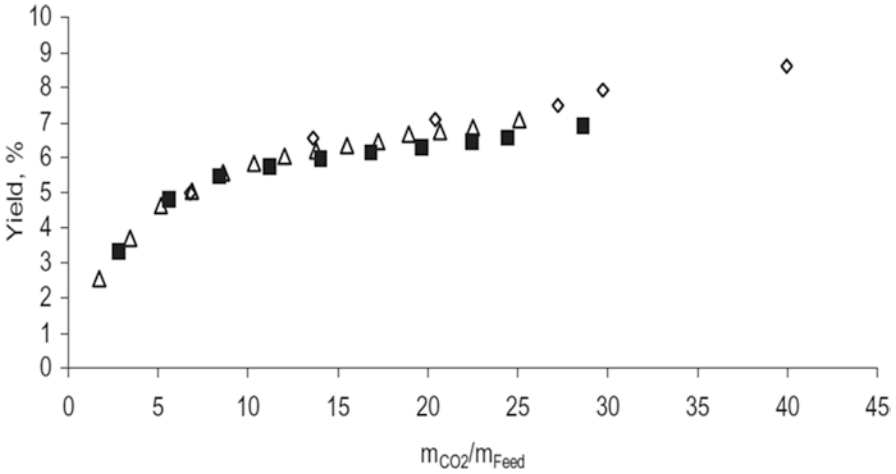

Fig. 20.12 OECs for turmeric SFE at different H_b/d_b ratios: 1.8 (◊), 3.6 (■) and 5.4 (Δ). Reprinted from Journal of Food Process Engineering, 30, M.E.M. Braga, M.A.A. Meireles, Accelerated solvent extraction and fractionated extraction to obtain the *Curcuma longa* volatile oil and oleoresin, 501–521, Copyright 2007, DOI: 10.1111/j.1745-4530.2007.00133.x, http://onlinelibrary.wiley.com/doi/10.1111/j.1745-4530.2007.00133.x/abstract, with permission from Wiley

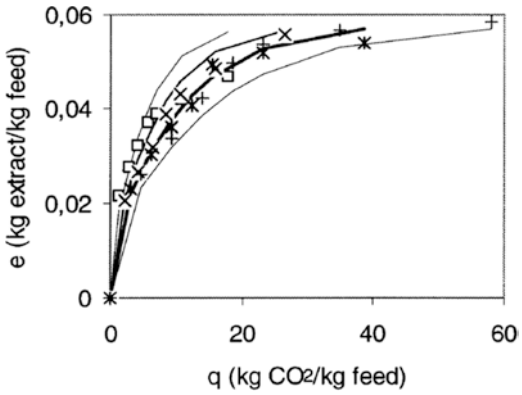

Fig. 20.13 OECs for orange peel SFE at different H_b/d_b ratios: 2.6 (□), 1.8 (×), 1.2 (✳), 0.8 (+) and model (–). Reprinted from Journal of Supercritical Fluids, 18, A. Berna, A. Tárrega, M. Blasco, S. Subirats, Supercritical CO_2 extraction of essential oil from orange peel; effect of the height of the bed, 227–237, Copyright 2000, with permission from Elsevier

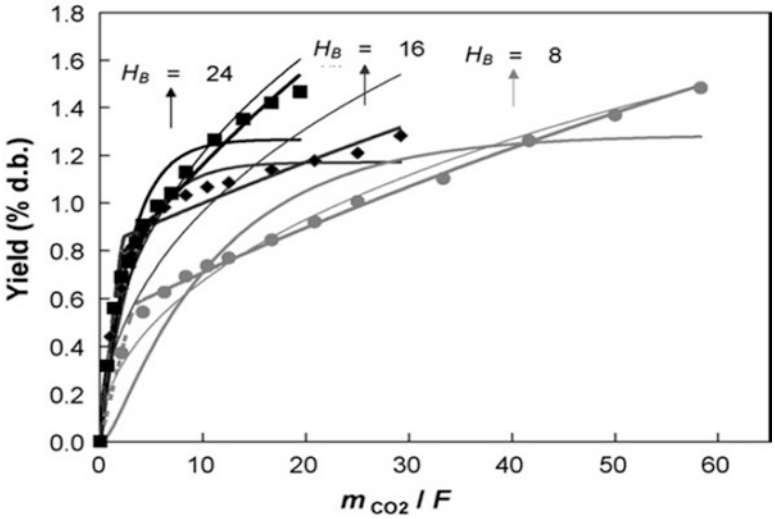

Fig. 20.14 OECs for *Cordia verbenaceae* leaves SFE at different H_b/d_b ratios: 0.04 (gray color ●), 0.08 (◆) and 0.12 (■). Reprinted from Journal of Supercritical Fluids, 46, S. Quispe-Condori, M.A. Foglio, P.T.V. Rosa, M.A.A. Meireles, Obtaining β-caryophyllene from *Cordia verbenacea* de Candolle by supercritical fluid extraction, 27–32, Copyright 2008, with permission from Elsevier

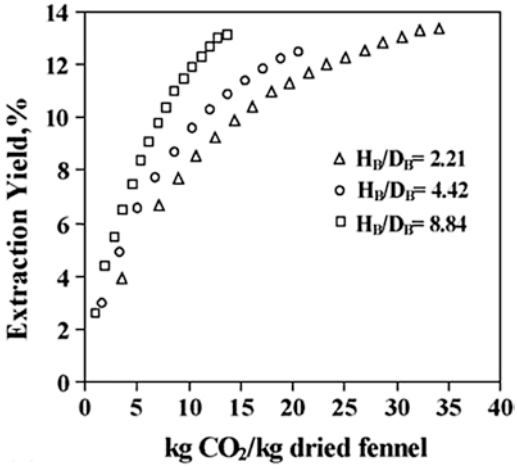

Fig. 20.15 OECs of SFE of fennel for different H_b/d_b ratios. Reprinted from Journal of Supercritical Fluids, 35, L.S. Moura, R.N. Carvalho Jr., M.B. Stefanini, L.C. Ming, M.A.A. Meireles, Supercritical fluid extraction from fennel (*Foeniculum vulgare*): global yield, composition and kinetic, 212–219, Copyright 2005, with permission from Elsevier

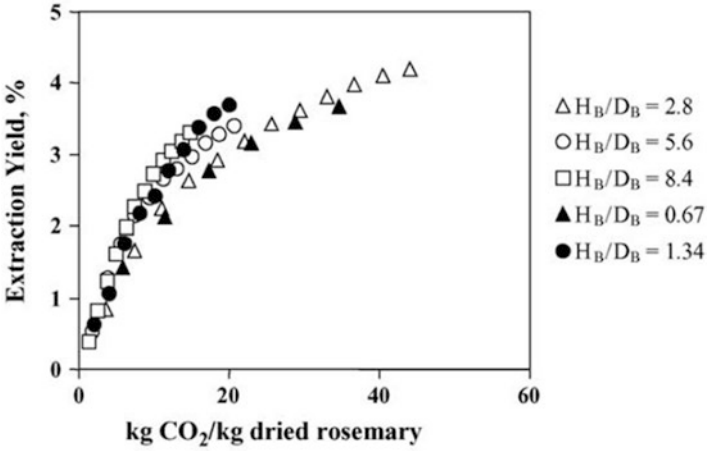

Fig. 20.16 OECs of SFE of rosemary for different H_b/d_b ratios. Reprinted from Journal of Supercritical Fluids, 35, R.N. Carvalho Jr., L.S. Moura, P.T.V. Rosa, M.A.A. Meireles, Supercritical fluid extraction from rosemary (*Rosmarinus officinalis*): Kinetic data, extract's global yield, composition, and antioxidant activity, 197–204, Copyright 2005, with permission from Elsevier

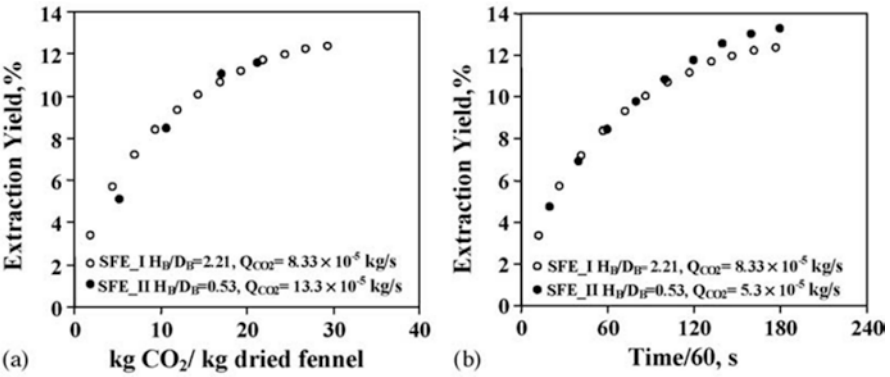

Fig. 20.17 OECs of SFE of fennel determined in extraction beds of different geometries using Eq. (20.5) (**a**) and Eq. (20.6) (**b**). Reprinted from Journal of Supercritical Fluids, 35, L.S. Moura, R.N. Carvalho Jr., M.B. Stefanini, L.C. Ming, M.A.A. Meireles, Supercritical fluid extraction from fennel (*Foeniculum vulgare*): global yield, composition and kinetic, 212–219, Copyright 2005, with permission from Elsevier

$$\frac{Q_{CO_2 2}}{Q_{CO_2 1}} = \left(\frac{F_2}{F_1}\right)^2 \left(\frac{H_{b_1}}{H_{b_2}}\right)\left(\frac{d_{b_1}}{d_{b_2}}\right) \tag{20.5}$$

$$\frac{Q_{CO_2 2}}{Q_{CO_2 1}} = \left(\frac{F_2}{F_1}\right)^2 \left(\frac{H_{b_1}}{H_{b_2}}\right)\left(\frac{d_{b_1}}{d_{b_2}}\right)^3 \tag{20.6}$$

where Q_{CO_2} is the solvent massic flow rate, F is the feed mass, H_b is the extraction bed height, d_b is the bed diameter, and the subscripts 1 and 2 refer to the different SFE equipment.

However, Moura et al. (2005) and Carvalho et al. (2005), as well as Martínez et al. (2007), used only small vessels (smaller than 300 mL) in their study. Takeuchi and Meireles (2007), using Eq. (20.6) to study the scale-up of SFE process for *Achyrocline satureioides*, observed that although the criterion was appropriate for reproducing OECs for different bed geometries when the feed mass was the same, when increasing the feed mass, the equation did not satisfactory predict the kinetic behavior of the process (Fig. 20.18). Therefore, these equations need further evaluation and development so they can be used in the scale-up of SFE processes.

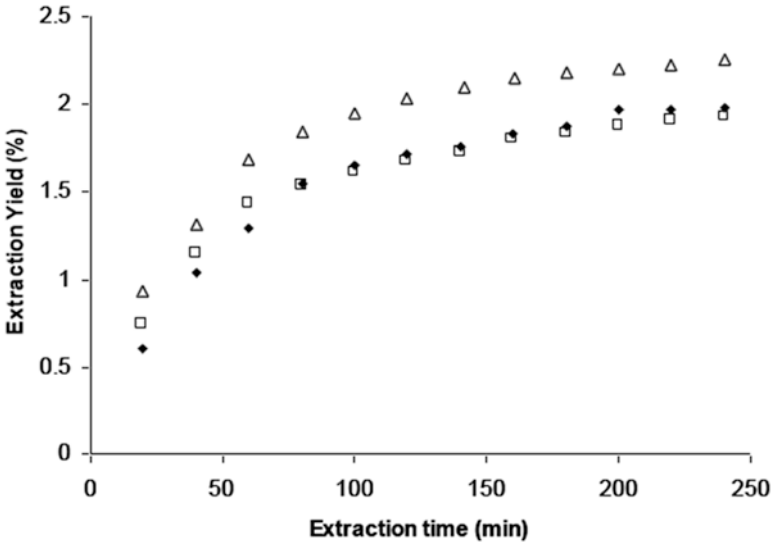

Fig. 20.18 OECs for *Achyrocline satureioides* flowers SFE determined at different H_b/d_b ratios using Eq. (20.6) as scale-up criterion: 5.15 (◆, $F_1 = 15$ g), 1.01 (□, $F_2 = 15$ g) and 2.03 (Δ, $F_2 = 30$ g) (Takeuchi and Meireles 2007)

20.3.1.3 Criteria Based on Mathematical Modeling

The objectives of mathematical modeling are to simplify the information, to describe a process and to predict a process. Mathematical models based on the transport phenomena of the SFE process, or even with merely empirical basis, are useful tools in the scale-up study. Modeling OECs can help to comprehend the process kinetics, through the definition of extraction rates, steps, time, and even parameters with strong physical meaning that may be useful to estimate the behavior of large scale procedures. Models based on transport mechanisms deserve great attention, since their use can provide useful information to be applied in scale-up and their parameters have well-defined physical meaning. These models are elaborated from diverse interpretations of mass transfer and equilibrium inside the extraction bed and use the concentration gradient between the solvent phase and the substrate surface as the driving force for mass transfer (Martínez and Silva 2013).

Considering these aspects, scale-up studies based on mathematical modeling were conducted (Alonso et al. 2002; del Valle et al. 2004; Kotnik et al. 2007; Han et al. 2009). However, none of these studies were successful in reproducing OECs at different scales when using the models, and some of them reported lower yield with scale increase (del Valle et al. 2004; Kotnik et al. 2007).

del Valle et al. (2004) used a two-stage model with extraction rate controlled by oil solubility initially, and by mass transfer in the solid phase at the end, to describe a 30-fold scale-up of SFE of rosehip oil, from $F = 26$ g to $F = 800$ g. It was observed that extraction was slower at the pilot plant than laboratory scale when the solvent superficial velocity (ν) was used as scale-up criterion. The authors suggested that this behavior may be associated with partial drag of solutes in the recycling stream, which limits the solubility, or heterogeneous flow in the extractor due to uneven packing or temperature profiles inside the extractor. Casas et al. (2007, 2008), Martínez et al. (2007) and Martínez (2005) also found that constant ν is not a suitable kinetic parameter for scale-up of SFE.

Kotnik et al. (2007) also used a two-stage model, divided into constant extraction rate and falling extraction rate periods to describe the OEC of SFE of chamomile flowers. For laboratory scale experiment using $F = 15$ g and $Q_{CO_2} = 0.13 \text{kg/h}$ the yield was around 3 % for 90 min of process. When the experiment was conducted at pilot scale with $F = 1$ kg (67-fold increase) and $Q_{CO_2} = 13 \text{kg/h}$ (100-fold increase), the yield was 2.5 % for 90 min of process. Therefore, in this case the performance at pilot scale was poorer than at laboratory scale, indicating that the scale-up criterion adopted was not appropriate.

Han et al. (2009) used the Sovová's extended Lack's Model to try to describe and correlate data determined at small (500 mL) an large (260 L) scales for SFE of safflower. Although the model successfully described the data, the mass transfer coefficients in the solid and fluid phases were different for small and large scales, implying that different kinetics behaviors were obtained. The authors did not use a specific scale-up criterion; therefore, the model was not used to predict the kinetics behavior, but only to adjust the data at different scales.

Alonso et al. (2002) used a scale-up criterion consisting of constant ν, mass transfer coefficient and equilibrium parameter to simulate the OECs of SFE at large scale, but they did not test it experimentally. Instead, they used mathematical modeling to determine the ideal "bed length vs. time" for the extraction of pollutants from soil.

Many scale-up works have strong theoretical basis, concentrated on modeling and simulation of large scale processes based on laboratory results (Martínez and Silva 2013). However, the criteria they used to scale-up the SFE process (constant ν, equilibrium parameter, or no specific criterion) limits their application. In order to improve the relevance of the data determined by mathematical modeling, these studies should focus on appropriate scale-up criteria based on kinetic parameters, which have shown better results.

The three approaches discussed (determining empiric correlations based on bed geometry, maintaining kinetic parameters constant or using mathematical modeling) have not provided conclusive results on SFE scale-up, since it was not possible to reproduce the OECs at different scales. Besides, it can be noticed that most studies on scale-up presented some experimental limitation, as the size of the extractor, which precludes the conclusion about the criterion tested. Nevertheless, so far, the criteria that have shown best results are related to keeping kinetic parameters, mainly the mass transfer rate, constant by using constant S/F and/or Q_{CO_2}/F . Even so, more studies on the scale-up of SFE of natural products are needed, especially using larger equipment.

20.3.2 UAE

Although ultrasonic devices have been used for years in research and diagnostics, major advances have been made in the last 10 years, turning this laboratory-based prototype technology into fully operational commercial processes throughout Europe and the USA (Patist and Bates 2011). Even so, few applications have been carried out on an industrial scale of operation, owing to the lack of unified design and scale up strategies, especially for heterogeneous systems, as the presence of a solid phase leads to additional complexity in terms of mixing, energy dissipation and mass transfer (Gogate et al. 2011).

One extremely important aspect for the development of UAE is that laboratory scale experiments must be able to be up-scaled for industry and this is an aspect of sonochemistry that has been of interest for many years. Ideally, this requires understanding the appropriate design parameters for UAE equipment, and some of these are still under development (Leonelli and Mason 2010). Although there are many laboratory data for UAE, the scale-up of this process has not been extensively researched.

In UAE the experimental outcome (extraction yield and/or rate) is a function of the extractor geometry (extractor shape and number and location of transducers); the energy dissipated per volume of treated material; the intensity (actual power

output per surface area of the sonotrode); and the frequency of irradiation (Gogate et al. 2011). Both energy and intensity are independent of scale, that is, they are intensive parameters, and thus any ultrasonic process will be scalable using these two parameters (Patist and Bates 2008, 2011). The large scale extractor, on the other hand, must be carefully designed to achieve uniform distribution of the cavitational activity, to ensure that all the plant material is affected by the ultrasound. To achieve this goal it is recommended that multiple transducers are used, possibly with multiple frequencies, which usually represents a great difference from laboratory scale process (Shirsath et al. 2012; Gogate et al. 2011). Therefore, the scale-up of UAE processing needs study.

Theoretical work is required for efficient optimization of the large scale design of the sonochemical extractor. Several models are available from the very fundamental involving cavitation bubble dynamics and its chemical effects through radical formation, to more empirical ways of trying to reproduce the same acoustic pressure field in larger scale. Based on theoretical analysis, one can obtain the pressure field distribution in any new extractor with different geometries and operating conditions, which can aid in optimization for maximum and uniform cavitational activity. Modeling studies can be extended to quantification of other useful parameters such as distribution of temperature, mass transfer coefficient, liquid streaming, etc., which can influence the extraction process (Shirsath et al. 2012; Leonelli and Mason 2010).

The major problems identified in the effective design of large scale extractors are: local existence of cavitation events very near to the irradiating surface; wide variation of the energy dissipation rates in the bulk volume of the extractor coupled with the inability of the existing tools to accurately predict the cavitating zones in the extractor and link it with the observed chemical or physical effects of cavitation; erosion of the sonicator surfaces at the high power intensities required for industrial scale operations; and lack of robust design and scale-up strategy establishing the importance of different aspects such as hydrodynamics, mixing and mass transfer, which control the effectiveness in the physical/chemical processing applications. Suitable levels of power dissipation and lower frequencies of irradiation are recommended to ensure that required levels of mass transfer rates are obtained in the larger scales of operation. Furthermore, additional work is required for quantifying the mass transfer as well as the mixing characteristics for multiple frequency/multiple transducer operation, as such data is lacking in literature (Gogate et al. 2011).

Next, studies on scale-up of UAE found in the literature are presented. Boonkird et al. (2008) studied the UAE of capsaicinoids from *Capsicum frutescens* at laboratory and pilot scales. The authors reported 85 % of recovery of capsaicinoids at laboratory scale using indirect sonication in an ultrasonic cleaning bath, working frequency of 35 kHz, *S*/*F* of 5 (v/w), 95 % (v/v) ethanol as solvent, 45 °C, and 3 h. At pilot scale using a 20 L extraction tank (1000-fold scale-up) equipped with transducers bonded to the walls at the fixed ultrasonic frequency of 26 kHz and 70 kHz, recovery of capsaicinoids was 76 % and 70 %, respectively.

Paniwnyk et al. (2009) scaled-up the UAE of rosemary from 1 kg of raw material in 20 L of ethanol using a submersible transducer at the basis of the bath to 6.25 kg

of raw material in 125 L of ethanol using six submersible transducers placed vertically into the separate compartments of the extraction bath. The yield of carnosic acid and rosmarinic acid increased with the scale increase, especially for rosmarinic acid.

Achat et al. (2012) used UAE to extract olive leaves using olive oil as solvent, with the objective to enrich the olive oil with oleuropein. The laboratory scale experiments were performed using a stainless steel jug equipped with one external transductor. The best extraction conditions determined for 25 kHz were 60 W, 16 °C and 45 min. A tenfold scale-up was performed to a 30 L pilot unit equipped with four ultrasound transductors using the same operational conditions of the laboratory scale experiment. The yield of polyphenols at pilot scale was equal to laboratory scale. Pingret et al. (2012) and Virot et al. (2010) used the same equipment to study the scale-up of UAE of polyphenols from apple pomace. Polyphenol yields in the pilot ultrasound extraction were comparable to the laboratory scale experiments.

There have been few studies on the scale-up of UAE of natural products using external transductors, and there are no studies on scale-up of UAE process using a probe. The lack of studies in this field, which are much less than for SFE, even if scale-up study on SFE is not exhausted yet, shows that the industrial application of UAE for obtaining bioactive compounds from plants is far from being optimized. But despite the difficulties in scale-up, a number of larger scale sonochemical extractors have been developed. Vinatoru (2001) reported the use of ultrasonic reactors of 700–850 L for UAE extraction from plant material. Leonelli and Mason (2010), Chemat et al. (2011) and Adam et al. (2011) reported industrial UAE systems of up to 1000 L, located in Romania, Italy, France and Australia.

20.4 Economic Evaluation of Extraction Processes

To evaluate the feasibility of a process it is necessary to demonstrate that the technology is capable of meeting the product specifications and the process requirements defined by the customer or the market. In the natural product industry the requirements are usually a minimum concentration or purity of specific compounds and minimum extraction or recovery efficiency (Martínez and Vance 2008). To achieve these requirements, the extraction process should be optimized for operational conditions such as temperature, pressure, solvent flow rate, time and S/F. This is done at laboratory scale. Then, from this data the process can be scale-up to the industrial level. A general workflow for the industrial implementation of SFE and UAE is illustrated in Fig. 20.19.

The velocity of technological innovation leads the industry to look for new and improved manufacturing processes that can be used to design innovative and unique products. In today's market flexibility and low cost are important when developing a new process. Therefore, to transfer any technology from the academic environment to the industry, tools that allow rapid and accurate estimates of technical and economic feasibility are critical (Prado 2009). In the field of natural product

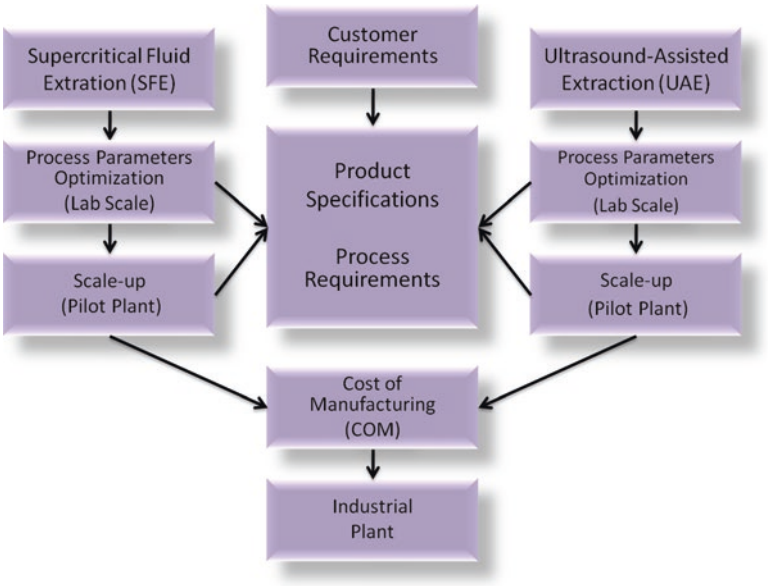

Fig. 20.19 Work flow for SFE and UAE industrial implementation. Adapted from Martínez and Vance (2008)

extraction, it is known that LPSE techniques have low investment cost. Emergent SFE and UAE technologies must compete with existing products; therefore, developing an economical process is of paramount importance. Within this context, economic evaluation of extraction processes provides necessary support for the selection of one specific technique among all of the existing options. In addition, cost analysis, especially capital estimation, facilitates decisions related to in-house manufacturing versus outsourcing (Papavasileiou et al. 2007).

For a precise estimate of the cost of manufacturing (COM) a determined product in a specific industrial plant, the cost of most equipment pieces must be known. Therefore, it is necessary to know the flow diagram of the process to be evaluated, containing information on the mass and energy balances and sizing of the equipment. Sometimes not all of the information is available. Therefore, the cost estimation aims at evaluating the feasibility of a project taking into account the factors that are known by the evaluator and estimating factors that are unknown (Prado 2009). Hence, precision of the estimate depends on the amount of information available.

According to the Association for the Advancement of Cost Engineering International (AACE International), the cost estimate can be divided in five classes. A Class 5 estimate is an order-of-magnitude (also known as ratio or feasibility) estimate that is normally used when "go or not go" decisions must be made, or when evaluating several process alternatives for the same end. It typically relies on cost information for a complete process based on previously built plants. This cost information is then adjusted using appropriate scaling factors, for capacity and inflation,

to provide the estimated capital cost. This normally requires only a block flow diagram, and some data on the cost of industrial processes that are already installed and operating. Therefore, Class 5 estimates are based on the lowest level of definition of the project and thus have low accuracy level. Moving forward, Class 2–3 estimates are applied to processes considered feasible from the initial study, and they require more process information to be performed. Class 1 estimates, on the other hand, are close to full definition of the project, i.e., a high level of maturity, which leads to detailed estimation with 50–100 % accuracy. This classification considers that cost estimation is a dynamic process that involves multiple estimations until a final one approximates the real value (Turton et al. 2003; Leal et al. 2008).

Some methods for evaluating the economic feasibility of SFE and UAE extraction processes have been proposed in the literature; these methods are presented in the next section.

20.4.1 SFE

Two technologies that compete with SFE are SD and LPSE. Despite the high investment cost of SFE technology when compared to traditional processes, SD and LPSE may require several subsequent operation units that can be avoided in SFE, such as centrifugation, solvent removal and pigments removal, among others (Meireles 2003). Moreover, conventional technologies are usually high energy consuming. Considering this scenario, economic evaluation of the SFE process is especially important, because there is a lot of preconception of investors due to the high investment cost of this technology. On the other hand, studies on its economic feasibility have demonstrated that SFE presents low operational cost, and allows production of high quality extracts. Thus, when evaluating the economic feasibility of the SFE process, the COM must be determined considering yield, productivity, selectivity, product quality and all of the costs involved in the process, including both investment and operational costs (Al-Jabari 2002; Rosa and Meireles 2005). The optimization of this process seeks to reach the highest yields and mass transfer rates while consuming the lowest amount of solvent and energy, resulting in a product of high chemical and sensory quality containing minimal residual solvent (Badalyan et al. 1998).

Several economic issues influence the feasibility of the SFE process. The initial cost estimate is defined by taking into account data from three important items: type of raw material, operational conditions and industrial requirements. In this context, techno-economic feasibility studies initially require process design, selection of unit operations, calculation of material properties and material balances. Next, equipment cost, fixed capital investment, total capital investment and total operating cost must be estimated. Economic feasibility is assessed using appropriate criteria such as net present value, internal rate of return and payback time. Sensitivity analysis is carried out to evaluate the cost contribution of the key parameters.

Whenever possible, simple tools that need the least amount of experimental information should be used to optimize the process. Using this principle, some COM estimation methods have been proposed for SFE.

20.4.1.1 Class 4–5 Estimate

Until approximately 10 years ago there was no simple and rapid COM estimation method that allowed considering SFE among other options when selecting an extraction process for a determined raw material. Rosa and Meireles (2005) performed a pioneering work in which they evaluated the economic feasibility of SFE technology using a rapid Class 4–5 estimation method based on the methodology of Turton et al. (2003), which defines the cost of manufacturing (COM) as a weighed sum of five factors: capital investment cost (FCI), cost of operational labor (COL), cost of raw material (CRM), cost of waste treatment (CWT), and cost of utilities (CUT). The details and considerations for each cost are presented by Rosa and Meireles (2005), and the COM can be calculated using Eq. (20.7).

$$\mathrm{COM} = 0.304\mathrm{FCI} + 2.73\mathrm{COL} + 1.23\left(\mathrm{CRM} + \mathrm{CWT} + \mathrm{CUT}\right) \tag{20.7}$$

Several studies on the COM of SFE were conducted for various plant matrices using this methodology (Table 20.1). These studies demonstrated the economic feasibility of SFE technology to process natural products. An observed trend is the decrease of capital costs over the years due to competition among manufacturers. Moreover, these studies showed that frequently the fraction that has major impact on COM is the CRM, not the FCI, as previously thought (Prado and Meireles 2010; Pereira and Meireles 2007a, 2010; Rosa and Meireles 2005; Leal et al. 2008, 2010; Albuquerque and Meireles 2012; Prado et al. 2009a, b; Santos et al. 2010). CRM can represent up to 90 % of COM, whereas FCI usually is below 50 % of COM. This happens because most SFE applications use high added value raw materials, commonly found in the natural product industry. These materials have high purchase cost and are consumed in large quantity in the industrial process. The high CRM can also be related to low availability of the raw material and/or low amount of the desired compound in the raw material. If the raw material is a residue, on the other hand, the CRM has less important impact on the final cost (Pereira and Meireles 2007a; Prado et al. 2012). Thus, when working with residues, FCI and COL gain more importance.

When a large quantity of raw material is processed, like in the coffee and tea decaffeination industries, SFE operational costs are below US$ 3.00/kg raw material, which is a value much lower than that found for conventional technologies, which are high energy-consuming. CUT share is usually below 1 % for SFE, while COL fractions are intermediate between CRM/FCI and CUT. CWT is usually neglected in the SFE process. Because of the low operational cost, the COM for SFE process is often lower than the COM of conventional processes. These figures

Table 20.1 Class 4–5 cost of manufacturing (COM) estimate of natural extracts obtained by SFE

Raw material	Extract yield (%)	COM (US$/kg extract)		Reference
		SFE	Conventional Process	
Anise (*Pimpinella anisum*)	7.9	14.34 (extract)[a]	51.31 (essential oil)	Pereira and Meireles (2007a)
		14.34–28.68 (essential oil)[a]		
Banana peel (*Musa* spp.) dried	6.2	0.08[a]	–	Comim et al. (2010)
	6.0	0.15[b]		
	6.5	0.08[a]		
	6.9	0.16[b]		
Black pepper (*Piper nigrum*)	4.3	144.00–1112.00[a]	232.00–3345.00[a]	Leal (2008)
Brazilian ginseng (*Pfaffia glomerata*)	0.6	1648.00		Leal et al. (2010)
Buriti (*Mauritia flexuosa*) fruits	7.5–15.7	22.56–125.55 (oil)[a]	15.00 (oil)	Prado et al. (2010)
		2550–5380 (carotenoids)[a]		
Croton zehntneri Pax et Hoffm	4.14	238.00–243.00	–	Leal (2008)
Clove (*Eugenia caryophyllus*) buds	12.9–14.1	9.18–10.97[a]	40.00	Rosa and Meireles (2005)
Cordia verbenacea	0.14	914.00[a]	10,350.00–17,000.00[a]	Leal (2008)
Fennel (*Foeniculum vulgare*) leaves	12.5	7.72 (extract)[a]	24.40 (essential oil)	Pereira and Meireles (2007a)
		7.72–15.44 (essential oil)[a]		
Fennel (*Foeniculum vulgare*) leaves	12.12	38.50–40.00	–	Leal et al. (2007)
Ginger (*Zingiber officinalis*) rhizome	2.7	99.80[a]	100.00	Rosa and Meireles (2005)
Grape seed oil	12	8.0	–	Fiori (2010)
Lemon verbena (*Aloysia triplylla*) leaves	1.49	95.79[a]	182.01	Pereira and Meireles (2007b)
Linseed oil (*Linum usitatissimum* L.)	28.8	13.21	-	Galvão et al. (2012)
Mango (*Mangifera indica*) leaves	3.04	151.01[a]	–	Pereira and Meireles (2007b)
Marigold (*Calendula officinalis* L)	–	611.12–785.85[a]	283.00–583.70	Mezzomo et al. (2011)
		730.93–728.27[c]		
Oregano (*Origanum vulgare*)	2.4	202.00[a]	450.00[a]	Navarro-Díaz et al. (2009)

(continued)

Table 20.1 (continued)

Raw material	Extract yield (%)	COM (US$/kg extract)		Reference
		SFE	Conventional Process	
Palm (*Elaeis guineensis*) pressed fiber	2.71–7.01	19.46–62.82 (oil)[a]	1.74 (oil)	Prado et al. (2010)
		3560–17,220 (carotenoids)[a]		
Peach (*Prunus persica*)	–	5.22–30.08[a]	40.00–150.00 (oil)	Mezzomo et al. (2011)
		4.64–26.37[c]		
Pink shrimp (*P. brasiliensis* and *P. paulensis*) residue	3.0	165.00[c]	–	Mezzomo et al. (2013)
Pupunha (*Guilielma speciosa*) fruits	6.6–13	17.15–22.39[a]	–	Prado et al. (2010)
Rosemary (*Rosmarinus officinalis*) leaves	5.0	30.29 (extract)[a]	76.50 (essential oil)	Pereira and Meireles (2007a)
		30.29–60.57 (essential oil)[a]		
Rosemary (*Rosmarinus officinalis*) leaves	4.2	214.00[a]	369.00[a]	Leal (2008)
Spearmint (*Mentha spicata* L.)	–	276.29–241.26[a]	574.60–1647.00	Mezzomo et al. (2011)
		328.86–288.22[c]		
Sweet basil (*Ocimum basilicum*)	1.1–2.0	572.82–1049.58[a]	–	Leal et al. (2008)
	7–11	107.37–152.45[a]		
	14–24	47.96–85.83[a]		
Tabernaemontana catharinensis branches	1.6	79.35 (extract)[a]	–	Pereira et al. (2007)
	–	440.31 (alkaloids)[a]		
	1.04	121.79[c]		
Thyme	11.0	190.00[a]	210.00 (volatile oil)[a]	Prado et al. (2009b)
Vetiver (*Vetiveria zizanoides*) roots	1.28–1.41	9.70 (National variety)[a]	151.79 (essential oil)	Pereira et al. (2008)
		24.26 (Bourbon variety)[a]		
Weakfish (*Cynoscion striatus*)	17.5	2,475,637.50–3999.518.91[d]	–	Aguiar et al. (2012)
		2,217,489.36–3,741,370.76[e]		

[a]SFE unit composed by 2×400 L extractors
[b]Unit composed by 2×500 L
[c]SFE unit composed by 3×300 L extractors
[d]Unit composed by 2×500 L extractors in American market
[e]Unit composed by 2×500 L extractors in Chinese market

demonstrate that SFE is not an expensive technique per se and can be competitive with conventional processes, as long as a proper economic assessment is performed (Leal et al. 2008).

20.4.1.2 Class 2–3 Estimate

Process simulation tools have been used in the chemical and petrochemical industries since 1960 (Papavasileiou et al. 2007; Toumi et al. 2010). Computer-aided process design can result in easier development, scale-up, and economic evaluation of processes. Moreover, it helps reduce costly and time-consuming laboratory and pilot plant assessments, and allows comparison of a number of process alternatives in a short time (Rouf et al. 2001; Papavasileiou et al. 2007; Jully et al. 2004). Therefore, the major application of process simulators is to evaluate and optimize integrated processes, by adjusting the operational conditions to reduce costs (Leal et al. 2006). Additionally, when a process is ready to move from development to manufacturing, simulators can ease the technology transfer and process fitting. Moreover, simulators can be used in the projection of new processes, allowing estimation of all of the costs involved in the processes (Veggi 2009).

Bravi et al. (2002) studied the optimization of SFE of sunflower oil using simulation tools. They determined the ideal number of separators, which allowed reduction of electrical energy consumption from 6678 to 1820 kW. Leal et al. (2007) and Takeuchi et al. (2008) simulated different operational conditions of the separator of an SFE process to evaluate the impact of these conditions on the COM of extracts due to solvent loss in the extract stream and extract loss in the solvent stream. They found differences of 2–4 % on COM depending on the pressure and temperature applied to the separator.

The process simulator SuperPro Designer® is a Windows-based simulation software for modeling biochemical, food, pharmaceutical, specialty chemical, as well as other continuous and batch manufacturing processes. It allows sizing equipment, calculating material and energy balances, and estimating the preliminary COM of processes, as well as integrating processes. It has a wide database of chemical compounds and unit operations, and delivers detailed technical and economic evaluation reports (Flora et al. 1998).

Cost estimates performed by this software can be considered Class 2–3. It is necessary to have a complete flow diagram of the process, and all equipment must be known. The software algorithm estimates the COM as the sum of five costs, similar to those described by Turton et al. (2003), FCI, COL, CUT, CWT, CRM, and extra cost related to quality control and research and development (CQC). SuperPro has been used in process simulation and cost estimation by many authors for various processes (Athimulam et al. 2006; Alshekhli et al. 2011; Kamuri et al. 2012; Kawachale and Kumar 2011; Vázquez and Rodríguez 2011).

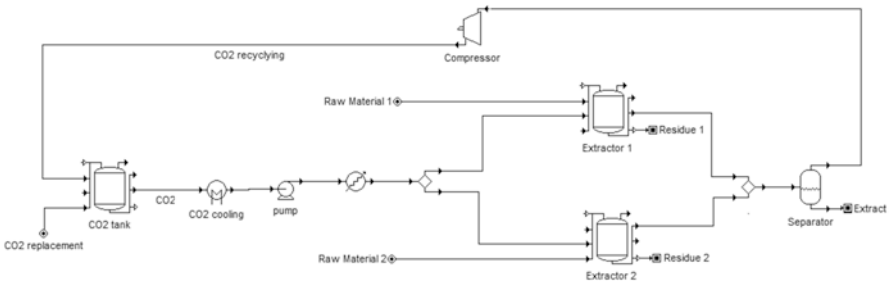

Fig. 20.20 Scheme of SFE built in SuperPro Designer, used for the economic evaluation of the process

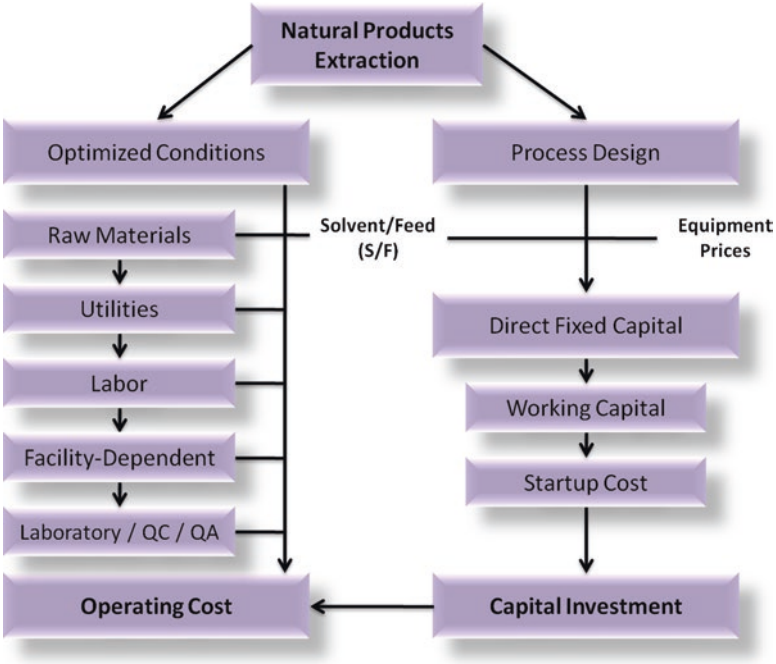

Fig. 20.21 Fractions composing the manufacturing cost of natural products

Misailidis et al. (2009) successfully used SuperPro to examine three bio-processing alternatives designed for co-production of arabinoxylan in a wheat bio-refinery operation. They concluded that the economics of the biorefinery improved as a result of extraction of arabinoxylan as a co-product and incorporation of a recycling loop for other critical components. Kotoupas et al. (2007) investigated the economics and environmental impact of treatment of cheese whey wastewater using SuperPro. It was demonstrated that procedures using membrane ultrafiltration and reverse osmosis can be easily simulated by this software. SuperPro Designer was

also efficient for economic evaluation of the production of a biodrug when compared to Aspen BPS software, and was considered a simpler tool (Rouf et al. 2001).

Despite the fact that SuperPro has mostly been used in the bioprocesses industry, Prado et al. (2009a) developed a methodology for simulating SFE, which has been used for the economic evaluation of the process (Fig. 20.20). Departing from optimized technical data obtained in laboratory, industrial equipment can be designed. Then, the various inlet and outlet streams of the process should be specified, with their associated economic values, which correspond to the purchase cost of raw material, selling price of revenue, and treatment cost of waste streams (Alshekhli et al. 2011). Figure 20.21 shows how all of these issues interrelate in COM estimation of natural products.

SuperPro's Economic Evaluation Report provides information on fixed capital cost and operating cost, along with a profitability analysis. In general, the costs are divided into direct, indirect and general expenses. Direct costs take into account expenses that depend directly on production rate. Some of the items that contribute to direct costs are raw material, utilities and operation. Fixed or indirect cost does not depend directly on production rate, and should be considered even if production is stopped; this includes taxes, insurance and depreciation. General expenses are necessary to keep a business running, and include administrative cost, sales, marketing, research and development, etc. (Pereira and Meireles 2007a). Subtracting these costs from sales income results in the gross-profit.

Total Capital Investment

Total capital investment cost includes direct fixed capital (DFC), working capital and start-up cost. DFC costs are related to equipment, its installation, territorial taxes, engineering, etc. It represents the sum of the total plant cost (TPC) and the cost of contractors' fees and contingency. TPC consists of total plant direct cost (TPDC) and total plant indirect cost (TPIC). While TPIC depends on the cost of engineering and construction, TPDC includes the cost of equipment purchase, installation, process piping, instrumentation, insulation, electrical, building, yard improvement, and auxiliary facilities (Turton et al. 2003).

Working capital represents the operating liquidity available to a business. Finally, start-up cost is associated with the beginning of operation and the validation of the process. All of these costs are associated with the FCI fraction of COM.

For SFE equipment, TPC varies strongly according to the market. In China, it can be US$ 1,500,000 for a 2×500 L equipment, while the same equipment in the European market would cost US$ 2,625,000. American market prices are usually between those of Europe and China.

Operating Cost

Operating cost represents costs that depend directly on the production rate of the industrial plant. It consists of cost of raw material and solvent lost during the process (CRM), cost of utilities (CUT), cost of operational labor (COL), cost of waste treatment (CWT), and cost of quality control and quality assurance (CQC).

Operational Labor Cost (*COL*): The number of personnel required to operate an industrial plant depends on its size, configuration and level of automation, besides the batch time (Martínez and Vance 2008). When working with conventional extraction technologies the number of operators is usually higher than for innovative technologies, because the latter tend to be equipped with automatic control devices. Therefore, not much man power is required for loading and unloading the extractors in SFE (Shariaty-Niassar et al. 2009). In general, one supervisor and two operators are required for a fully automated large plant. Moreover, when long-duration batches are required, it is possible to avoid manpower operation during the night, leading to important savings. Because of this feature, in SFE processes the COL fraction tends to have short participation in the COM, despite often being the most important operating cost of SFE, except for very large units (coffee, tea, hops, etc.), because raw and spent material handling cannot be totally automated. Therefore, optimization of the unit design must take into account the manpower cost depending on local considerations and batch duration (Perrut 2000).

Raw Material Cost (*CRM*): This cost includes plant material, its preparation and transportation, and solvent lost in the process. Often, pretreatment of the raw material is required before extraction. Drying and/or milling may be required, as these procedures increase the yield and quality of the extract. In SFE, maximum moisture content of the plant material is usually 15 %. In addition, depending on the type of raw material (root, fruit, flower, leaf, granule, etc.), it should undergo other preparation procedures, such as cutting, cleaning, and classifying (Shariaty-Niassar et al. 2009). These steps are independent and must be considered in the process cost. The amount of material loaded per batch depends on the bed apparent density and particle size. In the SFE process, the raw material is packed in a compact fixed bed, which allows the best use of the extractor volume available.

Utilities Cost (*CUT*): Utilities required for a typical extraction process include electricity, steam, and cooling water. These components are used to heat the extraction vessel and the solvent to the desired temperature, to run the pump and other electrical devices, and to cool the water needed in the condenser, separators, etc. Reducing operating costs requires minimizing energy requirements, which also implies a reduction in the associated capital cost of the auxiliary equipment. In SFE, the higher the pressure and the temperature used in the process, the higher the utilities cost. Moreover, CUT in SFE is directly related to recirculation of the solvent, which depends on the operational conditions used in the separators. The higher the separator pressure, the lower is the energy required to recirculate the CO_2. On the other hand, the separator pressure must be low enough to allow for efficient separation between the solvent and the extract and avoid solvent loss in the extract stream and extract loss in the solvent stream.

Waste treatment cost (*CWT*): CWT includes the cost of treating and/or disposing of certain process outputs, such as undesirable solid, aqueous, organic, or gaseous by-products. Residue from the SFE process is the exhausted plant material, which can be disposed of directly into the soil without harm to the environment, or it can sometimes be reused by other industries (Rosa and Meireles 2005). Moreover, the CO_2 lost in the process is not toxic in small quantities (Brunner 2005). Even when cosolvents are used, these are usually GRAS; therefore, the same considerations for CO_2 apply after the solvent is evaporated from the exhausted raw material. For these reasons, CWT is usually disregarded in SFE processes.

Quality control cost (*CQC*): CQC accounts for the costs of off-line analysis and quality control. Chemical analysis and physical property characterization, from raw materials to final product, is a vital part of chemical operations.

Indirect Costs

Some facility-dependent indirect costs associated with the FCI fraction include depreciation, equipment maintenance, insurance, local (property) taxes and possibly other overhead expenses. Factors that contribute to depreciation are physical and functional. Physical depreciation arises from the actual use of a plant asset. Functional depreciation is due to obsolescence factors such as technological advances and less demand for a product. The purpose of recording depreciation is to show the decline of usefulness of an asset, not a decline of its market value. Depreciation merely reduces the value of plant asset accounts; it does not reduce the cash account or affect cash flows (Gilbertson and Lehman 2011). In economic evaluations the depreciation time of equipment is usually stipulated as 10–15 years, representing a share of 10–15 % of the FCI (Turton et al. 2003). Taxes depend on local legislation. Insurance is associated with the cost of protecting the company; it usually represents 1–3 % of the FCI (Turton et al. 2003).

Some other general expenses that must be considered and are not directly related to the operating or fixed costs include sales, marketing, research and development, as well as administrative costs.

Class 2–3 Estimate Applied to SFE

SuperPro Designer has been used as a software tool to evaluate the economic feasibility of SFE (Table 20.2) and other processes like ultrasound, pressurized liquid extractions and low pressure solvent extraction (Santos et al. 2010, 2012; Veggi and Meireles 2010).

Some studies demonstrate the efficiency of using this simulator. Shintaku (2006) performed Class 1 and Class 5 estimates of COM for the SFE of sugarcane filter cake, a residue generated in the sugar/ethanol industry. He assumed the use of an industrial unit with three extractors of 7740 L each, which would be necessary to process all filter cake generated by one sugar/alcohol processing plant. For this

Table 20.2 Class 2–3 cost of manufacturing (COM) estimate of natural extracts

Raw material	Operational conditions	Extract yield (%)	COM (US$/kg extract)			Reference
	T (K); *P* (MPa); *t* (min); *F* (kg raw material/batch); *S* (kg/s); ρ (kg/m^3); PW (W); *r* (rpm)		SFE	UAE	Conventional Process	
Annatto (*Bixa orellana* L.) seeds	(1) *T*: 333; *P*: 31; *t*: 250; *F*: 0.0203; *S*: 17.3×10^{-5}; ρ: 656	(1) 1.89	(1) 1781.62[a]	–	–	Albuquerque and Meireles (2012)
			382.00[b]			
			258.54[c]			
	(2) *T*: 300; *P*: 20; *t*: 40; *F*: 0.0203; *S*: 17.3×10^{-5}; ρ: 656	(2) 2.12	(2) 292.50[a]			
			124.58[b]			
			109.27[c]			
Beans (*Phaseolus vulgaris*)	(1) *T*: 323; *P*: 35; *t*: 40; *S*: CO_2	(1) 0.4	(1) 938	–	–	Veggi et al. (2011)
	(2) *T*: 323; *P*: 35; *t*: 77; *S*: CO_2 + ethanol (10 %, v/v)	(2) 0.5	(2) 7000			
Brazilian ginseng (*Pfaffia glomerata*) roots	*T*: 303; *P*: 20; *t*: 360; *F*: 0.020; *S*: 7×10^{-5} (CO_2) + 10 % (v/v, ethanol)	0.53	2766	–	–	Leal et al. (2010)
Buriti (*Mauritia flexuosa*)	*T*: 313; *P*: 20; *t*: 120; *S*: 3.1×10^{-3}; ρ: 590	6.3	44.43[d]	–	–	Prado et al. (2009a)
Clove (*Eugenia caryophyllus*)	*T*: 313; *P*: 15; *t*: 52; *S*: 3.0×10^{-3}	14.19	30.00[b]	–	–	Pereira et al. (2013)
			40.00[c]			

Raw material	Operational conditions	Extract yield (%)	COM (US$/kg extract)			Reference
	T (K); *P* (MPa); *t* (min); *F* (kg raw material/batch); *S* (kg/s); ρ (kg/m^3); PW (W); *r* (rpm)		SFE	UAE	Conventional Process	
Flame vine (*Pyrostegia venusta*) leaves	(1) *T*: 323; *P*: 35; *t*: 40; *S*: CO_2	(1) 0.6	(1) 1773	–	–	Veggi et al. (2011)
	(2) *T*: 323; *P*: 35; *t*: 77; *S*: CO_2+ethanol (10 %, v/v)	(2) 1.5	(2) 22,000			
Grape (*Vitis vinifera*) seeds	*T*: 303; *P*: 35; *t*: 300;*F*: 4.677; *S*: 2.14×10^{-3}; ρ: 908	13.42	179.91–290.17[a]	–	4.85 (hexane extracted oil)	Prado et al. (2012)
			43.02–70.85[b]		40.00–80.00 (pressed oil)	
			11.88–21.13[c]			
Grape (*Vitis vinifera*) bagasse	(1) *T*: 313; *P*:20; *t*: 180; *F*:0.002; *S*: CO_2+ethanol (10 %, v/v); *S*: 9.8×10^{-5}; ρ: 617.6	(1) 3.1	(1) 133.16[c]	–		Farías-Campomanes et al. (2013)
	(2) *T*: 313; *t*: 360; *S*: EtOH; *r*: 168	(2) 8.2			(2) 334[c,h]	
	(3) *t*: 180; *S*: EtOH	(3) 10.4			(3) 126[c,j]	

(continued)

Table 20.2 (continued)

Raw material	Operational conditions	Extract yield (%)	COM (US$/kg extract)			Reference
	T (K); *P* (MPa); *t* (min); *F* (kg raw material/batch); *S* (kg/s); ρ (kg/m^3); PW (W); *r* (rpm)		SFE	UAE	Conventional Process	
Heteropterys aphrodisiaca roots	(1) *T*: 323; *P*: 35; *t*: 40; *S*: CO_2	(1) 0.8	(1) 4170	–	–	Veggi et al. (2011)
	(2) *T*: 323; *P*: 35; *t*: 77; *S*: CO_2+ethanol (10 %, v/v)	(2) 2.5	(2) 30,000			
Ice-cream-bean (*Inga edulis*) leaves	(1) *T*: 323; *P*: 35; *t*: 40; *S*: CO_2	(1) 1.5	(1) 3004	–	–	Veggi et al. (2011)
	(2) *T*: 323; *P*: 35; *t*: 77; *S*: CO_2+ethanol (10 %, v/v)	(2) 2.7	(2) 47,000			
Jabuticaba (*Myrciaria cauliflora*) skins	(1) *T*: 323; PW: 81; *t*: 60; *S*: EtOH	(1) 11.93	–	(1) 401.21[g]		Santos et al. (2010)
	(2) *T*: 303; *t*: 60; *S*: EtOH; *r*: 150	(2) 9.01[h]			(2) 422.18[g,h]	
	(3) UAE: *t*: 10; *S*: EtOH; PW: 81	(3) 10.08[i]		(3) 387.20[g,i]		
	ABE: *T*: 303; *t*: 60; *S*: EtOH; *r*: 150					
	(4) *t*: 480; *F*: 0.025; *S*: EtOH	(4) 9.92[j]				
	(5) *t*: 480; *F*: 0.025; *S*: EtOH; pH: 3 (HCl)	(5) 9.5[k]			(4) 778.42[g,j]	
					(5) 1001.00[g,k]	

Raw material	Operational conditions	Extract yield (%)	COM (US$/kg extract)			Reference
	T (K); *P* (MPa); *t* (min); *F* (kg raw material/batch); *S* (kg/s); ρ (kg/m^3); PW (W); *r* (rpm)		SFE	UAE	Conventional Process	
Jackfruit (*Artocarpus heterophyllus*) leaves	(1) *T*: 323; *P*: 30; *t*: 120; *F*: 0.024; *S*: 8.33×10^{-5}	(1) 1.77	(1) 320.05[e]	–	(2) 219–145.28[e]	Veggi et al. (2009)
	(2) *t*: 240; *F*: 0.010; *S*: EtOH; *r*: 165.2	(2) 7.24				
Jatoba (*Hymenaea courbaril*) bark	(1) *T*: 323; *P*: 35; *t*: 40; *S*: CO_2	(1) 1.3	(1) 16,130	–	–	Veggi et al. (2011)
	(2) *T*: 323; *P*: 35; *t*: 77; *S*: CO_2 + ethanol (10 %, v/v)	(2) 2.4	(2) 48,000			
Mango (*Mangifera indica*) leaves	(1) *T*: 323; *P*: 30; *t*: 90; *F*: 0.1079; *S*: 8.3×10^{-5}	(1) 3.6	(1) 92.00[c]	–	(2) 32.00[g]	Prado et al. (2013)
	(2) *t*: 120; *F*: 0.01; *S*: EtOH; *S* 1.39×10^{-4}	(2) 9.3				
Palm (*Elaeis guineensis*) pressed fiber	*T*: 328; *P*: 25; *t*: 45; *S*: 3.3×10^{-3}; ρ: 176	6.2	61.82[d]	–	–	Prado et al. (2009a)

(continued)

Table 20.2 (continued)

Raw material	Operational conditions	Extract yield (%)	COM (US$/kg extract)			Reference
	T (K); *P* (MPa); *t* (min); *F* (kg raw material/batch); *S* (kg/s); ρ (kg/m^3); PW (W); *r* (rpm)		SFE	UAE	Conventional Process	
Pomegranate (*Punica granatum* L.) Leaves	(1) *T*: 313; *P*: 30; *t*: 300; *F*: 0.02; *S*: 0.84×10^{-4}; ρ: 448	(1) 0.52	(1) 182.43[d]	–	–	Cavalcanti et al. (2012)
			141.34[e]			
			134.38[f]			
	(2) *T*: 323; *P*: 30; *t*: 300; *F*: 0.02; *S*: 0.84×10^{-4}; ρ: 448	(2) 0.67	(2) 153.17[d]			
			119.87[e]			
			114.36[f]			
Sugarcane residue (filter cake)	*T*: 333; *P*: 35; *t*: 30–180; *F*: 1.339; *S*: 1.84×10^{-3}; ρ: 260	2.88	1250.00–1731.04[a]	–	310–1090 (tablets with 3 % policosanol)	Prado and Meireles (2012)
			301.79–424.33[b]			
			83.39–116.55[c] (extracts with 3–6 % policosanol)			

T=Temperature (K); *P*=Pressure (MPa); *t*: time (min); *F*=Feed (kg raw material/batch); *S*=Solvent flow rate (kg/s); ρ=Bed density (kg/m^3)
Extractor capacity: [a]: 2×5 L; [b]: 2×50 L; [c]: 2×500 L; [d]: 2×100 L; [e]: 2×400 L; [f]: 2×1000 L; [g]: 2×300 L; [h]: Agitated bed extraction (ABE); [i]: ABE+UAE; [j]: Soxhlet extraction; [k]: Soxhlet extraction with acidified solvent

plant size, the COM was estimated as US$ 13.63/kg extract for Class 1, and US$ 229.29/kg extract for Class 5. The COM obtained by Prado and Meireles (2012) for the same raw material using SuperPro (Class 2–3 estimate) and assuming the use of an industrial unit with two extractors of 500 L was US$ 84.00/kg.

Takeuchi et al. (2009) calculated the COM of LPSE process for the extraction of *Achyrocline satureoides* using a Class 4–5 procedure, reaching COM of US$ 858.53/kg extract for a 2×400 L plant. Veggi (2009), conducting the same evaluation using a Class 2–3 procedure (SuperPro) for a 2×300 L plant, estimated the COM as US$ 573.34/kg extract, a value 40 % lower than that found by Takeuchi et al. (2009). Thus, the trend is a substantial reduction in COM as the level of detailing of the project increases. The Class 4–5 economic evaluations, performed with low amount of information, are to be used only as a guide when there are several alternatives. With further development of the industrial project, when more precise technical information is available, it is necessary to reevaluate the COM.

The main factors that have been shown to influence the COM (plant size and design, and operational conditions) are detailed in the next section.

Plant Size and Design

Equipment cost is extremely important for the implementation of new technologies, especially in SFE, where the initial investment is high. Equipment sizing should be as accurate as possible, as underestimating or overestimating production capacity could result in unnecessary costs of equipment and utilities. For this reason, COM evaluations are usually performed for multiple plant sizes. These economic evaluations are based on laboratory or pilot scale data, but as scale-up studies have found that the yield of SFE process can increase with the scale, it can be expected that in industrial application the COM can be further reduced (Prado et al. 2011).

Depending on the raw material evaluated, the economic feasibility of SFE processes can be reached for extractors departing from 2×50 L. Increase in extractor capacity promotes a linear increase of the raw material needed, while increase in equipment costs does not follow the same proportionality, thus diluting the FCI and decreasing the COM (Prado et al. 2012). The COM of SFE of sugarcane filter cake decreased from US$ 1250/kg extract for a 2×5 L plant to US$ 84/kg extract for a 2×500 L plant (Prado and Meireles 2012). The same behavior was observed for SFE of clove (Pereira et al. 2013): for a 2×5 L plant the process was considered economically unfeasible, but for 2×50 L or 2×500 L plants the industry would operate with COM far below the product's selling price. Therefore, as the operation scale increases, the COM substantially decreases. This is a strong incentive to use large capacity multi-products units in time-sharing rather than operating small capacity units dedicated to a single product (Perrut 2000).

Traditional production units are composed of at least two extractors, wherein one is unloaded/loaded while the other is used for extraction, but three or more extractor configurations are often used to reduce dead time and increase extraction efficiency. For a given production capacity, increasing the number of extractors will decrease energy consumption and operating costs, but increase investment cost (Clavier and

Perrut 2004). Mezzomo et al. (2011) studied the economic viability of SFE of peach almond, spearmint and marigold, evaluating two different extraction units (2×400 L and 3×300 L) for different cycle times. The lowest COM for peach almond was obtained in a 2×400 L for cycle time of 30 min (US$ 4.64/kg extract). For spearmint the same extraction unit provided the most viable process, with a specific cost of US$ 242.26/kg extract. The SFE from marigold was not economically viable, probably because of the imprecise operational parameters assumed (not optimized) and due to the high cost of raw material. In general, it was demonstrated that the plant with two extractors presented higher COM than for three extractors. Even so, it was considered more advantageous because investment cost was lower (Mezzomo et al. 2011, 2013).

Operational Conditions

The yield and quality of SFE extracts is determined by temperature, pressure, *S*/*F*, solvent flow rate and extraction time, as well as the characteristics of the plant material. While temperature and pressure determine the solubility of the extract in CO_2, solvent flow rate determines the kinetics behavior of the process. The optimization of these parameters influences the COM.

As an example, for the same temperature, increasing pressure may favor the process yield, decreasing the COM. Prado et al. (2010), performing an economic evaluation of SFE of pressed palm fiber, observed that the COM decreases with pressure because of the higher yields that are obtained at elevated pressures. The same behavior was observed for SFE of thyme and sweet basil, where higher pressure resulted in lower COM (Prado et al. 2009b; Leal et al. 2008).

Cavalcanti et al. (2012) evaluated the COM of processing pomegranate leaves by SFE at different temperatures and pressures. Lower COM was obtained for larger plant size at higher pressure and temperature. However, according to the authors, although the COM is a direct function of temperature and pressure, mainly due to energy costs, extraction yield was the factor that exhibited the most influence, causing a significant decrease in the COM even with temperature and pressure increase. Other studies reported similar observations (Albuquerque and Meireles 2012; Prado et al. 2010, 2012).

Another parameter that influences COM is the *S*/*F*, because low *S*/*F* implies lower operating costs and higher production capacity due to a high number of batches being possible per year when short extraction time is used. Generally, industrial processes target *S*/*F* below 30. However, higher *S*/*F* is justified for high added value products. In specific cases, $S/F > 100$ has been used in commercial applications (Martínez and Vance 2008).

S/*F*, solvent flow rate (Q_{CO_2}), and cycle time (t) are interrelated by Eq. (20.8).

$$\frac{S}{F} = \frac{Q_{CO_2} t}{F} \tag{20.8}$$

High solvent flow rates imply high operating and capital costs. However, they could increase production capacity. Therefore, optimization of solvent flow rate or residence time of the solvent in the extraction vessel provides attractive COM. High residence time implies a long batch time. Conversely, short residence time may result in shorter contact time between the solvent and solute, resulting in loading of the solvent much lower than the saturation concentration at the selected operating conditions (Martínez and Vance 2008). When evaluating the COM for pink shrimp residue using 2 × 400 L and 3 × 300 L units, Mezzomo et al. (2013) found lower COM for 2 × 400 L configuration, since it uses higher solvent flow rate due to the larger column volume (400 L), increasing the mass of extract recovered and reducing the specific cost. In clove oil extraction by SFE, the economic viability of the process was significantly improved by adjusting the solvent flow rate and the cycle time for the same *S*/*F*; higher solvent flow rate resulted in lower COM (Pereira et al. 2013). Prado et al. (2010) found that higher pressure (30 MPa) and solvent flow rate (1.04 kg/s) influenced the decrease of specific COM of carotenoids from dried shell and pulp of buriti.

Another important detail that can be obtained from cost analysis is the best cycle time. To evaluate optimized cycle time, the yield, chemical composition and cost data should be considered. From an industrial point of view, operation time and extraction efficiency play an important role in the manufacturing cost estimate, once they are also related to the number of extraction cycles that can be performed per year (Santos and Meireles 2011). The number of cycles provides data about the amount of material to be processed per year, the energy to be used per year, the labor required to do it and the extract productivity. Shorter extraction time increases the number of cycles leading to a reduction in COM because the depreciation of the investment and other costs are diluted in the productivity. Long extraction times, on the other hand, imply having fewer cycles within 1 year, leading to greater impact of FCI on the COM (Rosa and Meireles 2005; Mezzomo et al. 2011; Comim et al. 2010; Aguiar et al. 2012). Furthermore, process time directly influences the CRM. Cycle time reduction leads to higher CRM due to the increase in the number of batches performed per year (Aguiar et al. 2012; Mezzomo et al. 2013). In this context, economic evaluation allows finding the balance between yield and cost, taking into consideration product quality, to determine the best cycle time for each raw material.

In SFE processes, independently of the raw material, 70–90 % of the yield is achieved during the CER period, which is the shortest period of the OEC (Meireles 2003, 2008). Therefore, interrupting the process before the extraction bed is exhausted is usually economically advantageous (Rosa and Meireles 2005). In general, optimal extraction time practically coincides with the end of the CER period. This suggests that it would be economically recommended to perform industrial SFE processes only during the convective step, while DC extraction causes a significant increase in the specific cost (Aguiar et al. 2012). This trend has been observed for SFE of several plant materials (Prado et al. 2010; Comim et al. 2010; Mezzomo et al. 2011).

Prado et al. (2010) also demonstrated that the SFE process was more advantageous for extracting buriti oil, pupunha oil, and pressed palm fiber using t_{CER} as cycle time. However, these authors also evaluated the cycle time for a process reaching the DC step, and concluded that since the carotenoids were mostly solubilized in this step, when a carotenoid-rich extract is the goal the process should be carried out up to the DC period.

Albuquerque and Meireles (2012) presented another example of the effect of time on COM. The authors evaluated the extraction of bixin from annatto by SFE and observed that when the process time was prolonged 15 min after the t_{CER}, the extract yield increase 25 %, while the COM increased 8 %; however, the specific cost of the bixin fraction decreased around 7 %, due to its content increase in the extract. They also demonstrated that the COM considering t_{FER} as cycle time significantly increased, making the process economically unfeasible.

Considering all of these aspects, it can be noticed that cycle time is one of the parameters that presents the most influence on the economic feasibility of the SFE process. Therefore, operational conditions should be determined to allow for high extraction rates, so that high yield is obtained in a short time, which allows increasing the number of batches per year and thus processing a high amount of raw material. Furthermore, the extract quality must be taken into account, since the extracts obtained in different periods of the OEC (steps CER, FER and DC) may show large differences in chemical composition between them (Prado et al. 2010; Leal 2008).

Another aspect that needs to be considered is the use of cosolvents. Some research has reported that using cosolvents increases the yield of the process and therefore reduces the COM of the product (Leal et al. 2008, 2010). On the other hand, other authors have demonstrated that despite increasing yield, sometimes the use of cosolvents is not economically attractive due to extra costs associated with solvent removal (Pereira et al. 2007; Veggi et al. 2011). Therefore, economic evaluation should be conducted for several operational conditions so that the best combination of parameters is obtained.

One important parameter to be evaluated in economic assessments is the payback (investment cost over the benefit). In many industries maximum payback time is shorter, for example, 2 years instead of 4 years, when the technology risk is high (Patist and Bates 2011). For SFE payback time can be as low as 0.5–1.5 years (Prado et al. 2012; Pereira et al. 2013).

20.4.2 UAE

High power ultrasound has only become an efficient tool for large scale commercial applications in the last 10 years. Like most innovative food processing technologies, it is not an off-the-shelf technology and therefore needs to be developed and scaled up for each application (Patist and Bates 2008). As economic evaluation using simulation software SuperPro Designer is becoming a valuable tool to determine the

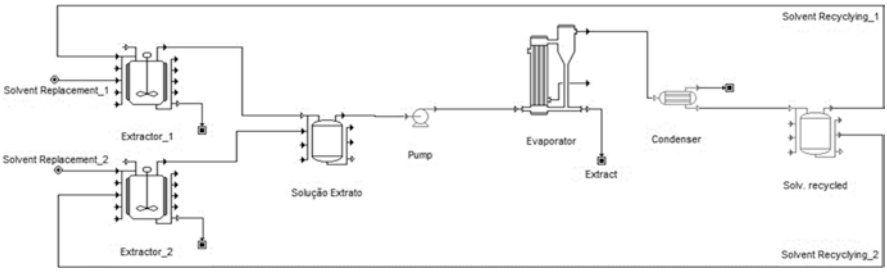

Fig. 20.22 Scheme of UAE built in SuperPro Designer, used for the economic evaluation of the process

feasibility of SFE, this same methodology has been applied to other extraction processes, including UAE. However, a few differences apply to these methods.

The cost of UAE equipment is lower than for SFE equipment. The price of equipment varies between € 10,000 (5 L) and € 200,000 (1000 L), which represents only 25 % more than for conventional LPSE equipment. The great advantage of UAE is the short extraction time, which usually decreases the energy spent by a factor of 10 when compared to LPSE. This feature makes the COM of UAE competitive in the natural product market (Adam et al. 2011).

The same behavior of SFE is observed for UAE processes in terms of influence of plant size; the COM is inversely proportional to the extractor capacity, which represents an advantage for investment in a large multipurpose industrial unit (Fig. 20.22) (Santos et al. 2010; Veggi et al. 2011).

As in SFE, UAE processes are mostly automated, which decreases the number of operators needed compared to traditional processes. However, in UAE there is an extra unit operation compared to SFE: the removal of solvent from the extract. This step requires extra labor compared to SFE, but still lower when compared to LPSE. Therefore, as in SFE, in UAE the COL tends to have low participation in the COM.

As for the CRM, the largest difference from SFE is that in UAE the raw material is immersed in the solvent inside the extractor tank; therefore, the real density of the particles is considered in calculations (Santos et al. 2010), instead of apparent bed density that is considered in SFE. A lower amount of raw material can be loaded in a UAE extractor than in an SFE extractor for the same extractor size since in UAE the solvent must be loaded with the raw material, whereas in SFE the solvent flows continuously through the porous packed extraction bed. According to Peters et al. (2003) for general processes, raw material cost varies from 10 to 80 % of COM, which can be found in UAE processes.

UAE has competitive energy cost when compared to LPSE. Depending on the application, the amount of energy required per liter of material treated (often defined as kWh/L) is comparable to any other unit operation in the industry, such as homogenization, milling, heat shock, etc. For an extractor of 1 L, the energy consumption

of LPSE is 5 kWh, while for UAE it is only 0.25 kWh (Adam et al. 2011). A typical thermal 100 L heating bath operates at 2.8 kWh with operating cost of approximately € 0.41/h (UK domestic rate) with an energy density of 112,000 kJ/m^3. A 125 L ultrasonic bath has a comparable operating cost of approximately € 0.53/h at 35 °C without additional heating with an energy density of 115,200 kJ/m^3. As the yield when using the 125 L ultrasonic bath is higher when compared to the thermal process at 35 °C for the same extraction time, or the extraction time is shorter for the same yield, ultrasound appears to be a viable option for scale-up purposes (Paniwnyk et al. 2009). Energy consumption is higher in UAE than in SFE, though, because of the power that must be added to the extraction medium and of the extra solvent elimination step. Therefore, the CUT share tends to be larger for UAE processes than for SFE.

In UAE processes, CWT can usually be neglected for the same reasons detailed for SFE. The only accumulated waste is the exhausted solid, which, being constituted of vegetable material, can be incorporated into the soil after solvent removal. As a result, there is no harmful waste to be treated (Takeuchi et al. 2009).

The studies on the economic feasibility of UAE processes are scarce. Santos et al. (2010) evaluated the UAE of jabuticaba skins for the recovery of anthocyanin-rich extracts. They performed a Class 2–3 economic evaluation using SuperPro. Four different extraction methods were studied: UAE; agitated bed extraction (ABE); combined UAE+ABE; and Soxhlet extraction (acidified or not), for three different extractor capacities (50, 100 and 300 L). UAE and ABE presented similar COM (US$ 387.20/kg and US$ 401.21/kg, respectively), and their combination did not improve yield; therefore, the COM increased when ABE+UAE was used (US$ 422.18/kg), for an extractor of 300 L. In this study the CRM did not represent a large fraction of the COM due to the low price of jabuticaba skins.

For UAE payback time is in general less than 1 year (Patist and Bates 2011). Patist and Bates (2008) described an example of the industrial application of the UAE technology. They reported that for a total capital investment of US$ 700,000, payback time was only 4 months, with a US$ 2,000,000 benefit per year, due to yield increase when compared to LPSE. However, the process was not specified. Cavitus, an Australian company, reported payback of 2 years for UAE of grape pigments and flavor for must, attributed to increased extraction yield by 30 % and improved sensory properties of the final product (Cavitus 2013).

Literature on the economics of UAE is even poorer than that on SFE, despite this technology being used at industrial scale. If proper economic evaluations are performed, it is possible that the COM would decrease for this technology, as has been happening for SFE.

20.5 Combining SFE and UAE

SFE has slow dynamics even when solute free solvent is recirculated, and therefore improvements in mass transfer are required. The use of high-intensity ultrasound represents a potential efficient way of enhancing mass transfer processes. Therefore, the addition of ultrasound to SFE (USFE) has been proposed as a mechanism to enhance both the mass transfer rate and the yield of the SFE process, and it seems to be a promising technique (Shirsath et al. 2012). This is probably the only practical way to produce agitation in SFE because the use of mechanical stirrers is not possible in this process (Chemat et al. 2011). The deep agitation produced by ultrasonic energy enhances the mass transfer due to radiation pressure, streaming, agitation, high amplitude vibrations, cellular damage, which increases intra-particle diffusivity, etc. (Riera et al. 2010; Balachandran et al. 2006).

The ultrasonic field is integrated inside the supercritical extractor without losing a significant volume fraction. The USFE process was used to extract oil from almond at laboratory and pilot (5 L) scales, reaching yields 15–90 % higher than for SFE only; the kinetics of the SFE process was improved by 30 % (Blasco et al. 2010; Riera et al. 2004, 2010; García-Reverter et al. 2008). Riera et al. (2010) also studied the USFE of cocoa cake oil in a pilot plant (5 L), reaching yields 43 % higher than when using SFE alone.

Balachandran et al. (2006) studied adding ultrasound to SFE of ginger, and found that both the extraction rate and the yield increased compared to simple SFE. The yield was up to 30 % higher at the end of the extraction.

Hu et al. (2007) studied the extraction of oil and coixenolide from adlay seed by SFE and USFE. The most favorable conditions for SFE were 45 °C, 25 MPa, 4 h and 3.5 L/h of solvent flow rate. When ultrasound was applied (USFE), the following parameters were preferred: 40 °C, 20 MPa, 3.5 h and 3.0 L/h. Therefore, USFE reduced the temperature, pressure, CO_2 flow rate and time needed for the process, which represents energy savings, besides increasing the yield in 14 %.

Luo et al. (2007) studied the SFE and USFE of ginsenosides from ginseng in supercritical CO_2 reverse microemulsions formed by bis(2-ethylhexyl) sodium sulfosuccinate. Ultrasound significantly enhanced the extraction process, improving the yield 2.6 times.

Gao et al. (2009) used USFE to recover lutein esters from marigold. The mass transfer coefficient in the solid phase was increased from 3.1×10^{-9} m/s for SFE to 4.3×10^{-9} m/s for USFE due to the use of ultrasound. Therefore, the yield of lutein esters increased significantly with the presence of ultrasound.

USFE is a promising technique; however, it is still in the early stages of development. Scale-up issues are not solved yet for either SFE or UAE techniques. It is expected that USFE is even more complex than the separate techniques and will thus require further study.

20.6 Conclusions

Scale-up studies of SFE and UAE are under development, but there is still no certitude in these fields. Most of the SFE studies in the available literature are inconclusive, and there is no consensus on a scale-up criterion applicable to SFE of solid matrices. Nevertheless, keeping S/F and/or Q_{CO_2} / F constant have been showing promising results as scale-up criteria. Mathematical modeling should be also used more often in the near future for this purpose. For UAE there are even fewer studies on scale-up than for SFE. So far, the focus has been on keeping the energy dissipated and the intensity constant. Different extractor geometries and using multiple transducers have also been evaluated, showing good results. Even so, both SFE and UAE processes are far from having a universal scale-up criterion established. Therefore, more studies in this field are needed.

Adequate scale-up of a process is of paramount importance to a further and not less important step: cost estimation. Studies on COM of natural products obtained by emergent technologies have been considerably increasing in the past 10 years. Economic evaluation has allowed demonstration of the feasibility of both SFE and UAE compared with conventional extraction processes, often with lower COM for the emergent technologies. Nevertheless, further development is necessary so that more precise estimates can be carried out, which would be of great help in decision making processes.

Finally, a new approach that gathers the advantages of both SFE and UAE technologies is the novel method of ultrasound assisted supercritical fluid extraction (USFE), which has demonstrated promising results. Scale-up studies and economic evaluation of this process have yet not been carried out.

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Chapter 21
Multiphysics Modelling of Innovative Food Processing Technologies

Pablo Juliano and Kai Knoerzer

21.1 Introduction

The food industry is an increasingly competitive and dynamic arena with consumers being more aware of what they eat and, more importantly, what they want to eat. Important food quality attributes such as taste, texture, appearance, and nutritional content are strongly dependent on the way foods are processed (Knoerzer et al. 2011b).

In recent years, a number of innovative food processing technologies, also referred to as "emerging" or "novel" technologies have been proposed, investigated, developed, and implemented with the aim to improve or replace conventional processing technologies. These technologies take advantage of other physics phenomena such as static high hydrostatic pressure or dynamic pressure waves, or electric and electromagnetic fields, and provide the opportunity for the development of new foods, but also for improving the quality of established food products through gentle processing. The physical phenomena utilized by these technologies can potentially reduce energy and water consumption and, therefore, can play an important role towards environmental sustainability of food processing and global food security by expanding the shelf stable product spectrum (Knoerzer et al. 2011b).

Apart from the underlying thermo- and fluid-dynamic principles of conventional processing, these innovative technologies incorporate additional Multiphysics dimensions, for example, pressure waves, electric and electromagnetic fields, among others. To date, they still lack an adequate, complete understanding of the basic principles of intervening in temperature and flow evolution in product and equipment during processing (Barbosa-Canovas et al. 2011). The development and optimization of suitable

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equipment and process conditions that provide the adequate uniformity still remains a challenge. Computational Fluid Dynamics (CFD) is an established tool for characterizing, improving and optimizing traditional food processing technologies; the partial differential equations solved are those describing the conservation of mass, momentum, and energy (i.e., Continuity, Navier–Stokes, and Fourier equations). Innovative technologies, however, provide additional complexity and challenges for modellers because of the concurrent interacting Multiphysics phenomena; further partial differential equations need to be solved simultaneously, such as the Maxwell's and the constitutive equations for problems involving electromagnetics (e.g., microwave and radiofrequency processing), charge conservation (e.g., pulsed electric field processing), and wave equations (e.g., Helmholtz wave equations) for ultrasonic and megasonic processing (Knoerzer et al. 2011b). These equation systems can increase in complexity when not only the process variables are to be predicted, but also process outcomes such as microbial/enzyme inactivation or food matrix modification. In such cases the numerical problem is coupled to equations describing the dynamics of occurrence of such phenomena, e.g., inactivation, the development of (acoustic) forces leading to transport of concentrated species, among others.

21.2 Simulating Innovative Food Processing Technologies

A common problem of innovative food processing technologies is the nonuniformity of the treatment, which can be caused by gradients of process variables such as temperature, electric field strength, or sound pressure fields in the processing chambers. A nonuniform distribution of a certain process variable leads to nonuniformities in the resulting outcomes of the process (e.g., microbial inactivation).

While trial-and-error optimization is always an option to improve equipment and process design, it is the least preferred way, as it is very cost-, labor-, and time-intensive and a good performance may be missed, as not all possibilities can be tested following this approach. On the other hand, numerical modelling using CFD can be used exactly for this purpose at reduced cost and time of equipment use. This way, advantages and disadvantages of the respective technology can be identified and either utilized or minimized.

Numerical modelling studies have been reported across the range of innovative food processing technologies, such as microwave and radiofrequency, ultraviolet light, high pressure (thermal), pulsed electric field, and ultrasonics/megasonics processing.

The common objective of microwave and radiofrequency processing is the temperature increase in the treated product, e.g., for the purpose of thermal pasteurization, sterilization, preheating, cooking, thawing, and drying. Numerical studies on microwave processing have been reported, for example by (Birla et al. 2008; Tiwari et al. 2011; Geedipalli et al. 2007; Knoerzer et al. 2008).

Unlike microwave and radiofrequency processing, ultraviolet light for treating liquid products or product surfaces is a nonthermal process utilized mainly for the

purpose of inactivation of microorganisms. Modelling studies have been reported, for example by (Huachen and Orava 2007; Unluturk et al. 2004).

The following sections highlight in more detail the latest advances in modelling high pressure thermal, pulsed electric field and ultrasonics/megasonics processing. These technologies have been investigated in more detail by our group and therefore become the focus of the following sections.

21.2.1 High Pressure Thermal Processing

High pressure thermal processing is a technology that is effective in inactivating not only vegetative organisms but also microbial spores, due to the elevated temperatures involved. Because the process time can be reduced compared to thermal-only processing through rapid compression heating and decompression cooling, quality attributes, such as color, nutrients, flavor, and texture can be better retained (Olivier et al. 2011).

A number of studies have been reported on the utilization of Multiphysics modelling for equipment and process characterization in terms of process temperature and flow field distributions (Knoerzer et al. 2007; Knoerzer and Chapman 2011), prediction of microbial (spore) inactivation (Juliano et al. 2009), and equipment optimization (Knoerzer et al. 2010) (Fig. 21.1).

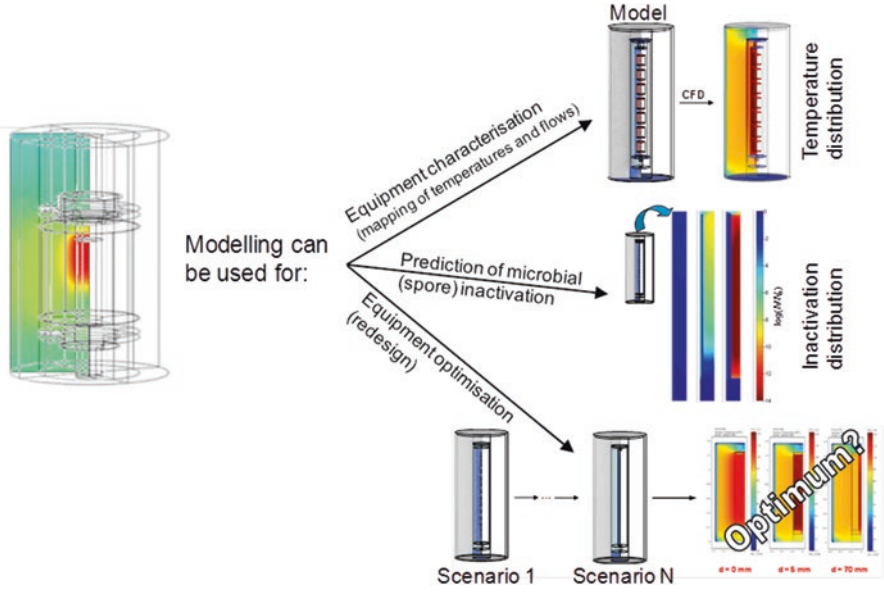

Fig. 21.1 Applications of numerical models describing high pressure thermal processing

Knoerzer et al. (2007) reported on the use of a numerical model to describe temperature and flow distribution in a 35 L pilot scale high pressure sterilization system (Avure Technologies Inc., Seattle, WA, USA) and evaluated the differences of the process variables for a number of different product carriers made of metal and insulating plastic material. Figure 21.2 shows the temperature distributions in three investigated scenarios at the end of pressurization, namely a cylindrical steel high pressure vessel without carrier, one with a metal carrier and one scenario where a carrier made from insulating PTFE was placed into the vessel.

As shown in the figure, the temperature distribution achieved in scenario (a) shows nonuniformities and relatively low temperatures compared to the scenarios where carriers are included, which avoid pronounced cooling down caused by the incoming pressurization fluid. Temperatures in scenario (b) are more uniform but still lower than in scenario (c). Furthermore, during pressure hold time, scenarios (a) and (b) exhibit pronounced heat losses, whereas the PTFE carrier in scenario (c) was able to retain the heat inside the carrier.

As expected, only the insulated carrier provided process conditions feasible for sufficient and uniform product sterilization through microbial spore inactivation (Fig. 21.3).

Juliano et al. (2009) applied more detailed models (Fig. 21.4a) describing the process variables (i.e., pressure, temperature, and flow) and evaluated the differences in the extent and distribution of predicted inactivation of *Clostridium botuli-*

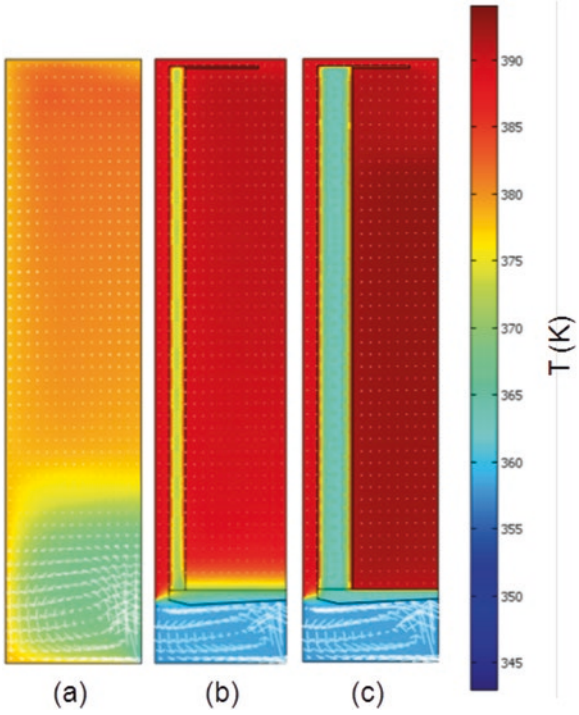

Fig. 21.2 Temperature distribution in an axis-symmetric section of the cylindrical high pressure vessel; (**a**) no carrier in the vessel, (**b**) inclusion of cylindrical metal carrier, (**c**) inclusion of cylindrical PTFE carrier; at the end of pressurization

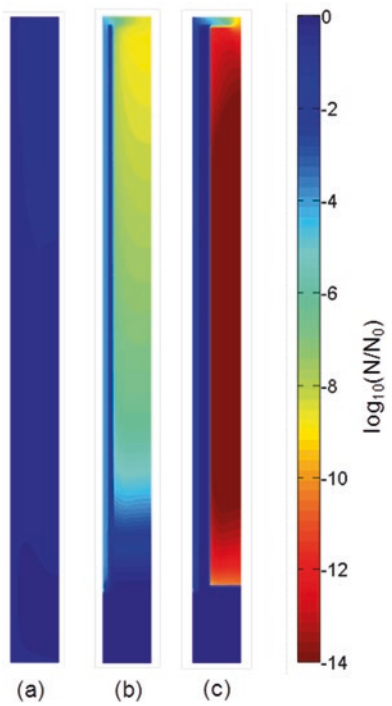

Fig. 21.3 Distribution of *Clostridium botulinum* spore inactivation as predicted by the log-linear model in (**a**) the vessel without product carrier, (**b**) the vessel including a steel carrier, and (**c**) the vessel including a PTFE carrier

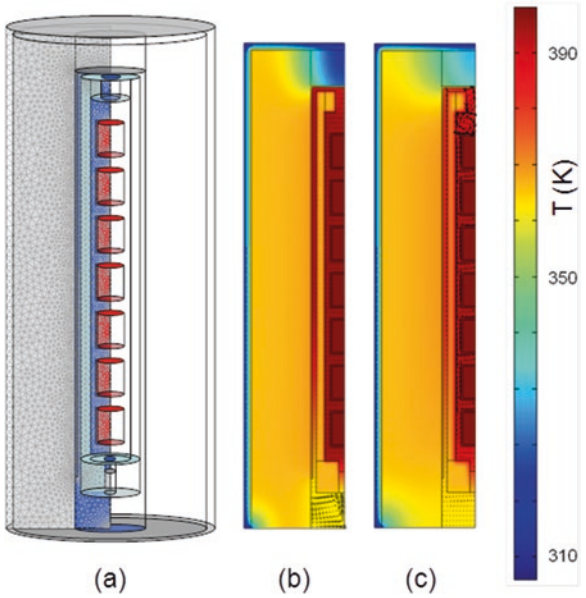

Fig. 21.4 Model geometry (**a**) and predicted temperature distributions at the end of pressurization (**b**) and pressure hold time (**c**)

num spores in food packages. In a first step, the CFD models were able to show heat retention inside the food packs and temperature magnitudes of ~121 °C during pressure hold time (Fig. 21.4b, c).

The predicted transient temperature distributions were then coupled to selected predictive microbial inactivation models, namely, the commonly known log-linear model, an *n*-th order model and a Weibull distribution model. The different inactivation models predicted very different levels of spore inactivation for the same pressure and temperature conditions. For example, the log-linear model predicted inactivation of *C. botulinum* spores in the order of 16 $\log_{10}$ after 3 min processing at 600 MPa and 121 °C (Fig. 21.5a), whereas the Weibull model indicated spore inactivation of only 9 $\log_{10}$ for the same process (Fig. 21.5b). The inactivation model used ultimately will affect the selection of optimum process conditions for a HPT process. The determination of the most appropriate microbial inactivation model for HPT products manufacture needs further study.

Knoerzer et al. (2010) then used a modified version of the model to optimize the wall thickness of the insulating carrier while increasing product load capacity. The carrier supplied by the manufacturer was designed with a wall thickness such that sufficient heat retention was ensured during processing. The authors derived a dimensionless parameter, referred to as the Integrated Temperature Distributor (ITD) value (Eq. 21.1) to evaluate temperature uniformity, and the temperature magnitude expressed relative to a target temperature and heat retention during processing.

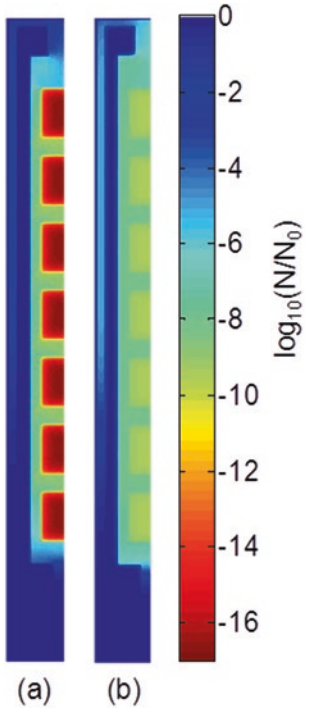

Fig. 21.5 Indication of inactivation of *C. botulinum* spores as predicted by the log-linear model (**a**) and the Weibull distribution model (**b**)

$$\mathrm{ITD} = \frac{\int_{r_{\min}}^{r_{\max}} \int_{z_{\min}}^{z_{\max}} 10^{\frac{\frac{\int_0^t T(t)\mathrm{d}t}{t_{\mathrm{process}}} - T_{\mathrm{target}}}{z_T}} \mathrm{d}r\mathrm{d}z}{\left(r_{\max} - r_{\min}\right) \cdot \left(z_{\max} - z_{\min}\right)} \tag{21.1}$$

where $r_{\min}$, $r_{\max}$, $z_{\min}$, $z_{\max}$ cover the region of interest (the carrier volume), t_{process} is the process time of interest (in this case, the pressure holding time where most of the heat loss is expected) and T_{target} is the targeted holding temperature of the process under pressure.

An iterative strategy was applied, which consisted of a model that automatically changed the carrier wall thickness in a range of 0–70 mm, and evaluated temperature performances and load capacities for the respective scenarios. Figure 21.6 shows the modified model geometry with variable carrier wall thickness and the predicted temperature distributions at the end of pressure hold time for a wall thickness of 0, 5 and 70 mm.

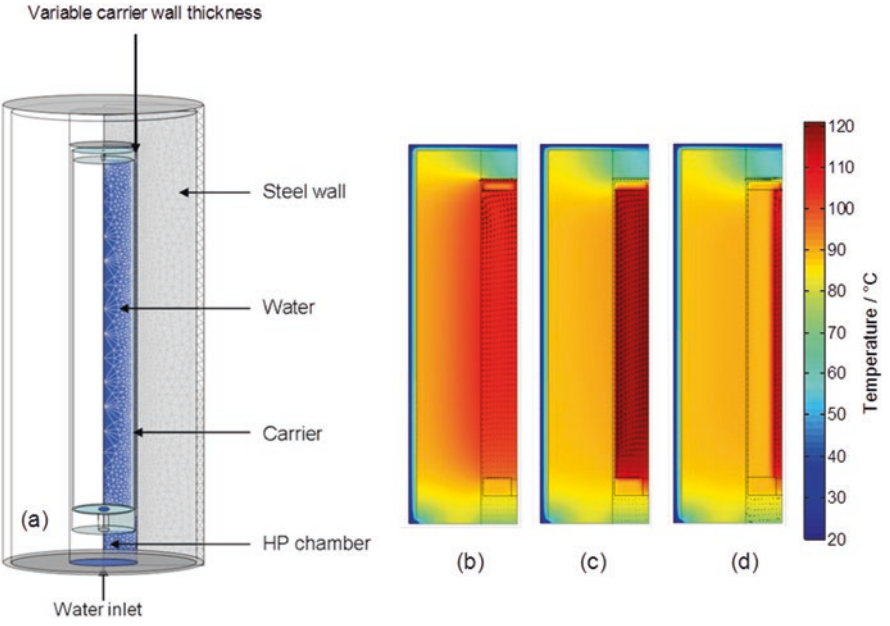

Fig. 21.6 Depiction of modified model geometry (**a**) and the predicted temperature distributions at a wall thickness of 0 mm (**b**), 5 mm (**c**), and 70 mm (**d**)

The study showed that the wall thickness can be reduced from 28 mm to approximately 4 mm without compromising temperature performance, leading to an increase of carrier load capacity by more than 100 % (Fig. 21.7).

21.2.2 Pulsed Electric Field Processing

Pulsed Electric Field (PEF) processing is a technology that can be applied for cold or low temperature pasteurization of liquid products. It is able to inactivate vegetative microorganisms through the application of electric fields in the order of several ten thousand volts per centimeter for a very short time, leading to cell poration and cell death (Heinz et al. 2003). Overall treatment times are in the order of microseconds. Other potential applications of this technology are for enhanced extraction processes or softening of fruit and vegetable tissue, for example for improving cutting performance and reducing cutting losses. Also, this technology can improve the quality attributes of foods compared to conventional thermal processing, such as flavor, color, and nutrients, among others. Being a continuous process, high throughputs are possible.

Published studies on numerical modelling of pulsed electric field processing include the utilization of such models for equipment characterization with respect to electric field, temperature and flow distribution (Buckow et al. 2010, 2011), the

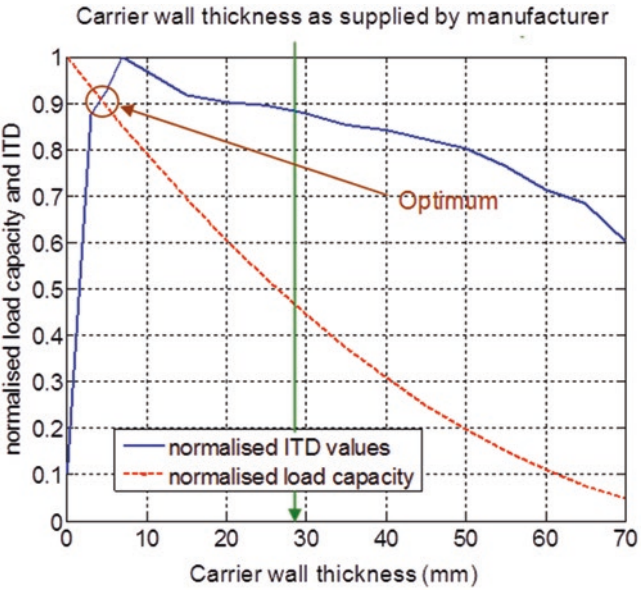

Fig. 21.7 Determination of optimum carrier wall thickness by evaluating temperature performance (ITD) and load capacity of the carriers with varying wall thickness

prediction of microbial or enzyme inactivation (Buckow et al. 2012; Knoerzer et al. 2011a) and equipment optimization to ensure uniform and effective processing (Knoerzer et al. 2012) (Fig. 21.8).

Buckow et al. (2010) reported on the development of a 3D model for a pilot scale pulsed electric field system (Diversified Technologies Inc., Bedford, MA, USA) to predict electric field strength, flow, and temperature distributions (Fig. 21.9) and an extensive validation of the model predictions through temperature measurements within the constrained space of the treatment chamber's active zone and the second ground electrode (Fig. 21.10). The authors investigated the treatment of salt solutions with different conductivities and whole milk, processed at two flow rates, and five different inlet temperatures. Pulses were applied at two different voltage settings, four pulse repetition rates, and three pulse widths. They were able to utilize this model to characterize and evaluate the performance of the system as supplied by the manufacturer with respect to electric field strength, temperature and flow distribution.

Buckow et al. (2011) applied this model further to derive simplified equations to estimate accurate electric field strengths and specific energy inputs from treatment variables such as voltage, pulse frequency and duration, among others. Also evaluated were the effects of changing treatment chamber geometry and configuration on these process variables. A common approach for estimating electric field strength is

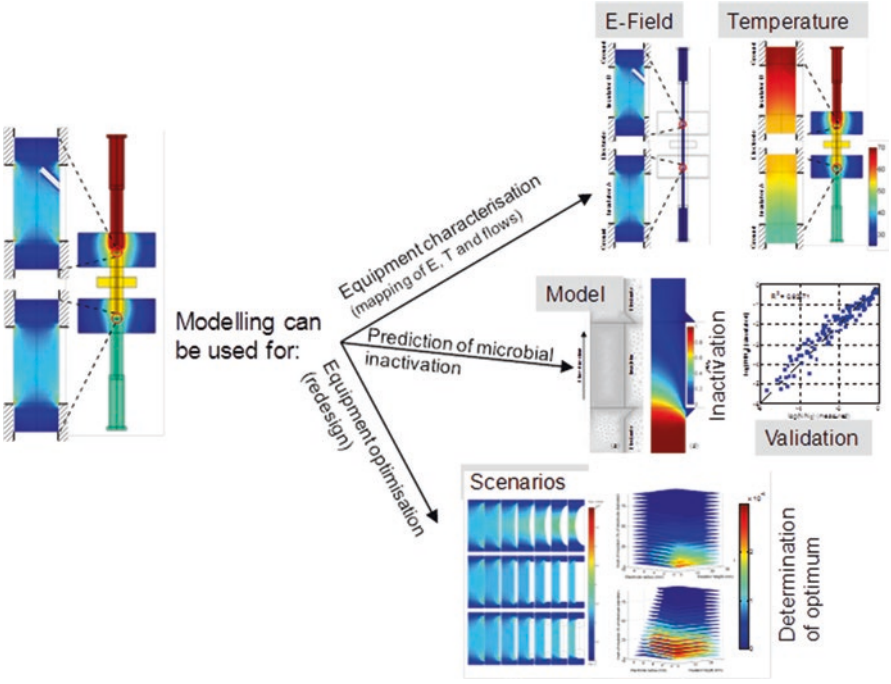

Fig. 21.8 Applications of numerical models describing pulsed electric processing

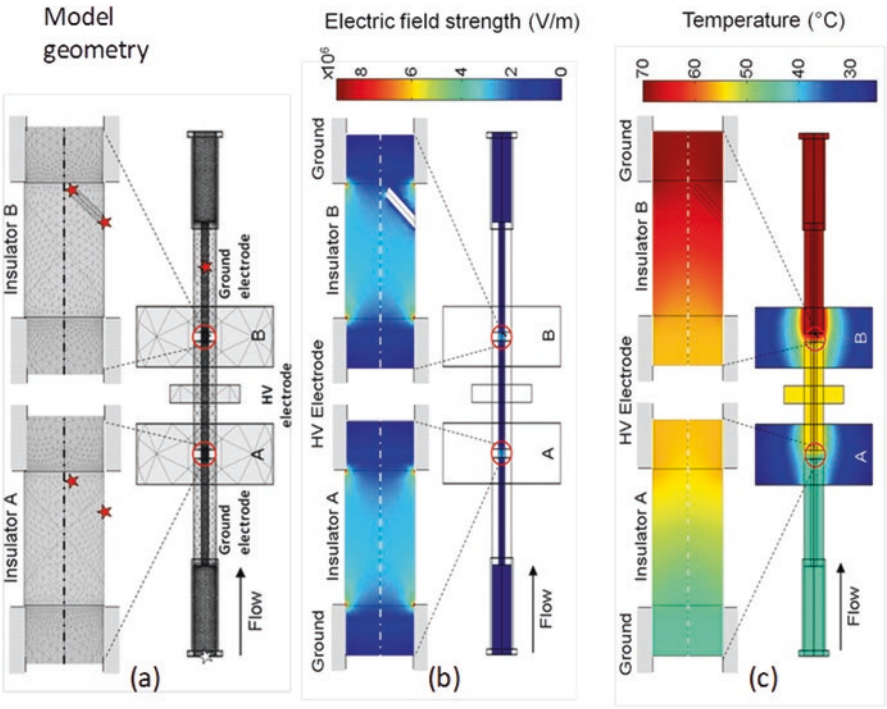

Fig. 21.9 Representation of the pilot scale treatment chamber including magnification of the treatment zones (model geometry **a**), electric field strength distribution in a salt solution at 4 mS/cm, flow rate of 4 L/min, inlet temperature of 45 °C, voltage of ~22 kV, pulse width of 5 μs, and frequency of 600 Hz (**b**), and temperature distribution at these conditions (**c**)

relating the applied voltage to the electrode gap. While this will give accurate predictions for parallel plate systems, it was found that for configurations used in continuously operating systems, such as co-field or co-linear design, this approach always over-predicts the actual electric fields. This is similar for specific energy input, commonly estimated by multiplying voltage, current, pulse width, pulse repetition rate and mass flow. Figure 21.11 shows (a) the correlation of relative electric field strength (i.e., actual electric field strength/estimated electric field strength for parallel plate configuration) and (b) the correlation of the relative specific energy input (i.e., the actual specific energy input/estimated specific energy input for parallel plate configuration) with the ratio of electrode radius and electrode gap for different chamber configurations. These configurations were: "no inset" (where the insulator bore diameter is equal to the inner electrode diameter), "rectangular inset" (where the insulator bore diameter is smaller than the inner electrode radius), "chamfer edge inset" (which is identical to the rectangular inset with rounded edges of the insulator bore), and "elliptical inset" (where the insulator bore has in inward concave shape); see also Fig. 21.13.

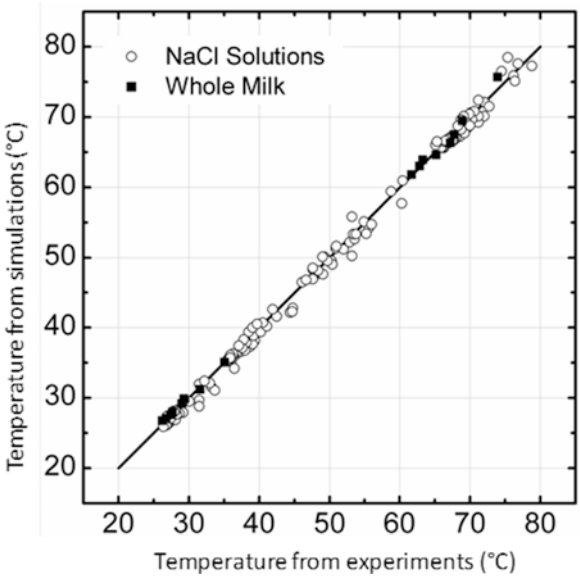

Fig. 21.10 Validation of the model for different fluids and ~50 process conditions (over 400 data points)

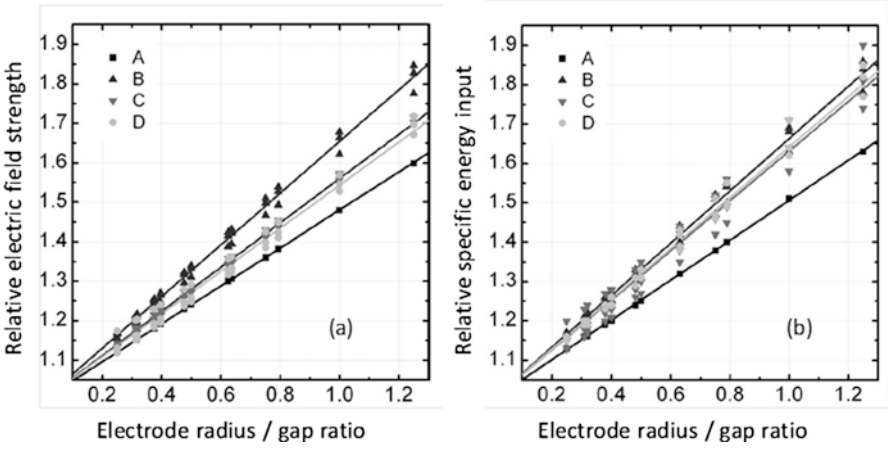

Fig. 21.11 Correlation of (**a**) the relative electric field strength and (**b**) the relative specific energy input with the ratio of electrode radius and gap for "no inset" (*A*), "rectangular inset" (*B*), "chamfer edge" (*C*), and "elliptical inset" (*D*) chamber configurations

Buckow et al. (2012) then developed and validated a model for a laboratory scale pulsed electric field system (Fig. 21.12a) and evaluated the effect of the electric field on lactoperoxidase (LPO) degradation (an indicator for pasteurization) by coupling the predicted temperature distributions (e.g., Fig. 21.12c) to predictive LPO degradation models accounting for the thermal component only.

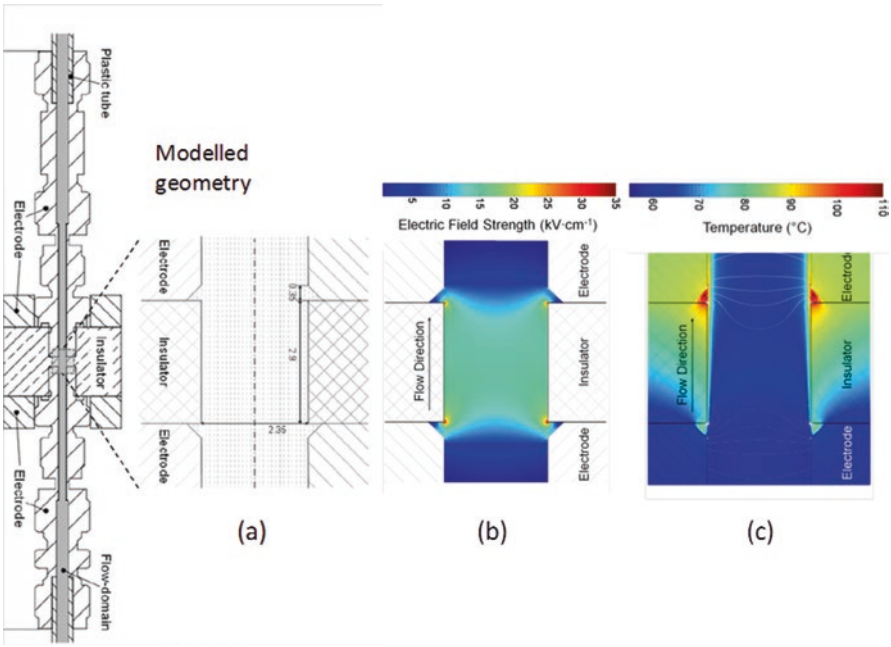

Fig. 21.12 Representation of the modelled geometry of the lab scale PEF system (**a**), predicted electric field distribution (**b**), and predicted temperature distribution (**c**)

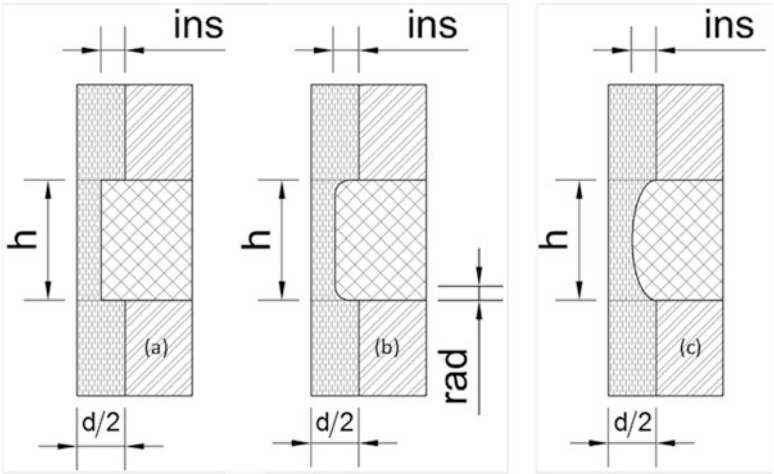

Fig. 21.13 Investigated chamber configurations, indicating the geometrical parameters varied in the model; (**a**) rectangular inset, (**b**) rectangular chamfered edge inset, and (**c**) elliptical inset scenario

The study indicated that the major effect for LPO inactivation comes from the elevated process temperatures as the predictions (thermal only degradation) were close to the measured degradation (combined thermal and PEF) for a number of process conditions; however, they found that some additional inactivation was also caused by the electric field of up to 12 % potentially caused by the high intensity electric pulses and induced electrochemical reactions.

Lastly, Knoerzer et al. (2012) developed an iterative algorithm that was capable of automatically changing the treatment chamber configuration and dimensions in the Multiphysics models and to identify, out of more than 100,000 scenarios, the one that showed the highest degree of electric field uniformity, together with sufficient throughput, lowest pressure drop, among other evaluation characteristics. The evaluation of the performance of the models was based on a parameter, referred to as the Dimensionless Performance Parameter (DPP), calculated by an equation derived by the authors (Eq. 21.2), accounting for the treatment volume, pressure drop estimations, electric field magnitude related to that achievable in parallel plate systems, electric field uniformity, and peaks of the electric field strength.

$$\mathrm{DPP} = \left(\frac{V_{\mathrm{zone}}}{V_{\mathrm{max}}}\right)^{a_1} \cdot \left(\frac{(d-\mathrm{ins})^4}{d^4}\right)^{a_2} \cdot \left(\frac{\frac{E_{\mathrm{av}}}{V_0}}{h_{\mathrm{min}}}\right)^{a_3} \cdot \left(\frac{n_{\mathrm{av}\pm 10\%}}{n_{\mathrm{total}}}\right)^{a_4} \cdot \left(\frac{E_{\mathrm{av}}}{E_{\mathrm{max}}}\right)^{a_5} \tag{21.2}$$

where V_{zone} is the volume of the treatment zone (insulator region) of the respective scenario, V_{max} the volume of the largest treatment zone considered, E_{av} is the average electric field strength of the insulator region, V_0 the applied potential, h_{min} the minimum electrode distance (gap) of all scenarios investigated, $n_{\mathrm{av}\pm 10\%}$ the number of elements in the treatment zone with electric field strengths within 10 % of the average electric field strength, n_{total} the total number of elements in the treatment zone and E_{max} the maximum electric field strength in the respective scenario; a_1–a_5 are weighing parameters adjustable depending on the importance on the respective performance variable.

Three different chamber configurations (Fig. 21.13) were studied and for each of these, four different geometry parameters were varied, with the internal diameter d of the electrodes ranging from 2 to 20 mm, the height h of the electrode gap ranging from 1 to 30 mm, a total inset ins (i.e., the internal diameter of the insulator) in a range of 0–90 % of the electrode diameter d, and for the "rectangular rounded edge inset" models also the chamfer radii rad ranging from 0 to 40 % of the diameter reduction ins (Fig. 21.13).

The algorithm first generated the models, then solved them, applied the performance evaluation by utilizing the DPP equation and then identified the scenario which yielded the highest DPP value, which was found for configuration (b). The authors then set up a full 3D model of this configuration, built the new chamber and performed validation studies of the model for a salt solution and apple juice and for

various process conditions. They found that the new design could be predicted well with respect to the temperatures generated in the treatment chamber (Fig. 21.14).

21.2.3 Ultrasonics and Megasonics Processing

Ultrasound processing spans over a wide range of acoustic frequencies, starting as low as 18 kHz, up to several MHz. Applications are as diverse as the frequency spectrum is wide. At the lower frequency (18–200 kHz) end (also known as ultrasonics) the effects are caused mainly by instable cavitation. Traditional applications such as emulsification, cleaning, extraction (Gogate and Kabadi 2009), and more novel applications used for improved drying (Sabarez et al. 2012) and beverage defoaming (Rodriguez et al. 2010) in airborne ultrasound systems can be listed. When using higher frequencies (>0.2 MHz, also known as megasonics), the effects can be either mechanical through standing pressure waves and microstreaming, and/or sonochemical (radical driven) or biochemical (stress response in living tissue). Novel high frequency applications include the separation of particles in standing wave systems (Juliano et al. 2013), and texture improvement of processed fruits and vegetables, through produce-internal stress responses (Day et al. 2012).

Published studies on numerical modelling of ultrasonics and megasonics processing include the utilization of Multiphysics models for equipment characterization with respect to acoustic pressure, temperature, and flow distribution (Trujillo

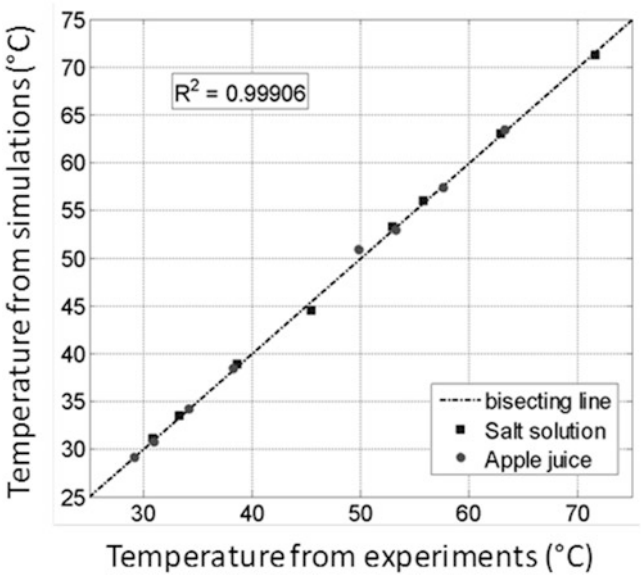

Fig. 21.14 Parity plot of predicted and experimentally determined temperature values in the new treatment chamber

and Knoerzer 2009, 2011), equipment optimization (Trujillo and Knoerzer 2009) and for predicting particle separation in megasonics standing wave applications (Trujillo et al. 2013) (Fig. 21.15).

Trujillo and Knoerzer (2011) reported on the development of a Multiphysics model capable of simulating the formation of a sound pressure jet produced by a sonotrode placed in water in a low frequency (20 kHz) high power ultrasound application. The acoustic power was dissipated within close proximity to the horn and the acoustic energy was completely converted into kinetic and thermal energy leading to a jet being formed and directed away from the sonotrode while the temperature was increasing in the bulk of the treated fluid. The model was validated by utilizing published data (Kumar et al. 2006) where fluid movement was measured by Laser Doppler Anemometry.

Figure 21.16 shows the computational representation of the system investigated by Kumar et al. (2006) and Trujillo and Knoerzer (2011) in 3D and 2D. Full 3D and axis-symmetric 2D models predicted almost identical values; therefore, and the fact that the computational demand of the 3D model was very high, all further models for comparison with the LDA data were solved in 2D only. Figure 21.17 shows a direct comparison of the velocity profile predicted by the model and the one measured by LDA for a specific power input of 35 kW/m^3. Both prediction and measurement show the jet being formed underneath the horn tip with much lower velocities throughout the rest of the reactor.

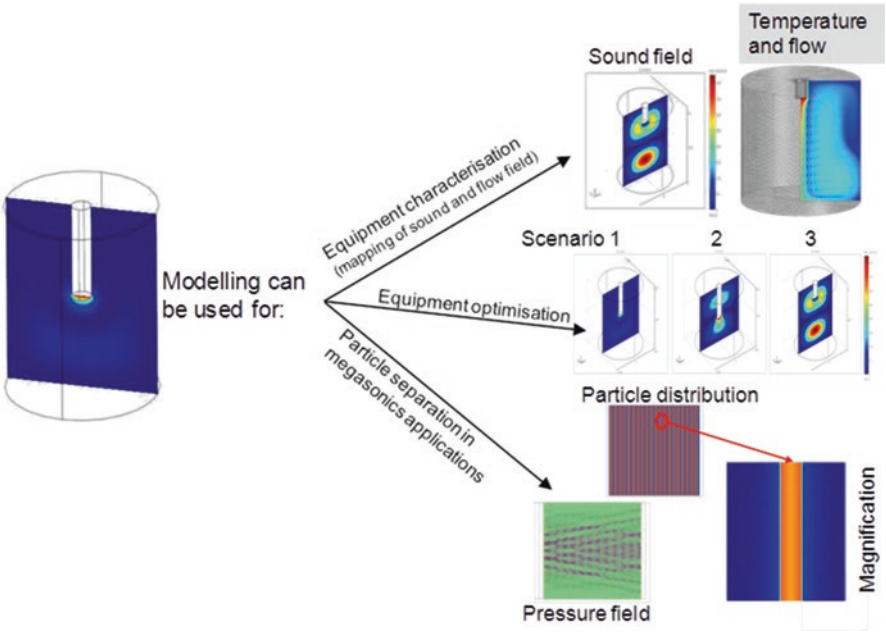

Fig. 21.15 Applications of numerical models describing low and high frequency ultrasound processing

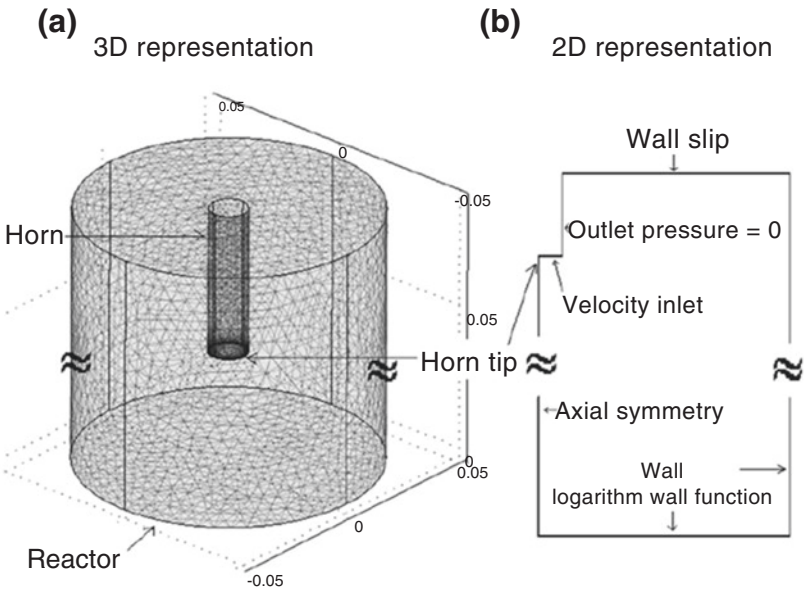

Fig. 21.16 Depiction of the geometry of the investigated system; (**a**) 3D representation, (**b**) 2D axis-symmetric representation, including the boundary conditions of the model

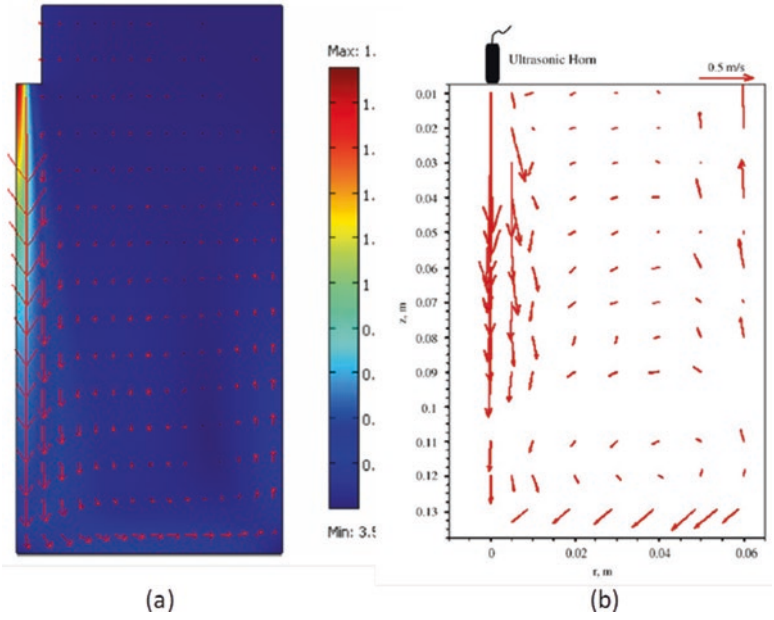

Fig. 21.17 Visual comparison of the predicted (**a**) and measured by LDA (**b**) flow profiles in the investigated system at a specific power input of 35 kW/m^3. The scale is defined by an *arrow* at the top right hand corner with a unit value of 0.5 m/s

In addition to visual comparisons, the authors also performed a quantitative validation of the model by comparing the predicted values of the axial velocity at a number of heights and radii (Fig. 21.18) and found good agreement.

Apart from low frequency ultrasound applications, Trujillo et al. (2013) have also published research on the development of a Multiphysics model for a high frequency ultrasound application for separation of particles out of a continuous water phase. The simulated separation reactor is shown in Fig. 21.19a. The model included solving for the mechanical displacement of the reactor walls, leading to the formation of an acoustic pressure field (indicated in Fig. 21.19a for a fixed frequency of 1.54 MHz as a thin band in the reactor and a magnified view in Fig. 21.19b, *p*), followed by predicting the acoustic radiation force acting on suspended particles (Fig. 21.19b, F_{Rad}). Finally, this (transient) force was utilized to solve for the movement of the particle phase to the nodes of the ultrasonic standing wave (Fig. 21.19b, X_{P}) and frequency ramping, leading to active separation of the particles away from the transducer plate towards the reflector.

The authors then compared digitized images of the actual process at discrete times of 0, 40, and 120 s (Fig. 21.20a) with the predicted particle band formation and transient band movement. As shown in Fig. 21.20b at a discrete time of 120 s, measurement and predictions agreed well. Figure 21.21 shows a parity plot of the measured and predicted band locations for all three time steps; as can be seen, very good agreement was found.

21.3 Summary and Outlook

It is widely established that innovative technologies are the means to meet a need and capture an opportunity, particularly around the manufacture of attractive, new, high quality products with fresh-like quality attributes, ensured safety, and long shelf-life, and more sustainable manufacturing. The main incentive for applying

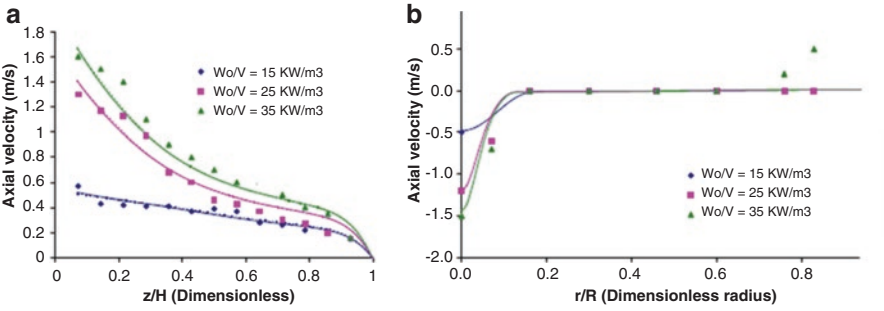

Fig. 21.18 Quantitative comparison of model predictions and experimentally determined values of (**a**) the axial velocity at different height levels under the horn tip and (**b**) different radii at height level of 13 % of the total reactor height

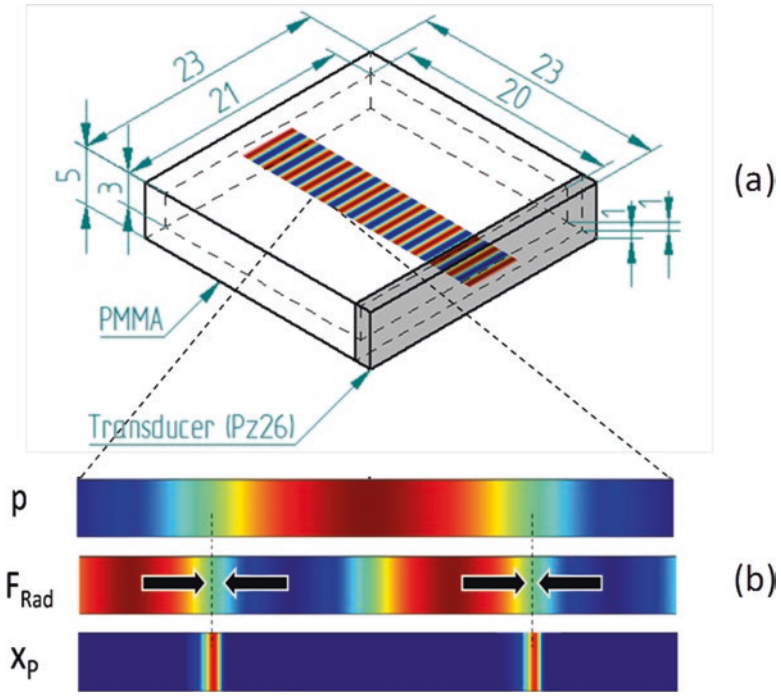

Fig. 21.19 Schematic representation of the investigated treatment chamber with pressure distribution for a fixed frequency of 1.54 MHz (**a**), magnification of one wavelength of the pressure distribution (**b**; p), the resulting acoustic force (**b**; F_{Rad}) and the particles concentrated at the nodes of the pressure wave at the fixed frequency (**b**; X_P)

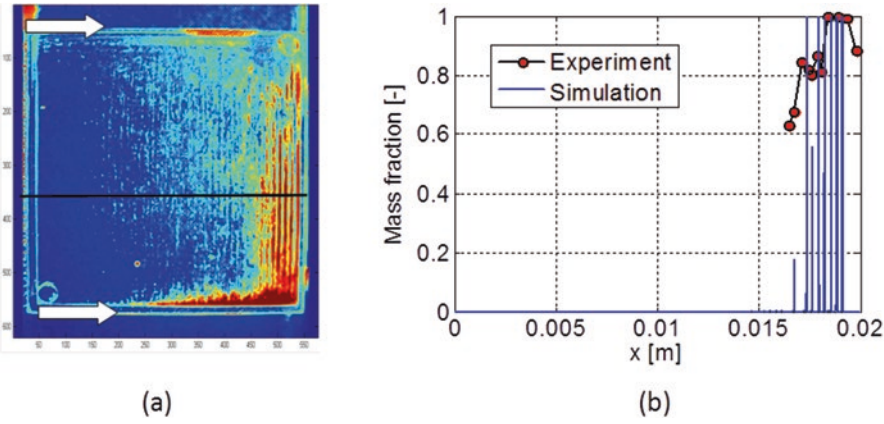

Fig. 21.20 (**a**) Digitized image of actual process at a discrete time of 120 s (false color representation; the *black line* indicating the area for comparison with the model prediction); (**b**) comparison of the mass fraction of the separated particles measured and predicted

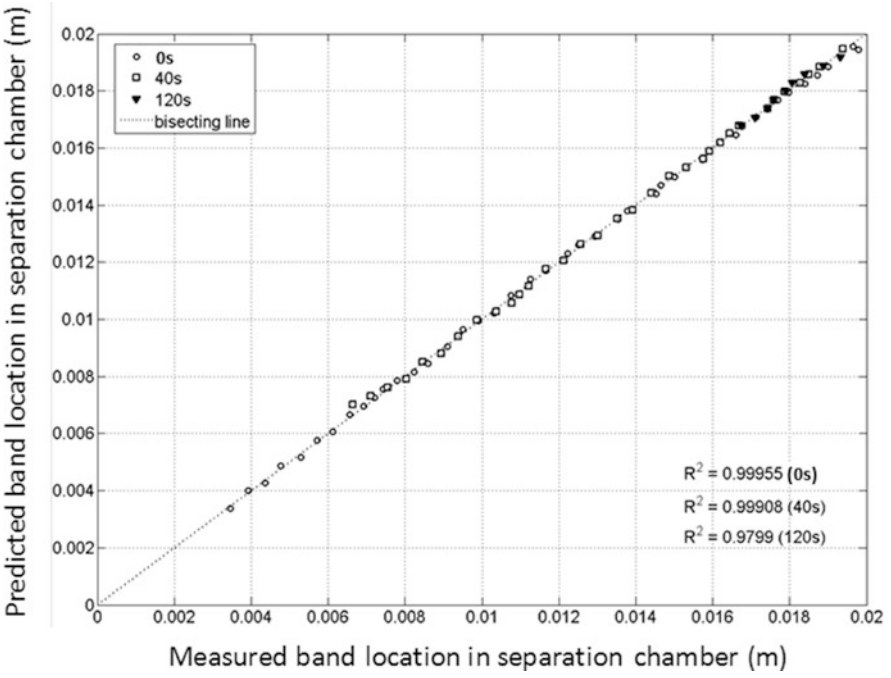

Fig. 21.21 Parity plot of the measured and predicted band locations at discrete time steps of 0, 40, and 120 s

these new technologies should focus on inducing disruptive innovation in the food manufacturing industry rather than providing merely incremental improvements of existing processes.

Validated Multiphysics models have been used to characterize, evaluate, and optimize existing equipment for innovative food processing technologies and such modelling strategies will assist in further developing these technologies for effective and efficient implementation in the food manufacturing industry. Without such modelling capabilities, relying on the traditional approach of trial and error, development will be slow and in some instances a sufficient performance justifying utilization in industry may never happen.

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Chapter 22
Ultrasound-Assisted Freezing of Fruits and Vegetables: Design, Development, and Applications

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Nomenclature

a	Characteristic length
Bi	Biot number
C_f	Specific heat capacity after freezing J/kg/K
C_p	Specific heat capacity of liquid J/kg/K
C_u	Specific heat capacity before freezing J/kg/K
h	Heat transfer coefficient W/m^{2}K
k_f	Thermal conductivity W/mK
L	Latent heat of freezing J/m^3
m	Mass kg
P_{diss}	Dissipated power W
P_{in}	Actual power W
Ste	Stefan number
T_c	Final product temperature °C
t_F	Freezing time
T_f	Initial freezing temperature °C
T_{fm}	Mean freezing time
T_i	Initial product temperature °C
T_{ref}	Reference temperature °C
t_T	Thawing time

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ΔH_{ref} Enthalpy change kj/kg
P_f Density after freezing kj/kg
P_u Density before freezing kj/kg

22.1 Introduction

Fruits and vegetables provide a balanced and nutritious diet as they are rich in antioxidants, vitamins, and minerals (World Health Organization 2003). The antioxidant, vitamin, and mineral contents of fruits and vegetable can reduce the risk of obesity, cardiovascular diseases, type 2 diabetes, and cancer. Fruits and vegetables are highly perishable and their utilization may be limited due to seasonality, poor postharvest handling, and high cost of storage. Decay, shriveling, and loss of quality of fresh fruits and vegetables are major issues in food and agriculture industries (Alvarez and Trystram 1995).

Several methods of preservation such as drying, chilling, freezing, canning, and modified atmospheric packaging (MAP) are available for extending the shelf-life of fruits and vegetables. Freezing, especially using temperatures lower than −18 °C, is a simple method of preservation that preserves fruits and vegetables for a long time while maintaining many of their fresh-like qualities (Prochaska et al. 2000; Fennema et al. 1973; Campañone et al. 2002). Lower temperatures (4 to −18°C) retard microbial growth and slow the chemical reactions responsible for quality deterioration (George 1993; Dinçer 1997; Reid and Fennema 2007).

The global frozen food market is expected to grow from $218.41 billion in 2010 to $261.50 billion in 2015 at an estimated compound annual growth rate of 3.7 % (MarketsandMarkets 2011), whereas the global frozen fruits and vegetables market is estimated to reach 22.6 million tons by 2015 (Global Industry Analysts 2012). In Japan, the consumption of fresh-cut fruits and vegetables has increased steadily from the late 1980s (Kim 2007). Japan also leads Asian countries in terms of consumption of frozen foods, consisting of 7.82 million tons in 2010 (MarketsandMarkets 2011). In China the amount of consumed fruits and vegetables has increased to 369 g per person per day since 1992 (World Health Organization 2003). The frozen food industry has also greatly expanded in China in the past decade. Market research has reported that sales of frozen foods in China increased to a value of $15.03 billion in 2008, which represents a 140 % increase in value since 2002 (Access Asia Ltd 2009).

A number of advanced technologies have been applied to preserve fruits and vegetables such as microwave, ohmic heating, ultrasound, and pulsed electric field, as reported by Barrett and Lloyd (2012), Duan et al. (2010), Mothibe et al. (2011) and Zhang et al. (2006). Ultrasound technology is a nondestructive, fast, and reliable technique used in the food industry for extending shelf life (Mizrach 2008). Ultrasound technology is being applied recently along with conventional food processing technologies (Jambrak et al. 2007; Deng and Zhao 2008a, b; Soria et al.

2010; Mothibe et al. 2011; Rawson et al. 2011; Kek et al. 2013; Lagnika et al. 2013; Islam et al. 2015b; Xin et al. 2014). Knorr et al. (2004) mentioned that ultrasound technology, especially ultrasound-assisted thermal treatment (UST), has promising effects in food preservation and product modification. These emerging technologies are also being applied in the freezing sector. Sanz et al. (1999) introduced high pressure assisted freezing, which is based on lowering the melting point of water with the help of pressure and allowing uniform nucleation of ice in the product. Li and Sun (2002b) also suggested that high pressure freezing and dehydrofreezing are novel methods for preserving fruits and vegetables. As a novel technology, ultrasound has been introduced to facilitate the freezing process for the last three decades. Mason et al. (1996) and Islam, Zhang et al. (2014b) reported that ultrasound reduces freezing time and enhances freezing efficiency. Acton and Morris (1997) reported that if a liquid mass is subjected to ultrasound, nucleation, and crystal growth during solidification of liquid can be altered.

Kennedy (2003) reported that crystallization of water in the freezing process is one of the key issues in maintaining shelf life and quality of frozen foods. The crystallization process and formation of ice crystals is driven by the freezing rate. Delgado and Sun (2008), Delgado et al. (2009), Islam et al. (2014b) and Li and Sun (2002a) reported that the application of ultrasound significantly enhances freezing rate and impacts the way fruits and vegetables are frozen. It has been proven that ultrasound-assisted freezing is a novel technology in terms of crystallization and recrystallization (Kiani and Sun 2011; Petzold and Aguilera 2009; Zheng and Sun 2006; Feng et al. 2011).

In the context presented above, this chapter presents an overview of recent advances in ultrasound-assisted freezing technology of fruits and vegetables. Mathematical models applied to quantify freezing and thawing time in an ultrasound-assisted freezing system are also presented in considerable detail. In addition, the structure–function aspects of ultrasonic transducers used to generate power ultrasound have also been illustrated.

22.2 Sonication

The benefit of application of ultrasound energy in various physical and processing systems has been continuously investigated since the 1970s. The application of ultrasound in processing systems such as emulsification, homogenization, extraction, crystallization, dewatering, pasteurization, degassing, foaming, activation/inactivation of enzymes, particle size reduction, and alteration in viscosity has been reviewed (Cheng et al. 2015; Islam et al. 2014a; Patist and Bates 2008). Leadley and Williams (2006) reported a number of processes that can be greatly enhanced by power ultrasound, such as crystallization of sugar solutions, hardening of fats, and the rate of production of chocolate and margarine. Ultrasound plays an important role in food technology in terms of processing, preservation, and extraction (Chemat et al. 2011).

Ultrasound is defined as a sound wave having frequencies higher than those that are audible to human ear (>16 kHz). These ultrasound waves can be broadly classified into low frequency and high frequency ultrasound. High frequency ultrasound operates at a frequency range of 2–20 MHz and a sound intensity range of 0.1–1 W/cm^2. This high frequency ultrasound finds its application in food quality analysis, medical imaging, and non-destructive inspection. Low frequency ultrasound that operates at a frequency range of 20–100 kHz and at a power intensity range of 10–1000 W/cm^2 is known as power ultrasound. Power ultrasound is more commonly used in food processing operations than high frequency ultrasound.

Lorimer et al. (1991) introduced a model to determine power ultrasound. In this model, P_{in} is the actual power consumed by the generator, which can be determined directly by using a high precision Wattmeter (W) in the input line. P_{diss} is the power dissipated in or delivered to the treatment vessel. This parameter can be measured by noting the rise in temperature in a batch treatment vessel. This power can be estimated using Eq. (22.1), given below.

$$P_{diss} = mC_p \frac{dT}{dt} \tag{22.1}$$

where m is mass of liquid (kg), C_p is the specific heat capacity of the liquid (J/kg/K), and (dT/dt) is the initial slope (K/s) of the temperature versus time curve measured within the first 30 s of sonication. Another parameter P_{rad}, which is the fraction of P_{diss}, used to form cavitation-induced free radicals, can be determined by chemical dosimetry (Contamine et al. 1995; Kimura et al. 1996).

Ultrasound frequencies ranging from 20 to 100 kHz are used in chemically important systems in which chemical and physical changes are desired (Hall et al. 2000; Ruecroft et al. 2005). A number of compression and rarefaction cycles occur when ultrasound passes through a medium. Cavitation bubbles are created when rarefaction exceeds the attractive forces among molecules in a liquid phase at high power condition (Soria and Villamiel 2010). The ultrasonic cavitation system is shown in Fig. 22.1.

When ultrasound is applied to cells, they undergo rapid contraction and expansion which produces a sponge effect. Gallego-Juárez et al. (2007) reported that the rate of moisture transfer (migration) was faster in vegetables subjected to power ultrasound. Natural pores were enlarged and new pores were created due to the rapid series of contractions and expansions caused by ultrasound propagation. Enlargement of existing pore size and creation of new pores resulted in faster moisture release from the product.

Generation of power ultrasound can be achieved by using various transducers. Mason and Povey (1998) mentioned the availability of liquid driven, magnetostrictive, and piezoelectric transducers. Among these transducers, magnetostrictive transducers were the first to be used on an industrial scale to produce power ultrasound. Most power ultrasound applications use piezoelectric or magnetostrictive transducers, as these are liquid driven and have additional advantages in homogenization and mixing (Mason and Povey 1998; Knorr et al. 2004).

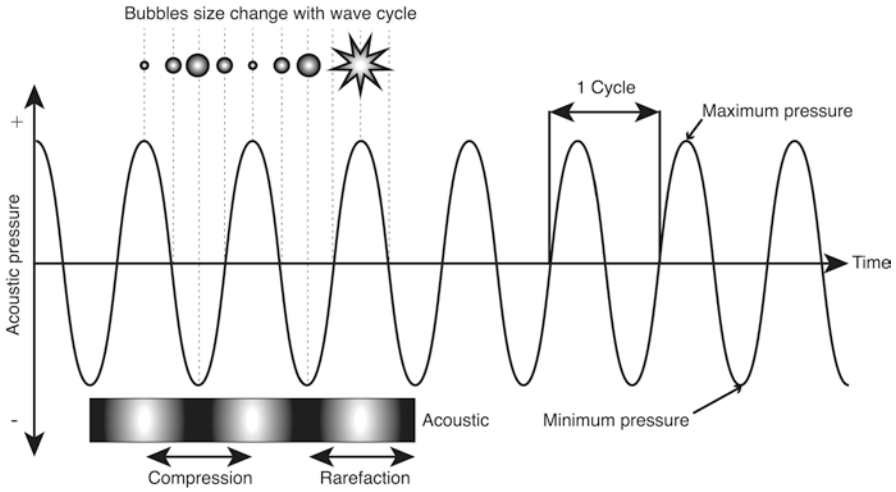

Fig. 22.1 Ultrasonic cavitation adapted from Soria and Villamiel (2010)

22.2.1 Ultrasonic Probe

Ultrasonic probes are used to generate power ultrasound. In a probe system, the transducer is attached to one or more metal shaped horns and used to convert AC power into mechanical vibration. A conveying probe delivers this mechanical vibration to the medium to which power ultrasound is being applied. A schematic diagram of an ultrasonic probe is presented in Fig. 22.2. The horn is usually constructed using titanium, aluminum, or steel, and may take the shape of a rod, plate, bar, or sphere (Zhang et al. 2011). The shape and dimension of the horn is very important, as they are responsible for creating movement of the horn tips in correct amplitude. A stepped horn is easy to design and offers the highest amplitude. In a cylindrical horn, there is no amplitude gain. When the diameter of a cylindrical horn exceeds 102 mm, it usually requires slots to reduce superfluous resonance (Keil Frerich and Swamy Kodavanti 1999; Mitchell et al. 1996).

The probe system has some drawbacks such as high probability of contamination by metal ions or particles and free radicals formed during the treatment (Zhang et al. 2011). An ultrasonic probe is suitable in pretreatment processes such as ultrasonic blanching. It has also been widely used in laboratory scale research work.

22.2.2 Piezoelectric Transducer

A piezoelectric transducer is the most commonly used transducer (Mason and Povey 1998; Aleixo et al. 2004; Gallego-Juárez et al. 2003) in which the immersion of the transducer in the treatment medium is required. A schematic diagram of a

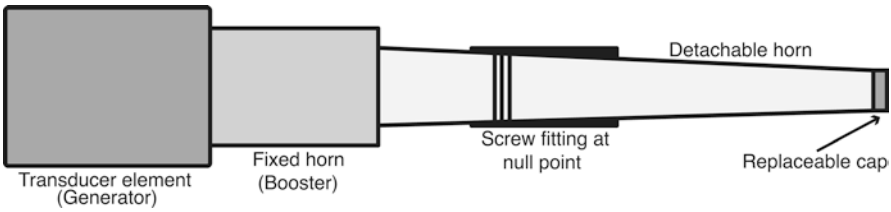

Fig. 22.2 Ultrasonic probe adapted from Mason (1998)

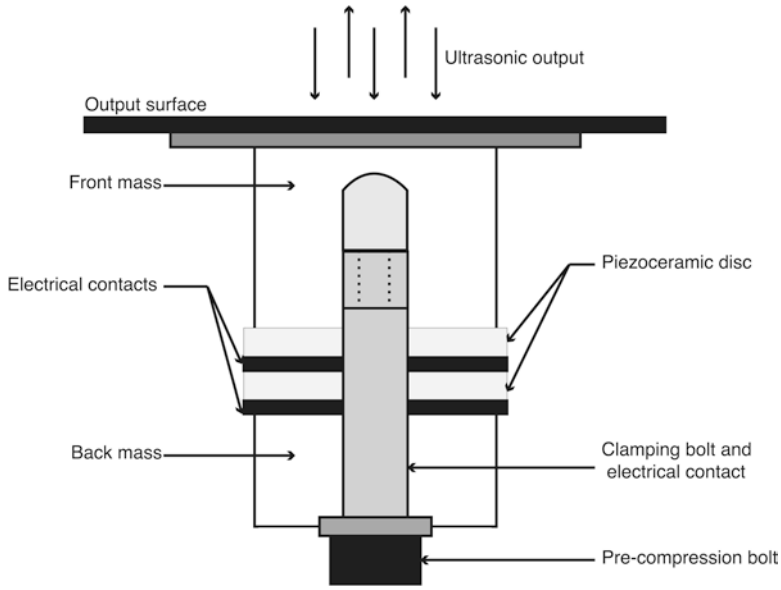

Fig. 22.3 Piezoelectric transducer

piezoelectric transducer is presented in Fig. 22.3. It consists of a single or double layer thick piezoelectric ceramic disk made of piezoelectric materials such as barium titanate ($BaTiO_3$), lead metaniobate ($PbNb_2O_6$), and the compound of lead zirconate and titanate (Mason and Lorimer 2003; Keil Frerich and Swamy Kodavanti 1999). Piezoelectric ceramics can be used in pairs as a composite in order to achieve additional efficiency. With changes in the polarity of the oscillating voltage, the ceramic assembly expands or contracts and thus sound waves are generated due to physical displacement.

Compared to other transducers, piezoelectric transducers function in a relatively wider ultrasonic range and offer higher output (Hamonic and Decarpigny 1988). Moreover, piezoelectric transducers are relatively inexpensive, smaller in size, lighter in weight, durable, and have better efficiency (about 95 %) (Thompson and Doraiswamy 1999). The output of piezoelectric transducers varies with the variation in temperature, depending on the ceramic composite. These transducers work well

within a humidity range of 35–85 %. Piezoelectric transducers are suitable for use in ultrasound-assisted freezing systems, especially in cryogenic and immersion freezing.

22.2.3 Magnetostrictive Transducer

A magnetostrictive transducer is a special type of solenoid in which a laminated metal or alloy forms the core and the copper wires are wound or coiled around the core (Mason and Lorimer 2003). This transducer is more suitable in frequencies higher than 100 kHz. A schematic diagram of a magnetostrictive transducer is presented in Fig. 22.4. In this kind of transducer, ferromagnetic materials such as nickel are used as construction materials. This is because the ferromagnetic materials expand when a magnetic field is applied, and return to normal size (contract) when the magnetic field is removed. When the alternating electric current is applied, the wrapped magnetostrictive materials generate an alternating magnetic field. This magnetic field causes the expansion and contraction of the magnetostrictive material, ultimately producing an ultrasound wave (Zhang et al. 2011).

Here, the energy conversion process occurs in three steps. Electrical energy is converted into magnetic energy in the first step. In the second step, this magnetic

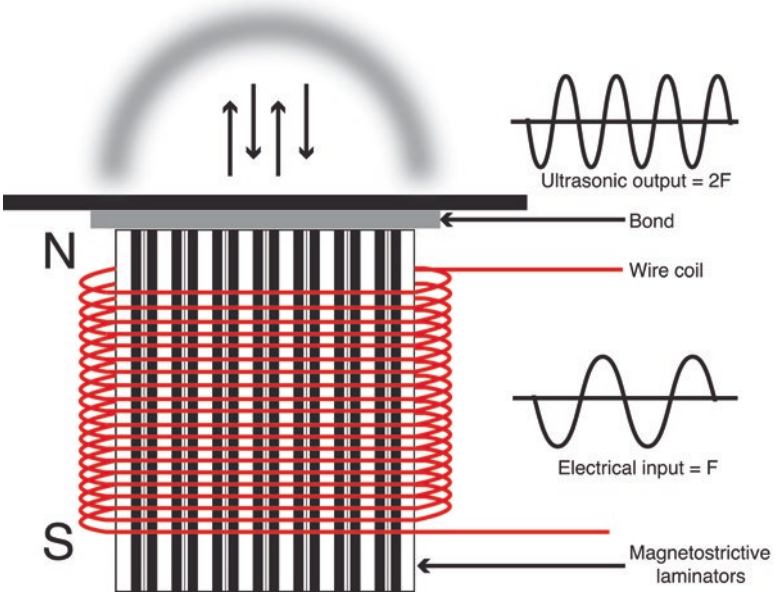

Fig. 22.4 Magnetostrictive transducer adapted from Fuchs (1999)

energy is converted into mechanical energy. Finally, the mechanical energy is converted to sound energy in the third step. About one half of the applied energy is lost due to this energy conversion process. However, the ultrasonic energy generated from this type of transducer is sufficient to be used in power ultrasound applications.

22.2.4 Stepped Plate Transducer

One of the most industrially suitable ultrasonic transducers is the stepped plate transducer. This transducer is also known as an airborne power ultrasound transducer, designed by Gallego-Juarez et al. (1978). This transducer consists of a circular flexible stepped shape vibration plate driven from its center by a piezoelectric vibrator. A schematic diagram of a stepped plate transducer is presented in Fig. 22.5. As the density of air is low, it thus presents very low specific acoustic impedance and high acoustic absorption. Therefore, to achieve satisfactory airborne power ultrasound, these transducers must generate very efficient ultrasound transmission (Zheng and Sun 2005). The stepped plate transducer based airborne power ultrasound systems are very efficient and powerful. Thus, this kind of transducer is expected to be more suitable in ultrasound systems used in air blast and fluidized bed freezers.

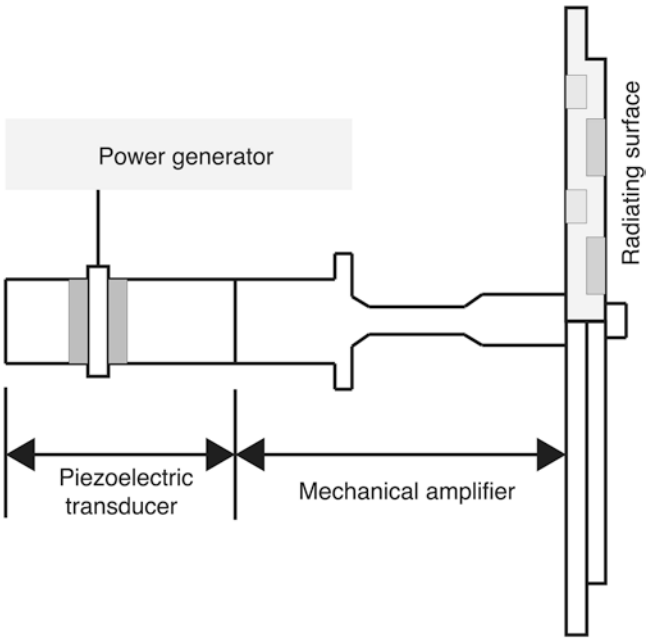

Fig. 22.5 Stepped plate transducer adapted from Gallego-Juarez (1988)

22.3 Freezing of Fruits and Vegetables

Freezing of fruits and vegetables involves a number of preprocessing or pretreatment operations. Figure 22.6 illustrates one example of such pretreatment operations. Freezing of fruits and vegetables is superior to canning and dehydration with respect to retention of sensory attributes and nutritive properties (Fennema 1977). Prochaska et al. (2000) reported that freezing of fruits and vegetables is the simplest method for preserving fresh-like quality. A study conducted by Bahçeci et al. (2005) showed that the freezing process alone cannot completely stop the activity of peroxidase (POD) and lipoxygenase (LOX), which are responsible for off flavor, off odor, and loss of nutrients.

Blanching of fruits and vegetables is a common pretreatment before freezing. Lin and Brewer (2005) studied the effect of microwave blanching on frozen peas. Results from this study showed that 97 % of peroxidase load was reduced in the treated product compared to that in the control. Gonçalves et al. (2011) reported that blanching of broccoli at −70 °C for 6.5 min before freezing significantly retained its characteristic green color during freezing. It was also found that the shelf life, color, aroma, and taste of frozen mushrooms can be significantly extended when blanching preceded freezing. For example, shelf life was extended from 4 to 12 months (Jaworska and Bernaś 2009). However, it is commonly accepted that blanching causes significant loss in ascorbic acid in fruits and vegetables (Abdel-Kader 1990; Howard et al. 1999).

The texture of fruits and vegetables after freezing is very important in terms of product quality. High temperature short time blanching followed by freezing at a rate of 4.5 °C/min has been reported as the optimal condition for improvement of textural quality (Roy et al. 2001). The effect of blanching, pretreatment, and frozen storage of mushrooms has been studied by Jaworska and Bernaś (2010). Results of this study showed that unbleached frozen mushrooms had less springiness,

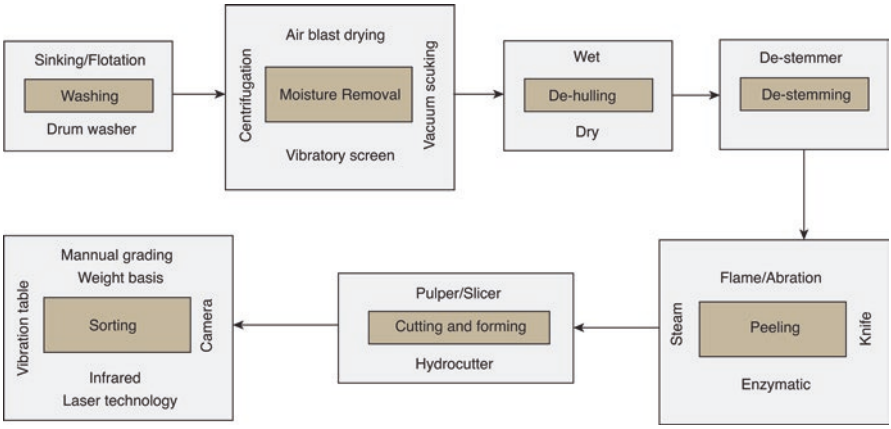

Fig. 22.6 Preprocessing operation of fruits and vegetables during freezing

chewiness, and gumminess compared to those of blanched mushrooms. Pectic polysaccharides, which are abundantly found in the primary cell wall and the middle-lamella between cells, are primarily responsible for most of the texture of fruits and vegetables.

The effect of freezing on the nutrients of fruits and vegetables has been studied by many researchers. A comparative study on the status of nutrients and minerals between frozen and canned fruits and vegetables was carried out by Rickman et al. (2007a) and Rickman et al. (2007b). Vitamin C content in frozen homogenates of raw fruits and vegetables was found to be stable for some period of time (No significant change in Vit C content of clementines over a year while potatoes and collard greens showed a cumulative decrease after 1 month) (Phillips et al. 2010). Gonçalves et al. (2011), Sikora et al. (2008) and Gębczyński and Lisiewska (2006) reported the retention of higher vitamin C content in frozen broccoli compared to that in the unfrozen control. Fruits and vegetables are good source of naturally occurring folate, primarily 5-methyltetrahydrofolate (5MTHF) (Konings et al. 2001; Vahteristo et al. 1997). 5MTHF is the most bioactive form of folate (Muller 1993). Phillips et al. (2005) reported that there was no loss of 5MTHF content in frozen fruits and vegetables during a 12 month storage period.

Slow freezing does not affect physicochemical reactions (George 1993). However, slow freezing results in the formation of large extracellular ice crystals. The higher proportion of large crystals in frozen fruits and vegetables increases the concentration of solutes; therefore, cell dehydration, membrane damage and cellular death occur due to osmotic plasmolysis (Cheftel et al. 2000). Thawing of frozen fruits and vegetables is a slower process compared to freezing. During the thawing process, extracellular ice does not reenter the cells, hence increasing the probability of extensive drip and softening of the texture (Cheftel et al. 2000). Thus, quick freezing of fruits and vegetables is preferable (Otero et al. 2000).

22.4 Ultrasound-Assisted Freezing

Ultrasound-assisted freezing can help overcome some of the problems encountered during normal freezing (Islam et al. 2014b; Cheng et al. 2014a, c, d; Xin et al. 2014). As ultrasound is capable of inactivating a wide range of enzymes (O'Donnell et al. 2010; Cheng et al. 2013; Islam et al. 2014a), ultrasound-assisted freezing will help to inactivate undesirable enzymes during freezing. This process eliminates the requirement for separate blanching pretreatment (Islam et al. 2014b). It has also been shown that ultrasound destroys microorganisms (Piyasena et al. 2003), thus minimizing the need for a separate pasteurization step. It has also been found that the application of ultrasound affects the texture profile during freezing. Xiaoyan. Li et al. (2011) studied the effect of power ultrasound on texture change of shrimp and found that the hardness increased due to application of power ultrasound. Also, cavitation and the sponge/interface effect of ultrasound

waves enhances mass transfer and freezing rates, thus improving the quality of frozen products.

Application of ultrasound during freezing affects every process/mechanism involved in freezing. The ensuing section discusses the main effects of ultrasound on the freezing process.

22.4.1 Crystallization Process

In the freezing process crystallization occurs due to supercooling or supersaturation. The crystallization process consists of two stages, i.e., formation of nuclei followed by the growth of the nuclei into crystals. Petzold and Aguilera (2009) reported that the crystallization process consists of three phenomena: nucleation, crystallization, and recrystallization.

The crystallization process is very important for freezing of fruits and vegetables (Kiani and Sun 2011; Islam et al. 2015a). The understanding of parameters such as crystal size distribution, crystal location and crystal morphology greatly helps to improve the freezing process. It has been shown that the power ultrasound is very useful in the crystallization process (Mason 1998; Acton and Morris 1997). Transmission of power ultrasound in a liquid medium causes cavitation (Ashokkumar and Grieser 1999; Zheng and Sun 2005); gas bubbles formed due to cavitation serve as nuclei for ice nucleation (Mason et al. 1996).

The effect of ultrasound on crystallization of water has been studied in considerable detail, including the effect of ultrasonic power, intensity, frequency, horn size, solution volume and duration on the crystallization process (Sigfusson et al. 2004; Luque de Castro and Priego-Capote 2007). A thorough review of literature on the crystallization of water in ultrasound-assisted freezing process has been made by Kiani and Sun (2011). All of the above studies suggest that the application of ultrasound-assisted freezing helps improve the crystallization of water in fruits and vegetables.

22.4.2 Nucleation

Nucleation is the process of formation of a new ice crystal. There are two kinds of nucleation, primary and secondary, or contact nucleation. When the formation of ice crystal occurs in a crystal free solution, it is known as primary nucleation, whereas when the ice crystal is formed in an existing crystal, it is called secondary nucleation. Chow et al. (2005) reported that nucleation occurs in a temperature range of 0 °C to −40 °C where supercooling occurs.

Ultrasound-assisted nucleation process in a number of model food such as sugar agar and mushroom was studied by Kiani et al. (2011), Kiani and Sun

(2011), Islam et al. (2015a) and (Islam et al. 2014b). Chow et al. (2003) and Fennema et al. (1973) reported the homogeneous and heterogeneous nucleation taking place in crystal-free and preexisting crystal systems, respectively. The effect of frequency (25 kHz) of power ultrasound on the nucleation process has been studied and found to be positive (Sun and Li 2003; Li and Sun 2002a; Zheng and Sun 2005). It has also been shown that power ultrasound accelerates the industrial nucleation process (Ruecroft et al. 2005; Saclier et al. 2010). Islam et al. (2014b) reported that Ultrasound at 0.39 W/cm^2 (20 kHz) reduced nucleation time by 24 %, 53 % and 34 % in *Lentinula edodes*, *Agaricus bisporus*, and *Pleurotus eryngii* respectively.

22.4.3 Crystal Growth

Crystal growth is the accumulation of mass of crystallizing components on a crystal. Crystal growth consists of two mechanisms or steps. The first mechanism involves diffusion of solid molecules from bulk to the surface of the growing crystal. The second mechanism or step involves incorporation of a growth unit into the crystal lattice (Sheere et al. 2004). The cavitation effect of ultrasound assists the bulk-phase mass transfer of solute to the surface of the growing crystal (Luque de Castro and Priego-Capote 2007).

The effect of ultrasound on crystal growth was investigated by Kapustin (1963), Arakelyan (1987) and Ruecroft et al. (2005). Results from these studies showed that the rate of crystal growth depends on the magnitude of supersaturation. Also the process of sonocrystallization has been studied by visualization method (Islam et al. 2015a; Chow et al. 2003, 2004; Christensen et al. 2005). At lower supersaturation, the application of ultrasound can increase growth rate by twofold, whereas at higher supersaturation, ultrasound does not show any effect on growth rate. One possible explanation for the former effect is that the quantity of available growth units in the vicinity of the crystal surface is small during lower supersaturation. Thus, there is diffusion limited bulk-phase mass transfer in supplying growth units to the crystal surface (Ruecroft et al. 2005; Luque de Castro and Priego-Capote 2007). The application of ultrasound enhances bulk phase mass diffusion, and hence the rate of crystal growth improves greatly.

22.4.4 Distribution of Ice Crystals

The size and size distribution of ice crystals greatly affects the quality of frozen fruits and vegetables. Larger crystals are implicated in the damage of cell structure. Slow freezing leads to the formation of large ice crystals, which occurs exclusively in the extracellular areas. Higher freezing rates produce small ice crystals evenly distributed across the tissue (Partman 1975; Persson and Lohndal 1993). The

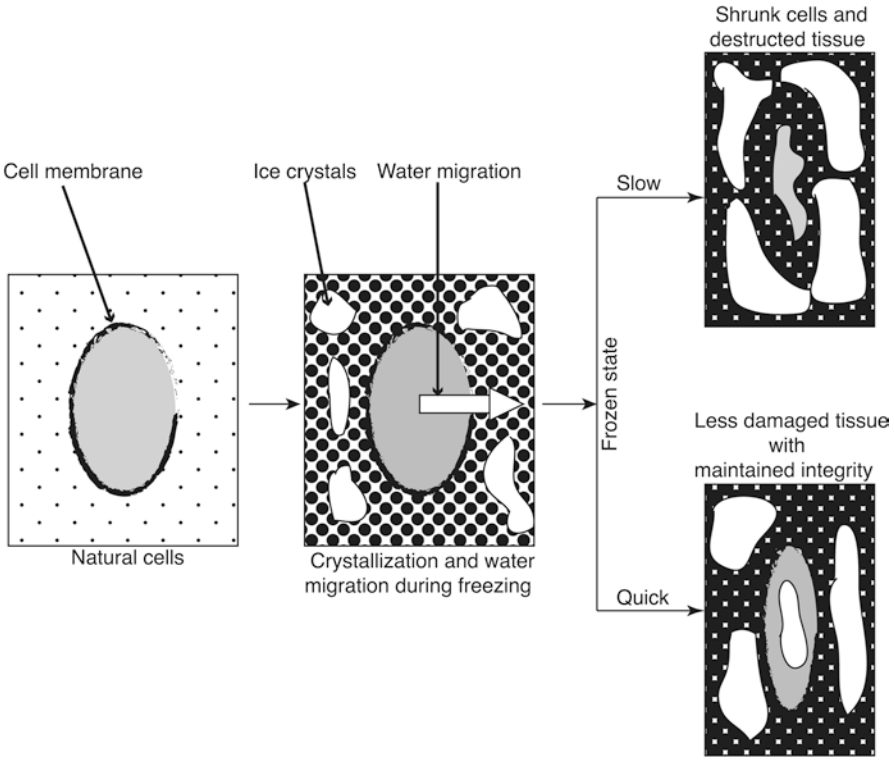

Fig. 22.7 Quality affected by rate of freezing adapted from Fikiin (2009)

process of formation of ice crystals in both slow and quick freezing is presented in Fig. 22.7. It is clear that quick freezing is preferred, as it preferentially produces small ice crystals and promotes better nucleation (Otero et al. 2000).

Factors such as nucleation, freezing rate and final temperature of the freezing process are responsible for the size and size distribution of ice crystals, which ultimately affect the color, texture, drip loss of frozen products (Norton et al. 2009; Martino et al. 1998). Inada et al. (2001) showed that ultrasonic vibration can actively control supercooling of water, which is a critical issue in cold-energy storage and transport systems. Ultrasonic vibrations strongly affect the phase transition from supercooled water to ice.

Ultrasonic power affects the size and size distribution of ice crystals. Acoustic stress of power ultrasound reduces the size of ice crystals during the crystal growth phase. For example, Gareth (1992) reported that smaller and evenly distributed ice crystals were formed during ultrasound-assisted production of ice lollypops. Similar results have been reported by Kiani et al. (2013), for solid model food (frozen agar gel) and by Islam et al. (2015a), for ultrasound-assisted frozen mushroom.

22.4.5 Effect of Ultrasound on Cell Structure

During ultrasound-assisted freezing, the cavitation effect helps to form uniform bubbles. Lepeschkin and Goldman (1952) first studied the effect of ultrasound on cell structure. Ultrasound waves were found to create microscopic channels in porous materials such as fruits due to sponge effect (De la Fuente-Blanco et al. 2006; Tarleton 1992; Fernandes et al. 2008, 2009; Tarleton and Wakeman 1998; Islam et al. 2015b).

Power ultrasound significantly affects the microstructure of frozen products (Islam et al. 2014b; Xin et al. 2013, 2014). Sun and Li (2003) reported better cellular structure in potatoes that were frozen using an ultrasound-assisted freezer. They used Cryo-SEM, which showed less extracellular void and cell disruption/breakage in the structure of the frozen potatoes. Fast freezing assisted by power ultrasound (Li and Sun 2002a), absence of intracellular nucleation caused by cavitation bubbles and reduced supercooling all help to preserve the microstructure of frozen products (Powrie 1973). Fernandes et al. (2008) and Fernandes et al. (2009) studied the effects of ultrasound on the cell structure of melon and pineapple. The results from this study showed that the application of ultrasound during freezing led to the formation of microscopic channels in the cell structure. Application of ultrasound reduces adhesion among cells, which leads to an increase in cell interspaces, which ultimately results in an increase in diffusion of water. Thus, ultrasound-assisted freezing helps to better preserve the quality of frozen products.

22.4.6 Effect of Ultrasound on Freezing Rate

The freezing rate of fruits and vegetables is influenced by ultrasonic power, intensity, pulsed or intermittent application, frequency and duration of application (Delgado and Sun 2008). It has also been shown that the application of ultrasound during freezing increases the heat transfer coefficient (Miles et al. 1999; Sastry et al. 1989).

Li and Sun (2002a) investigated the effect of power ultrasound (frequency 25 kHz, power 7.34, 15.85, and 25.89 W applied for 2 min) on the freezing rate of potato. Results from this study showed that freezing time was affected by ultrasonic power, exposure time and the freezing phase to which ultrasound was applied. Islam et al. (2014b) reported that overall up to 40 % reduction in total freezing time was observed in ultrasound-assisted freezing of mushrooms. Similar results have been reported by Comandini et al. (2013) and Hu et al. (2013). A reduction in the characteristic freezing time was observed when ultrasound at 25 kHz was applied during freezing of potato (Delgado and Sun 2008). The freezing rate of apple was enhanced when ultrasonic power level increased from 131 to 145.8 W at 40 kHz, whereas no significant enhancement of freezing time at 25 kHz and power level of 66.8 W was observed. The results were significant at the power level of 145.8 W (0.26 W/cm^2) (Delgado and Sun 2008).

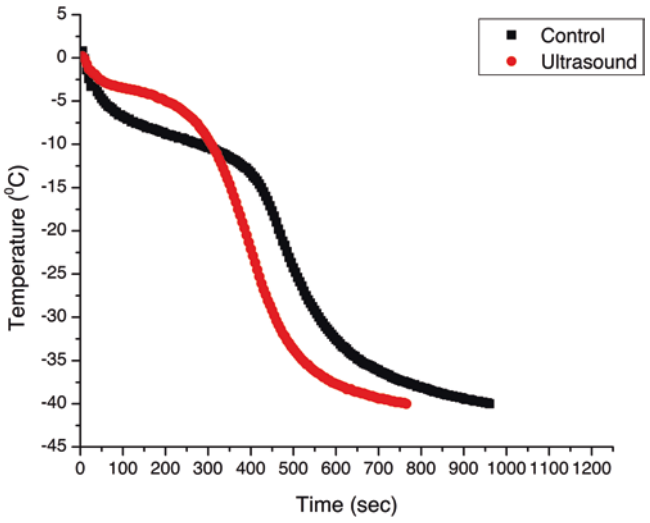

Fig. 22.8 Effect of ultrasound on freezing rate

Freezing rate affected by ultrasound-assisted immersion freezing of apple has been studied (Delgado et al. 2009). Results showed that an improvement in the characteristic freezing time up to 8 % was achieved due to the application of ultrasound. Figure 22.8 shows a trend of the ultrasound-assisted freezing rate.

22.5 Modeling of Freezing and Thawing Time

22.5.1 Freezing Time

Prediction of accurate freezing time is necessary to assess the quality, processing requirements, and economical aspects of food freezing. It has been reported by Pham (2001) that to ensure effective freezing, calculation of processing time, product temperature, heat load and water diffusion into and out of the product is very important. All of these parameters are influenced by operating process conditions and product characteristics. As the thermal properties of foods continuously change during freezing, it is very difficult to find an exact mathematical model to predict freezing of foods, although a number of models have been proposed in the literature for this purpose (Gauri 2005; Cleland 1990; Heldman et al. 1981; Ilicali and Saglam 1987; Pham 2006; Salvadori and Mascheroni 1991; Viviana 2012).

Hossain et al. (1992c) defined freezing time as the time to reach a particular temperature at the slowest cooling point. Time required to reach a temperature of −10 °C below the initial freezing point at the thermal center is the nominal freezing time for a given product, with a specified dimension and uniform initial temperature

of 0 °C. The time required to lower the product temperature from its initial value to a given final temperature at the thermal center is called effective freezing time (International Institute of Refrigeration 1972; Eek 1991; Delgado and Sun 2001).

The Plank equation as represented by Eq. (22.2) is one of the simplest equations to predict freezing time (Plank 1913; Delgado and Sun 2001; Viviana 2012).

$$t_{\text{Plank}} = \frac{\rho L}{T_f T_\infty}\left[\frac{PR}{h} + \frac{QR^2}{k_f}\right] \tag{22.2}$$

where P and R are constants which depend on geometry; P is equal to 0.5, 0.25, and 0.166 for slab, infinite cylinder, and sphere, respectively. R is equal to $P/4$. ρ (kg/m^3), T_f (K), T_α (K), L (J/m^3), h (W/m^{2}K), k_f (W/mK) represent the density, initial freezing temperature, ambient temperature, latent heat of freezing, heat transfer coefficient, thermal conductivity, respectively.

The dimensionless form of Plank's equation is represented by Eq. (22.3).

$$t_{\text{Plank}} = \frac{P}{Bi\,Ste} + \frac{Q}{Ste} \tag{22.3}$$

where P and Q are the shape factors. Bi (hR/k) and Ste represent Biot and Stefan numbers, respectively (Delgado and Sun 2001).

Cleland and Earle (1977, 1979a, 1979b, 1984a) were among the first authors who took the initiative to establish empirical models to describe freezing time. Cleland and Earle's (1984a) equation can describe the freezing time for three basic shapes (infinite slabs, infinite cylinders, and spheres), as represented by Eq. (22.4).

$$t_F = \frac{\rho\ H_{\text{ref}}}{E\left(T_f - T_\infty\right)}\left(\frac{2P_1R}{h} + \frac{4P_2R^2}{k_f}\right)\left[1 - \frac{1.65Ste}{k_f}\ln\left(\frac{T_c - T_\infty}{T_{\text{ref}} - T_\infty}\right)\right] \tag{22.4}$$

where Stefan number, $Ste = C_f(T_f - T_\alpha)/H_{\text{ref}}$; Plank's number, $Pk = C_u(T_i - T_f)/H_{\text{ref}}$; $P_1 = 0.5[1.026 + 0.5808Pk + Ste(0.2296Pk + 0.1050)]$; and $P_2 = 0.125[1.202 + Ste(3.41Pk + 0.7336)]$. C_u and C_f (J/kg/K) are the specific heat capacity before and after freezing, respectively. E is the shape factor (1 for slabs, 2 for infinite cylinders, 3 for spheres). Pk and Ste are dimensionless numbers that express the magnitude of precooling and postcooling effects, respectively. T_{ref} (°C) is the reference temperature and H_{ref} (kJ/kg) is enthalpy change in the temperature range of T_∞–T_f (°C). T_c (°C) is the temperature of the frozen product and T_i (°C) represents the initial product temperature.

Hossain et al. (1992a); Hossain et al. (1992b, 1992c) reported that the modified Plank's equation developed by Pham (Pham 1984, 1986) best fits the experimental data for materials having complex shapes when appropriate shape factors are used. The Pham (1986) equation (Eq. 22.5) for calculation of freezing time has

introduced terms such as precooling time $\Delta H_1/\Delta HT_1$, phase change-post cooling time $\Delta H_2/\Delta HT_2$, and Biot number *Bi*.

$$t_F = \frac{R}{Eh}\left(\frac{H_1}{T_1} + \frac{H_2}{T_2}\right)\left(1 + \frac{Bi}{2}\right) \tag{22.5}$$

where $\Delta H_1 = H_i - H_{fm} = \rho_u C_u (T_i - T_{fm})$; $\Delta H_1 = H_{fm} - H_c = \rho_f [L + C_f (T_{fm} - T_c)]$; $\Delta T_1 = (T_i + T_{fm})/2 - T_\alpha$; $\Delta T_2 = T_{fm} - T_\alpha$ and the mean freezing time, $t_{fm} = 1.8 + 0.263 T_c + 0.105 T_\alpha$; ρ_u and ρ_f (kg/m^3) represent density before and after freezing, respectively (Donald and Kenneth 1997; Pham 2008).

22.5.2 Thawing Time

Prediction of thawing time is very important in industrial freezing practices. It is necessary to realistically predict the thawing time for food products having different shapes. A considerable body of work has been published dealing with the prediction of thawing time for food products having simple as well as complex shapes (Ilicali 1989; Campañone and Zaritzky 2010; Cleland et al. 1986a, b, 1987; Salvadori and Mascheroni 1991; Lind 1991).

Gauri (2005, 2011) reported a power law approach to predict thawing time, which is a modification of Plank's equation proposed by Calvelo (1981) and Cleland (1990). The power law equation used for prediction of thawing time prediction is represented by Eq. (22.6), given below.

$$t_T = \frac{1.4921 \rho C_u a^2}{k_f}\left[\frac{0.5}{Bi\, Ste} + \frac{0.125}{Ste}\right]^{1.0248} Ste^{0.2712} Pk^{0.061} \tag{22.6}$$

where $Bi = ha/k_f$; $Ste = C_f(T_\alpha - T_f)/\Delta H_{ref}$; $Pk = C_u(T_f - T_i)/\Delta H_{ref}$ where ΔH_{ref} is the enthalpy change from T_f to T_α

An equation used to predict thawing time based on Plank's equation (Cleland and Earle 1984b; Gauri 2005) is represented by Eq. (22.7).

$$t_T = \frac{\rho C_u a^2}{k_f E}\left[\frac{P}{Bi\, Ste} + \frac{R}{Ste}\right] \tag{22.7}$$

where $P = 0.5[0.7754 + 2.2828\ Ste\ Pk]$; $R = 0.125[0.4271 + 2.1220 Ste - 1.4847 Ste^2]$; *E* and *a* represent shape factor and characteristic length, respectively.

Ultrasound-assisted freezing affects the microstructure, ice crystal size distribution, and also the freezing rate of fruits and vegetables. All of these parameters are expected to affect the rate and time of thawing (Cheng et al. 2014b). For instance, Islam, Zhang et al. (2014b) reported that ultrasound-assisted frozen mushrooms

required less thawing time than control samples. However, there are no mathematical models that are specifically used to predict the thawing time of food products which are frozen using ultrasound-assisted freezing systems.

22.6 Design of Ultrasound-Assisted Freezer

Air blast freezing, plate freezing, immersion freezing, cryogenic freezing, and their combinations are the most common methods used for food freezing (Barbosa-Cánovas et al. 2005; Da-Wen 2001; Fellows 2009). Donald and Kenneth (1997) compared commonly used freezers in terms of capital cost, fan/pump energy, overall operating cost, rate of freezing, unwrapped weight loss, relative size of facility, product size, product shape, and product types. Similarly, Fellows (2000) compared the performance of commonly used freezers in terms of heat transfer coefficient and freezing time.

While designing an ultrasound-assisted freezing system, it is essential to select an appropriate transducer, as discussed in sonication section of this chapter. In addition to the design aspects discussed in previous sections, it is essential to maintain the intensity of sound wave and proper distribution of the sound wave throughout the freezing conduit. Thus, it is essential to carry out rigorous analysis in order to be able to design and construct an ultrasound-assisted freezer. There is a general lack of literature dealing with the design of ultrasound-assisted freezers. Zheng and Sun (2005) and Zheng and Sun (2006) proposed the design of an ultrasound-assisted scraped surface freezer for ce cream manufacturing. In this section, we discuss the design aspects of a simple ultrasound-assisted freezer.

22.6.1 Air Blast Freezer

In an air blast freezer, food products are stacked into a tray (batch type) or into a movable trolley (continuous type). In the batch type, cold air (−30 °C to −40 °C) from the evaporator section of the freezer is circulated by means of fans at a speed of 1.5 m/s, either horizontally or vertically. In the continuous type of blast freezer, air can flow in co-current, countercurrent or in cross-flow modes. The moisture evaporates from food products due to sublimation, and the build-up of ice that takes place on the refrigeration coil requires frequent defrosting. Air blast freezers have the advantage of being low cost and delivering higher throughput. Air blast freezers can be used for a wide range of food products regardless of their type, size, and shape. A schematic diagram of an ultrasound-assisted air blast freezer is presented in Fig. 22.9. Here, products are stacked into trays or trolleys. Cold air from the evaporator is circulated through the commodities. Four sets of steeped plate transducers are placed at two opposite ends of the chamber. Ultrasonic power is transmitted through circulated air. The heat generated in the transducers is carried out by the forced cold air from the evaporator.

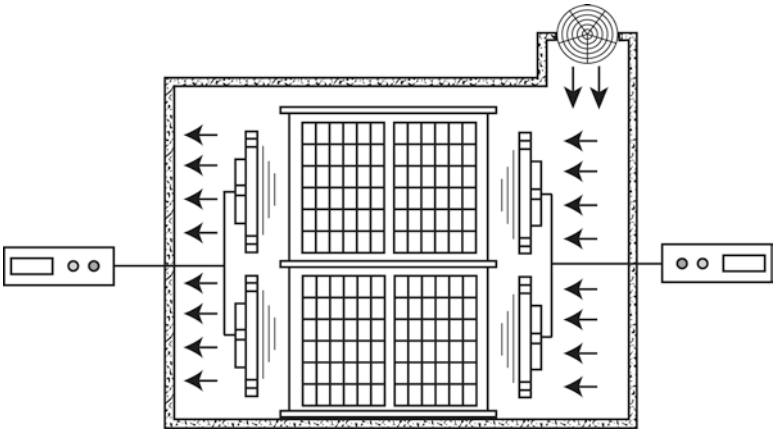

Fig. 22.9 Ultrasound-assisted freezing in air blast freezer

22.6.2 Fluidized Bed Freezer

A fluidized bed freezer is a modification of an air blast freezer, in which the commodities are placed on a perforated conveyor belt and the cold air is passed through the conveyor at a very high velocity. A fluidized bed freezer is best suited for fruits and vegetables such as peas, sweet corn kernels, strawberries or fried potatoes. The fluidized bed freezer requires less frequent defrosting compared to an air blast freezer, as less dehydration occurs in fluidized bed freezers. Additional advantages of the fluidized bed freezer include higher heat transfer coefficient, shorter freezing time, and higher production rate.

A schematic diagram of an ultrasound-assisted fluidized bed freezer is presented in Fig. 22.10. The fluidized bed freezer also uses steeped plate transducers to produce power ultrasound. The products are allowed to pass on a slightly inclined conveyor belt. Cold air is forced through the produce by means of fans. The loss of acoustic power in an ultrasound-assisted fluidized bed freezer is relatively low. A fluidized bed freezer has an advantage over air blast freezers, because the products in the former move continuously and the product is exposed to the sound wave more uniformly.

22.6.3 Plate Freezer

The plate freezer is the most common type of contact freezer. Food materials having regular and block shape are better suited for this type of freezer. Food materials are placed between two plates which are cooled by a circulating refrigerant. The plates can be arranged vertically or horizontally in the plate freezer, and pressure can be applied for good contact (Barbosa-Cánovas et al. 2005). Although plate freezers are capital intensive, they nevertheless have some distinct advantages (Donald and

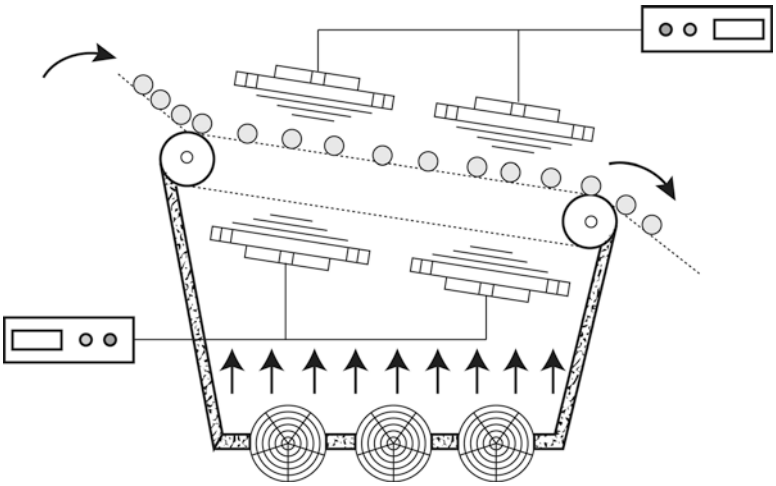

Fig. 22.10 Ultrasound-assisted freezing in fluidized bed freezer

Kenneth 1997) including high freezing rate, less defrosting, compact size and ease of transportation.

A schematic diagram of an ultrasound-assisted plate freezer is shown in Fig. 22.11. The ultrasonic transducer is attached to the plate, which is in contact with the refrigerant. Piezoelectric and magnetostrictive transducers can be used in this type of freezer. Products to be frozen are loaded and subjected to sound waves from both sides. Since the transducers are attached to the plate, the dissipated heat generated by the transducers is carried away or absorbed by the refrigerant. In this type of freezer, a certain amount of the acoustic power will be absorbed by the plate or by the refrigerant; thus, a certain amount of loss of acoustic power can be expected in this type of freezer.

22.6.4 Immersion Freezer

In this freezing system, fruits and vegetables are immersed into liquid coolant such as polyol, glycol, propylene glycol, and $CaCl_2$ solution. Delgado et al. (2009) studied the effect of ultrasound-assisted immersion freezing on apple. Due to the direct contact of food with the refrigerant, this type of freezer provides a high rate of heat transfer, fine ice crystals and high product throughput. Islam et al. (2014b) studied the physicochemical properties of mushrooms during ultrasound-assisted immersion freezing and reported that ultrasound-assisted immersion freezing is a suitable technology for freezing of mushrooms. Similar report has been made by Xin, Zhang et al. (2014), Xu et al. (2015b), Xu et al. (2014), Xu et al. (2015a), Cheng et al. (2014a), Cheng et al. (2014d) and Cheng et al. (2014c) for the freezing and frozen

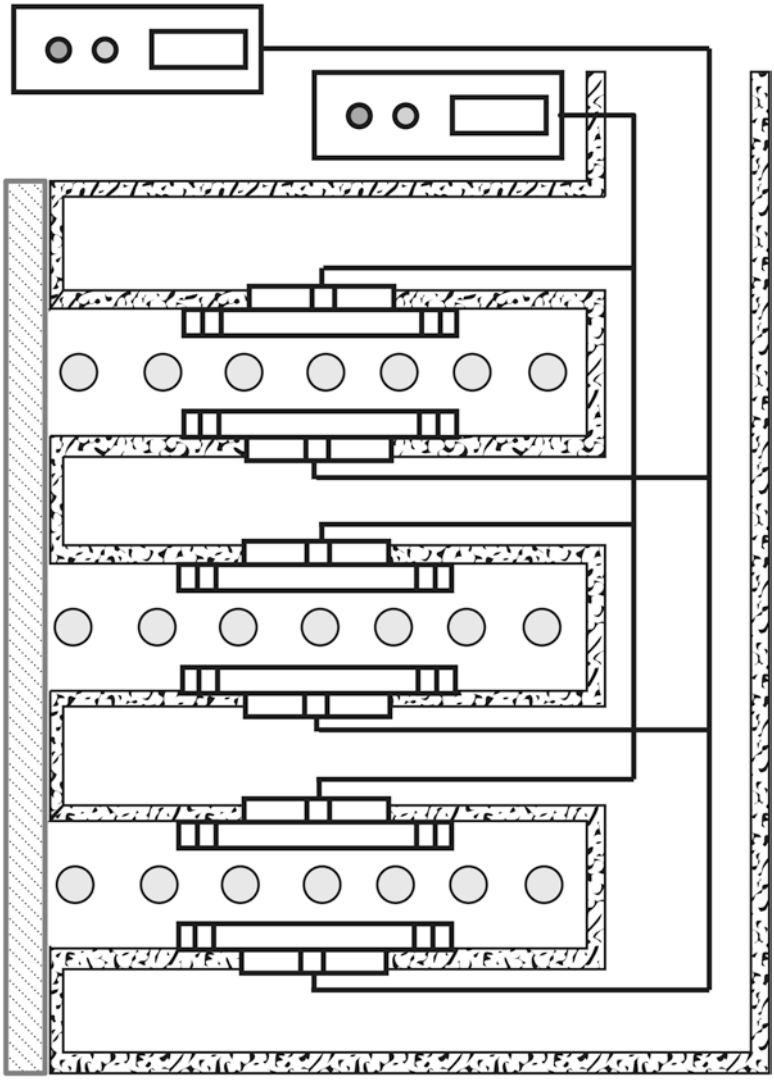

Fig. 22.11 Ultrasound-assisted freezing in plate freezer

storage different fruits and vegetables. In addition, instrumentation and operational costs are low in immersion type freezers (Tressler 1968; Fleshland and Magnussen 1990; Lucas and Raoult-Wack 1998). Agnelli and Mascheroni (2001) reported similar advantages when an immersion freezer was integrated combined with a common mechanical freezer. The application of immersion freezing is limited due to high viscosity at low temperature and also due to high organic contamination.

A schematic diagram of an ultrasound-assisted immersion freezer is presented in Fig. 22.12. Four sets of transducers are commonly used in immersion type freezers

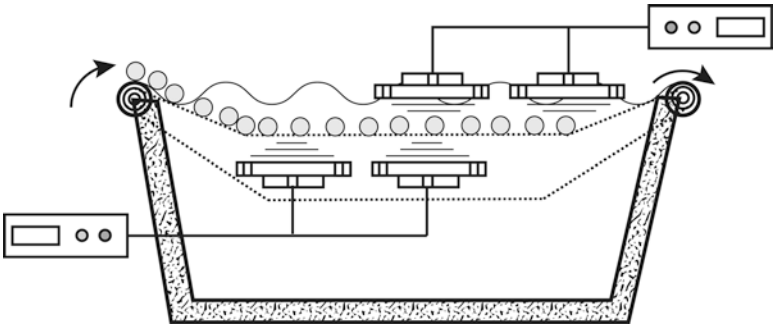

Fig. 22.12 Ultrasound-assisted freezing in immersion freezer

to provide sound waves. Islam et al. (2014b) presented a laboratory scale ultrasound-assisted immersion freezer where six sets of piezoelectric transducers were evenly attached to the bottom of the freezer cavity. In that model, samples were kept stable in the immersion fluid while immersion fluid was circulated by the aid of a low temperature circulation pump. Whereas, in the present model, food products pass through a conveyor belt which is immersed in immersion fluid and ultrasonic power transmitted through the liquid medium to the product.

22.6.5 Cryogenic Freezer

The cryogenic freezer was introduced to maximize product quality, and to minimize moisture loss and operating cost. In a cryogenic freezer, cryogenic liquids such as liquid carbon dioxide (boiling point −79 °C) or liquid nitrogen (boiling point −196 °C) are sprayed on food samples. Use of chlorofluorocarbon (CFC) is limited due to its negative impact on the environment. Food products pass through a belt, and cryogenic liquids are sprayed onto the foods at high pressure. Commodities such as fish fillets, seafood, fruits, and berries are suitable for this kind of freezer (Persson and Lohndal 1993; George 1993). The advantages of the cryogenic freezer are ease of operation, compact size, low cost of equipment, ease in installation and commissioning, mechanical simplicity, and low maintenance cost (Donald and Kenneth 1997).

A schematic diagram of an ultrasound-assisted cryogenic freezer is shown in Fig. 22.13. In this system four sets of airborne power ultrasound transducers are attached in opposite directions and cold air is supplied using motorized fans. Cryogens are sprayed onto the moving commodities. The nozzles are connected to the transducers in order to achieve better distribution of cryogenic liquid. When the fruits and vegetables are passing through the conveyor belt, they are subjected to both the liquid cryogens and also the ultrasound waves. The quality of fruits and vegetables frozen using this cryogenic freezing system is found to be better compared to the product quality in many other freezers.

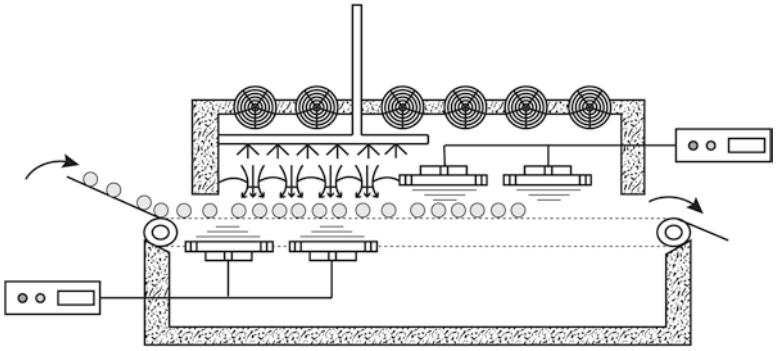

Fig. 22.13 Ultrasound-assisted freezing on cryogenic freezer adapted from McCormick and Zhai (2011)

22.7 Concluding Remarks

The demand for high quality frozen fruits and vegetables is steadily increasing. This is because it is not easy to supply seasonal fruits and vegetables throughout the year while maintaining their fresh-like quality. In this context, ultrasound-assisted freezing has advantages over conventional freezing systems in terms of faster crystallization and formation of smaller crystals, which are important quality parameters. Inactivation of enzymes and microbes can be achieved conveniently in ultrasound-assisted freezing systems. Freezing time is short, and throughput is high in ultrasound-assisted individual quick freezing of fruits and vegetables.

However, there is poor understanding of the thermodynamic process involved in ultrasound-assisted freezing. The effect of ultrasound-assisted freezing on cell structure, size distribution of ice crystals and other physiochemical properties of frozen fruits and vegetables have not yet been adequately studied. The factors involved in controlling crystal growth and recrystallization during ultrasound-assisted freezing require further investigation. Since ultrasound-assisted freezing is not applied on the industrial scale, more fundamental studies have to be undertaken on scaling up of ultrasound-assisted freezing technology to the industrial level. In addition, further development in design and functionality aspects of transducers and freezers must take place before this technology can achieve greater industrial acceptance.

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Chapter 23
Characterization of Microbial Inactivation by Microwave Heating

Pascale Gadonna-Widehem and Jean-Claude Laguerre

23.1 Introduction

Food heating processes contribute to providing safer products that help maintain health. Microorganisms and toxins are a major health risk. Therefore, microbial hazard characterization of foods must be carried out prior to defining the objectives of food processing.

The International Commission on Microbiological Specifications for Foods (ICMSF) has proposed a preventive scheme for managing microbial risks in foods. It introduces the important new concept of a food safety objective, FSO (Stewart et al. 2002). The FSO is the maximum frequency and/or concentration of a microbiological hazard in a food at the moment of consumption warranting the appropriate level of health protection (Codex Alimentarius Commission 2004). Heat processing is a fundamental step in the food chain for eliminating or reducing microbial populations and for reaching the FSO.

During food processing, the heat intensity necessary to kill microorganisms depends on the nature of the microorganism, the microbial load, the heat sensitivity of the product, and the requisite storage conditions. Microbial inactivation can be measured by the number of decimal reductions corresponding to the performance of the heat process.

These types of processes aim to reduce or to destroy pathogens (pasteurization) or all microorganisms, including spores (sterilization). These both treatments are generally defined by the duration of heating at a constant temperature.

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For security reasons, following the reduction of microorganisms purposely added to foods during an industrial process is not favored. Therefore, prediction of the evolution of the microbial load and, consequently, determination of the temperature and the time of processing are made using the key parameters: *D*- and *z*-values.

The decimal reduction time at a constant temperature *T* (*D*-value) is used to estimate the time required for reduction of a microbial load. The *z*-value allows for evaluation of the bacterial heat resistance and establishes equivalences between two heating treatments in order to obtain the same microbial reduction number.

Alternative processes like microwave heating are applied more frequently, particularly since it drastically reduces cooking times. On a commercial scale, microwave technologies are applied to cooking meat and poultry, tempering butter, baking dough, as well as pasteurizing/sterilizing ready meals (Mullin 1999; Schubert and Regier 2005). In the context of catering, pasteurization of foods during cooking helps ensure food safety and is a key point of the Hazard Analysis Critical Control Points (HACCP) plan. However, as pointed out by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF 2006), scientific data is lacking for the prediction of the degree of inactivation that might be achieved using microwave treatments.

During microwave heating, the applied temperature is not constant and thus the *D*- and *z*-values are not appropriate for determining time–temperature references. Optimization of microwave processes can be achieved by establishing specific power death time curves for microbiological markers. Therefore, based on the same strategy as for conventional heating, Laguerre et al. (2011) developed new parameters: D_p- and z_p-values are defined at constant specific power during infant formula sterilization.

In this chapter, an overview of microwave heating is presented in Sect. 2, followed by a discussion on food safety and microwave heating (Sect. 3). Characterization of microwave heating using specific key-parameters is presented followed by a summary of thermobacteriology in food processing in Sect. 4.

23.2 An Overview of Microwave Heating

23.2.1 Definition

Microwaves are the combination of an electric field and a perpendicular magnetic field propagating in free space (Fig. 23.1). The wave is characterized by its frequency (*f*) and wavelength (λ), as shown in Eq. (23.1):

$$\lambda = \frac{c}{f} \tag{23.1}$$

where *c* is the light velocity in vacuum space (3×10^8 m/s).

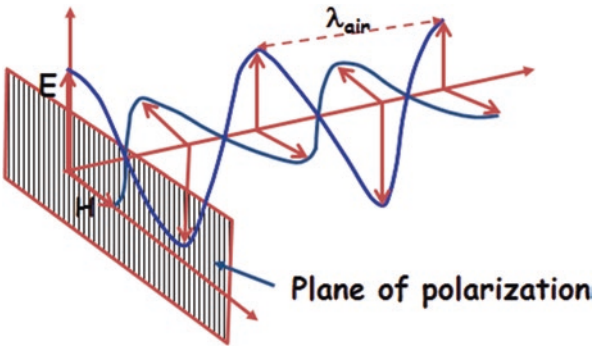


Fig. 23.1 Electromagnetic wave propagating in free space

Microwaves are positioned in the electromagnetic radiation spectrum between radiowaves (300 MHz) and infrared radiations (300 GHz) (Schubert and Regier 2005). The conveyed energy (E) by a wave is proportional to its frequency (f) according to Eq. (23.2), where h is Planck's constant ($h \approx 6.626 \times 10^{-34}$ J s).

$$E = h.f \tag{23.2}$$

It appears that the energy carried by microwaves is lower than that conveyed by infrared or visible radiations, since the frequency of the latter is much greater than that of microwaves. Thus, the absorption of microwaves by a molecule will affect its rotational energy, while the absorption of infrared and visible light will affect the vibrational energy and electronic transition energy of the molecule, respectively.

Only three frequencies: 433, 915, and 2450 MHz are allocated by the International Telecommunication Unions (ITU) to industrial applications for heating (Mullin 1999). The first frequency is not used in Europe. The remaining two can be used without restriction in industrial equipment and domestic ovens (Schubert and Regier 2005).

23.2.2 Heating Mechanism

Microwaves interact with matter through the electric field. Accordingly, if a polar molecule of a condensed medium (liquid water) is subjected to an alternating electric field varying from low frequencies (LF) to infrared frequencies (IR), the molecule will attempt to align itself to the field and therefore move at the same frequency as the latter as long as possible. Thus, a slight decrease of the dielectric constant (ε') is observed, while the loss factor or absorption coefficient (ε''), initially close to zero, will gradually increase (Fig. 23.2).

When approaching the microwave (MW) frequency, it becomes increasingly difficult for the water molecule to remain aligned with the field because of the high field frequency (2450 MHz) and the many interactions (H-bonds) with other molecules attempting to align themselves to the electrical field. A time lag is thus

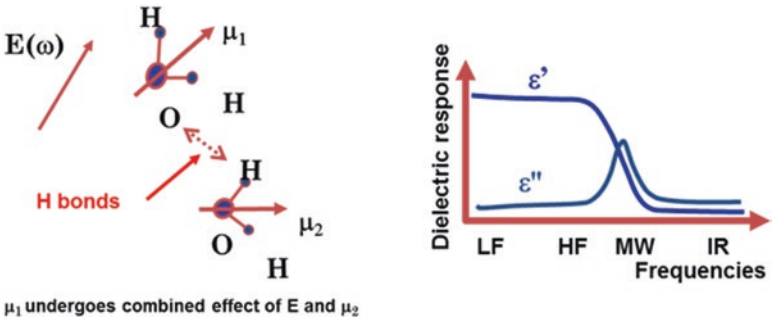


Fig. 23.2 Illustration of the dielectric response of a water molecule subjected to a frequency variation of an alternating electric field (LF: Low Frequencies, HF: High Frequencies, MW: microwaves, IR: InfraRed)

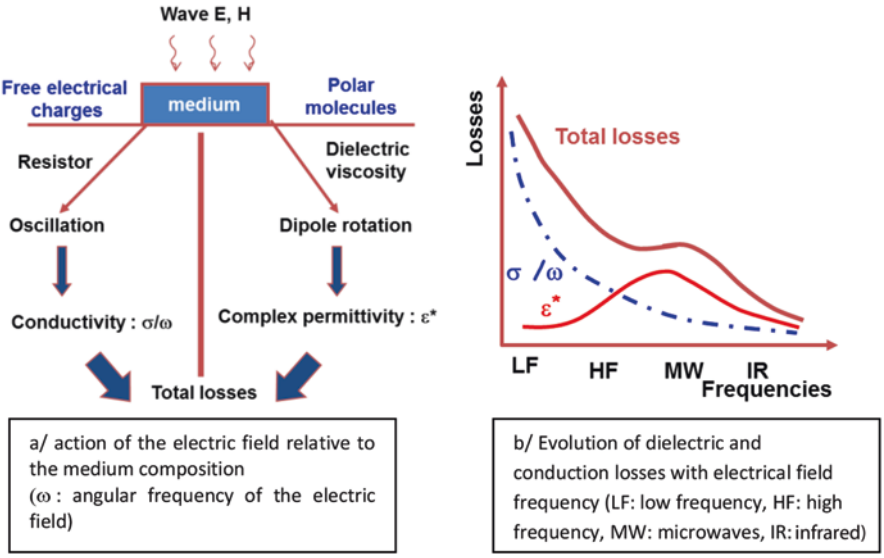


Fig. 23.3 Total losses of a medium subjected to an electromagnetic field

established between the dipole rotation of the molecule and the electrical field. The occurrence of ε'' is directly related to the random motion of the molecules after the lag, causing more friction between them, and therefore enhancing heat generation.

In more general terms, if a medium is subjected to an electromagnetic field, the heat generation will be the result of two phenomena. The first is conduction losses due to oscillations of free electrical charges (Joule effect) depending on the conductivity of the medium (σ). The second is due to dielectric relaxation losses of polar molecules depending on complex permittivity of the medium (ε^*) (Fig. 23.3a).

It is noticeable that total losses are mainly due to dielectric losses even if the conduction losses are not completely zero (Fig. 23.3b). Very often these conduction

losses are neglected for microwave heating of food. However, for products rich in salts, such a contribution should be considered.

23.2.3 Electromagnetic Waves Interaction with Food

23.2.3.1 Dielectric Properties

The absorption of the microwaves by food depends on its complex permittivity ε^*. This permittivity depends on the properties of the product, such as dielectricity (ε' and ε''), electrical conductivity (σ), and the frequency of the electric field. When the heat losses by conduction are neglected, the complex permittivity can be written as Eq. (23.3).

$$\varepsilon^* = \varepsilon' - j.\varepsilon'' \tag{23.3}$$

In this equation, ε' is the dielectric constant of the product, indicating its ability to store electrical energy when subjected to an electromagnetic field. Regarding ε'', the loss factor or dielectric loss, it informs on the ability of the conversion or dissipation of electrical energy into heat (Tang 2005).

Generally, the dielectric properties of food depend on several factors such as the frequency of the field, food temperature, moisture content, and composition (salt, fat, and other components) (Venkatesh and Raghavan 2004; Tang 2005).

23.2.3.2 Absorbed Power

When a product containing polar molecules and free electric charges is submitted to microwave radiation, it absorbs the wave and transforms its energy into heat. This absorption is mainly based on its loss factor ε''. The power dissipated per unit volume is given by Eq. (23.4).

$$P_{abs} = 5.563 \times 10^{-11} \varepsilon''.f.E_{int}^2 \left[\mathrm{W/m^3} \right] \tag{23.4}$$

with f: frequency of electric field (Hz) *and* E_{int}: product internal field (V/m)

The absorbed power, converted into heat by the product, induces a rise of its temperature ($\Delta\theta$), as shown in Eq. (23.5) (Tang 2005). This temperature rise depends on both the loss factor and on the electric field.

$$\rho C_p \frac{\theta}{t} = 5.563 \times 10^{-11} \varepsilon'' f E_{int}^2 \tag{23.5}$$

where C_p (J/kg °C) is the specific heat of the product, ρ (kg/m^3) is its density, and Δt (s) is the time increment.

23.2.3.3 Behavior of Electromagnetic Waves According to the Nature of the Material

When a body is subjected to microwave radiations, a portion of this radiation is reflected at the surface of the body and the rest is transmitted. The proportion of the reflected radiation compared to the transmitted radiation depends on the nature of the body. Thus, three main categories of material may be considered:

Materials transparent to microwaves (Fig. 23.4a): A portion of the incident wave is reflected at the surface of the body, while the other part is transmitted without being absorbed by the molecules of the material. Thus, transparent materials are advantageously used in the manufacture of containers for food heating by microwave, such as electrical insulators.

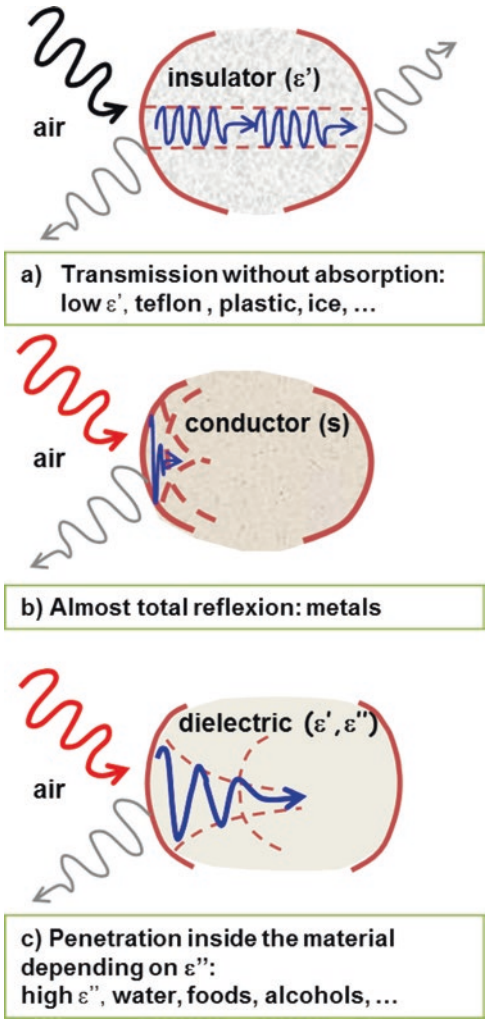


Fig. 23.4 Microwave–material interactions

Conductors (Fig. 23.4b): Almost all of the incident wave is reflected; the very small transmitted part is absorbed in its entirety on a very thin peripheral layer. For metals, its reflection property is used to advantage in the design of microwave equipment, particularly for the transport of the wave in the waveguide (rectangular metal tubes for conveying the wave of the magnetron to the treatment chamber) and in the applicators (enclosure in which the product undergoes the processing).

Dielectric material (Fig. 23.4c): These media are rich in polar or polarizable molecules such as food (water, alcohols, carbohydrates, proteins, etc.). Much of the incident wave is absorbed and converted into heat inside the product. After penetration, the wave undergoes more or less attenuation depending of the loss factor (ε'') of the medium.

23.2.4 Product Temperature Profiles During Microwave Heating Versus Conventional Heating

Microwave heating is generally faster than conventional heating. In the case of hot air, a heat exchange occurs between the air and the surface of the product by convection, and then heat diffuses by conduction from the surface to the core. This diffusion depends on the physical properties of the product (specific heat and thermal conductivity, porosity, etc.). However, it is known that foods are poor thermal conductors, thus, heat diffusion towards the core is usually slow.

In the case of microwave heating, when the size of the product is adapted to the depth of the penetration of the wave, the entire product will be heated very quickly. A comparison of the evolution of the core temperature of the product during heating using both methods is illustrated in Fig. 23.5. The core temperature of the

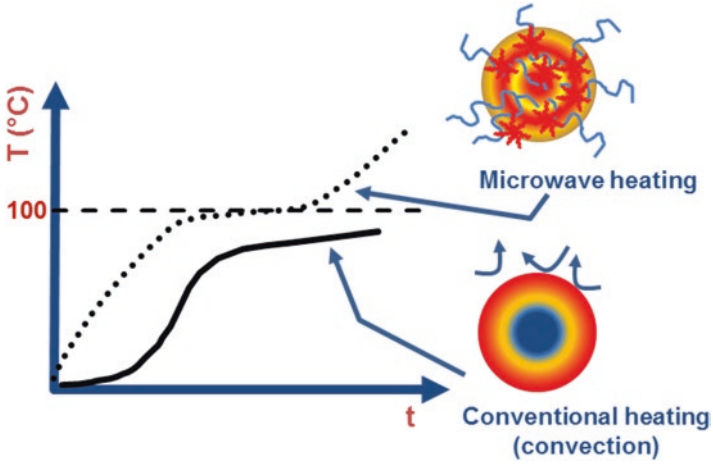


Fig. 23.5 Comparison of temperature profiles between a microwave heating and a hot air heating

product heated using microwaves increases rapidly to 100 °C. Such is the case of products with high water content. The temperature remains at 100 °C until the disappearance of the free water inside the product, and then rises again until the end of heating.

In the case of hot air heating, the core temperature of the product increases slowly at the beginning due to the low rate of heat transfer between the surface and the center of the product. Thermal inertia due to the thermal resistance of the product will result in heterogeneous heating between the surface and the center (Fig. 23.5). Unlike microwave heating, the temperature of the product is limited by the air temperature.

Thus, a question is raised: "Is the temperature of the product more homogeneous when it is heated by microwaves?" At first, it might be considered that microwave heating would be more homogeneous than hot air heating. In reality, however, the phenomena are more complex, and they will be discussed in Sect. 3.2.

23.3 Microwave Heating and Food Safety

23.3.1 Effects of Microwave Heating on Microorganisms

When a microorganism is subjected to conventional heating at a lethal temperature, it undergoes a number of cell damages and disruptions of metabolism related to the denaturation of proteins, inactivation of certain enzymes, and degradation of other thermolabile compounds (Rahn 1929). Since microwave radiation results in the heating of the medium, it can be expected to observe similar effects on microorganisms such as those for conventional heating.

Many authors have shown the lethal impact of microwave heating on different strains of microorganisms, including vegetative cells of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* (Najdovski et al. 1991), spores of *Bacillus subtilis* and *Geobacillus stearothermophilus* (Najdovski et al. 1991; Laguerre et al. 2011), *E coli* (Woo et al. 2000; Canumir et al. 2002), *Enterococcus faecalis* (Riva et al. 1991; Tessier et al. 2006), *Salmonella enterica arizonae* (Hanna Wakim 2008), *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (Tajchakavit et al. 1998). In all of the studies mentioned previously, microwave treatment induced a significant reduction of inoculated germs (2–6 log), depending on the applied microwave power.

Woo and others (2000) showed that microwave heating induced alterations in the cell walls of *E. coli* and *B. subtilis*. This deterioration of the cell membrane leads to leakage of cellular contents, particularly of nucleotides and proteins. The release of nucleotides was quantified by measuring the absorbance at 260 nm of the cell suspension. The authors highlighted that the leak was strongly correlated to microwave power. The effect of microwave heating on the integrity of the cells was studied using scanning electron microscopy and transmission electron microphotography.

According to their findings, microwave treatments caused damage to cell membranes by becoming rough and swollen. Protein denaturation and aggregation in the cytoplasm as well as the induction of heat shock proteins were suggested.

For many years, specific effects related to electromagnetic energy on the lethal action of microwaves were extensively studied by many authors and resulted in controversy. Some authors (Goldblith and Wang 1967; Lechowich et al. 1969; Hamrick and Butler 1973; Welt et al. 1994; Bates and Spencer 1995; Tong 1996; Göksoy et al. 2000) carried out comparative studies on the inactivation of microorganisms by microwave heating and by conventional heating. It was concluded that the lethal effect of the microwaves would be exclusively of thermal origin.

Other authors, on the contrary, highlighted athermal effects of microwaves on the inactivation of *Salmonella typhimurium* (Culkin et al. 1975), and *Aspergillus niger*, *Rhizopus nigricans*, and *Penicillium* sp. (Olsen 1965). Nevertheless, most of these studies applied temperature measurement techniques inappropriate to microwave heating, where the temperature was monitored at a single point of the product. However, the heterogeneity of microwave heating could lead to the presence of hot and cold spots within the same heated product. Consequently, microorganisms can be subjected to uneven heating inside this product.

Thus, it is difficult to conclude that the previous works reveal athermal effects of microwave heating. In fact, according to Risman (1996), athermal effects should not be explained by macroscopic temperature. By definition, the absorbed power threshold which does not cause an increase in the temperature of a biological system is considered to be 4 W/kg. According to Anantheswaran and Ramaswamy (2001), athermal effects can only be caused by power consumption ranging between 0.4 and 4 W/kg. Under these conditions, the thermoregulatory system of the organisms is able to compensate for the effect of the radiation. Therefore, beyond 0.004 kW/kg, there will be a thermal effect because the absorbed energy cannot be dissipated.

Regardless of the scale, industrial or domestic, microwaves are used with specific applied power ranging from 0.5 to 10 kW/kg. These values are significantly higher than the threshold of 0.004 kW/kg mentioned above. Therefore, even if athermal effects exist, they have no significant consequence on the microwave heating of foods.

23.3.2 Heating Heterogeneity and Microbiological Risks

Heterogeneity is a main concern when heating foods. Regardless of the considered operation, pasteurization or cooking, it is absolutely essential to achieve sufficient reduction of pathogens in order to obtain safer foods. However, uneven temperatures occur during conventional heating of solid or viscous liquid foods. Therefore, overheating is always needed to obtain a certain level of temperature at the coldest point, thus ensuring sufficient microbial reduction. Similarly, uneven temperatures are encountered in microwave heating due to numerous factors. Some of these factors are related to the product (dielectric properties, size and shape of the product,

state of the product: frozen or not, product composition, thermal properties), while others are related to the microwave cavity (type of microwave equipment: single mode or multimode cavity, size of the cavity, possible combination or not with hot air, steam, or infrared) (Schubert and Regier 2005). Therefore, the issue of food safety arises in the same way as for conventional heating, namely in managing temperature level at the coldest point of the product. In order to guarantee food safety, it must be ensured that the coldest point of the product (generally the center in the case of conventional heating) attains a certain temperature, so that the target sterilization or pasteurization value is reached at that point. The same applies to microwave heating. However, the difficulty with this type of heating resides in the determination of the location of the coldest point. This point is generally found at the product's surface. In fact, in microwave ovens, the air surrounding the product is initially at room temperature. Thus, the heat is not transferred towards the product's center from the surface as in conventional heating.

23.3.3 Actions to Minimize the Effects of Uneven Heating

Taking into account the previous observations, certain practices can be applied to microwave heated foods to ensure a safer end-product.

For instance, it is preferable to use small sized pieces of a food because the absorption of the waves depends on the thickness, volume, and shape of the product (Zhang and Datta 2001). Thus, a spherical product of small size (radius less than or equal to 1 cm) will be more evenly heated compared to a larger product.

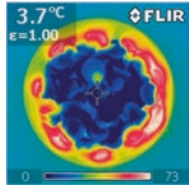
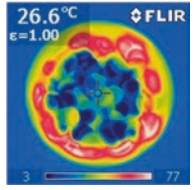
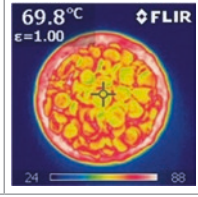
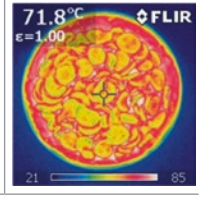
The starting material must have similar temperatures in order to ensure homogeneous heating. For example, since ice is transparent to microwaves, a frozen compound will absorb very few microwaves compared to a compound stored at room temperature (Meda and Raghavan 2005).

Cooking foods long enough ensures that the coldest point reaches a sufficient temperature. Indeed, the heterogeneity of heating tends to decrease with the duration of the treatment. Table 23.1 presents the evolution of the average temperature of the surface of a frozen zucchini dish subjected to microwave cooking. It can be noticed that the standard deviation of temperature is reduced from 22.5 °C after 8 min of cooking to 4.5 °C after 20 min of the same treatment. This implies a more homogenous heating of the dish.

Mixing the food during and at the end of the cooking operation will ensure better distribution of the temperature. Allowing the food to stand for 1–2 min at the end of the heat treatment helps equilibrate the product's temperature. In fact, the product's temperature still increases after the end of the microwave treatment.

Uniform heating is important from a microbiological perspective to ensure that the microorganisms found in the coldest point of the heated food are destroyed or reduced to an acceptable level.

Table 23.1 Illustration of microwave heterogeneity during cooking of zucchini

	Zucchini cooking			
Heating time (min)	8	12	16	20
Surface *T*°	21.5	37.8	73.6	76.8
±	±	±	±	±
SD (°C)	22.3	21.0	4.2	4.5
Pictures related to heating time				

23.4 Characterization of Microbial Destruction During Conventional and Microwave Heating

Before explaining the method for the *D*- and *z*-values determination adapted for microwave heating (Sect. 5), the conventional approach used for heat resistance characterization of microorganisms is presented.

23.4.1 Thermobacteriology of Food Processing During Conventional Heating

23.4.1.1 *D* and *z*-Values, Key Parameters for Microorganisms Heat Resistance Determination

Thermal Resistance at Constant Temperature

When microorganisms (vegetative cells or spores) are exposed to heat treatments at lethal temperatures, the population decreases exponentially with heating duration. When the residual microbial population is plotted on a semi-logarithmic scale against time, a linear curve is obtained (Fig. 23.6). This profile can be represented by a first order reaction (Stumbo 2006).

For this first order reaction, the relation is Eq. (23.6):

$$\log C = -\frac{k}{2.303}t + \log C_0 \tag{23.6}$$

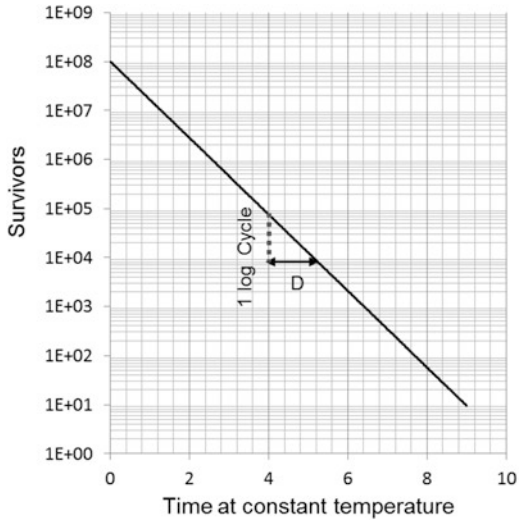


Fig. 23.6 Semi-log plot of survivor microorganisms versus time

where C_0 and C are the initial microbial counts and microbial survivors after the treatment, respectively, and k represents the first order rate constant.

The inverse of the slope is defined as the D-value (Eq. 23.7)

$$D \text{ value} = \frac{2.303}{k} \tag{23.7}$$

The decimal reduction time (D-value) is the treatment time required to reduce tenfold the microbial population at a constant temperature. Thus, D-value is used to characterize the heat resistance of the organism at this temperature. This parameter is useful for comparing the heat resistance of different species at a given temperature.

Using Eqs. (23.6) and (23.7), the duration required to reduce the number of logarithmic cycles of the bacterial population can be calculated (Eq. 23.8):

$$t = \log\left(\frac{C_0}{C}\right) D = n.D \tag{23.8}$$

The decimal reduction number or treatment effectiveness is presented in Eq. (23.9) (Hermier and Cerf 1991; Mafart 1991).

$$n = \log\left(\frac{C_0}{C}\right) \tag{23.9}$$

To explain this logarithmic destruction, several hypotheses are proposed. According to Stumbo (2006), the most likely hypothesis is the loss of the reproductive capacity of the germ due to distortion of a gene essential for reproduction proposed by Rahn (1929, 1945). Indeed, Rahn (1945) rejects the hypothesis of cell death due to the denaturation of enzymes. It is suggested that the decrease in concentration of certain enzymes is a consequence of growth inhibition due to the denaturation of the gene. The logarithmic denaturation of this gene can be thus explained by a first order reaction.

However, in some cases, during inactivation, a nonlinear behavior at the beginning of the treatment (shoulder pattern) can be observed, as shown in Fig. 23.7. In this example, a shoulder pattern is observed during a heating treatment of *Geobacillus stearothermophilus* spores in an oil bath at 113 °C with a lag phase preceding microbial destruction.

According to Stumbo (2006), this phenomenon can be explained by microorganism activation by heat before its destruction. Bacterial spores, for example, must be heated at a sublethal temperature to provoke spore germination.

The nonlinear model can be explained by heterogeneous microbial populations consisting of several subpopulations with their own inactivation kinetics (Hermier and Cerf 1991; Stumbo 2006). Two remarks can be made about the exponential

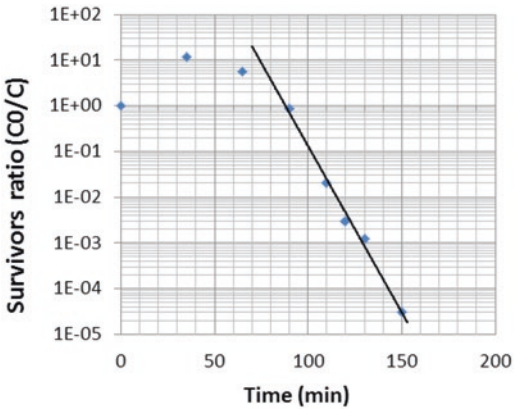


Fig. 23.7 Survivors ratio of spores of *G. stearothermophilus* submitted to a heating at 113 °C in a bath oil (unpublished data)

shape of the survival curve of a microorganism. First, the risk of microbial survival is reduced when the starting population is low. Second, it is not possible to achieve absolute sterility since the survival curve is exponential and tends asymptotically to zero (Cheftel and Thomas 1963). Hence, commercial sterility corresponds to a 12-decimal reduction of the target germ: *Clostridium botulinum* spores.

The *D*-value, an indication of heat resistance of a microorganism, may vary with strain, culture conditions, pH, water activity, and food composition (NaCl concentration, additives). It is therefore very important to specify the conditions of determination of the *D*-value (Sörqvist 2003).

Relation Between Thermal Resistance of Microorganisms and Temperature

When *D*-values are plotted on a semi-logarithmic scale against the temperature, a linear curve is obtained. The *z*-value, also representing the microbial heat resistance, can be determined as the inverse of the slope of the curve (Fig. 23.8).

The *z*-value is defined as the increase in temperature that would produce a reduction in *D*-value of a factor of 10, as shown in (Eq. 23.10).

$$D_{T2} = D_{T1} \times 10^{\frac{T_1 - T_2}{z}} \tag{23.10}$$

A similar curve to that of Fig. 23.8 is obtained when the heating time for a complete microbial destruction is plotted against the temperature on a semi-logarithmic scale. The slope of this curve, called thermal death time (TDT) also corresponds to the inverse of *z*-value (Cheftel and Thomas 1963). This straight line shows the time–temperature couples, which gives the same destruction rate of the target organism. Hence, the high temperature and short time treatment (HTST) can be equivalent to that of low temperature and long time.

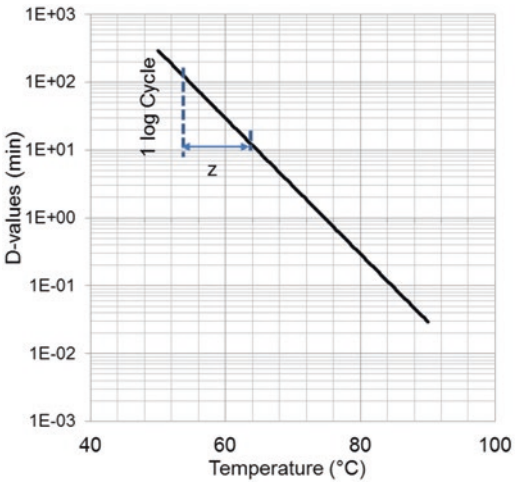


Fig. 23.8 Semi-log plot of *D*-values versus temperature

In these conditions, a reference temperature must be chosen for the calculation of the time to achieve pasteurization or sterilization. However, at an industrial level, the temperature of the product is variable during pasteurization or sterilization. A new time–temperature relation according to the reference temperature is developed and the heating time required to obtain an equivalent bacterial destruction can be calculated (Eq. 23.11):

$$F = \int_0^t 10^{\frac{T - T_{\mathrm{Ref}}}{z}} \, \mathrm{d}t \tag{23.11}$$

where F is the pasteurization or sterilization value and T_{Ref} is the reference temperature (70 °C for pasteurization and 121.1 °C for sterilization).

Several methods have been suggested for D-and z-values determination (Stumbo 2006). The majority of these methods are based on the monitoring of a population of microorganisms subjected to a heat treatment at a constant temperature at different times by the enumeration of survivors.

The thermal death time (TDT) tube method uses small diameter tubes (7–10 mm) containing a culture medium or food (1–4 mL) with target microorganisms (Bigelow and Esty 1920). Tubes are sealed and heated in water baths. The American Can Company (1943) method proposes to put microorganism-inoculated products in small sealed cans and autoclaving them at controlled temperatures. In this case, a very small sample must be taken in order to have a homogeneous temperature and the coming up time (CUT) and coming down time (CDT) of the autoclave must be short and fast.

In spite of precautions, bacterial populations can be modified by culture conditions (phase of growth, culture medium, etc.) or by the preparation of the suspen-

sion. Therefore, it is difficult to obtain the same physiologic state of microorganisms before determining *D*-values. To avoid problems of reproducibility, Reichart (1979) proposes a new procedure for modeling microorganism inactivation during increasing temperature protocols. This study proposes to submit a large bacterial suspension (400 mL) to a variable temperature heat treatment. A 1 mL sample is then taken every 30 s and cooled rapidly by injection into the dilution tubes provided for enumeration. With each sample taken, temperature is also measured. Survivor curve and temperature curve are obtained, as shown schematically in Fig. 23.9.

Next, the derivative of log *C* at each point of the curve, for a given time and a given temperature, is calculated and the *D*-value is calculated for the given temperature by Eq. (23.12).

$$-\frac{\mathrm{d}\log C}{\mathrm{d}t} = \frac{k}{2.303} \tag{23.12}$$

23.4.1.2 Use of *D* and *z*-Values for the Optimization of Heat Treatment

In conventional heating, *D*-values are used to calculate the time required at a constant temperature to kill the target microorganism. The conventional approach for establishing pasteurization or sterilization scales is presented in Table 23.2. For a target microorganism and a reference temperature (*T*), the thermal death time (*F*) is determined and corresponds to the time required to cause an adequate reduction of a microbial population.

However, this treatment can also lead to quality degradation, particularly the destruction of vitamins A, B1, and C. Thus, pasteurization should not have the sole purpose of destroying microorganisms. A number of secondary objectives such as preserving the nutritional quality of the food and its organoleptic quality or the destruction of harmful enzymes should be taken into account (Mafart 1991).

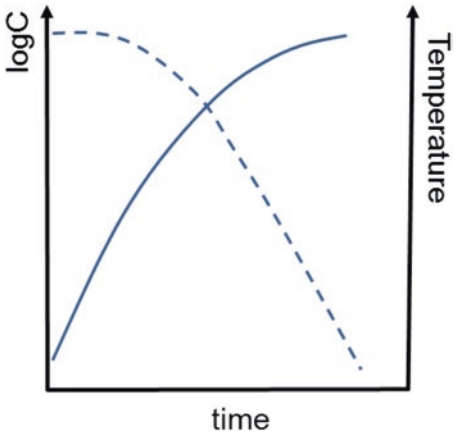


Fig. 23.9 Representation of survivors during heating treatment expressed in log *C* (---) and temperature (——) versus time adapted from Reichart (1979)

Table 23.2 Pasteurization and sterilization values used in France (adapted from Martin 1984 and Stumbo 2006)

Description	Pasteurization	Sterilization
Target germ	*Enterococcus faecalis*	*Clostridium botulinum*
Reference temperature (T)	70 °C	121.1 °C
D_T and z values	$D_{70}=2.95$ min	$D_{121.1}=0.21$
	$z=10$ °C	$z=10$ °C
Initial number of bacteria in the product	10^7 UFC/g	1 spore/container
Final number desired in the product	10^{-6} UFC/g	10^{-12} spores/container
Treatment effectiveness	$n=\log(10^7/10^{-6})=13$	$n=\log(1/10^{-12})=12$
$F=n\times D$	$F=13\times 2.95=38$ min	$F=12\times 0.21=2.52$ min

These secondary reactions generally follow a first order kinetics. It is therefore possible to characterize the sensitivity to heat of these indicators by using D and z-values. More specifically, the z-value characterizes the impact of temperature variation on the kinetics of thermal destruction of the indicator. If z-value is low, a small increase in temperature is sufficient to amplify the destruction. Inversely, if this value is high, a small temperature variation will have little effect on destruction (Bimbenet et al. 2002). Thus, the thermal destruction of most microorganisms in a vegetative state or spores has a z-value generally lower (5–10 °C) than that of certain chemical indicators such as vitamin C (25 °C) (Mafart 1991) or lysine (21 °C) (Bimbenet et al. 2002). It is therefore possible to optimize heat treatment by drawing on the TDT graph of the target microorganism the time/temperature curves of the indicators of interest, as shown in Fig. 23.10. In this example, to ensure a decimal reduction (n) greater than or equal to 12 on the target microorganism while limiting destruction of indicator 1–10 % and those of indicator 2–15 %, we must choose a time–temperature couple located in the gray area, namely inside the optimal zone.

23.4.1.3 *D*- and *z*-Values for Alternative Technologies

These two key parameters can be used for the characterization of alternative technologies when the intensity of the treatment is constant and when the inactivation of microorganisms is logarithmic during treatment (Lado and Yousef 2002). For alternative technologies, the D-value is also determined by setting the intensity of the treatment at a constant level. Some examples are presented in Table 23.3.

In the case of microwave heating, treatment intensity corresponds to the applied specific power (W/mL or W/g).

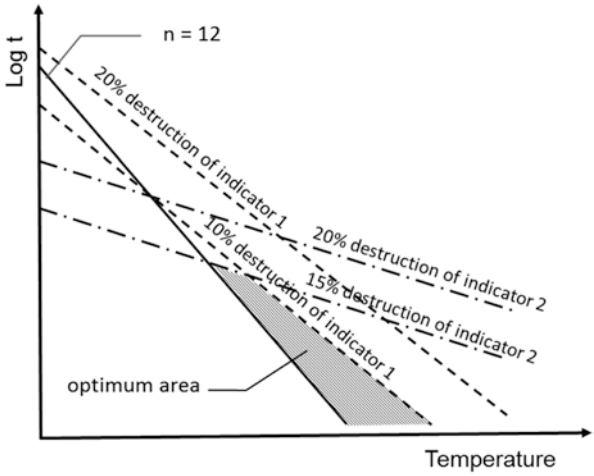


Fig. 23.10 Use of the thermal death time (TDT) approach for the optimization of a heat treatment

Table 23.3 Examples of alternative technologies using *D*- and *z*-values at constant intensity

Technologies	Intensity	Unit
Conventional heating	Temperature	°C or °F
Ionizing radiation	Radiation dose	kGy
High pressures	Pressure	MPa
Pulsed electric field	Electric field strength	kV/cm
Ultraviolet radiation	UV dose	ergs/mm^2
Ozone	Concentration	ppm or mg/L
Microwave heating	Specific power	W/g or W/mL

23.4.2 Adaptation of the Concept of D- *and* z*-Values for Microwave Heating Processes*

23.4.2.1 The Notion of D_p- and z_p-Values

The temperature of the product continues to increase throughout the microwave treatment, in opposition to conventional heating where the product reaches and maintains a certain level of temperature. Thus, conventional *D*- and *z*-values are not applicable to this type of technology. To overcome this difficulty, some authors determined a *D*-value for a final temperature (Tajchakavit and Ramaswamy 1997; Tajchakavit et al. 1998; Tessier et al. 2006) or tried to maintain the temperature at a constant level (Riva et al. 1991). During pasteurization of apple juice, Canumir et al. (2002) determined *D*-values at constant power levels and proposed a *z*-value

in Watts to represent the resistance of a target microorganism during microwave heating. However, for extrapolation purposes, it would be better to take into account the weight of the product during microwave heating. Therefore, a new model based on specific power per weight unit coupled to a determined duration is more adequate for validating pasteurization or sterilization processes by microwave heating (Laguerre et al. 2011).

The decimal reduction time (D_p-value) is the treatment time (t) required to reduce microbial population (C) by 90 % (or one decimal reduction) at a constant applied specific power expressed in W/g or in W/mL (Eq. 23.13), i.e., the applied power related to the weight or the volume of the product.

$$D_p \quad \text{value} = \frac{t_2 - t_1}{\log C_2 - \log C_1} \tag{23.13}$$

The z_p-value is the change of specific power (P) necessary to cause a tenfold change in the D_p-values of microorganism under specified conditions (Eq. 23.14).

$$z_p \quad \text{value} = \frac{P_2 - P_1}{\log D_{p2} - \log D_{p1}} \tag{23.14}$$

Applications of these key parameters on sterilization, pasteurization, and cooking are presented in the following subsections.

23.4.2.2 Microwave Sterilization

The concept of D_p and z_p-values have been tested for sterilization of infant food in a lab-scale microwave pilot (Laguerre et al. 2011). For sterilization, the microbial reference is well established and heat treatments must ensure a 12 decimal reduction of *Clostridium botulinum* spores or per equivalence a 5 decimal reduction of *Geobacillus stearothermophilus* spores (Cheftel and Thomas 1963). Samples of infant milk formula were submitted to a constant power in a lab-scale microwave pilot and the bacterial population was determined at a specific applied power (W/mL). The inactivation curves of *G. stearothermophilus* spores in the milk at constant specific powers follow first-order kinetics and the D_p-value can be determined by using the slope of the straight line (Fig. 23.11). The z_p-value is finally determined by the inverse of the slope obtained by plotting the log D_p versus specific power. Similarly, vitamin C degradation in milk was also followed during microwave treatments at constant specific power (W/mL) in the lab-scale pilot. First order kinetics were obtained and used to determine D_p and z_p-values (Table 23.4).

Some additional markers were used to qualify the impact of microwave treatments on the quality spoilage of milk, such as tryptophan and carboxymethyllysine. Finally, the Fast index was used to measure neo-formed contaminants related to the

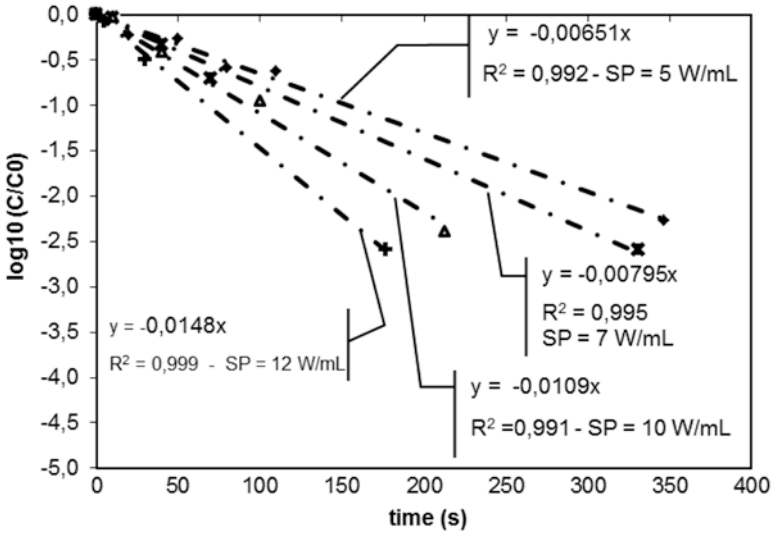


Fig. 23.11 Survival kinetic curves of *G. stearothermophilus* spores for different microwave specific powers (◆ experimental and — calculated data) (Laguerre et al. 2011)

Table 23.4 *D* and *z*-values of spores of *G. stearothermophilus* and vitamin C (Laguerre et al. 2011)

Indicator	SP[a] (W/mL)	D_p-value (s)	R^2	z_p-value (W/mL)	R^2
Spores	5	157.8	0.992		
	7	123.7	0.994		
	10	85.8	0.991	20.0	0.995
	12	67.2	0.999		
	15	46.8	0.950		
Vitamin C	5	1475.6	0.998		
	7	860.6	0.957		
	10	745.0	0.899	14.1	0.937
	12	353.1	0.918		
	15	280.9	0.964		

[a]SP: specific microwave power

Maillard reaction (Tessier et al. 2006). Based on these results, microwave sterilization of infant formula was optimized by establishing the thermal destruction curves for each marker.

The optimal area (gray area in Fig. 23.12) is in the region of high specific power and low processing time, which is equivalent to HTST heat treatments. The optimal condition for microwave sterilization of infant food was determined using the same approach as that of conventional thermal treatments (Fellows 1992; Bimbenet

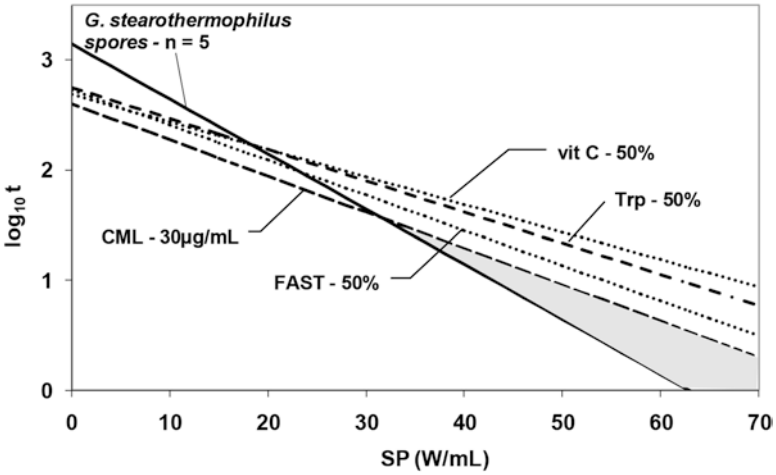


Fig. 23.12 Determination of the optimal zone of formulated milk sterilization treated at constant specific power (SP) by microwave heating (Laguerre et al. 2011)

et al. 2002). However, the levels of specific power required for this operation are not compatible with current commercial devices for which the maximum specific power is around 10 W/mL.

23.4.2.3 Microwave Pasteurization

Choice of Target Microorganism for Pasteurization

Pasteurization is defined as any process, treatment, or any combination of both applied to food to reduce the most resistant microorganism(s). Pasteurization must ensure a contamination level that does not present a public health risk under normal distribution and storage conditions (NACMCF 2006). Generally, a representative bacterium is selected for a food product following a hazard and risk evaluation for health. For example, *Salmonella senftenberg* is chosen for the pasteurization of eggs, as these pathogenic bacteria are the most representative in this product. For milk, *Mycobacterium tuberculosis* or *Coxiella burnetti* are the target bacteria. For meat and poultry, the NACMCF recommends heat treatments ensuring a 4-log reduction in *Listeria monocytogenes* of chilled foods containing cooked or uncured meat (NACMCF 2006). In the same context, Mackey and Bratchell (1989) proposed to cook food to an internal temperature of 70 °C for 2 min to ensure the destruction of *L. monocytogenes*.

For cooked foods, French regulations recommend more extensive thermal processing by choosing a very resistant germ, *Enterococcus faecalis* (Rosset and Poumeyrol 1986). This bacterium was chosen as a reference during industrial ham processing because it is a non-sporulated, heat resistant bacterium and is responsible for food spoilage. Thermal processes for vacuum packed foods practiced in

Europe are designed to achieve a 12–13 log reduction (12–13*D*) of *E. faecalis*. According to the calculation presented in Table 23.2, the pasteurization value for cooked foods is 38 min at 70 °C and is the minimal process to obtain a shelf life of 6 days (Ministry of Agriculture 1974, 1988).

Several authors have used these bacteria as a target microorganism in their studies; a summary is presented in Table 23.5. The *D*-values at 70 °C vary from 0.2 to 7.9 min.

Reported *z*-values for *E. faecalis* vary from 5.0 to 17 °C with a mean of 8.3 °C. These results indicate the high resistance of this bacterium because *z*-values for pathogens are generally lower than 7 °C (Cerf et al. 1996; Sörqvist 2003). The *D* and *z* values depend on strain, medium or foods and treatment conditions (Moats 1971). Sörqvist (2003) has collected and analyzed data from the literature to evaluate heat resistance of bacteria in liquids with pH values of 6–8. This review shows that in these conditions, all bacteria *L. innocua*, *L. monocytogenes*, *E. coli*, *Yersinia enterolitica*, *Salmonella* spp. (except *S. senftenberg* 775 W), *Campylobacter jejuni/coli* were more sensitive to heat treatments than *E. faecalis* and *E. faecium*.

Table 23.5 Examples of *D*- and *z*-values found in literature for *E. faecalis*

Strain conditions	D_{70} (min)	D_{72} (min)	*z*-value (°C)	references
E. faecalis	0.35[a]	na	5.6[a]	Ott et al. (1961)
E. faecalis liquids pH values 6–8	0.61[a]	0.38	9.5	Gardner and Patton (1975); Sanz Pérez et al. (1982); Magnus et al. (1986); Sörqvist (2003)
E. faecalis ATCC 19433	0.44	na	6.8	Joffin and Joffin (2010)
E. faecalis ATCC 19433 milk	0.20[a]	na	5.7	McAuley et al. (2012)
E. faecalis Ham	7.0	na	17	Joffin and Joffin (2010)
E. faecalis Cured meat	2.95	na	10	Rosset and Poumeyrol (1986); Mossel and Thomas (1988); Cerf et al. (1996)
E. faecalis P1a ham	7.9	na	7.5	Joffin and Joffin (2010)
E. faecalis 2350p1 milk	0.6[a]	2.7	7.5	McAuley et al. (2012)
E. faecalis 2356p1 milk	0.82[a]	0.3	5.0	McAuley et al. (2012)
E. faecalis CIP 76.117 BHI medium pH 7.3	0.39	0.4[a]	9.3	Gadonna-Widehem et al. (2012a)

[a]Values were calculated from data collected in the literature

E. faecalis and Microwave Treatment

Few studies have reported the impact of microwave treatments on *E. faecalis*. Lechowich et al. (1969) have investigated this impact on *E. faecalis* suspensions containing 10^9 UFC/mL by using a 2450 MHz modified microwave oven. After treatment of 30 min at 1500 W, very low reduction (less than one decimal reduction) was obtained. Moreover, it appeared that the lethal effect was only due to the heat production. Knutson et al. (1988) compared the impact of conventional and microwave heating on *E. faecalis* inoculated in milk. Very low reduction was observed after microwave heating compared to an LTLT treatment (62.8 °C for 30 min). The killing activity of microwaves was also tested by Najdovski et al. (1991) at constant power (325 and 600 W). Unlike *E. faecalis*, *Staph. aureus*, *Streptococcus pyogenes* group A, *E. coli*, and *Ps. aeruginosa* were promptly killed in less than 5 min. In addition, *E. faecalis* was more resistant to microwave treatments in dried suspensions than in aqueous ones. Riva et al. (1991) have compared the effects of conventional and microwave heating on *E. faecalis* survival by maintaining a constant temperature. Both heating technologies gave comparable *D*- and *z*-values. Gadonna-Widehem et al. (2012a) have used the same approach as that of conventional heating to determine D_p- and z_p-values at constant specific power after microwave heating (Table 23.6). They established the inactivation kinetics for *E. faecalis*. A shoulder pattern with an initial lag phase followed by a logarithmic death rate was observed (Fig. 23.13). The absence of mortality during the lag phase is attributed to an insufficient temperature of the medium (<60 °C). In the second part of the curve, the bacterial population decreased linearly with time. The *D*-values at constant specific power (D_p) could be determined by the slope of these straight lines.

Using this new approach, specific parameters for microwave heating have been determined (D_p and z_p values) and could be extended to other microorganisms.

23.4.2.4 Microwave Cooking in Catering

It is common that pasteurization or even sterilization occur during cooking (Ball and Olson 1957; Pittia et al. 2008). The cooking step in food preparation is often

Table 23.6 D_p- and z_p-values obtained for *E. faecalis* CIP76.117 in BHI at pH 7.3 after microwave heating at constant specific power in a lab-scale pilot (Gadonna-Widehem et al. 2012b)

Specific power (W/g)	Equations	R^2	D_p-values (s)
4	$y=-0.023x+13.1$	0.990	44.1
8	$y=-0.063x+15.4$	0.954	15.9
11	$y=-0.102x+17.0$	0.966	9.8
14	$y=-0.154x+19.8$	0.924	6.5
z_p-value			
12.1	$Y=-0.083x+1.9$	0.977	

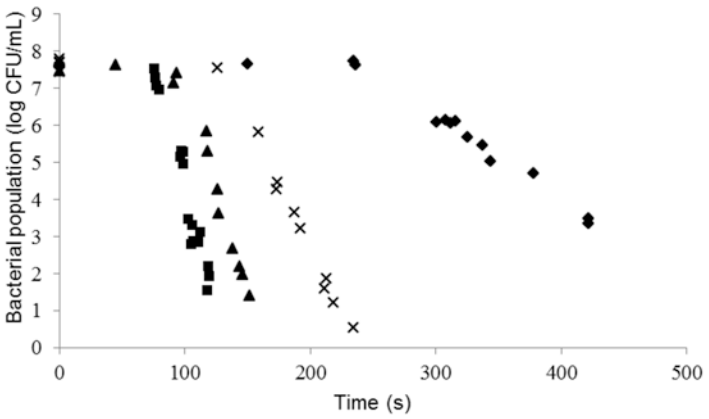


Fig. 23.13 Cumulative data of survival bacteria ($n=4$) obtained after microwave heating of *E. faecalis* CIP 76.117 suspensions in BHI medium pH 7.3 at different specific powers: 4 (♦), 8 (×), 11 (▲), 14 (■) W/g performed on a laboratory-scale pilot (MES Technologies Company)

a critical control point in HACCP plans in industries or in catering services (Codex Alimentarius Commission 2003; Regulation EC 852/2004). As an example, in French law, minced steaks must be cooked at a temperature higher than 65 °C (DGAL 2007). For safety reasons, the authorities recommend maintaining a core temperature of 70 °C for 2 min in order to eliminate *E. coli* STEC (ANSES 2011). These recommendations for cooking minced steak in catering are based on *D*- and *z*-values. Microwave ovens became interesting for fast cooking on a catering scale ever since high power microwaves (up to 3000 W) were commercialized. To study pasteurization during microwave cooking, Gadonna-Widehem et al. (2012b) evaluated *E. faecalis* survival during the cooking of a hotpot dish (beef, potatoes, onions, and white stock solution) using a microwave catering scale oven (Fig. 23.14). After mixing with a sterile spoon, 1/5 of the dish was collected and analyzed on Slanetz and Bartley agar or after enrichment in BHI broth. Despite the high inoculum (6×10^{11} bacteria per dish), no bacteria were detectable after 25 min of microwave heating. This result confirmed that pasteurization occurred during the cooking operation of the hotpot. A shoulder pattern was observed in the first part of the curve. Low bacterial destruction was obtained because temperature was lower than 60 °C. During this phase, only heat sensitive bacteria were killed. During the second phase, a fast and linear decrease was observed. D_p-value of 63 s at a specific power of 1 W/g was observed. Pasteurization was also proved during beef Burgundy cooking in a microwave oven adapted for catering (Jouquand et al. 2015).

From these results, Gadonna-Widehem et al. (2012b) concluded the possibility to give recommendations for microwave cooking based on product weight, applied microwave power, and cooking time. Furthermore, final temperature of the product must be measured after mixing.

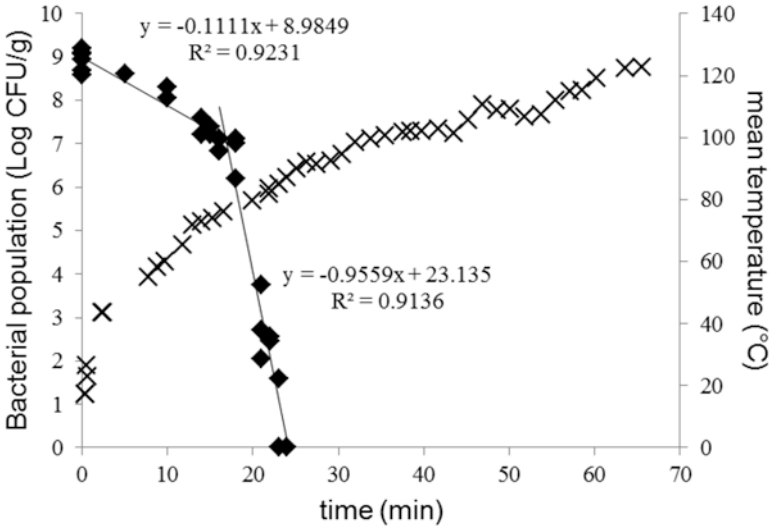


Fig. 23.14 Cumulative data of *E. faecalis* population (♦) and mean temperature (×) versus time during the hotpot cooking in microwave catering scale oven Electrolux 260153 Air-o-speed™ at 1 W/g

23.5 Conclusion

Regardless of the finality of the microwave treatment (sterilization, pasteurization, or cooking), microbial populations can be reduced or destroyed using this alternative technology. However, optimized conditions are required for this type of heating in order to reduce the microbial hazard to an appropriate level that ensures food safety. Specific destruction parameters, D_p- and z_p-values, can be determined to qualify this heating method. The acquisition of such scientific data could help authorities to provide time-specific power references for the reduction or destruction of a target organism. Nevertheless, data are still required to achieve the same level of knowledge as for conventional heating, keeping in mind the already existing difficulty of choosing the appropriate target germ for validating pasteurization operations.

With microwave heating, care must be taken to guarantee food safety. The first recommendation is to improve the distribution of temperature. To achieve better results when using microwave ovens:

- power and time of heating must be adapted to the weight of the product
- regular mixing during cooking must be applied to increase homogeneity of temperature
- waiting for a few minutes after the end of the cooking to obtain a better distribution of temperature is necessary
- mixing at the end of cooking and measuring the final temperature are needed

The second recommendation is to train staff using microwave technology in order to achieve better control. The staff must be aware of the microbial risks associated with the product, but also with the cooking method. For example, from a food safety point of view, microwave ovens are more adapted for reducing cooking time of simmered dishes rather than for fast preparation.

In addition, microwave technologies need improvements mainly in the areas of higher oven power, convective modes, and temperature measurements. For example, commercial ovens could be equipped with an infrared sensor in order to manage cooking operations as a function of product surface temperature.

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