Jingdun Jia · Donghong Liu · Haile Ma *Editors*

Advances in Food Processing Technology

ZHEJIANG UNIVERSITY PRESS
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Chapter 1
Innovation of Food Physical Processing Technology in China

Jingdun Jia, Yiqiang Ge and Xun Wei

Abstract With the development of society and the improvement of consumer's living standards, healthy, safe, and high-quality foods are preferred. As an emerging technology in food industry, food physical processing has attracted significant attention in recent years. This chapter introduces the current status of various food physical processing technologies—such as high hydrostatic pressure, irradiation, microwave, ultrasound, pulsed electric field and cold plasma—in some countries and regions like the United States of America (USA), Europe, and China. The last 10-year data relating to physical food processing technology has been collected from scientific research, research platforms, and industrial support and analyzed to evaluate the development and innovation in food physical processing technologies around the world, and specifically in China. This is expected to help the reader to understand the present advances and future trends of typical food physical processing technologies.

Keywords Food processing technology · Scientific research · Current status · Future trends

1.1 Introduction

Food processing technology is constantly developing as a response to a number of different challenges. Increasing customer demand for healthy, safe, and high-quality foods with natural flavor is partly responsible for the evolution of established food processing technology. Further, owing to the introduction of chemistry and biology, nutritional science and food processing technology has been widely promoted. With the increasing demand for high food quality, safety, and nutritional value, as well as greater product variety, the food industry faced with the challenge to improve production efficiency and product quality. Since 1990, a great deal of research has been

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carried out to develop methods using physics to solve problems in food processing; this includes acoustics, optics, electrics, and magnetism.

Today, a number of emerging food physical processing technologies have already been applied in the food industry, such as high hydrostatic pressure (HHP), radiation, microwave, ultrasound, pulsed electric field (PEF), and cold plasma. These technologies reduce the processing time, enhance food quality, and improve the operating conditions.

High-pressure processing (HPP) is a food physical processing technology that uses elevated pressure—with or without the addition of heat—to deactivate vegetative cells of pathogenic and spoilage microorganisms, modify enzymatic activity, and reduce the loss of desirable compounds, thus preserving the freshness and nutritional value of food (Huang et al. 2014). HPP treats food using pressures of 100–1000 MPa at room temperature or mild heat. The process is also commonly referred to as HHP processing, and ultra-high-pressure (UHP) processing. HPP has been considered to be an innovative technology in food processing because of its "minimized processing" of the technical characteristics of food products. With an increasing number of customers demanding fresh and natural products, HHP treating food has become a hot research topic. HPP is used as an alternative technology for killing pathogens such as Escherichia coli, Salmonella, Listeria, and Vibrio without additional heat processing (Balasubramaniam and Farkas 2008). It is effective on a wide variety of foods such as fruits, juices, vegetables, seafood, sauces, and ready-to-eat meats.

Microwave technology was developed in the 1940s and China began to carry out its own research and development in the 1970s. At present, this technology is widely applied in the food processing field; however, early development of microwave technology for the food industry was slow. In 1986, the industrial microwave equipment was successfully developed for food temperature control in products such as precooked bacon, poultry, patties, noodles, fast food, and dried fruits and vegetables, as well as for the disinfection of bread and yogurt (Zuo 2010). After the microwave sterilization process, foods can be stored at room temperature. Microwave also assists in thawing and re-heating frozen foods and convenient foods, such as some flavor snacks (Sheng 1999).

The food irradiation process, also known as ionizing radiation, uses radiation to irradiate foods and raw materials. The process is done to delay the development of certain physiological processes (germination and maturation), kill insects, and maintain quality while extending the storage time by disinfection, sterilization, and inhibition of mold. Due to the advantages of radiation process, such as low energy consumption, pollution-free operation, and nonthermal treatment, irradiation technology has a wide range of applications in the food industry (Zheng et al. 2012; Chen et al. 2007).

Ultrasound is a sound wave with a frequency higher than the upper limit of human hearing. From a food processing perspective, it can be considered as a high-frequency vibration that causes fluid mixing and shear forces on a microscale. As a physical processing technology, ultrasound is considered to be an alternative to conventional thermal treatment based on its promising effects in food processing (Ashokkumar 2011). The applications of ultrasound in the food industry can be divided into two

categories: high-frequency ultrasound and power ultrasound. The former uses high frequencies of 2–20 MHz with low sound intensity (0.1–1 W cm^{-2}) and is mainly applied in quality analysis, medical imaging, and nondestructive inspection. Power ultrasound, also known as high-intensity ultrasound, refers to sound waves with low frequencies (20–100 kHz) and high sound intensity (10–1000 W cm^{-2}) (Kentish and Feng 2014). The formation, growth, and collapse of cavitation bubbles in liquid media induce mechanical effects such as microstreaming, high shear forces, shock waves, and sonochemical reactions, e.g., free radicals and hydrogen peroxide (Li et al. 2016; Rastogi 2011). This approach can alter the physicochemical properties or structure of a material, and it has been widely used in microbial and enzyme inactivation, emulsification, nebulization, sonocrystallization, and extraction of bioactive component(s) in foods and material (Kentish and Feng 2014).

Pulsed electric field (PEF) is one of the novel, nonthermal processing technologies used in the food industry; short period (1–100 μs), high field strength (typically 20–80 kV cm^{-1}) electric pulses are used to treat food stuffs located between two electrodes. Due to its properties of being environmentally friendly, high efficiency, easy handling, and low processing temperature, PEF technology has gained considerable attention from food researchers (Buckow et al. 2013). Moreover, PEF treatment is conducted at ambient, sub-ambient, or slightly above ambient temperature for short time (<1 s); this results in minimal undesirable changes in the sensory characteristics of the food. The first application of PEF technology dates back to the 1960s, but the first commercial application was not achieved until 2005 (Clark 2006). Currently, the PEF technology in food industry is mainly used for drying, extraction and diffusion of various biological materials, and inactivation of microorganisms and enzymes (Nowacka 2014; Puértolas and Barba 2016). PEF technology has also been successfully applied as an alternative to traditional thermal processing of bulk liquid food products, such as alcoholic beverages, milk, fruit juice, and liquid egg (Ait-Ouazzou et al. 2013; Salvia-Trujillo et al. 2017; Wu et al. 2015a, b; Espina et al. 2014). In addition, there is a synergistic effect between PEF technology and other mild preservation methods, thereby making it a topic of interest in recent years (Lü et al. 2016; Siemer et al. 2014).

Another promising nonthermal food processing technology is the cold plasma; it applies a mixture of various active species of charged particles, such as electrons, ions, atomic species, UV photons, and these charged particles can kill microorganisms (Patil et al. 2014; Fernández et al. 2012). The use of nonthermal plasma in the food industry has developed over the last 10 years, for applications such as microbial inactivation, modification of food packaging materials, modification of hydrophobicity of food surfaces, enzyme inactivation, and enhancing grain germination (Misra et al. 2011; Pankaj et al. 2014; Misra et al. 2016; Randeniya and De Groot 2015).

1.2 Development of Innovative Food Physical Processing Technology in the World

The food industry is increasing its efforts to enhance food quality and safety throughout the world. Data from high-pressure processing, microwaves, irradiation, ultrasound, pulsed electric field, and cold plasma have been analyzed. This will allow for a proper assessment of the level of development in innovative food physical processing technology around the world, which is said to have witnessed a rapid development in the last decade. The USA holds a dominant position both in scientific research and industrial applications. In terms of the number of published papers, it ranks first in food irradiation and ultrasonic processing technology, and second in high-pressure processing, microwave, pulsed electric field and cold plasma. As for the number of issued patents, the USA ranks first in all fields, except for microwave technology where it ranks second. Recently, physical processing technology in China has developed quickly and has achieved excellent outcomes, dominating the top of list in terms of the number of published articles in the fields of high-pressure, and microwave technology, and second in the rankings for food irradiation, with ultrasound and pulsed electric field in the third place. However, cold plasma technology in China is still at a preliminary stage, and the volume of published articles so far was not enough to place it in the top ten. Furthermore, China has no advantage in terms of published patents and is far behind other countries. Advances have been made in European countries, especially Spain, which ranks first in terms of pulsed electric field technology, and third in high-pressure and ultrasonic processing while Ireland has made advances in the field of cold plasma. Although Russia does not make the top ten in terms of published numbers for physical processing technology, the country dominates the world in terms of patents in the field of microwave technology. As for other Asian countries, only Japan and India perform well. In some developed nations, especially the USA, European countries, and Japan, basic research has been conducted into natural phenomena, and now more attention has been paid to commercialization, industrialization, and national competition.

1.2.1 High-Pressure Processing (HPP)

HPP technology was first reported in 1899 for milk preservation, and in Japan it was rapidly developed for use in the food industry. In 2010, there were approximately 150 high-pressure units worldwide. In total 250,000 tons of vegetables (33%), meat (30%), fish (15%), and juices and beverages (12%), have so far been processed across 71 industries in America, 26 in Europe, 22 in Asia, and 5 in Oceania (de Oliveira et al. 2017; Campus 2010; Mújica-Paz et al. 2011). In 2009, the Food and Drug Administration (FDA) of the USA approved the applications of high pressure as a preheated process for the commercial sterilization of low-acid foods (Juliano et al.

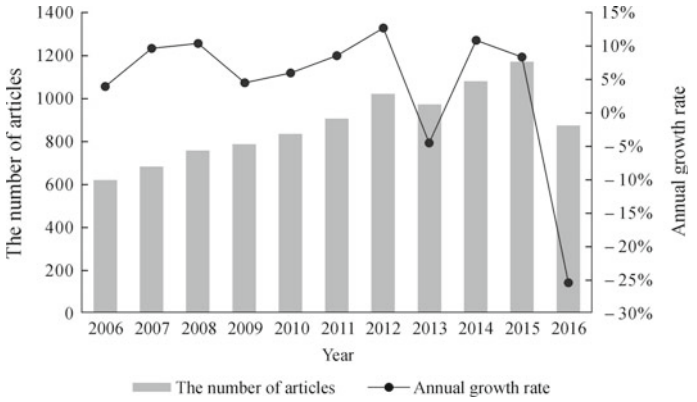

Fig. 1.1 The number of published articles and annual growth rate in the high-pressure processing field

2012; Stewart et al. 2016). This is a significant milestone for the commercialization of sterile foods preserved by pressure-assisted thermal processing (PATP).

Over the past decade, HPP technology has developed rapidly across the world. From 2006 to 2016, there was a rise in the number of published articles on the HPP of foods. As shown in Fig. 1.1 (with data from Scopus), the number of published articles on HPP technology was below 800 before 2010, but, from 2010, there was a rapid increase in the number of published papers. In general, the development of high-pressure technology in the food industry shows a steady upward trend globally, marking its increasing sophistication. The top ten countries for article publication were China, the USA, Spain, Italy, Germany, Japan, the Republic of Korea (hereinafter "Korea"), France, the United Kingdom (UK), and Brazil (Fig. 1.2). Considering the number of articles published over the last decade across the world, the number of articles from China accounted for 17% of the total, the USA 16%, and Spain 11%. The fact that China produced the largest amount of articles means that they have done abundant research in this field.

In general, the number of international patents issued in the countries under review relating to the application of HPP to food processing has shown an upward trend over the last decade. Figure 1.3 indicates that the number of patents has increased from 2006 to 2011. In particular, the largest number of patents was in 2011 and the growth rate of patents issued in 2008 was more than 50%. The number of issued patents showed the same trends between 2012 and 2016, as there was between 2006 and 2011; this number reached a maximum in 2016. The percentage of international patents issued over the last decade in different countries is summarized in Fig. 1.4.

In this period, the USA accounted for 15%, Canada 10%, China 8%, and Japan 5%, respectively. The majority of international patents are issued in the USA and Canada. Although the history of the development of HPP technology for the food industry is short in China, it is at the forefront in terms of international patents. The high-acid jam, marketed by the Japanese company Medi-Ya, was the first commercial

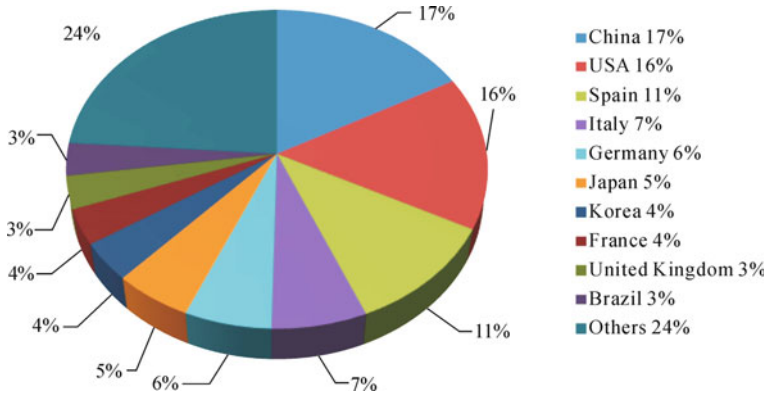

Fig. 1.2 The top ten nations in the published articles of high-pressure processing

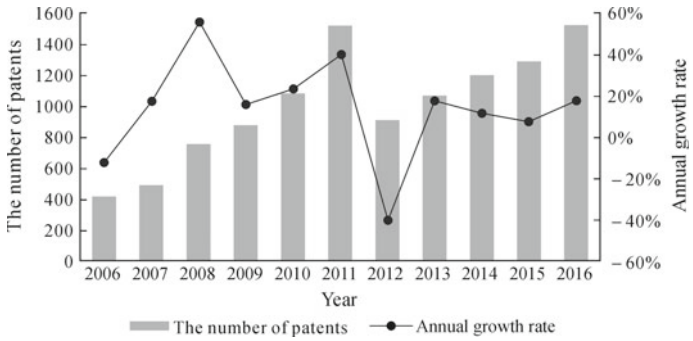

Fig. 1.3 The number of published patents and annual growth rate in the high-pressure processing field

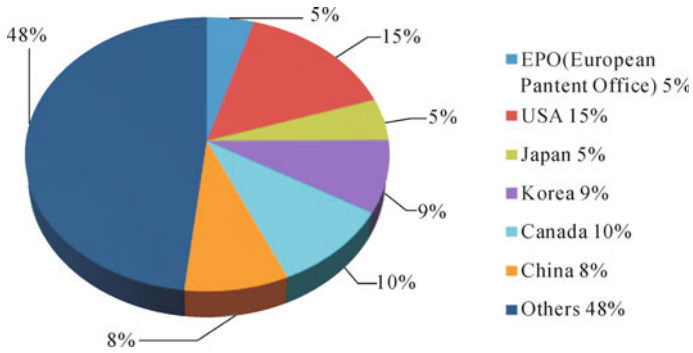

Fig. 1.4 The top ten nations with the published patents of high-pressure processing

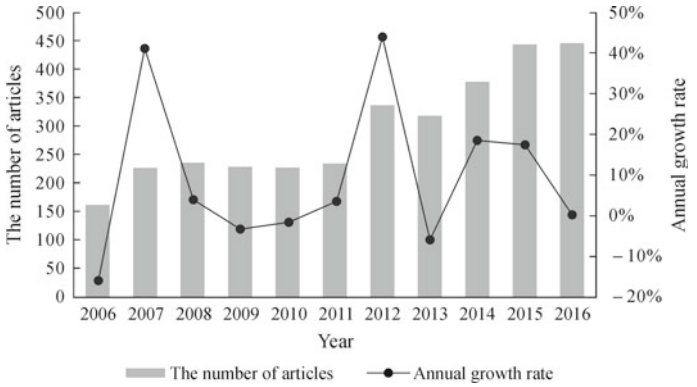

Fig. 1.5 The number of published articles and annual growth rate in the microwave field

food to which HPP technology was applied in the early 1990s (Mertens 1995). HPP technology has also been widely used for other products due to the commercial success of jams. These include HPP-treated jellies and shellfish in Japan; oysters and guacamole in the USA; and fruit juices in France, Mexico, and the UK (Smelt 1998; Torres and Velazquez 2005). The equipment capacity has developed as product demand has increased; for example, Avomex Inc. began treating avocado with HPP using a 25 L batch processing unit in 1996, and later a 50 L vessel. By 2000 the company had produced a semi-continuous unit and a larger 215 L batch processing vessel (Torres and Velazquez 2005).

1.2.2 Microwave

Since the 1980s, microwave technology for food processing has advanced rapidly in developed countries. As shown in Fig. 1.5, the number of articles on microwave technology used for food has shown a general trend of growth. Figure 1.6 shows the top ten nations in terms of the number of published articles on microwave technology, of which China has published the largest number, showing that a lot of research has been done in this field in china.

Figure 1.7 shows that there were a great number of patents issued around the world in the microwave field between 2006 and 2016, reaching its peak in 2010 and 2012. While the number of articles kept small between 2012 and 2015, and in 2016 it rose again to nearly 4600, indicating that research focus has been redirected at microwave food technology. Over the past decade, Russia has issued the highest percentage of patents on microwave food technology totaling 58%, the USA was second with 7%, and China was third with 5% (Fig. 1.8). The results show that although China is a global leader, there is a large gap when compared to Russia. In Japan's food market, microwave foods come in several varieties, while in the USA, they are mostly

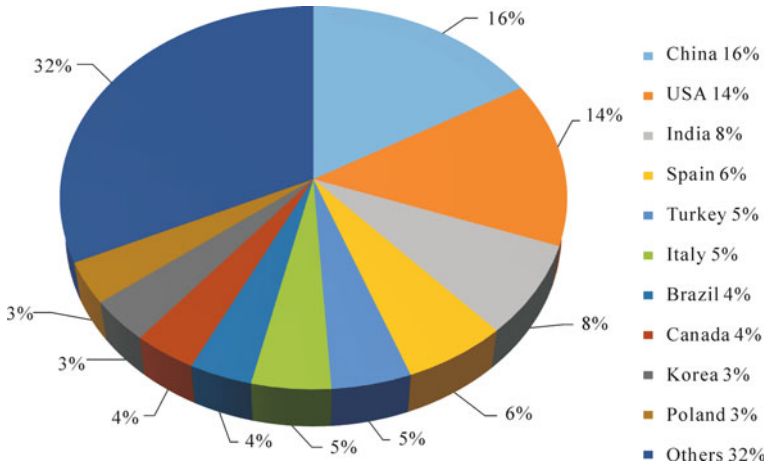

Fig. 1.6 The top ten nations with the published articles on microwave technology

prefabricated convenience foods. At present, there are more than 200 companies in the USA producing more than 300 kinds of microwave prefabricated food marked with a "microwave" label. The products include refined dishes, prefabricated soups, chilled small packs of fast foods, vegetables, side dishes, all kinds of dessert, frozen fast foods, pancakes, fried potatoes, crisp peanuts and so on. The Bormel Company is one of the top 500 enterprises in the world and produces microwave foods, including a variety of traditional American dishes, nutrition soups, children's nutrition foods, nutrition fast foods, and frozen microwave foods suitable for breakfast, lunch, and dinner. Asia is the largest producer and consumer of frozen foods in the world, the family consumption rate of microwave foods has reached 88.4%. There are more than 20 companies which produce nearly 200 kinds of microwave foods which can be divided into five major categories: fried meatloaf, rice, pasta, hamburger, and fried steak (Sheng 1999).

1.2.3 Irradiation

American researchers first treated hamburgers with radiation in 1943. In 1986, the FDA developed regulations for "irradiation of food production, processing, and treatment", and later several amendments were added regarding the radiation source, food category, purpose, radiation dose, logo, and packaging. In 1997, FDA approved a license to irradiate red meats such as beef, pork, and lamb.

As shown in Fig. 1.9, since 2006, the number of papers on irradiation techniques applied to food has fluctuated slightly, indicating that research into this technology is at a relatively mature stage. From the total number of articles on food irradiation technology, Fig. 1.10 shows that the USA, Korea, and China are the top three, indi-

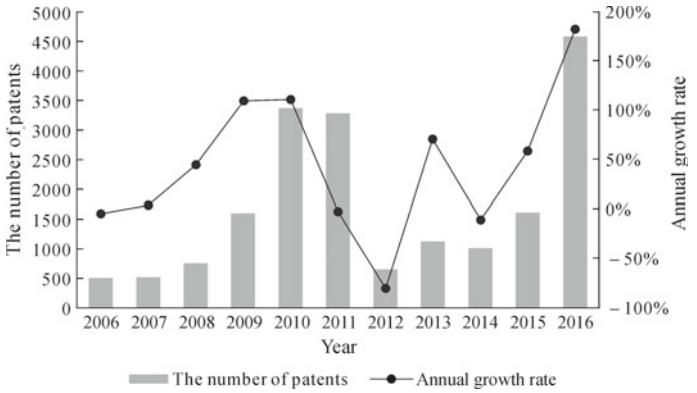

Fig. 1.7 The number of published patents and annual growth rate in the microwave field

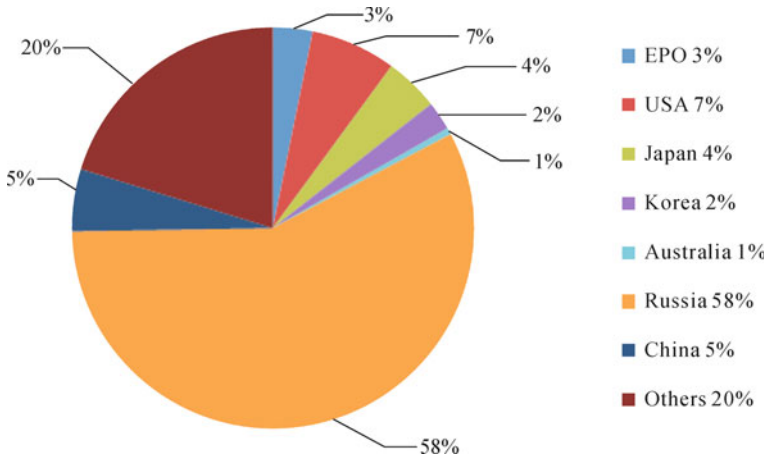

Fig. 1.8 The top ten nations with the published patents on microwave food technology

cating that China is also promoting the development of food irradiation technology and its applications.

The number of patents issued relating to the food irradiation technology in the world is presented in Fig. 1.11. Between 2006 and 2008, it showed a rapid growth, reaching a maximum of 229 patents in 2008. Following this, the global number of patents on the subject began to decline until 2012 when it began to rise gradually. This suggests that people were more concerned about the irradiation food, and this promoted more research in this area. Over the past decade, the USA has issued the largest number of patents on food irradiation technology totaling 23% of world patents; Japan was second with 12%, EPO was third, and China was fourth (Fig. 1.12). The results show that although China has made some advances in the field of food

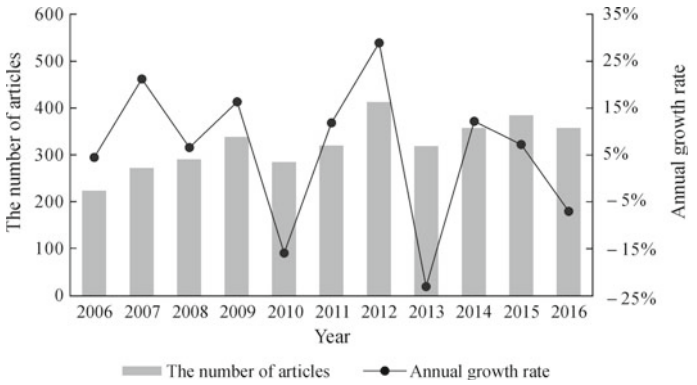

Fig. 1.9 The number of published articles and annual growth rate in the irradiation field

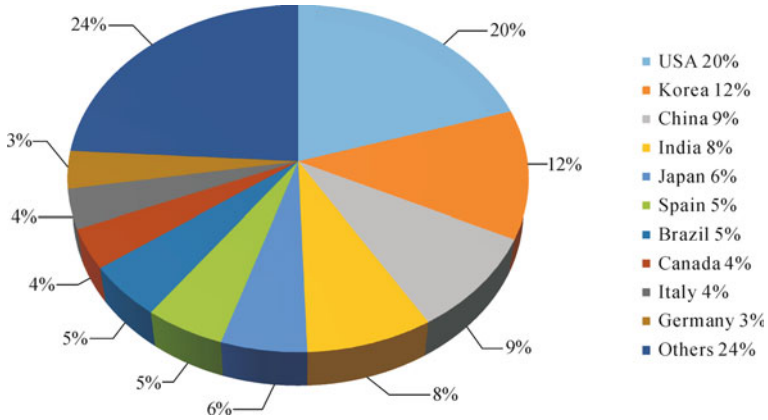

Fig. 1.10 The top ten nations with the published articles on food irradiation technology

radiation, compared with the USA, Japan and other countries, there is still room for improvement.

1.2.4 Ultrasound

Before the 1990s, ultrasound technology was mainly used for ultrasonic detection. Globally, there has been an upward trend in the number of published papers on ultrasound technology over the last decade. As shown in Fig. 1.13, the number of articles was comparatively small before 2010, less than 200 articles each year. Notably, after the European acoustics conference held in Greece in June 2010, the number of published articles started to increase significantly. In this conference, power ultrasound technology applied to food was considered as one of the four areas

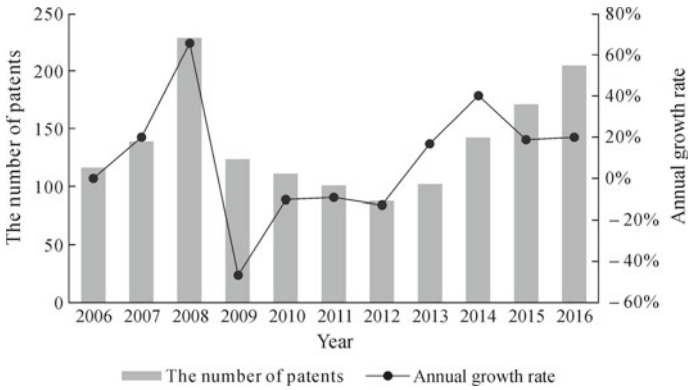

Fig. 1.11 The number of published patents and annual growth rate in the irradiation field

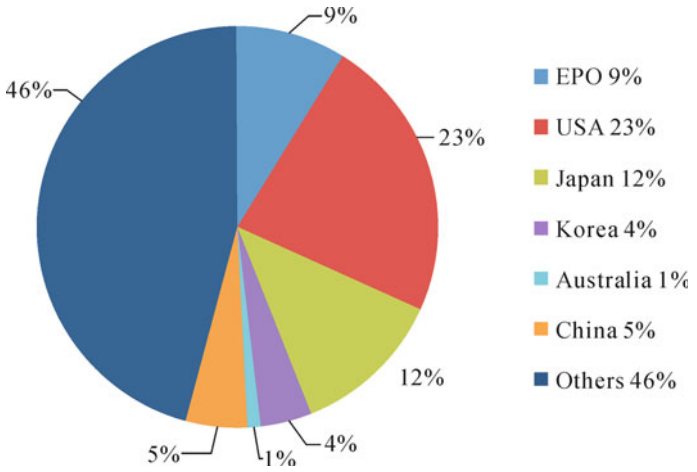

Fig. 1.12 The top ten nations with the published patents of irradiation

of focus. In 2006 alone, more than 450 articles were published. Comparing the total number of documents for ten countries (Fig. 1.14), the dominant positions in the field are occupied by the USA, which accounted for 19% of the total, China 14%, and Spain 9%, which was followed by Italy (5%), the UK (5%), Australia (5%), Brazil (4%), Germany (4%), Canada (3%), and India (3%).

As shown in Fig. 1.15, the number of relevant patents grew rapidly between 2006 and 2008 when it reached its peak. By now, the number of patent applications per year have been becoming stable. Figure 1.16 shows the proportions of international patent applications. The USA has the highest percentage at 34%, with Russia and the EPO approximately equal in proportions. However, Asian countries have recorded fewer advances in securing patents. Large-scale processing using power ultrasound is now commercially available in Europe and the USA. An example of a batch

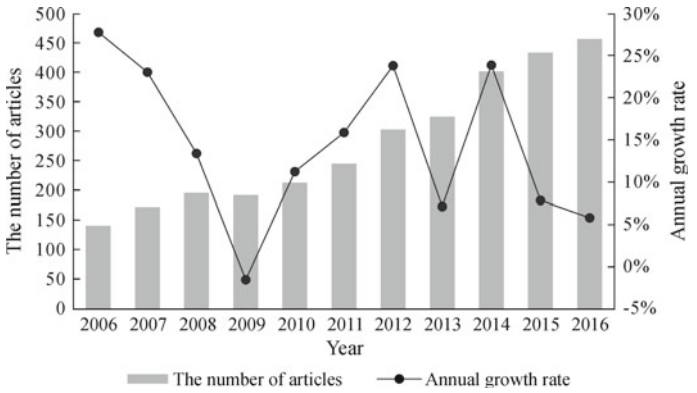

Fig. 1.13 The number of published articles and annual growth rate in the ultrasound field

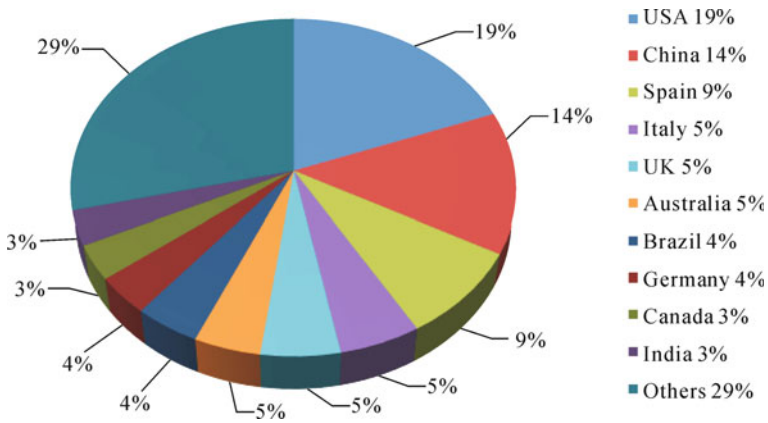

Fig. 1.14 The top ten nations in the published articles of ultrasound

industrial ultrasonic reactor was designed and built in Romania for the extraction and preparation of tinctures from various herbs. It can reduce the extraction times from 28 days to around 10 h with similar or better results. Hielscher has produced the UIP16000 operating at 16,000 W, which they claim to be the most powerful ultrasonic processor in the world. It can be used for homogenization, dispersion, and deagglomeration, processing up to 50 m^3 h^{-1} (Leonelli and Mason 2010).

1.2.5 Pulsed Electric Field (PEF)

The first application of PEF technology in the food industry was in the 1920s for processing of dairy products. Later, more studies were conducted to enhance the

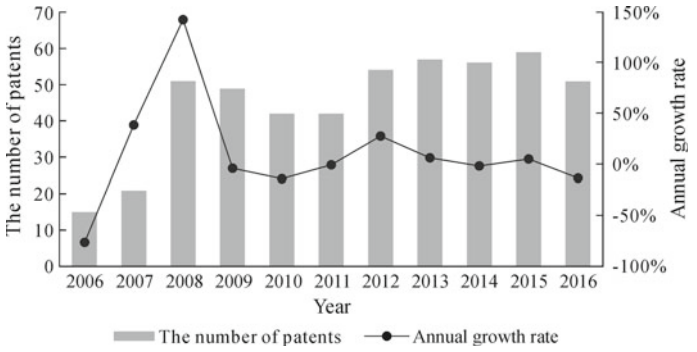

Fig. 1.15 The number of published patents and annual growth rate in the ultrasound field

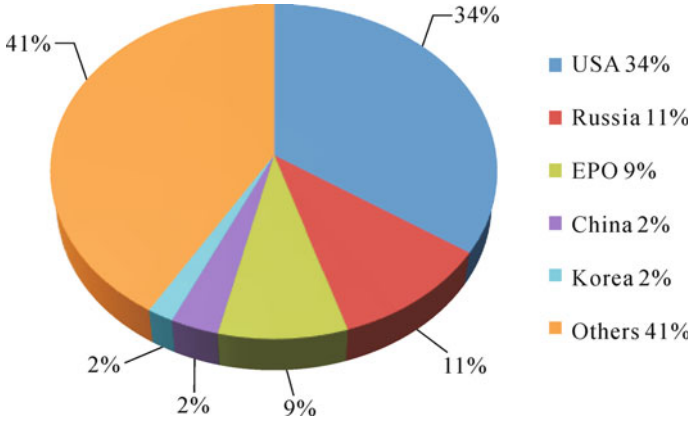

Fig. 1.16 The top ten nations in the published patents of ultrasound

efficient antimicrobial effect of PEF technology at room temperature with minimal loss of food quality. In the last ten years, the global number of scientific publications on the application of PEF in the food industry has increased considerably, from 37 papers in 2006, to 95 in 2016 (Fig. 1.17). The FDA of the USA approved the technical certification of PEF technology in 2005, promoting the development of PEF in the commercial market (Ravishankar et al. 2008; Barba et al. 2015). However, Fig. 1.18 shows that the development of PEF technology among different countries is imbalanced. In the last ten years, more than 50% of the articles about PEF technology in food processing were from Spain, the USA, and China with shares of 25%, 16%, and 13%, respectively.

Figure 1.19 presents the number of international patents relating to PEF technology across the world over the last decade, of which 57% are from the USA, ranking first in the world (Fig. 1.20). Before 2015, there were less than 10 relevant international patents each year, which has increased to 17 in 2015, and 36 in 2016, indicating the development of PEF technology around the world. Several international research

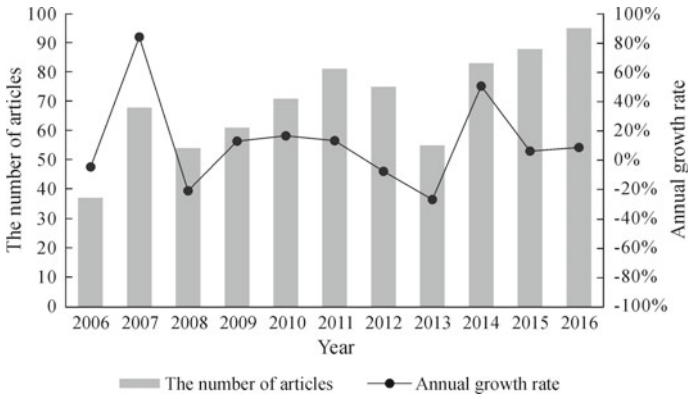

Fig. 1.17 The number of published articles and annual growth rate in the field of PEF

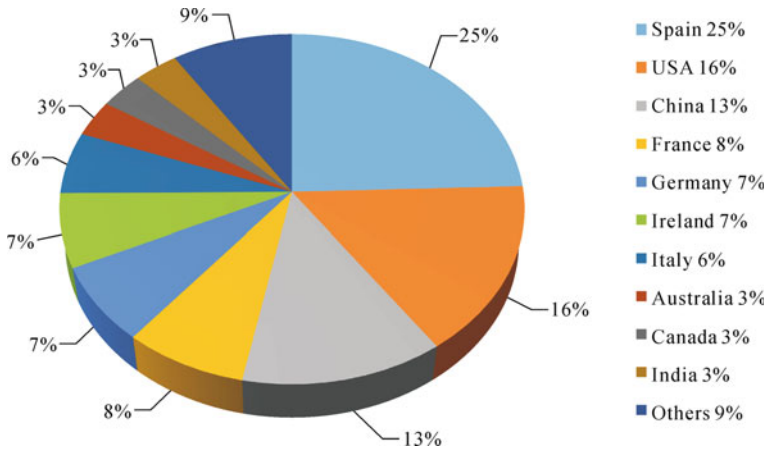

Fig. 1.18 The top ten nations with the published articles on PEF

groups and enterprises aim to promote the development of PEF technology around the world, including Ohio State University in the USA, Diversifie Technologies Inc. (DTI) in the USA, Berlin University of Technology in Germany, Elea GmbH in Germany, the German Institute of Food Technology, University of Adelaide in Spain, University of Guelph in Canada, Washington State University in the USA, Nestle Co. in Switzerland, and University of Zaragoza in Spain (Misra et al. 2017; Agcam et al. 2014). Ohio State University, in particular, has developed the first commercial PEF bench-scale system (OSU-4F), with six co-field chambers for the continuous processing of liquid food at a rate of 200–500 L h^{-1} (Sánchezmoreno et al. 2004; Odriozola-Serrano et al. 2009). In October 2010, Europe launched the 2-year "Smart-milk" project, which has successfully developed a high-voltage PEF processing system for the process of milk sterilization at a rate of 6000 L h^{-1} (Odriozola-Serrano

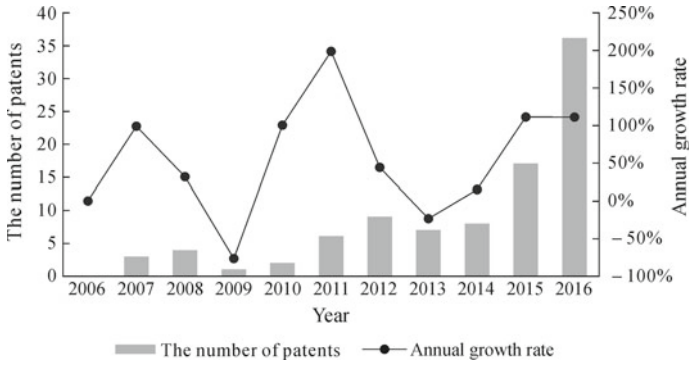

Fig. 1.19 The number of published patents and annual growth rate in the field of PEF

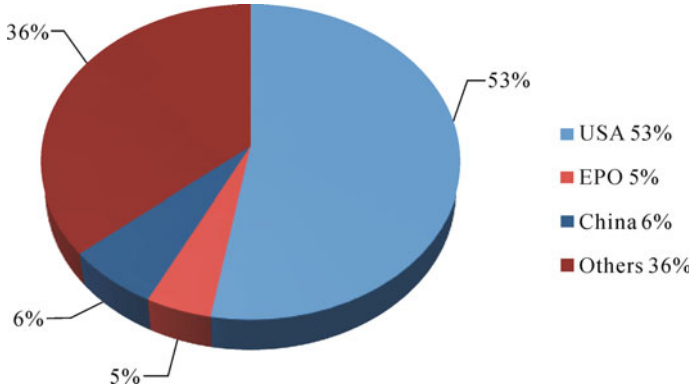

Fig. 1.20 The top ten nations in the published patents of PEF

et al. 2009). In 2015, DTI introduced a scalable PEF pilot system for pasteurizing juices, lysing algal cells, and extracting compounds from plant materials, processing up to 100 L h^{-1} (DTI 2015). Elea GmbH, a famous supplier of PEF systems in Germany, has developed more than 70 kinds of PEF systems with different capacities and that can be used to process various food products (e.g., juices, dairy, veggie chips, potato chips, etc.) (Elea GmbH 2017).

1.2.6 Cold Plasma

Cold plasma technology was a newcomer to the food industry in the last decade. As shown in Fig. 1.21, the development of cold plasma technology for food applications remains at a preliminary stage throughout the world and requires more research into further applications. Before 2008, there were very few papers about cold plasma

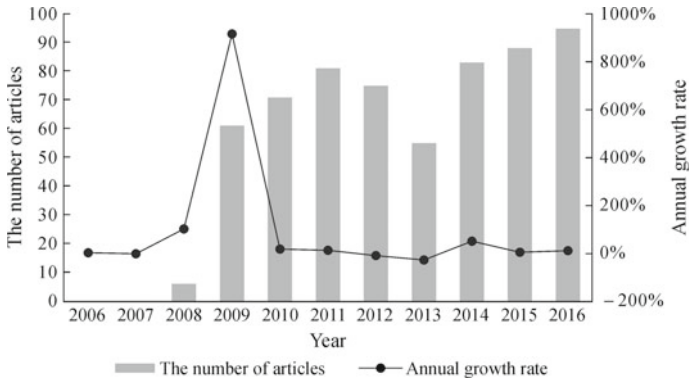

Fig. 1.21 The number of published articles and annual growth rate in the cold plasma field

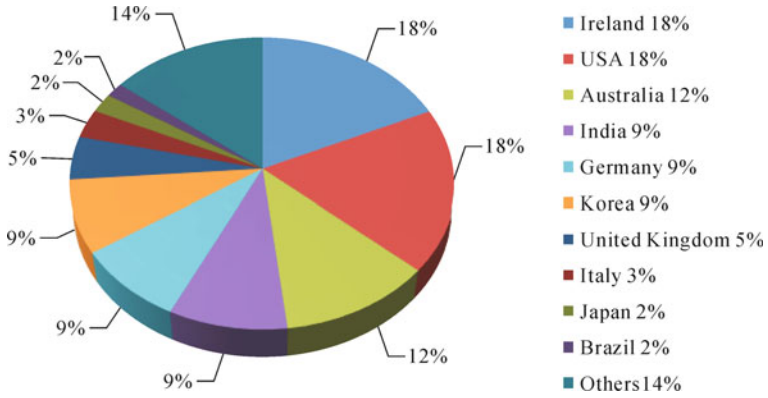

Fig. 1.22 The top ten nations in the published articles of cold plasma

technology relating to food processing. From 2008 to 2016, the number of scientific publications in this area has increased. Ireland, the USA, and Australia are the major countries for cold plasma research, which is indicated by the number of scientific papers from these three countries (Fig. 1.22). Currently, the research groups investigating in the applications of cold plasma technology in the food industry include the Institute of Chemical Technology in India, University of Bologna in Italy, Dublin Institute of Technology in Ireland, Seoul National University in Korea, Nagoya University in Japan, Purdue University in the USA, Loughborough University in the UK, Wageningen University and Walailak University in Thailand (Sarangapani et al. 2016; Tappi et al. 2016; Misra et al. 2015; Ishikawa and Hori 2014; Keener et al. 2012; Veen et al. 2015; Shaw et al. 2015).

1.3 Innovation of Food Physical Processing Technology in China

A number of studies have focused on physical methods to solve the problems of food processing, such as acoustics, optics, electrics, and magnetics. In the last ten years, there has been a leap in the development of food processing technology in China. High-pressure processing, microwave, irradiation, ultrasound, and pulse electric field are of interest for academic study and food industry applications. In order to promote the technical advances of food physical processing and to enhance the technical level of domestic food physical processing, four seminars on "food physical processing technology" have been successfully held between 2014 and 2017 under the direction of the China Rural Technology Development Center, the Ministry of Science and Technology of the People's Republic of China. These seminars explored the existing situation and opportunities for scientific and technological innovation of food physical processing and promoted cooperation between domestic institutes and researchers. Moreover, the Annual Conference of Chinese Institute of Food Science and Technology (CIFST), and the Chinese Society of Agricultural Engineering (CSAE) have held forums on food physical processing technology since 2015. There are many food companies, research institutes, and universities working together on this emerging technology.

Taking high-pressure processing technology as an example, which is used widely in food industry, many fruit and vegetable juice manufactures cooperate with key laboratories and research centers in order to conduct their research, and use emerging technology to replace traditional production methods. This is with the aim of improving the flavor and nutritional value of food products, as well as extending shelf life and other benefits.

1.3.1 Scientific Research in China

Food processing technology in China has grown substantially over the last decade, as a result of considerable investments into research. The number of articles and patents is increasing, and China's international contribution to the food physical processing technology has made great progress. In scientific research, as shown in Fig. 1.23, the number of published articles and patent applications has risen since 2006. The number of domestic articles on high-pressure processing, pulsed electric field, irradiation, ultrasonic, and microwave technology has been summarized in Fig. 1.24. It had increased since 2006, peaking in 2013, and then decreasing year on year. Meanwhile, from 2006 to 2016, the number of articles published in the international journals was increasing annually, though the total is still less than the number of domestic articles. As shown in Fig. 1.25, the annual number of domestic patents applicable to food physical processing technology shows an upward trend. However, the number of international patent applications is more complex, with a

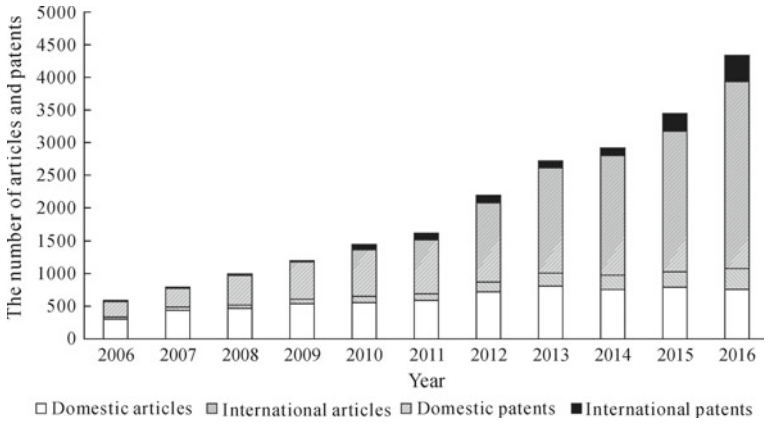

Fig. 1.23 The number of published articles and patents in food physical processing technology

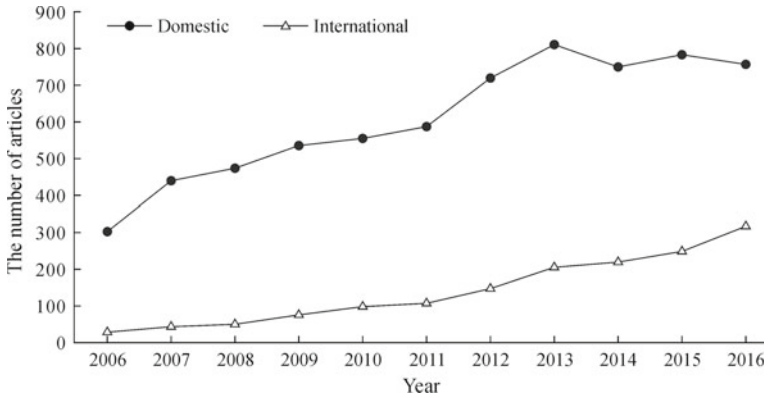

Fig. 1.24 The number of international and domestic published articles in food physical processing technology

small increase between 2006 and 2009, more applications from 2009 to 2011, and a slow increase from 2011 to 2014. The number of international patents increased substantially from 2015 to 2016, although the number of international patents was far less than that of domestic patents.

1.3.1.1 High-Pressure Processing

In recent years, the development of HPP for food has made significant progress in China. Figure 1.26 indicates that the number of articles published by Chinese researchers has increased, and that the number of domestic published articles was substantially larger than the number of international articles over the last decade.

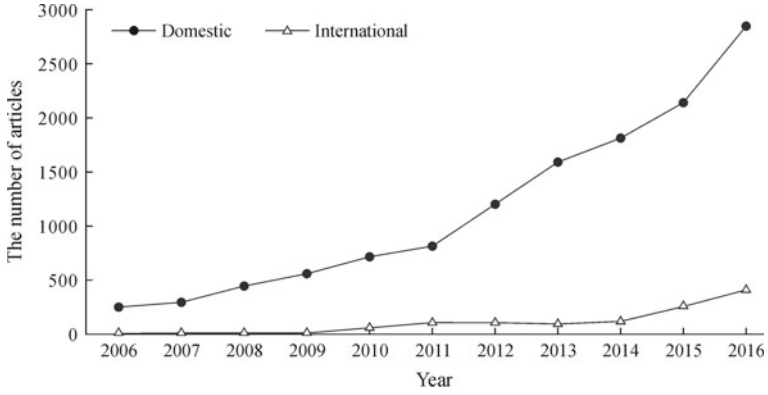

Fig. 1.25 The number of international and domestic patents on food physical processing technology

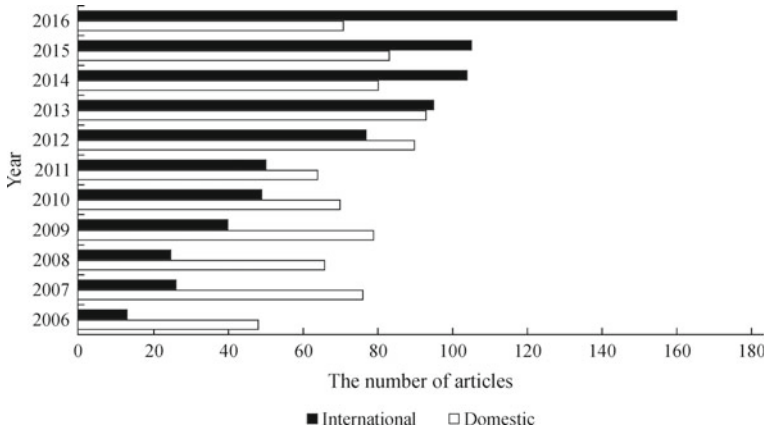

Fig. 1.26 The number of international and domestic published articles in the high-pressure processing field

Basic research into HPP in the food field in China has been increasing. However, compared with the advanced level around the world, China still has less high-impact research results. The development of HPP in food manufacturing has grown dramatically since 2009. As shown in Fig. 1.27, the number of patents issued in China has grown rapidly, and reached 178 cases in 2016. Although the number of patents issued was large, there were a small number of international patent in the food industry. Therefore, we should pay more attention to in international patent applications this industry.

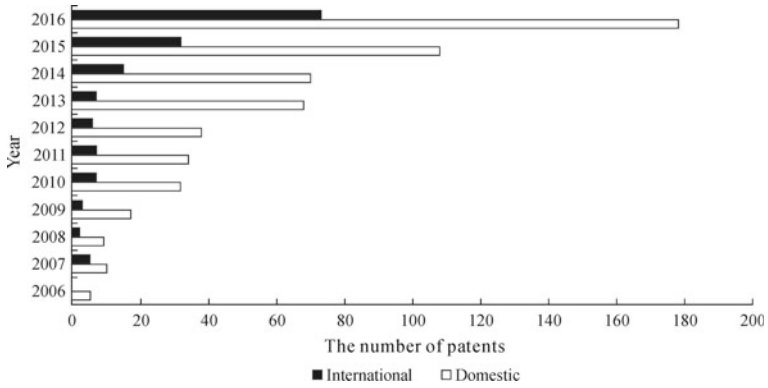

Fig. 1.27 The number of international and domestic patents in the high-pressure processing field

1.3.1.2 Microwave

The number of domestic articles on the microwave food technology has been on the rise over the last decade. Although the number has dropped from 2013 to 2016, it still remains at approximately 300 articles per year; this indicates that microwave technology in China has been well developed. At the same time, with high demands from the food industry, China's research into the microwave food technology is making innovative progress to seek for industrial applications. As shown in Fig. 1.28, between 2006 and 2016, the number of SCI articles published in the area of the microwave food technology by Chinese researchers increased significantly. From 2006 to 2009, there were only five to six SCI articles published each year, but by 2016, this number had increased to 43. In the past ten years, the number of domestic patents on the microwave food technology has shown a steady growth. At the same time, the number of international patents has also increased significantly. Figure 1.29 shows that from 2006 to 2009, only about ten domestic patents were issued annually, but this was nearly 100 in the years between 2011 and 2014, and 303 patents were issued in 2016.

1.3.1.3 Irradiation

As shown in Fig. 1.30, from 2006 to 2016, the number of domestic papers published on the food irradiation technology has fluctuated slightly, indicating that irradiation is a well-recognized technology in China, and that good progress has been made. In recent years, China's research into the food irradiation technology has become more popular, and the number of high-level research papers has continued to increase. From 2006 to 2016, the number of SCI papers on the food irradiation technology published by Chinese researchers has been growing. From 2006 to 2008, there were only five to six SCI papers published, but in 2016 this number had reached 43.

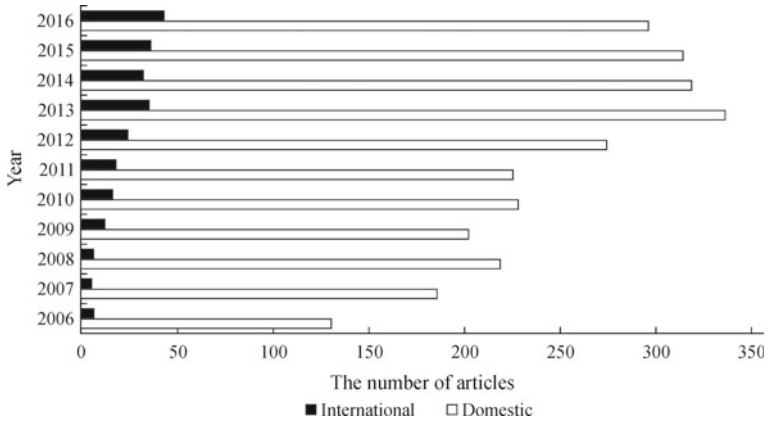

Fig. 1.28 The number of international and domestic published articles in the microwave field

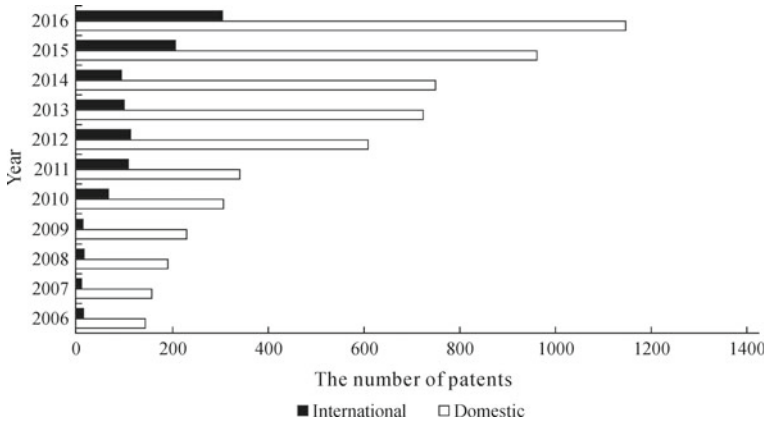

Fig. 1.29 The number of international and domestic patents in the microwave field

From 2014 to 2016, the number of domestic patents on food irradiation technology has increased. The number of international patents has also increased significantly. From 2006 to 2008, only one to three international patents were issued annually, but nine were published in 2014 and 18 in 2015, compared to 31 in 2016, as shown in Fig. 1.31.

1.3.1.4 Ultrasound

In the field of the ultrasonic food processing technology, the number of articles published by Chinese researchers has increased (Fig. 1.32). It is also shown that the articles published in Chinese academic journals represent a high proportion of the

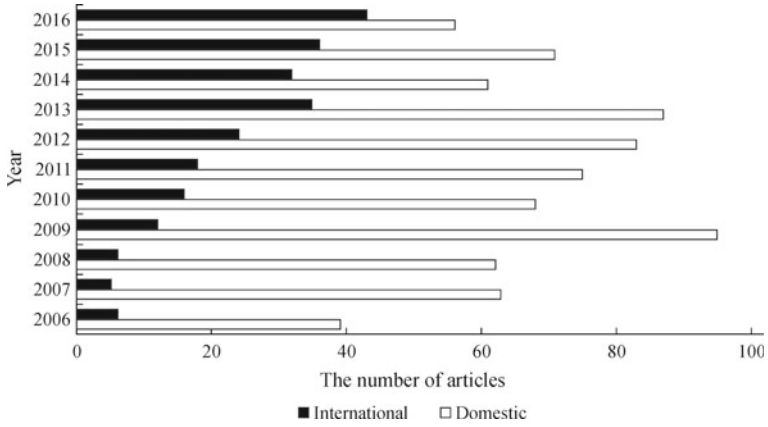

Fig. 1.30 The number of international and domestic published articles in the irradiation field

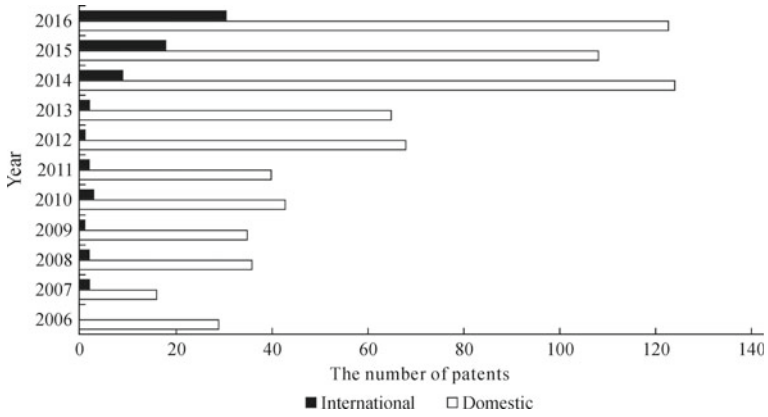

Fig. 1.31 The number of international and domestic patents in the irradiation field

total number of articles published by Chinese researchers. These scientific articles are of high-level in academic quality. Figure 1.33 shows that the number of patents has increased dramatically from 2006 to 2016; however, the number of international patent applications is still low. Patent applications are related to national development interests and international competitiveness. Therefore, more attention needs to be paid to international patent applications in China. With the support of national policy, the technology of ultrasonic food processing will develop rapidly in the future.

1.3.1.5 Pulsed Electric Field

The development of PEF technology in China only started in the 1990s, and remained at laboratory scale with limited studies. The research mainly focused on two aspects,

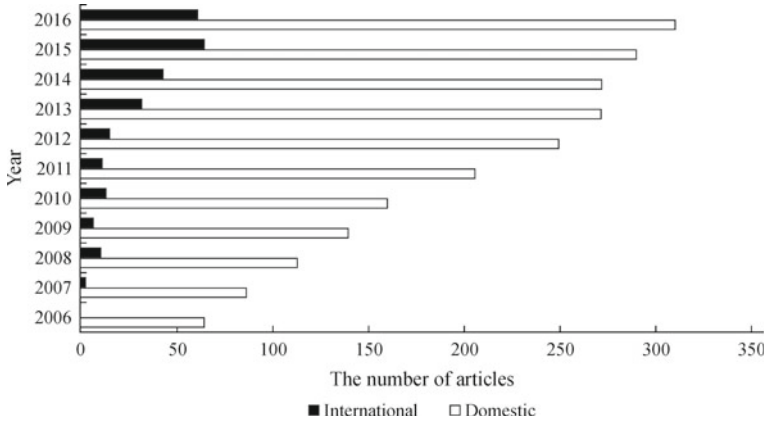

Fig. 1.32 The number of international and domestic published articles in the ultrasound field

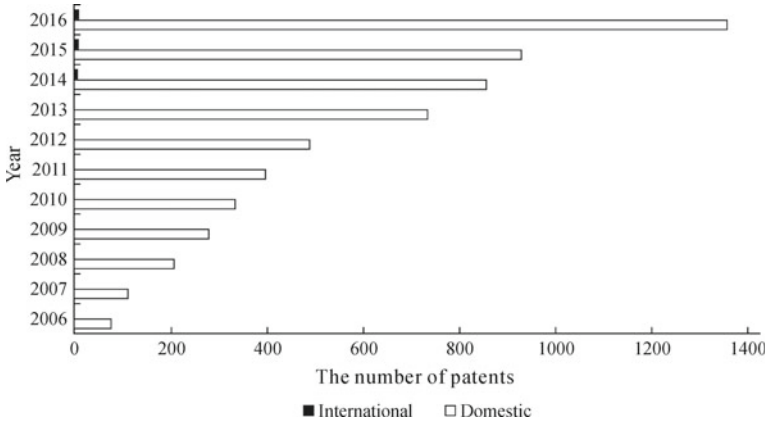

Fig. 1.33 The number of international and domestic patents in the ultrasound field

including the design of PEF processing systems and the inactivation mechanisms. As shown in Fig. 1.34, the published articles on PEF technology for food processing in China have made continuous progress over the last decade. The number of both international and domestic publications in China was only 18 in 2006 and increased to 34 in 2016. As for the related patents, the amount increased from 2 in 2006 to 55 in 2016. There are many more papers published in domestic journals than those in international journals according to the science citation index (SCI). Figure 1.35 reflects that domestic Chinese patents are more numerous than international patent applications, indicating that the international competitiveness of Chinese patents on the PEF technology is still in a weak condition.

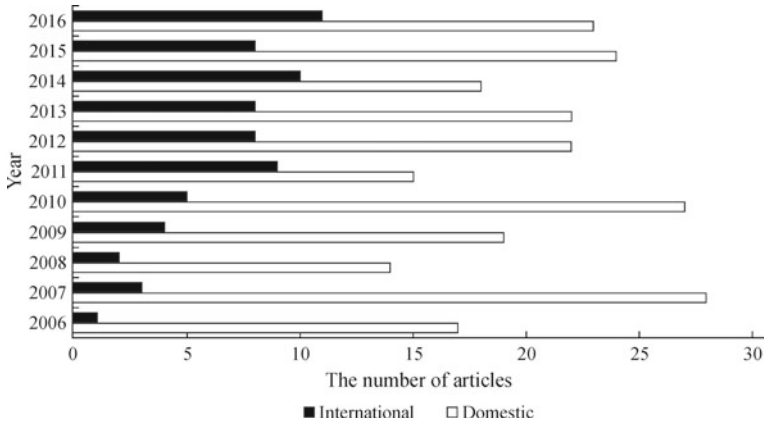

Fig. 1.34 The number of international and domestic published articles in the field of PEF

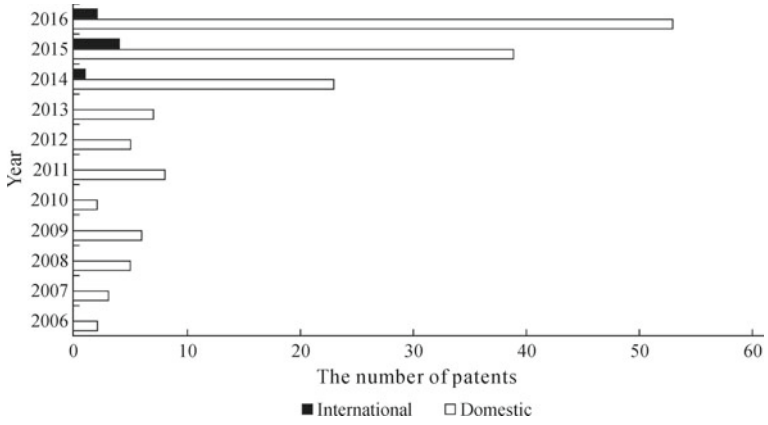

Fig. 1.35 The number of international and domestic patents in the field of PEF

1.3.1.6 Industrial Support

With the development of food physical processing technology, more food companies accept this new technology and upgrade the traditional processing methods for high-quality products.

High-pressure processing is an innovative and promising technology. Industrial applications of HPP in China have also made a major breakthrough. The concept of "HPP+" presented by a team from China, combines HPP with heat and other technology. HPP + technology has broken through the technical bottlenecks that restrict the processing of high quality, not from concentrate (NFC), fruit and vegetable juice. China has established a high-pressure processing line of fruit and vegetable juice, and developed a number of new products from NFC fruit and vegetable juice. Beijing

Heju Network Technology Co., Ltd. produces kale, cabbage, and apple juice; carrot, and ginger juice; and other cold pressed fresh fruit and vegetable juice products. All of these products are treated by "HPP+". "Hey Juice", a famous brand of "Perse" also produces the HPP-treated juice. "HPP+" technology will be promising for processing China's vegetable products to enhance the nutritional value of these value-added products. Research into HPP equipment in China has started in recent years, but compared with other countries, there is a big gap between the basic research and industrial applications, for there has been lack of development in domestic nonthermal processing hardware equipment, and it is difficult to conduct relevant research into industrial applications. Limited research funding is another barrier to conducting extensive research and developing advanced food physical processing technology. In terms of HHP equipment, China has attempted to move to the forefront of the world. At present, some companies, such as Inner Mongolia Baotou KeFa High-Pressure Technology Co. Ltd., and Tianjin Huatai Sen Miao Biological Engineering Technology Co. Ltd., have the capacity to manufacture HPP equipment. Currently, industrial applications of HPP in China are mainly for the treatment of fruit juice. Multiple applications of HPP technology should be developed further, such as for HPP-treated aquatic products and ready-to-eat foods with high nutritional value.

Since 2006, the microwave food industry has developed rapidly. The world's microwave food processing equipment is also growing quickly, with applications such as vacuum-drying, freeze-drying, disinfection and sterilization, and baking. Nanjing Sanle Microwave Technology Development Company Limited in China is mainly engaged in research, equipment design, manufacturing, and technical services for microwave energy application technology. The company now produces microwave equipment with 915–2450 MHz categories. There are 30 different models, mainly for food and agricultural products in drying, sterilization, puffing, and preservation. As a new technology in the food processing industry, microwave technology can be used to develop high quality food to meet consumers' demands.

Food irradiation technology has many advantages such as no pollution, no residue, low cost, and less energy consumption, thoroughly sterilizing and retaining the integrity of food nutrients and flavor. Therefore, the applications of food irradiation technology have become extensive in the food industry. Guizhou Jinnong Irradiation Technology Co. Ltd., which is supported by Guizhou Academy of Agricultural Sciences, conducts scientific research and radiation processing system design. The company has a laboratory for microbiological testing, dose monitoring, and quality analyses of agricultural products; their daily irradiation processing capacity is 30–100 tons. Shanghai Gaoying Technology Co. Ltd. is supported by National Synchrotron Radiation Laboratory of the University of Science and Technology in China. The company has become a modern large-scale radiation processing chain and has built two electron beam irradiation centers in Shanghai and Shenzhen.

In China, ultrasound-assisted enzymatic hydrolysis and extraction have been successfully applied in the food industry. The first functional peptide production line using ultrasonic-assisted enzymatic hydrolysis technology in the world was built in 2010 by Jiangsu Tianqi Bio-tech Co. Ltd., which is also the first company conducting large-scale production of rapeseed peptides. In addition, a second functional peptide

production line using ultrasonic-assisted enzymatic hydrolysis technology was built at Jiangsu Wukesong Bio-tech Co. Ltd. in 2011. So far, more than 20 kinds of peptide products have been developed and commercialized by Chinese companies. The development of high-grade, high value-added products is good for ensuring that there are high quality food products on the market.

Supercritical CO_2 combined ultrasound-assisted extraction technology has been used to extract propolis flavones for high yields and profits. This technology has been applied by a number of companies as Jiangsu Jiangda Yuanshengtai Bio-tech Co. Ltd., Jiangsu Fengao Bio-tech Co. Ltd. and Heilongjiang Yingchun Bee Product Co. Ltd.

1.3.2 Research Platform and Future Development

In recent years, the state has paid more attention to food physical processing technology. A number of key national laboratories were established to promote food physics processing research and potential applications. For example, Jiangsu University, one of the organizers of the "Food Physics Processing Technology Seminar" supported by China Rural Technology Center, established "Jiangsu Provincial Key Laboratory for Food Physical Processing" in 2010. These laboratories are mainly engaged in basic research and development of the technology for food physical processing, and the development of physical processing equipment. The main aims of future research will be to improve food quality, ensure food safety, improve production efficiency, reduce cost, reduce energy consumption and emission, and to realize automation.

A research network for high-pressure technology in food processing has been established in China. The members are mainly from colleges and universities, such as China Agricultural University, Jiangnan University, South China University of Technology, Jilin University, and Zhejiang University. Research laboratories have also been set up, such as National Engineering Technology Research Center for Fruit and Vegetable Processing in China Agricultural University, and the Key Laboratory of Fruit and Vegetable Processing in National Agriculture and Engineering Research Center of Ministry of Education. These centers largely focus on research into the quality changes of high pressure-treated fruits and vegetables. The State Key Laboratory of Food Science and Technology in Jiangnan University is mainly engaged in the research of high pressure-treated aquatic products. In recent years, the government has increased financial support for research into high-pressure technology; for example, research into high-pressure technology for food has been supported by the National Key Project of Scientific and Technical Supporting Programs, and the National Natural Science Foundation of China. Some research has also been funded by local organizations. Currently, research into high-pressure technology in China is mainly focused on sterilization, extraction, and modification of food. Most studies remain at the level of technical applications, with less research into the aspects of the systematic mechanisms (Zhang et al. 2015). Few researchers have investigated and

addressed the mechanisms of high-pressure processing in the food system, which limits further development in this field in China.

In addition, the working conditions of high-pressure equipment are stable, and the equipment lacks real-time temperature detection. In the future, these aspects should be taken into consideration during the development of such technology. Other modern technologies, such as PCR, should also be used to carry out systematic research into the high-pressure mechanism. A multidisciplinary approach involving university, industry, and government collaborations are required to address the various research needs in order to improve quality.

There are many laboratories and research institutes in colleges and universities that are engaged in research into microwave technology for use in food. For example, a research laboratory in Jiangnan University has developed a microwave and vacuum frying integrated device for conditioning food, and a method for efficient frying, in order to solve problems such as low efficiency, high energy consumption, poor uniformity, and low yield. South China University of Technology and the Midea Group have jointly established the "Microwave Food Research Center", which has contributed to the development of the microwave food industry. "Chinese Laborers—Media Group Microwave Food Research Center" is located in South China University of Technology, and has received investment of more than 10 million yuan for research funding. The main research topics include: microwave food processing characteristics, microwave heating food uniformity, the impact of microwave processing for food nutrients, microwave food safety assessment, and other aspects of the basic research and construction of the relevant standard systems. The research team was joined by university professors, graduate students, and industry experts. The Chinese Academy of Agricultural Mechanization Sciences has undertaken research into "microwave energy sterilization technology and device development", and has successfully developed continuous microwave vacuum-drying equipment, as well as microwave powder dryer, sterilization equipment, and far infrared microwave vacuum dryer.

The main organizations in China conducting research into irradiation technology are the Institute of Agricultural Products Processing, the Chinese Academy of Agricultural Sciences, the Zhejiang University, the Institute of Atomic Energy and Agricultural Utilization, the Jiangsu Academy of Agricultural Sciences, the Beijing University, and the Jiangnan University. The Radiation Center of the Institute of Agricultural Products Processing, the Chinese Academy of Agricultural Sciences, conducted research into radiation processing in the Asia Pacific Region with research funding support from the former State Planning Commission and the Ministry of Agriculture, and by the International Atomic Energy Agency. The Center began construction in 1993 and was officially open in June 1995. The Center is mainly engaged in radiation preservation of agricultural products, food seasoning irradiation processing, health supplies and disposable medical radiation sterilization, irradiation sterilization of pet food and animal feed on an industrial scale and commercial applications. The Center has a hanging chain design source capable of loading a 500 thousand Curie cobalt 60 irradiation device, realizes safe operation of the system, the detection and control automation. It is equipped with advanced experimental equip-

ment and ancillary facilities, according to customer requirements for irradiation dose, dose control, and strict quality assurance, to guarantee the quality of radiation. In the future, the establishment of standards of food hygiene, and food irradiation process specification, will be highly requested. In accordance with international standards and guidelines, and irradiated food laws and regulations, it is necessary to promote the development of food irradiation processing industry.

Organizations working on ultrasonic food processing technology research are mostly located in Chinese universities. The research of "Intelligent Sensing and Process Control of Cold Chain Foods, Engineering and Technological Research Centre of Guangdong Province" in South China University of Technology mainly focuses on ultrasound-assisted freezing and crystallization. The organization of "Key Laboratory of Physical Processing and Agricultural Products of Jiangsu Province" in Jiangsu University primarily focuses on ultrasound-assisted enzymatic hydrolysis and extraction. They have successfully designed low power consumption, high field strength and multimode ultrasonic equipment, with 15 different ultrasonic laboratory equipment models which can meet a variety of production requirements. The research of "Zhejiang Key Laboratory for Agro-Food Processing" in Zhejiang University mainly focuses on ultrasound-assisted degradation, modification, and sterilization, with funding supports from the National Natural Science Foundation of China, the National Science and Technology Support Program, and local organizations. Some research into ultrasonic food processing technology is conducted into the effects and process optimization, but the mechanism of ultrasonic processing in the food system has not been studied enough yet, limiting further development and industrial applications. A great amount of effort needs to be devoted to developing intellectual-property rights in large-scale ultrasonic processing equipment for industrial productions. On the other hand, in order to improve the efficiency in transforming scientific research achievements into applied technology, universities and enterprises should strengthen their cooperation with each other to make full use of individual advantages, resources, knowledge, and expertise.

The main organizations in China working on the PEF technology are Jiangnan University (Jin et al. 2015), Zhejiang University (Zhao 2010), South China University of Technology (Yang et al. 2016), China Agricultural University (Zeng 2009), Tsinghua University (Li et al. 2011), Dalian University of Technology, and Jilin University (Zhang et al. 2014). Jiangnan University designed a fixed processing chamber (flat plate type) combined with a set of OSU-4L high-voltage PEF system, collaborating with Ohio State University, USA (Cheng-Wen et al. 2012; Wu et al. 2015a, b; Ping et al. 2010; Wei and Yang 2008; Zhao and Yang 2009). Jilin University developed a high-voltage PEF continuous processing flow system for extraction of polysaccharides, accelerating the aging of liquor and microbial inactivation (Wu et al. 2015a, b; Zhou et al. 2014). Tsinghua University has successfully designed the four generations of the PEF food sterilization equipment, including THU PEF1, THU PEF2, THU PEF3, THU PEF4 (Yin 2006; Chen et al. 2010; Li et al. 2010). Nevertheless, the capacity of the THU series of high-voltage PEF food sterilization equipment is relatively low, only at laboratory scale. In 2008, research groups from Zhejiang University designed a high voltage pulse generator-based ARM embedded

system for sterilization of food equipment. In the next two years, based on the pulse generator, Zhejiang University developed a PEF food sterilization processing system. This equipment had an adjustable output voltage of 0–30 kV, three processing chambers, adjustable pulse frequency of 0–400 Hz, and a processing capacity of 60 L of liquid food per hour. However, commercial and industrial scale PEF systems are still not available in China. Although the progress of the theoretical researches and the equipment design is limited, the research into PEF technology for food processing is still underway. In the future, the following aspects should be taken into consideration during the development of the PEF technology in China. Processing devices should be designed and optimized in order to meet high efficiency, stabilization, and safe control requirements for industrial-scale applications; the safety of PEF-treated food products should be evaluated through experiments; and the identification of reasonable costs, quality and benefit are essential for the industrial applications.

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Chapter 2
The Basic Concept and Research Progress of Food Physical Processing

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Abstract This chapter provides an introduction to interdisciplinary food physical processing as well as applications of food processing in research and industry production, and the effects of food physical processing on food quality. This chapter introduces the main research work on food physical processing by Professor Haile Ma's team from Jiangsu University; this includes ultrasonic-assisted enzymatic hydrolysis of protein, ultrasonic-assisted fermentation, ultrasonic-assisted extraction, ultrasonic cleaning of food, ultrasonic degradation of polysaccharides, ultrasonic-assisted transesterification of triglycerides, ultrasonic-assisted osmotic dehydration of vegetables, ultrasonic-assisted aging of vinegar, magnetism-assisted fermentation, infrared blanching and dehydration of fruit and vegetables, combined Laser and UV irradiation breeding of microorganism, and pulsed light processing of food.

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2.1 The Birth of Interdisciplinary Food Physical Processing

Traditional food processing technology is based on chemistry and biology; however, after long-term research and parameter optimization, there are still improvements to be made to key parameters, such as processing efficiency, product yield, product activity, and energy consumption. Extensive research has been conducted all over the world, from different angles, to explore new physical methods using sound, light, electricity, magnetism, and force in the various elements of food processing. A large number of studies have shown unexpected effects of the application of physical techniques, with the prospect of a wide range of applications (Fig. 2.1). Food physical processing technology, as a new kind of food processing technology, has drawn much attention across the world.

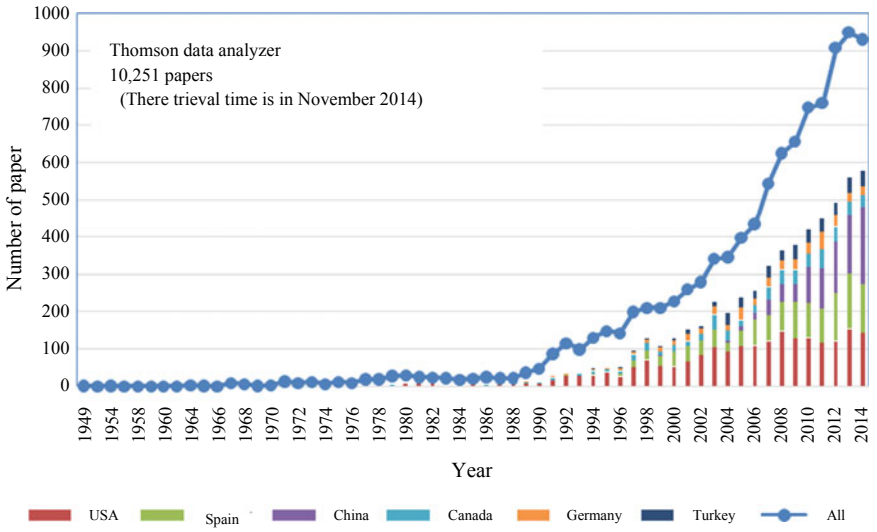

Fig. 2.1 The number of articles published in the SCI journals on application of physical fields in food processing (The number of articles has increased dramatically since 1990 and entered a new growth period after 2003) (Jia et al. 2016)

2.2 Basic Structure of Physical Food Processing Technology

Food physical processing technology can be divided into three sections: nonthermal, thermal, and rapid detection. A categorization of different food physical processing technologies is shown in Fig. 2.2.

According to the different physical fields, food physical processing technology can be divided into ultrasonic, microwave, ultra-high pressure, electric field, magnetic field, infrared, and radio frequency processing. In the last 30 years, ultrasonic, microwave, ultra-high pressure, and electric field processing were listed in the top four in terms of the number of published articles (Fig. 2.3). In the past 24 years in China, the number of published articles related to microwave processing technology was much higher than those on other technologies (Fig. 2.4).

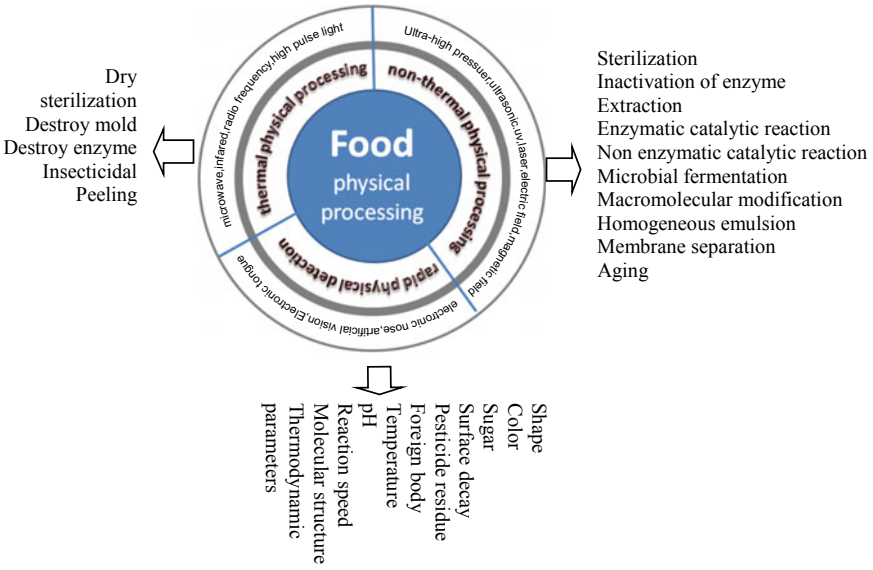

Fig. 2.2 Basic structure of food physical processing technology (Jia et al. 2016)

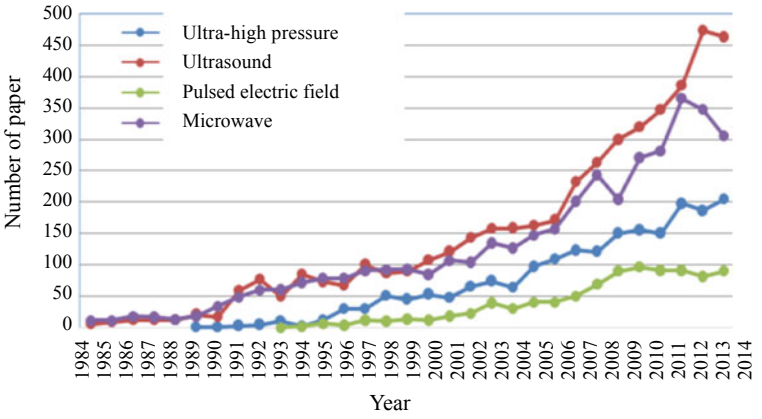

Fig. 2.3 The number of articles published in the SCI journals on application of physical fields in food processing (Jia et al. 2016)

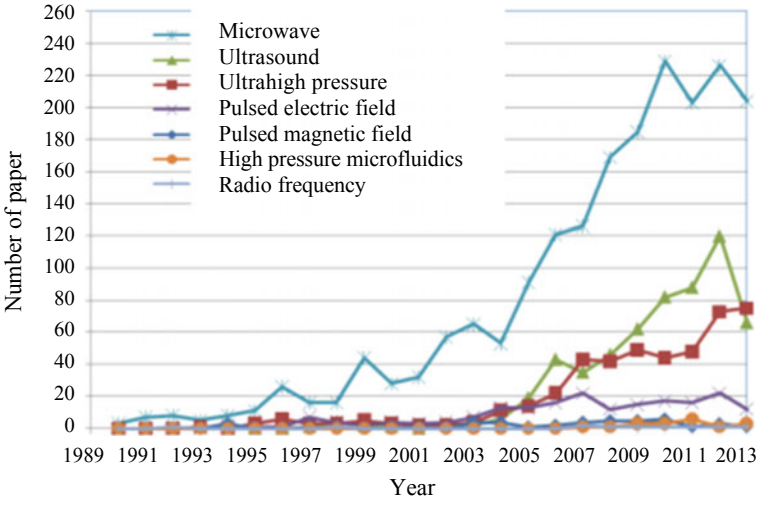

Fig. 2.4 The number of articles published in Chinese journals on application of physical fields in food processing (Jia et al. 2016)

2.3 Research Progress of Several Typical Physical Food Processing Applications

2.3.1 Ultrasonic-Assisted Enzymatic Hydrolysis of Protein

2.3.1.1 Background

Angiotensin converting enzyme (ACE) inhibitory peptides from plant proteins, prepared with traditional enzymolysis, have a high activity (Matsui et al. 2000; Xing 2003). They are good-quality and safe food-based functional peptides. However, traditional enzymolysis has some disadvantages including long enzymolysis time, low utilization rate of the enzyme, and low conversion rate of the substrate. This is mainly due to a low contact level caused by uneven stirring, decreased enzyme activity, and aggregation and deposition of the protein during enzymolysis (Dadzie 2013; Qu et al. 2013a; Zhou et al. 2015). This is also due to low contact level between enzyme and substrate, and unsuitable protein structures. There is a great demand for more efficient enzymolysis methods to be developed in order to overcome these shortcomings.

Ultrasound technology, as a novel nonthermal physical processing technology, has many applications in food science and related fields. Acoustic cavitation, resulting from the mechanical interaction between sound waves and bubbles in a liquid, is considered to be the major effect responsible for the initiation of most sonochemical reactions in liquids. The collapse of cavitation bubbles, which are formed rapidly and explode violently during sonication, can generate violent physical phenomena, such as micro jets, shear forces, shock waves, and turbulence (Mason and Weissler 1994; Soria and Villamiel 2010; Pierre et al. 2014; Mason et al. 1996; Chandrapala et al. 2012). In order to solve the problems with traditional enzymolysis, ultrasonic-assisted enzymatic hydrolysis was studied with the aim of improving efficiency. This was achieved by treatment of the extract with ultrasound before adding protease, application of ultrasound in the enzymatic hydrolysis process, and pretreatment of protease by ultrasound. We chose byproducts from the processing of agricultural product as the main raw materials for the substrate for enzymatic hydrolysis in our research.

At present, many researchers carry out experiments using a simple ultrasonic cleaning tank and ultrasonic cell crusher to replace the divergent ultrasonic and energy accumulation type machines. The working mode of ultrasonic is relatively single. The main working modes include continuous and pulsed ultrasound, contact and noncontact, single-frequency ultrasound, and multifrequency ultrasound. In order to use ultrasound effectively for enzymatic hydrolysis, we proposed to use the multimode ultrasound process which can work in mono-, dual-, or tri-frequency, sweeping frequency, simultaneous, and sequential working modes for food industry applications.

2.3.1.2 Methods and Results

- **Pretreatment of the Raw Protein Material by Ultrasound Before Adding Protease**

The rapeseed protein (protein content, 60.5 g/100 g), wheat germ protein (protein content, 27.6 g/100 g), corn gluten meal (protein content, 58.6 g/100 g), rice dreg (protein content, 57.9 g/100 g), wheat gluten (protein content, 76.0 g/100 g), and oat protein (protein content, 58.3 g/100 g) were used as the raw material. The raw proteins were pretreated under the optimal ultrasonic pretreatment conditions (Dadzie 2013; Qu et al. 2013a, b; Jin et al. 2015a; Yang et al. 2017a; Ren et al. 2014; Zhang et al. 2015a; Wang et al. 2015a, b, c), and then once the protease was added, they were hydrolyzed in optimal enzymolysis conditions. To determine the effects of ultrasound pretreatment on enzymatic hydrolysis for peptide production, we investigated changes in the activity of protein hydrolysates, and the enzymatic reaction rate of the raw proteins. In order to study the effects of ultrasound pretreatment on the activation of enzymolysis, and the molecular structure, secondary structure, and microscopic morphology of proteins we used the infrared spectrum, circular dichroism spectrometry, fluorescence spectrum, scanning electron microscope, atomic force microscope, and other chemical methods.

The effects of ultrasound pretreatment on the ACE inhibitor activity of the hydrolysates of wheat germ protein, wheat gluten protein, oat protein, and corn gluten meal are shown in Table 2.1. From this we can see that these effects were significant and after the pretreatment of wheat germ protein by probe ultrasound, the half maximum inhibitory concentration (IC_{50}) of the hydrolysate was 25.8% lower than that of the control. The reduction was 36.9% for wheat gluten hydrolysates, 43.4% for oat protein hydrolysates, and 74.1% for corn gluten meal hydrolysates. Therefore, the pretreatment of the raw protein substrates by probe ultrasound is beneficial for the release of antihypertensive peptides. This treatment can significantly increase the bioactivity of the hydrolysates.

Table 2.1 The effects of ultrasound pretreatment on the ACE inhibitor activity of protein hydrolysates (Jia 2009; Dadzie 2013; Wang et al. 2016c; Zhang et al. 2016a, b; Ma et al. 2010; Ding et al. 2009) (Adapted by permission from Springer)

	Wheat germ protein	Wheat gluten	Oat protein	Corn gluten meal
IC_{50} of control (mg mL^{-1})	0.62	0.65	0.53	0.45
IC_{50} of ultrasound pretreatment (mg mL^{-1})	0.46	0.41	0.30	0.26
The percentage increase (%)	−25.8	−36.9	−43.4	−74.1

Table 2.2 The effects of ultrasonic pretreatment on the initial rate of enzymatic hydrolysis (Jia 2009; Zhang et al. 2015a; Wang et al. 2015a, b, c) (Adapted by permission from Springer)

	Wheat germ protein (1.5 g L^{-1})	Wheat gluten (10 g L^{-1})	Oat protein (15 g L^{-1})
Rate/V of control (g L^{-1} min^{-1})	0.016	0.074	1.520
Rate/V of ultrasound pretreatment (g L^{-1} min^{-1})	0.022	0.089	2.330
The percentage increase (%)	37.50	20.27	53.29

Table 2.3 Effects of ultrasonic pretreatment on the kinetic parameters for the enzymatic hydrolysis of protein (Zhang et al. 2015a; Mao 2007; Jin et al. 2015b) (Adapted by permission from Springer)

	Wheat gluten		Rapeseed protein		Corn gluten meal	
	K_M (g L^{-1})	K_A (min L^{-1} g^{-1})	K_M (g L^{-1})	K_A (min L^{-1} g^{-1})	K_M (g L^{-1})	K_A (min L^{-1} g^{-1})
Control	45.580	0.416	49.170	1.410	8.390	0.178
Ultrasound pretreatment	37.928	0.425	29.680	1.550	6.190	0.191
The percentage increase (%)	−16.78	2.16	−39.64	9.93	−26.22	7.30

At the initial stage of enzymatic hydrolysis, linear regression was used in Excel to calculate the different concentrations of protein, where the slope of the linear equation gives the initial rate of the enzymatic hydrolysis. Here, the effects of ultrasonic pretreatment on the initial rate (V) of enzymatic hydrolysis were studied using wheat germ protein, wheat gluten, and oat protein. These results are shown in Table 2.2.

From the results in Table 2.2, we can see that the regression correlation coefficient R_2 was higher than 0.996, indicating a strong linear relationship. Meanwhile, we also note that in the initial reaction stage, the enzymatic hydrolysis of protein is a first-order reaction. Moreover, ultrasonic pretreatment can significantly improve the initial rate of enzymatic hydrolysis, promote the hydrolysis of enzymes, and accelerate the process of enzymatic hydrolysis. Kinetic parameters of the enzymatic reaction can be estimated by the direct linear method of the Line Weaver–Burk plot. It is found that $1/V$ is linear to $1/S_0$ by plotting $1/V$ versus $1/S_0$. The results of linear regression analysis are shown in Table 2.3. K_A represents the binding frequency between the substrate and enzyme, and K_M represents an apparent constant analogous to the Michaelis–Menten constant, as shown in Table 2.3.

As shown in Table 2.3, there were no significant differences in K_A between the ultrasonic pretreatment and the conventional enzymatic reaction (control). The maximum reaction rate is similar when the enzyme is saturated with the substrate. However, the K_M value of enzymatic hydrolysis significantly decreased after ultrasonic pretreatment compared with the traditional enzymatic hydrolysis (control). The K_M of wheat gluten, rapeseed protein, and corn gluten meal decreased by 16.79%, 39.64%, and 26.22%, respectively. The results show that the affinity between the enzyme and substrate increased quickly, and the effect of ultrasound on the kinetics of the enzymatic reaction was affected by changes of the affinity between the enzyme and substrate.

The main reasons for the improvement in the ACE inhibitory activity of the hydrolysates after ultrasound pretreatment were the changes in fluorescence intensity, surface hydrophobicity, and free sulfhydryl (SHF) and disulfide bond after ultrasound pretreatment of wheat germ protein. For the corn gluten meal, the sweeping frequency pretreatment could significantly increase the degree of hydrolysis and ACE inhibitory activity of zein hydrolysis by altering the second structure of zein and rupturing the smooth surface of the protein (Ren et al. 2014; Yang et al. 2017b; Wang et al. 2016a; Zhang et al. 2016a, b). For wheat gluten, in order to characterize the relationship between ACE inhibitory activity of hydrolysates, free sulfhydryl, the disulfide bond, and surface hydrophobicity, there were changes in the secondary structure elements by ultrasound pretreatments. Stepwise multiple linear regression (Stepwise MLR) was performed to describe the quantitative relationships between the structure of wheat gluten and the ACE inhibitory activity of hydrolysates. The results show that free sulfhydryl, α-helix, disulfide bonds, surface hydrophobicity, and random coils were significantly correlated to the ACE inhibitory activity of hydrolysate. The standard partial regression coefficients were 3.729, −0.676, −0.252, 0.022, and 0.156, respectively (Zhang et al. 2015b). The main mechanisms of ultrasound pretreatment on the activation of enzymolysis can be described as: (a) the surface characteristics of proteins can be altered rapidly by ultrasonic pretreatment, the surface of the protein was damaged which exposed more restriction sites; (b) the helix structure of protein can be stretched; (c) the sulfhydryl content and hydrophobicity of the protein can be increased. These effects can change the structure of proteins by enhancing the extensibility of proteins and exposing more enzyme sites. These changes can promote enzymatic hydrolysis by increasing the combination of the enzymes and proteins (Zhang et al. 2017; Zhou et al. 2013a, b).

- **Application of Ultrasound to the Enzymatic Hydrolysis Process**

Using wheat germ protein as the experimental material, alcalase was added to the protein solution, and the mixture was immediately treated using a sweeping frequency ultrasound for 90 min at a frequency of (24 ± 2) kHz with power 600 W L^{-1}. Taking the ACE inhibitory action as the main index, the effects of ultrasound pretreatment on the bioactivity of the hydrolysates was determined.

The ultrasound had a significant effect on the initial rate of enzymatic hydrolysis, which was increased by 14.32% when using the probe ultrasound, compared with the control group. When the sweeping ultrasound was applied to the process of

wheat germ protein hydrolysis, the initial rate increased from 56.02% to 83.52%. The kinetic parameters of enzymatic hydrolysis for wheat germ were changed by ultrasonic treatment. This illustrates that the application of ultrasound could promote the enzymatic hydrolysis of wheat germ, and that the effect of the sweeping plate was better than the fixed frequency plate ultrasonic treatment. During the process of enzymolysis of wheat germ protein, the kinetic parameter K_A obtained with the ultrasound probe, sweeping ultrasound, and flat plate ultrasound increased by 22.2%, 66.7%, and 33.3%, respectively compared to the control. K_M decreased by 13%, 6.9%, and 13.4%, respectively, compared with the control.

The application of ultrasound to the process of enzymatic hydrolysis could significantly improve the ACE inhibitory activity and protein conversion rate. The ACE inhibitory activity of protein hydrolysates increased by 41.2%, and protein conversion rate increased 35.5% compared with the control when sweeping ultrasound was used in the enzymolysis process. There was no significant changes in ACE inhibitory activity of the protein hydrolysates when using the probe ultrasound compared with the control in the enzymolysis process; however, the conversion rate increased by 8.2% (Zhou et al. 2013a).

In conclusion, the application of ultrasound to the process of enzymatic hydrolysis can effectively improve the ACE inhibitory activity of hydrolysates. The higher the substrate concentration is, the more obvious the effect of ultrasonic treatment gets. Therefore, using ultrasound in the enzymolysis process is an effective means of preparing ACE inhibitory peptides.

- **Pretreatment of Protease by Ultrasound**

In order to explore the effects of ultrasound treatment on enzyme activity and its activation mechanism, alcalase was used to determine the effects of energy-gathered ultrasound on the activity, kinetics, thermodynamics, and molecular structure of alcalase, which was done with the aid of the chemical reaction kinetics model, Arrhenius equation, Eyring transition state theory, fluorescence spectroscopy, and circular dichroism (CD) spectroscopy.

The highest alcalase activity was achieved when the sample was treated with energy-gathered ultrasound at 80 W for 4 min, when the enzyme activity increased by 5.8% over the control. The parameters of changes in enthalpy, enthalpy, and free energy for the process of ultrasonic activation of the enzyme are denoted as ΔH, ΔS, and ΔG. After treatment, the thermodynamics parameters *Ea*, ΔH, ΔS, and ΔG were reduced by 70.0%, 75.8%, 34.0%, and 1.3%, respectively (Ma et al. 2011a). Fluorescence and CD spectra revealed that ultrasonic treatment had increased the number of tryptophan on the alcalase surface slightly, increased number of α-helix by 5.2%, and reduced the number of random coils by 13.6% (Ma et al. 2011a, b; Huang et al. 2017a). This result indicates that ultrasound had a positive impact on the activity of protease. The mechanism of ultrasonic action on protease was that the treatment made alcalase exhibit more regularity and flexibility, which is helpful for the improvement of alcalase activity.

- **The Creation of the Ultrasonic Operation Mode for Enzymolysis**

Corn gluten meal and rice protein were used as the treated raw material to improve the enzymolysis efficiency and to determine the effect of sweeping frequency ultrasound (SFU), sequential dual -frequency ultrasound (SDFU), and muti-mode frequency ultrasound (MFU) assisted enzymatic hydrolysis on the ΔH, ACE inhibitory activity, and protein conversion rate (CR).

SFU is an advanced ultrasound technology in the field of physics. It differs from fixed frequency ultrasound (FFU) because its frequency is periodically increased from the lower frequency $f - \Delta f$ to the upper frequency $f + \Delta f$, and then decreased back down to $f - \Delta f$ around the center frequency f; this is expressed as $f \pm \Delta f$. Ren et al. (2014) studied the effects of sweeping frequency ultrasound treatment on enzymatic preparations of ACE inhibitory peptides from zein. They found that the SFU and FFU both increased the degree of zein hydrolysis by approximately 11.5% more than the control. SFU pretreatment also increased ACE inhibitory activity of zein hydrolysates by 12.3%–116.7% over the control. Qu et al. (2012) showed that sweeping frequency pulsed (SFP) ultrasound-assisted enzymolysis significantly increased the efficiency of enzymolysis and the activity of ACE inhibitory peptides at different substrate concentrations and enzymolysis times. Compared to traditional enzymolysis, the SFP ultrasound-assisted method significantly increased the protein conversion rate by 35.5%, and the ACE inhibitory activity of the peptide by 35.6%.

Simultaneous working mode has different ultrasound frequencies with simultaneously pulsed on-time and off-time. Sequential working mode has different ultrasound frequencies generated in a successive mode without interval. When the one pulses is on-time, the other one (DFU) or two (TFU) pulses are off-time (Qu et al. 2012).

Jin et al. (2015b) found that both sweeping frequency pulsed ultrasound (SFPU) and sequential dual-frequency ultrasound (SDFU) pretreatments improved ΔH and the protein conversion rate (CR) significantly ($P < 0.05$) when compared with the control. In respect of ΔH, the SDFU pretreatment resulted in a significantly ($P < 0.05$) higher value compared than the SFPU pretreatment, which may be due to the SDFU made the particle size of the corn gluten meal (CGM) much smaller as the acoustic power delivered to the system was greater than the other techniques. On the other hand, the cavitation yield of SDFU was higher because the implosion of the cavitation bubbles coming from lower frequency (20 kHz) irradiation provides new cavitation nuclei for the other ultrasound (40 kHz) irradiation.

Yang et al. (2017a, b), investigated the effects of ultrasound pretreatment on the enzymolysis and structure characterization of rice protein at different frequencies and working modes. The results show that the MFU of 20 kHz had higher ACE inhibitory activity compared with other MFUs. The ACE inhibitory activity of sequential DFU was higher than that of simultaneous DFU with the same frequency combination.

2.3.1.3 Conclusion

The ultrasound pretreatment of protein substrates can significantly increase the bioactivity of hydrolysates and accelerate the enzymatic hydrolysis because ultrasound changes the molecular structure of the proteins. This treatment can also significantly accelerate the enzymatic hydrolysis process and increase the conversion rate of protein. Ultrasound treatment of protease was helpful for decreasing the activation energy and increasing the activity of protease (Zhou 2015).

2.3.2 Ultrasonic-Assisted Fermentation

2.3.2.1 Background

Some research results indicate that appropriate ultrasound treatment can promote the growth of microbial cells and effectively promote the microbial fermentation process (Dai et al. 2017; Huang et al. 2014; Li 2014; Wang et al. 2016a, b, c, d; Zhang 2014; Zhang et al. 2014). Low-intensity ultrasound produces steady cavitation and provides repairable damages to cells. It changes the living state of microbial cells leading to acceleration of their proliferation and more products of metabolism. High intensity ultrasound, on the other hand, cannot lead to accelerating proliferation of microbial cells due to the irreparable damage to the cells (Xiong 2017).

2.3.2.2 Methods and Results

- **Ultrasonic Effects on the Growth of *Saccharomyces cerevisiae***

Saccharomyces cerevisiae was the first yeast species used by people; it was mainly applied to fermentation for ethanol production. The biomass of *Saccharomyces cerevisiae* cultured to the latent anaphase increased by 127.03% compared with the control group, when a frequency of 28 kHz and power of 140 W L^{-1} were applied for 1 h. Both fixed frequency ultrasound and scanning frequency ultrasound could accelerate the growth of *Saccharomyces cerevisiae*, but a high mortality rate was observed when using scanning frequency ultrasound (Xiong 2017; Dai et al. 2017).

The yield of ethanol increased by 19.33% over the control when an ultrasound frequency of 23 kHz was employed and fermented for 48 h, the content of β-phenethyl alcohol and other volatile metabolites such as esters also increased at the same time. The growth rate and cell density of *Saccharomyces cerevisiae* cultured in a 5 L fermentation tank were higher than in a flask, while the consumption rate of sugar in broth was low, accordingly, the eventually yield of ethanol was only 10.48%. The Logistic model generated a satisfactory mathematical model that accurately explained the behavior of the system with a coefficient of 0.995, allowing one to

predict ethanol fermentation process with *Saccharomyces cerevisiae* (Xiong 2017; Dai et al. 2017).

- **Ultrasonic Effects on the Growth of Candida Tropicalis**

The effects of ultrasound on the growth of *Candida tropicalis* are explored. When that of 28 kHz ultrasound irradiation with power intensity of 120 W L^{-1} was applied for 1 h, the *Candida tropicalis* culture biomass addition reached 148.5% at mid logarithmic phase (Xing 2015).

The Fluo-4/AM fluorescent probe method was used to explore the transmembrane behavior of intracellular Ca^{2+} under ultrasonic treatment. After a short period of low intensity ultrasound treatment, the size of the *Candida tropicalis* colony increased, and the Ca^{2+} fluorescence intensity decreased (the maximum value decreased to 25.8% after 2 h), which demonstrated that ultrasonic treatment can change the permeability of the cell membrane and that short-time ultrasound was good for cell proliferation (Xing 2015; Huang et al. 2017a).

Transcriptome sequencing results showed that the influence of low-intensity ultrasonic irradiation treatment on the proliferation of *Candida tropicalis* can be described by gene ontology. The genes involved were *CTRG-02711*, *CTRG-03249*, *CTRG-02500*, *CTRG-00817*, *CTRG-00897*, *CTRG-01717*, and *CTRG-05491*, which made up about 6.7 times, 5.5 times, 1.7 times, 1.9 times, 1.7 times, 14.4 times, and 1.5 times, respectively. These genes refer to the cell membrane, Golgi apparatus, signal transduction, cell cycle regulation, and DNA replication initiation. Hence, we assumed that the ultrasound effects on *Candida tropicalis* began from the cell membrane, gradually extended to the cell interior, and finally affected DNA replication. During this process, the corresponding gene and pathway were up-regulated, which caused the proliferation of *Candida tropicalis* (Huang et al. 2017b; Xing 2015).

We speculated that *CTRG-01717* (phosphatidylinositol 3 kinase *TOR2*) was the key gene of proliferation combined with the differential expression analysis. The expression of the *TOR2* gene increased 14.4 times. This gene is related to cell cycle regulation, and the protein product phosphatidylinositol 3 kinase (PI3K). It also belongs to the protein serine/threonine kinase activity, PI3K-Akt signaling pathway, and AMPK signaling pathway. Protein serine/threonine kinase activity is an important regulator of apoptosis. The function of the PI3 K-Akt signaling pathway is to inhibit cell apoptosis and promote cell proliferation. These all reflected the effect of low intensity ultrasound on the proliferation of *Candida tropicalis* (Huang et al. 2017b; Xing 2015).

- **Ultrasonic-Assisted Fermentation of Soybean Meal by *Bacillus subtilis***

Ultrasound stimulation was added to the process of soybean meal fermentation by *Bacillus subtilis*. The results illustrated that the effect of low intensity pulsed ultrasound can increase the fermentation efficiency on the third day after inoculation. Using 0.08 W mL^{-1}, 33 kHz, and an ultrasonic time of 1 h, the biomass, the content of soluble protein, and peptide were improved by 141.97%, 15.06%, and 24.42% respectively, compared to the control without ultrasound. The results of UV absorption spectra, the fluorescence emission spectra, the infrared spectra, and atomic force

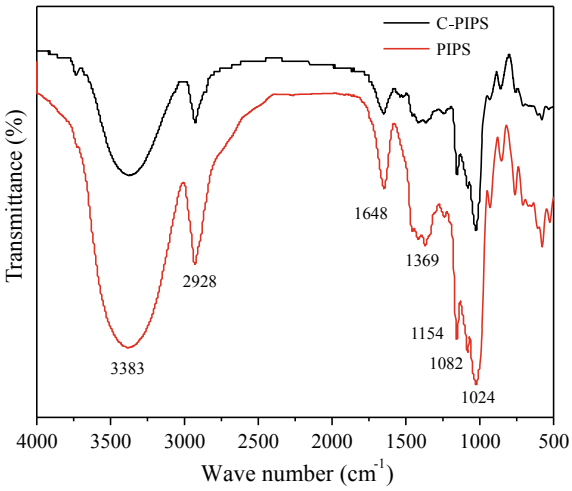

Fig. 2.5 FTIR spectra of PIPS and C-PIPS (Zhang et al. 2014)

microscope indicated that the ultrasound had reduced the size of the protein particles and altered the relative contents of the secondary structure. To summarize, ultrasound treatment can change the structure of soluble protein, loosening and stretching the protein molecules (Luo 2015).

- **Ultrasonic-Assisted Liquid Fermentation of *Phellinus igniarius***

In the study of ultrasonic-assisted *Phellinus. igniarius* liquid fermentation, a novel flat plate ultrasound technology was developed to stimulate polysaccharide production from *Phellinus igniarius* mycelial fermentation. Optimal conditions were found to be culture time of 3.8 days, ultrasound treatment time of 65 min, and duty cycle time of 25 s. This gave a maximum *Phellinus. igniarius* polysaccharide (PIPS) yield of 1.8002 g L^{-1}, which was an increase of 22.64% compared with the control (without any ultrasound). The Fourier transform infrared (FTIR) spectra of PIPS and C-PIPS (Fig. 2.5) implied that the ultrasonic irradiation mainly induced the cleavage of glycosidic bonds. Laser scanning confocal microscope (LSCM) observation suggested that ultrasound can change the morphology and structure of *Phellinus igniarius* mycelium, and accelerate the transfer of nutrients and metabolites (Fig. 2.6) (Zhang 2014; Zhou 2015).

2.3.2.3 Conclusion

The results of our research indicate that appropriate low-intensity ultrasound treatment can effectively promote the profilication of some species of yeast and bacteria, and that the proper ultrasonic irradiation during fermentation process can also accelerate the accumulation of products.

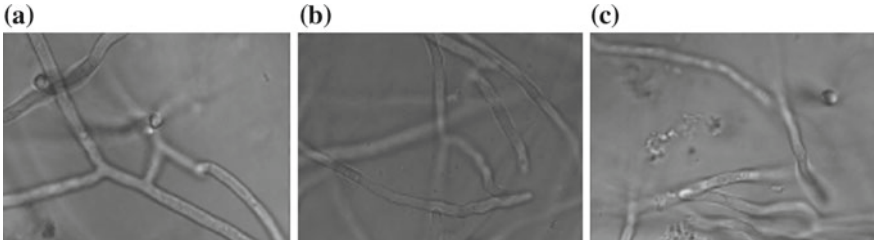

Fig. 2.6 Changes seen in laser scanning confocal microscopic images of *P. igniarius* mycelium. **a** Control treatment; Ultrasonic treatment at the optimal conditions (duty cycle time of 25 s, culture time of 3.8 d) for 60 min (**b**), and 120 min (**c**), respectively (Zhang et al. 2014)

2.3.3 Ultrasonic-Assisted Extraction

2.3.3.1 Background

The development of effective technology for the extraction of components has been closely studied by domestic and international scholars. At present, the main extraction methods include water or organic solvent extraction, microwave-assisted extraction, supercritical fluid extraction, and enzymolysis extraction. Traditional water and organic solvent extraction have low extraction efficiency and require the use of large quantities of organic solvent. Temperature control is difficult with microwave-assisted extraction making it unsuitable for heat-sensitive materials. For supercritical fluid extraction and enzymolysis extraction, the temperature is easy to control and the extraction efficiency can be guaranteed, but extraction costs are higher. These problems have always affected industrialization (Zhou et al. 2013b).

2.3.3.2 Methods and Results

To solve the problems describe above, ultrasound-assisted extraction has been applied more frequently in recent years for the extraction of the high-value components of agricultural products. This is because of its advantages such as short extraction time, high extraction efficiency, low solvent consumption, low operation cost, controlled temperature, and environmental protection. Ultrasound-assisted extraction can accelerate the breakdown of tissue cells, mainly due to the high frequency vibration of ultrasonic wave in the medium, and the instantaneous high temperature and pressure produced by ultrasonic cavitation bubbles, which is favorable for the release, diffusion, and dissolution of intracellular substances. In addition, the mechanical vibration of ultrasound can increase the flow rate of the solvent, and the collision frequency between the material and the solvent, thus speeding up the permeation and diffusion processes of the solvent in the solid raw material. Moreover, when the water content of the raw material is lower, ultrasound can accelerate the infiltration process of the

extracts. Therefore, ultrasound-assisted extraction technology has been extensively studied in the food engineering field.

Professor Haile Ma's team at Jiangsu University has carried out research into the extraction of high-value components such as plant polyphenols (Duan et al. 2009; Jiang et al. 2014; Liu et al. 2015; Pan et al. 2011), flavonoids (Fu et al. 2015; Guo et al. 2016; Zhang and Ma 2005), proteins (Gu et al. 2015; Li and Ma 2016; Ma et al. 2007a; Ma and Zhang 2006; Ma et al. 2007b; Wang et al. 2014a, b), polysaccharides (He et al. 2012; Huang et al. 2011; Kong et al. 2016; Li et al. 2011; Ma et al. 2011a, b; Qu et al. 2013a, b; Wang et al. 2013; Wang et al. 2015a, b, c; Wang and Ma 2007; Xiao et al. 2007; Zhang et al. 2010; Zhang 2014; Zhang et al. 2014), allicin (He et al. 2015), nucleotides (Cao et al. 2012), and other agricultural products using advanced ultrasound-assisted extraction technology. Compared with the traditional solvent extraction technology, and simple ultrasound-assisted extraction technology (using an ultrasonic cell crusher), the advanced ultrasound-assisted extraction technology developed by Professor Ma's team greatly improved extraction efficiency, the activity of high-value components, and industry applications. Accordingly, a systematic study on the advanced techniques of mono-, dual-, and tri-frequency pulsed ultrasound-assisted extraction is carried out for the first time. The unique technological advances and results are as follows.

Pulsed ultrasound-assisted extraction (PUAE) was studied in the extraction of polyphenols from pomegranate peel. The previous research showed that the PUAE significantly improved the extraction yield and shortened the extraction time. Compared with the conventional extraction (CE), the polyphenol yield increased by 22% and the extraction time was reduced by 87%. Compared with continuous ultrasound-assisted extraction (CUAE), the PUAE with frequency of 20 kHz, intensity of 59.2 W cm^{-2}, on-time of 5 s and off-time of 5 s saved 50% of electrical energy (Pan et al. 2011).

PUAE was applied in the extraction of proteins from double-low defatted rapeseed meal. Compared with the traditional alkaline method (TE), after the PUAE treatment under frequency of 28 kHz, power of 875 W, on-time of 3 s and off-time of 2 s, the extraction rate and yield of proteins increased by 38% and 95% respectively, and the extraction time decreased by 15%. The content of sodium hyposulfite in the extracts was 0.06%, lower than the index in the National Standard of China (content of sodium hyposulfite should be less than 1.85%) (Ma et al. 2007a).

Alternating dual-frequency countercurrent ultrasound-assisted extraction (TUAE) was used in the extraction of proteins and polysaccharides from Porphyra yezoensi. The previous research showed that under dual-frequency of 15/20 kHz and alternating working time of 4 s, the protein yield increased by 239%, the polysaccharide yield increased by 121%, he total yield increased by 168%, and the extraction time decreased by 64% when compared with TE. In addition, the protein yield was increased by 81%, the polysaccharide yield by 27%, and the total yield by 50% while the extraction time was reduced by 18% compared with mono-frequency countercurrent ultrasound-assisted extraction (MUAE) (Qu et al. 2013a).

The different working modes of energy aggregation counter flow single-frequency ultrasound (SFU), energy aggregation counter flow dual-frequency ultrasound

(DFU), and divergence triple-frequency ultrasound (TFU) were used in the extraction of polysaccharides from Maca (*Lepidium meyenii*), and compared to the traditional hot water extraction method. The previous research showed that the best extraction method was DFU with alternating two-frequency of 20/35 kHz. With this working mode and an ultrasound time of 15 min, the polysaccharide content was 57.64%. Compared with the traditional hot water extraction method (51.37%), the polysaccharide content increased by 12% (Kong et al. 2016). However, the structure of Maca polysaccharide did not change when it is observed by FTIR determination.

2.3.3.3 Conclusion

The experimental results confirmed that, compared with the commonly used extraction methods, advanced ultrasound-assisted extraction technology has a good extraction performance for components in agricultural products. Compared with the CE, PUAE significantly improved the extraction yield of polyphenols from pomegranate peel and shortened the extraction time. Compared with the CUAE, PUAE saved 50% energy. Compared with the TE, PUAE significantly increased the extraction rate and yield of proteins from double-low defatted rapeseed meal and decreased the extraction time. TUAE significantly increased the yields of proteins and polysaccharides from Porphyra yezoensi and reduced the extraction time compared with MUAE. Finally, compared with the traditional hot water extraction, energy aggregation counter flow DFU significantly increased the content of polysaccharides from Maca (*Lepidium meyenii*) (Kong et al. 2016).

2.3.4 Ultrasonic Cleaning

2.3.4.1 Background

Ultrasonic cleaning is not only the largest branch of power ultrasound, but also the most widely used method in practical applications. Compared with the traditional cleaning methods, ultrasonic cleaning has the obvious advantages of less remaining residue after cleaning, faster cleaning speed, saving water resources, green environmental protection, reduced damage to the objects, better quality cleanout, and easier automation. This energy-saving and environmentally friendly ultrasonic cleaning technology has been extensively applied in many fields such as food processing, mechanical, electrical, light, textile, railways, analytical, and medical industries. In terms of food processing, it is particularly well known for fruit and vegetable cleaning, and has developed the function of pesticide elution.

China has a great variety of vegetables and a wide planting area with an annual output of 580 million tons. Its planting area and production ranks first in the world (Han 2008). Vegetable cultivation requires the use of pesticides, including several highly toxic pesticides which have been banned by some countries, in order to con-

trol pests and weeds in pursuance of high production (Leng et al. 2009). Vegetable products are transported from the fields where they are harvested to supermarkets under complex circulation regulation. Despite the fact that China has taken drastic measures to control pesticide residues in vegetables, food poison and other negative consequences of excessive pesticide residues in fruits and vegetables are still a big challenge (Xu et al. 2008).

2.3.4.2 Method and Result

With the view of reducing pesticide residues in fruit and vegetables, extensive research has developed some methods for the degradation of pesticide residues including water washing, ozone degradation, ultraviolet degradation (Lu and Song 2009), photocatalytic treatment (Li et al. 2006) and ultrasonic treatment (Jiměez et al. 2007; Wang et al. 2010). Compared with other methods, ultrasound has low energy consumption as well as being simple, convenient, and producing no secondary pollution. The reduction of pesticide residues in fruit and vegetables by ultrasound is based on the micro-vibrations produced in the vegetables which increase the dissolution of pesticides. Another reason is that ultrasonic waves can radiate the water solution to stimulate production of -H, -OH, -OOH, -HO_2, and other highly active particles which form ozone, dioxygen, and other strongly oxidizing substances. These active particles can react with the pesticide molecules, thereby eliminating the toxicity of pesticide. Therefore, ultrasonic degradation of pesticide residues draws more attention for food safety.

Considering the above challenges, Professor Haile Ma's team studied the technology of cleaning agent degradation of pesticides in vegetables and developed special sweeping and dual-frequency ultrasonic vegetable cleaning equipment (Ma et al. 2012). The result is that the amount of pesticide residues on the surface of vegetables after ultrasonic cleaning is significantly less than after washing with water. All frequencies caused the degradation of deltamethrin but 40 kHz provided the best results. When the frequency was fixed at 40 kHz and the sweep frequency amplitude set to 2 kHz, the optimum result was indicated to be at a sweep cycle of 100 ms (Fig. 2.7). For ultrasonic frequency optimization, the effects of the ultrasonic double-frequency combination on the degradation of deltamethrin are shown in Fig. 2.8. The results reflect that the degradation rate of deltamethrin was highest at the double-frequency of 33 kHz/40 kHz (Fig. 2.9). Under optimum conditions, the degradation rate of deltamethrin solution (2 mg/L) was 55.88% after 60 min. The main degradation products of dimethoate and deltamethrin solution were analyzed by gas chromatography-mass spectrometry (GC-MS). The results show that dimethoate mainly degraded into small molecular substances during the complete oxidation reaction. Deltamethrin also degraded into small molecular weight substances. Such results can reduce water consumption, environmental pollution, and improve vegetable product safety. Additionally, the uniformity, frequency characteristics, and impedance characteristics of the sound field container in the ultrasonic cleaning machine also impacted the degree of cleaning and pesticide degradation.

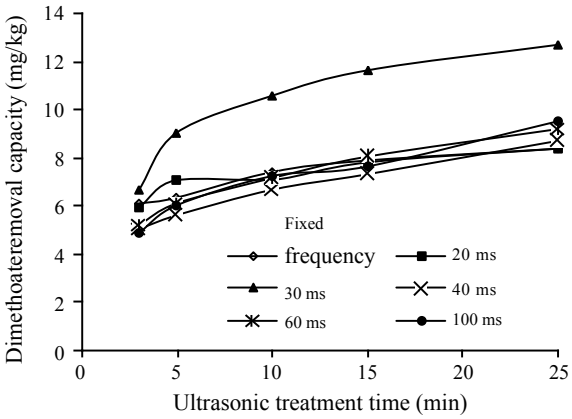

Fig. 2.7 Influence of ultrasonic sweep frequency cycle on dimethoateremoval capacity (Ma et al. 2012)

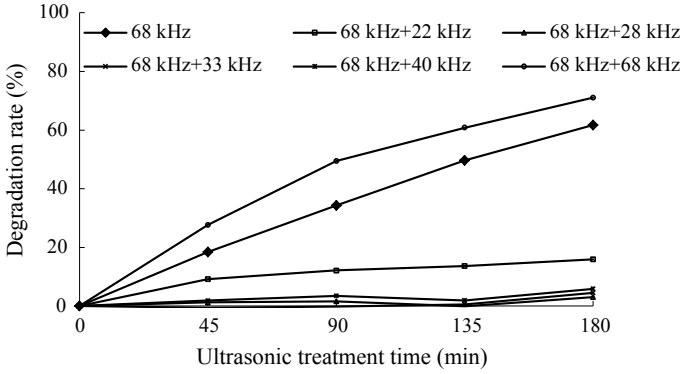

Fig. 2.8 Effects of the frequency combination under sweep mode on dimethoate degradation (Ma et al. 2012)

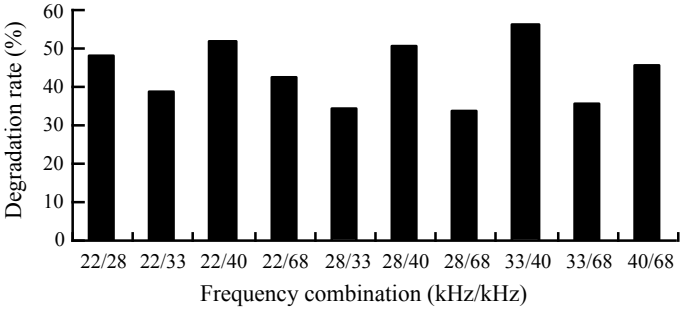

Fig. 2.9 Effects of frequency combination under sweep ultrasonic mode on deltamethrin (Ma et al. 2012) (With permission of Journal of Jiangsu University)

2.3.4.3 Conclusion

Ultrasound applications have been shown to be very effective for cleaning materials. This superior cleaning ability has drawn recent attentions as researchers seek new way to explore and advance its application. Various ultrasound frequencies were employed to study the effects on the cleaning and degradation of deltamethrin. The results show that various frequencies caused the degradation of deltamethrin but the best results were achieved at 40 kHz. Additionally, the degradation rate of deltamethrin was highest at the double-frequency of 33 kHz/40 kHz and under optimum conditions. The degradation rate of deltamethrin solution (2 mg L^{-1}) was 55.88% after 60 min.

2.3.5 Ultrasonic Degradation of Polysaccharides

2.3.5.1 Background

Polysaccharides form the central component of most organisms and possess many important biological roles. It is widely distributed in plants, animals, and microorganisms. Naturally occurring polysaccharides have been used in the pharmaceutical, medicinal, and food industries for many years due to properties such as antihyperlipidemia, immune activity, and antitumor. Most naturally occurring polysaccharides are applicable when water solubility and biological activities are improved. Physical, chemical, and biological technology has been applied successfully to improve the functionality of these polysaccharides, but which often result in low yield and/or high cost. Research on ultrasonic degradation of polysaccharides initially focused on organic pollutants in water, but the degradation of polysaccharides can be traced back to the 1930s. This technique is becoming promising because others, such as biological processes, have limits (Zhou and Ma 2006; Yu et al. 2015; Zhou et al. 2013b, 2015).

2.3.5.2 Methods and Results

One new challenge is the alteration of polysaccharides during ultrasonic degradation. This is because the physiological, physicochemical, and functional properties are highly dependent on the polysaccharide structure. In order to determine the effect of ultrasonic degradation on this structure, studies focus on degradation indexes such as viscosity, molecular mass distribution, infrared spectroscopy, differential thermal analysis, reductive, antioxidants, and various biological activities.

Mao and Ji (2010) studied the effects of ultrasound on the extraction and molecular structure of polysaccharides in *Ganoderma lucidum*, licorice, *Ficus*, Chinese yam, lentinan, and seaweed (Zhang et al. 2010; Huang et al. 2011; Wang and Ma 2007; Li et al. 2005; Xiao et al. 2007; Ma et al. 2007b, c). The results show that the molecular weight of polysaccharides has a significant correlation with antitumor

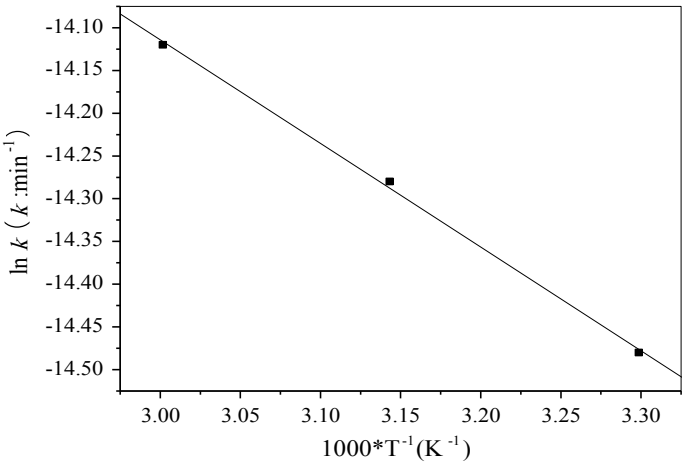

Fig. 2.10 Temperature dependence of the rate constant *k* for degrading PSPY solution with ultrasound Reprinted with permission from Zhou, C. S., Ma, H. L. Ultrasonic degradation of polysaccharide from a red algae (Porphyra yezoensis). Journal of Agricultural and Food Chemistry, 54: 2223–2228. Copyright (2006) American Chemical Society

and antithrombotic activity, plant growth, and molecular mass range. Yoshizawa (Yoshizawa et al. 1993; 1995) used agarose (β-agarase) to partially hydrolyze the polysaccharides in *Porphyra yezoensis* and found that both immunological activity and water solubility were improved. Zhou and Ma (2006) used 20 kHz ultrasound to treat polysaccharides from *Porphyra yezoensis* and established an ultrasonic degradation kinetic equation based on the intrinsic viscosity. It was discovered that there is an exponential relationship between the reaction rate constant and reaction time (Figs. 2.10 and 2.11). The activation energy of the degradation reaction calculation is based on the Arrhenius equation and indicates that the value is less than that of the two enzymatic activation energies (Zhou and Ma 2006).

In order to obtain structural changes, the molecular mass distribution was studied with infrared spectroscopy. It was found that the molecular mass distribution was shifted towards the lower molecular weights, whereas the infrared spectrum did not change significantly. The inhibition of tumor cell activity indicates that there was a significant improvement of SGC-7901 and U937 tumor cells, while no particular change occurred in 95D and 293 cells, which were affected by the degradation of *Porphyra yezoensis* polysaccharide. Furthermore, polysaccharide purification and structural analysis showed that the molecular structure of seaweed did not change, but there was degradation in some polysaccharides with specific molecular weight (such as 610,000 kDa) (Zhou and Ma 2006).

Wang et al. (2014a, b) investigated the effect of ultrasound on the structure of Ficus carica polysaccharide and its activity. The results show that the optimum extraction conditions were 21 min, 90 °C, and a liquid ratio of 49 mL g^{-1}. Under these conditions, the first extraction rate of Ficus Carica polysaccharide recorded was 3.03%,

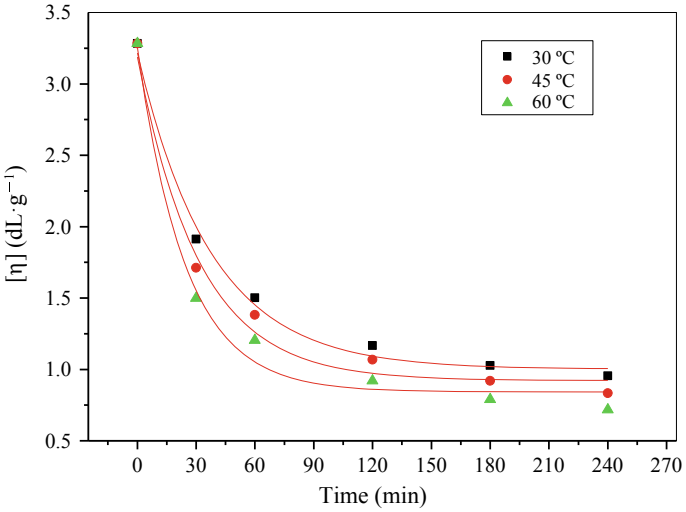

Fig. 2.11 Regression analysis on the effect of reaction temperature on intrinsic viscosity [η] of degradative PSPY Reprinted with permission from Zhou and Ma (2006). Ultrasonic degradation of polysaccharide from a red algae (Porphyra yezoensis). Journal of Agricultural and Food Chemistry, 54: 2223–2228. Copyright (2018) American Chemical Society

while the second extraction rate and yield were 3.86% and 94.62%, respectively. The results of mid-infrared spectroscopy showed that ultrasonic waves broke a large number of C-O-C and C-O-H bonds. Size exclusion chromatography multi-angle light scattering analysis suggested that the average molecular weight was reduced from 536,800 and 1,061,000 Da to 46,410 and 93,870 Da, respectively after ultrasonic treatment. Changes in polysaccharide structure, physical, and chemical properties under ultrasound treatment led to changes in its functional properties. These results show that polysaccharides have a certain biological activity and more excellent processing characteristics.

2.3.5.3 Conclusion

Ultrasonic degradation of polysaccharides revealed a significant correlation between molecular weight, immunological activity, and water solubility. Also, immunological activity and water solubility were improved under ultrasonic degradation. For the reaction rate of degradation, an exponential relation was established between the rate constants. There was also an improvement in the inhibition in the activity of SGC-7901 and U937 tumor cells, while no particular change occurred in 95D and 293. Under infrared spectroscopy, molecular mass distribution was shown to be shifted towards the lower molecular weight direction, whilst the infrared spectrum

did not change significantly. For optimum extraction conditions, 21 min, 90 °C, and 49 mL/g are proposed.

2.3.6 Ultrasonic-Assisted Transesterification of Triglycerides

2.3.6.1 Background

There is a growing interest in biodiesel as a renewable alternative to diesel fuels (Ganesh et al. 2013). A reaction occurs at the interfaces between two immiscible phases of triglyceride and methanol. Conventional mechanical agitation faces various problems related to the immiscible nature of the reactants which causes a poor mass transfer rate. The major problem is the process has a long reaction time and low reaction rate which means that the process is energy intensive (Ali et al. 2012). Considering the current drawbacks in conventional biodiesel production, new technology has been developed based on a process intensification approach (Pin et al. 2012). The use of ultrasound has gained interest in biodiesel production as it provides the mechanical energy for mixing, and the energy required to initiate the transesterification reaction (Singh et al. 2007). Hence, the reaction time is shortened and the biodiesel yield increased (Hanh et al. 2009; Ramachandran et al. 2013).

2.3.6.2 Methods and Results

In order to select a better method for preparing biodiesel through transesterification of sunflower oil which requires less catalyst, energy consumption, and time to reach equilibrium four different enhancement methods were considered. These included mechanical stirring (MS), flat plate ultrasonic irradiation (FPUI), flat plate ultrasonic irradiation with mechanical stirring (UIMS), and probe ultrasonic irradiation (PUI). Results showed that under the same condition, UIMS and PUI used less catalyst, less methanol, shorter time and less energy consumption than MS and FPUI with the same biodiesel conversion (Yin et al. 2012). Biodiesel production from soybean oil deodorizer distillate, enhanced by countercurrent pulsed ultrasound, was studied. The effects of the static probe ultrasonic enhanced transesterification (SPUE) and counter-current probe ultrasonic enhanced transesterification (CCPUE) on the biodiesel conversion were compared. The results indicated that the CCPUE was a better method for enhancing transesterification (Yin et al. 2015). The ultrasonic reactor is also capable of delivering a high biodiesel yield with a reduced amount of catalyst and methanol required in the reaction. Furthermore, the ultrasonic reactor shows outstanding energy efficiency, when compared to conventional mechanical stirring.

The chemical effect of ultrasonic treatment comes from local hotspots produced by cavitation. At the moment when a bubble collapses, a huge amount of energy is released that cannot be immediately transferred to the surroundings. As a result, local

hotspots are developed that have extremely high temperatures (ca. 5000 °C), high pressures (ca. 50 MPa), and high rates of heating and cooling in the bubbles (>109 °C s^{-1}). The ultrasonic energy can cause the formation of short-life time reactive radicals such as H· and HO· from reactants or solvent molecules at the moment of bubble collapse (Jia et al. 2014).

2.3.6.3 Conclusion

The introduction of ultrasonic energy has a positive impact on transesterification. The current barriers that hinder the large-scale application of ultrasonic energy are questions for science, such as the real mechanism of ultrasonic intensification and its integration with chemical energies; and engineering, such as how can ultrasonic reactor design be improved and parameters optimized. Therefore, ultrasound-assisted processes require further study both at the fundamental and application levels to realize practical ultrasonic systems.

2.3.7 Ultrasonic-Assisted Osmotic Dehydration of Vegetables

2.3.7.1 Background

Ultrasound technology has been applied to the dehydration of fruit and vegetables. Osmotic dehydration is the partial removal of water from food due to the difference in osmotic pressure. Ultrasound produces cavitations which cause alternate expansion and compression stress in the tissue of solid food samples, thus assisting the release of water from the tissue. Just like other food processing, the application of ultrasound to the osmotic dehydration of fruit and vegetables can enhance the mass transfer, save time, minimize the use of osmotic solution, and result in an eco-friendly process.

2.3.7.2 Method and Results

Recently, studies into the effects of ultrasound on the osmotic dehydration of sweet potato were carried out to optimize different ultrasound probe frequencies (20, 35, and 50 kHz), pretreatment times (10, 20, and 30 min), and sucrose concentration (20%, 40%, and 60%) (*W*/*V*). The objective was to achieve maximum water loss, maximum weight reduction, and minimum solid gain. The optimized results show that an ultrasound probe frequency of 33.93 kHz, pretreatment time of 30 min, and sucrose concentration of 35.69% (*W*/*V*) gives high water loss, high weight reduction, and low solid gain of 21.62%, 17.23%, and 4.40% (Ayobami et al. 2017; Oladejo and Ma 2016).

With an ultrasound probe of frequency 28 kHz, pretreatment time of 20, 30, 45, and 60 min, and sucrose concentration of 35% (*W*/*V*), the results show that

ultrasound can significantly enhance the moisture diffusivity of osmotic-dehydrated sweet potatoes. As a result of the cavitational effects of ultrasound, microscopic channels were created in the structure of the sweet potatoes, which provided passage for water to leave the tissue as shown in Literature by Oladejo et al. (2017a). Also the solute diffusivity of ultrasound-assisted distilled water (UD) and ultrasound-assisted osmotic dehydration (UOD) was found to be higher than that without ultrasound (OD) (Oladejo et al. 2017a).

The effects of ultrasound on the nutritional qualities of osmotic-dehydrated sweet potato were also determined. The vitamin C retention of ultrasound-assisted distilled water (DWU) and ultrasound-assisted osmotic dehydration of sweet potato were higher than that OD (Oladejo et al. 2017a). The results show that ultrasound helped to expel dissolved oxygen in the solution which can degrade the vitamin C content (Ayobami et al. 2017; Oladejo et al. 2017b).

2.3.7.3 Conclusion

Ultrasound is a promising technology, which can be applied to the dehydration of fruit and vegetables. It can enhance mass transfer, minimize the use of raw materials (osmotic agents), and preserve the nutritional quality (vitamin C content) of osmotic-dehydrated sweet potatoes. Further research is recommended on the effect of ultrasound pretreatment on sweet potato flour and starch.

2.3.8 Ultrasonic-Assisted Aging of Vinegar

2.3.8.1 Background

Zhenjiang vinegar is a traditional local product of Jiangsu Province, famous for its excellent taste and unforgettable aroma; the unique flavor is popular with both domestic and foreign consumers. High-quality Zhenjiang vinegar must be aged for a period of time to make up for the shortcomings of the freshly fermented vinegar, such as pungent odor, miscellaneous taste, non-pure, and mild taste. Traditional aging methods require large processing equipment and space for aging containers. The difficulty of management, the low productivity of vinegar, and the challenges of maintaining hygienic conditions, have resulted in a long production cycle and low production efficiency for Zhenjiang vinegar. Traditional methods have caused lots of adverse effects on the processing of vinegar, which cannot meet the needs of the current market and the fierce competition. Sonication of vinegar was studied in an attempt to speed up the aging process and improve the economic benefits of vinegar production.

A numbers of studies have been launched to investigate the artificial aging of wine and liquor. Some new technologies such as ultrasonic, high pressure, microwave, infrared, and laser have been successfully applied in the aging process of vinegar.

Artificial aging techniques can effectively solve the problems of traditional methods, greatly reducing the aging time of fresh vinegar and improving the flavor, which has enhanced competition in national and international markets.

2.3.8.2 Methods and Results

Ultrasound was used to age vinegar and principal component analysis (PCA) was used to evaluation its quality (Wang et al. 2015a, b, c), according to reasonable methods for measuring vinegar quality and established regulations. Eight samples of sonication aged vinegar, freshly fermented vinegar, and vinegar naturally aged for 1, 2, 3, 4, 5, and 6 years were used for quality comparison. Seven quality factors including the total esters, acidity, reducing sugars, alcohols, ketones, esters, and amino acids were investigated. PCA was used to filter the main ingredients and a comprehensive evaluation model was established based on PCA, which was used to determine the index weights and sample distribution diagram. The conventional sensory evaluation method was used to assess and classify the quality of vinegar. The results showed that the frontal two principal factors contained 87.24% of the information about the organic variable contents in the eight vinegar samples. The test results indicate that the model evaluation result is consistent with conventional sensory evaluation. The sample distribution diagram showed that the sonication aged vinegar was similar to the 4-year naturally aged vinegar.

We investigated how to improve the starch saccharification rate of the production progress of Zhenjiang vinegar, and to accelerate the aging process. As part of this large-scale application of amplitude modulation ultrasound in starch saccharification and vinegar aging has was also studied. The interaction between the indexes and conditions was explored to provide a reference for the application of ultrasonic technology in the vinegar industry. The experimental results showed that the cracks in the surface of starch granules increased, resulting in the degradation of starch and the destruction of the branched structure under ultrasound. During this process, the total amino acid content decreased, the content and types of heterocyclic compounds increased, and the content of the acids and alcohols decreased significantly. The overall trends in substance content were consistent with the naturally aged vinegar. The study has effectively distinguished and classified different years of vinegar and set up a comprehensive evaluation model capable of making an accurate assessment of the vinegar by using PCA. Although the study indicates that the aging technology applied in Zhenjiang vinegar is supported in theory, more in-depth research about aging mechanisms should be carried out in future.

Wang et al. (2017a, b) has explored the ultrasonic aging effect on Zhenjiang vinegar, which provides a theoretical basis for the industry regarding the aging of Zhenjiang vinegar by investigating the type and mechanism of free radicals, water molecules, hydrogen bond, and simulation systems. The results show that the content of volatile components in Zhenjiang vinegar changes; for example, the quantity of esters, heterocyclic compounds, and aldehydes increased, while the quantity of acids and alcohols decreased. At the same time, the contents of ketones and phenols

remained almost constant. The changes of the above indexes of sonication vinegar were consistent with the naturally aged vinegar and the effect of ultrasonic treatment was similar to aging for 2–3 years in Literature by Zhao (2014). The chemical changes caused by ultrasound treatment were measured for the content of iodine in potassium iodide starch solution. It has been confirmed that under ultrasound treatment the solution has a larger oxidation reaction capacity, indicating that ultrasound can accelerate chemical reactions in vinegar induced by free radicals through dissolved oxygen content and increased conductivity. A series of simulation systems were used to verify the mechanism of catalytic reaction for Maillard and oxidation reactions (Zhao 2014).

2.3.8.3 Conclusion

Generally speaking, ultrasound treatment can greatly reduce the aging time and improve the flavor of vinegar. However, research into the development of aging technology is still facing many difficulties. Our research group will devote resources to exploring the key problems, such as the use of comprehensive aging technology. The development of new technology will reduce the storage time, and maximize the economic benefits for the food industry.

2.3.9 Magnetic-Assisted Fermentation

2.3.9.1 Background

Valuable and rare medicinal fungi have attracted attention owing to their extensive applications and high commercial values. The fermentation of medicinal fungi has been widely used; however, there are some problems in the process of fermentation producing lower yields or medicinal components (Jiang et al. 2017). Researchers have aimed to solve these problems by altering the medium and culture conditions, as well as inducing mutations and screening new strains (Zhu et al. 2003). However, these methods do not have universal application and some are only applicable for specific species.

2.3.9.2 Methods and Results

Magnetic fields (MF) are one of the most important nonthermal technologies widely applied in food processing. High-intensity MF treatment has been found to be effective in inactivating microorganisms (Qian et al. 2016) and low intensity MF treatment can influence the biomass and secondary metabolites of fermentation (Santos et al.

2010). In our lab, two self-developed low-intensity MF devices were used to improve the fermentation of *Irpex lacteus* (*I. Lacteus*), which is a medicinal fungus with rich nutritional value and high bioactivity. Furthermore, the molecular mechanisms by which *I. lacteus* responds to low-intensity MF have been investigated.

- **On the Biomass**

According to the single factor experiments, the following optimal treatment conditions were obtained:

i. The first magnetic intervention time was 1 h after inoculation;
ii. The magnetic induction intensity was 35 Gs;
iii. The magnetic treatment was carried out for 3 h at the same time from the first day to the fourth day.

Under these conditions, the dry-weight of mycelia increased by 11.43% in shake flasks increased and 62.96% in a 5 L fermentor.

- **On the Cellular Morphology**

The scanning electron microscope (SEM) was used to observe the influence of low intensity MF on the mycelia of *I. lacteus*. The results showed that a low intensity MF significantly changed the mycelial morphology. After treatment, the mycelia had more wrinkles, folds, and branches.

- **On the Secondary Metabolites**

Low-intensity MF treatment also influences the contents of secondary metabolites. Compared to the control, low-intensity MF increased the fiber content of the mycelia 14.29% and the total sugar by 28.05%, meanwhile the crude protein content decreased by 3.18%, and the crude fat content, ash content, and polysaccharide production showed no significant changes. Importantly, a low intensity MF significantly increased the total amount of free and hydrolytic amino acids by 140.17% and 13.10%, respectively, with a prominent increase of Tyr (177.89%), Met (70.09%), Val (65.91%).

- **Molecular Mechanism of Low Intensity MF on the Fermentation**

RNA-Seq technology was used to analysis the mechanisms by which mycelia responds to low-intensity MF. Low-intensity MF treatment induced 3268 differentially expressed genes (DEGs) at 0 h, 1377 DEGs at 3 h, and 941 DEGs at 6 h after treatment relative to control. GO and KEGG analysis showed that many of the DEGs are involved in cell cycles (e.g. ORC2, RFC3/5) and amino acid biosynthesis (e.g., DHAP, homoserine kinase) which likely to be the factor responsible for the increase in biomass and amino acids (Fig. 2.12).

2.3.9.3 Conclusion

Low intensity MF is a promising technology which can be widely used in the fermentation of medicinal fungi. The biomass and many secondary metabolites increased

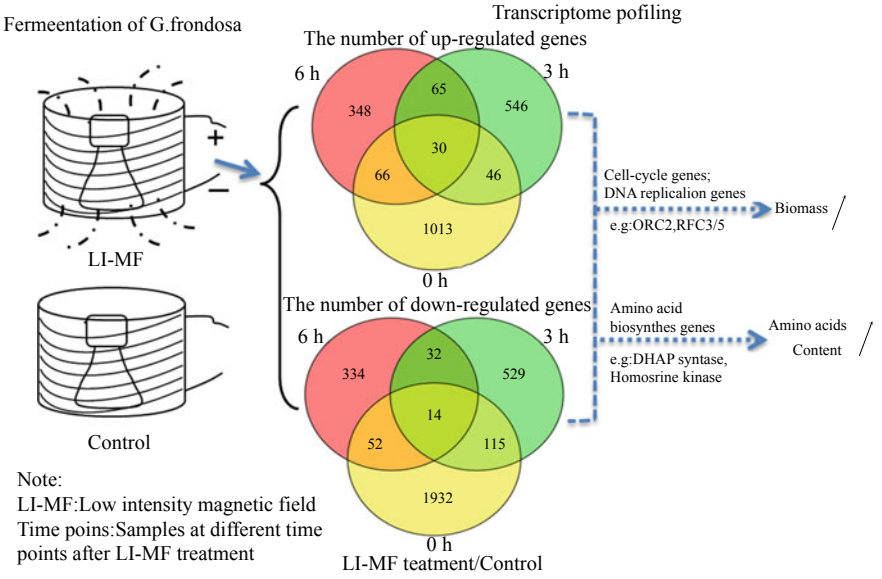

Fig. 2.12 A model of the molecular mechanism by which low intensity MF effects fermentation (Li 2017b)

after low-intensity MF treatment. RNA-Seq technology revealed that the treatment immediately induced up-regulation of genes involved in cell cycle and amino acid biosynthesis, which likely resulted in the increased biomass and amino acids content.

2.3.10 Infrared Blanching and Dehydration of Fruit and Vegetables

2.3.10.1 Background

Blanching is an important operation for most fruit and vegetable processing; it is a preprocessing operation, carried out before drying. The main purpose of blanching is to inactivate enzymes such as peroxidase, polyphenol oxidases, and phenolase which cause many adverse changes to products (Fellows 1990; Hiranvarachat et al. 2011), and to control microbial populations and keep colors stable for further processing. Conventional blanching involves processing with hot water, steam exposure, or acid treatment, but this has many drawbacks such as loss of water soluble nutrients (Lavelli et al. 2007), quality deterioration (Gornicki and Kaleta 2007), and environmental problems (Bomben 1977).

2.3.10.2 Methods and Results

Infrared (IR) technology is an innovative process of heat treatment in the food industry due to its considerable advantages such as high heat transfer rate, high drying rate, uniform temperature distribution, nutrient loss reduction, significant energy-saving, and environment friendly.

Several studies have shown that infrared blanching resulted in blanched products of better quality than those processed using conventional techniques (van Zuilichem et al. 1985; Ponne et al. 1994; Zhu and Pan 2009). Our group used the simultaneous infrared dry-blanching and dehydration (SIRDBD) method to blanch carrots and simultaneously remove a certain amount of moisture (Liu 2017; Wu et al. 2014). This method utilizes catalytic infrared energy to combine blanching and dehydration into a one-step process that is much more efficient than the conventional two-step process.

Wu et al. (2014) investigated the effects of various processing parameters on carrot slices exposed to infrared radiation heating for simultaneous blanching and dehydration. The influence of processing parameters on moisture reduction, drying rate, residual peroxidase (POD) activities, surface color change, and vitamin C retention was determined by a three-factor factorial design. Thin slices and/or high surface temperatures resulted in faster inactivation of enzymes and quicker moisture removal, compared to the thick slices and/or low surface temperature treatment.

The quality of carrot slices at different heating time with infrared drying-blanching, including residual POD, moisture reduction, and overall color change is shown in Fig. 2.13. The process which produced a 1 log reduction in POD activity resulted in moisture reduction from 40.2% to 88.8%, retention of vitamin C from 56.92 to 77.34 g/100 g and overall color change (ΔE) from 3.17 to 5.13 (Table 2.4). We concluded that simultaneous infrared blanching surface color change, retention of vitamin C, and retention of β-carotene of the carrot dice under SIRDBD were better than with traditional industrial hot water blanching. In addition, the cost of SIRDBD was lower than that of hot water blanching. Furthermore, the Midilli model performed well for describing drying behavior during SIRDBD and the Fractional conversion model fitted well for POD inactivation curves. Therefore, SIRDBD is a promising technique for simultaneous blanching and dehydration in the fruit and vegetable industry.

2.3.10.3 Conclusion

IR radiation heating technology is a new environment friendly heating technology with which is highly efficient and energy-saving. The processing parameters of SIRDBD show significant effects on the process characteristics and product quality of carrot slices. Appropriate processing conditions of SIRDBD can be used to blanch and partially or fully dehydrate fruit and vegetables.

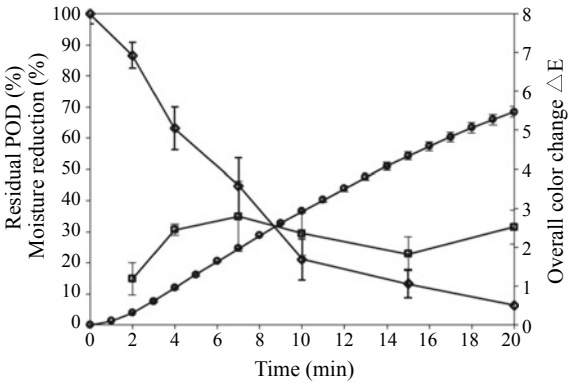

Fig. 2.13 Quality of carrot slices at different heating time with infrared dry-blanching at 90 °C (Wu et al. 2018)

Table 2.4 Required processing time, moisture reduction, surface color change, and retention of vitamin C in carrot slices at a 1 log reduction of POD with different surface temperatures (Reprinted from LWT-Food Science and Technology, 57(1), Wu, B. G., Pan, Z. L., Qu, W. J., et al., Effect of simultaneous infrared dry-blanching and dehydration on quality characteristics of carrot slices. 90–98. Copyright (2014), with permission from Elsevier)

Slice thickness (mm)	Surface temperature (°C)	Time for 1 log reduction of POD (min)	Moisture reduction for 1 log reduction of POD (g/100 g)	Top surface color change (ΔE)	Retention of vitamin C (g/100 g)
3	85	29.5	88.8	5.05a	61.90a
	90	15.5	67.7	3.28bc	74.32b
	95	11.7	55.0	4.58a	58.08c
5	85	29.5	71.1	4.00abc	56.92c
	90	17.5	54.0	3.17c	75.75b
	95	13.5	46.1	4.38ab	63.76a
7	85	\	\	\	\
	90	19.0	46.2	4.98a	77.34b
	95	16.0	40.2	5.13a	68.56d

Note Values within the same column followed by different letters are significantly different at $P < 0.05$

2.3.11 Combined Laser and UV Irradiation Breeding of Microorganism

2.3.11.1 Background

Medical mushrooms are used as folk medicine for a variety of human diseases in several Asian countries (Lemieszek et al. 2011; Lim et al. 2016). *Phellinus igniar-*

ius, a well-known Basidiomycete fungus belonging to the genus *Phellinus* in the Polyporaceae family, is a medicinal mushroom with significant biological activities (Mizuno 1999). In recent years, studies have revealed that polysaccharides are the main active compounds in *P. igniarius* and they have attracted more research to explore the pharmacological functions, which include antitumor, anti-inflammatory, hemostasis, invigorating the liver, promoting blood circulation, and reinforcing the spleen (Dai et al. 2010). However, wild or natural *P. igniarius* is becoming increasingly rare and the field-cultivation cycle is too long limiting the development and utilization of *P. igniarius* (Chen et al. 2007, Zhang et al. 2014). Up to now, traditional mutation is still the most effective strategy for improving the productive capacity of different strains (Khaliq et al. 2009).

Ultraviolet (UV) radiation is widely used in mutagenesis-selection protocol. The mutagenic and lethal mechanisms of UV radiation have been elucidated in several microorganisms (Ikehata and Ono 2011; Ravanat et al. 2001; Alifano et al. 2008). However, due to the lower density of the UV on positive variation, researchers have developed various methods of mutation screening by compound mutation (Yu et al. 2011). Low-power laser irradiation technology has been developed for microorganism mutation breeding. Based on a homemade XeCl 308 nm excimer laser, an innovative and effective mutagenesis protocol has been developed for an industrial strain producing an antibiotic (Alifano et al. 2008). A laser with a wavelength of 620 nm has a significant growth-stimulating effect, especially for He–Ne laser with a wavelength of 632.8 nm (van Breugel and Bä 1992). However, there were a few reports about the use of combined low-power He–Ne laser and UV irradiation for inducing microorganism mutation.

2.3.11.2 Method and Results

The combined low-power He–Ne laser and UV radiation as a novel microbial mutation technique was applied to screen the *P. igniarius* strains for a high production yield of endo-polysaccharides.

Protoplast from *P. igniarius* was initially irradiated using a low-power He–Ne laser and UV, and then after five generations mutant strains were selected which improved the yield of metabolites during submerged fermentation. In shake flasks, the dry weight of mycelia and the endo-polysaccharides derived from the screened mutant (JZx) fermentation were 20.715 g $L^{-1}$and 1.428 g L^{-1}, which increased by 40.31% and 56.58%, respectively, compared with the wild strain (CK) (Zhang et al. 2014).

Electrophoresis spectrogram analysis of antagonistic experiments between isozyme indicated that the genetic material of the screened mutants had changed.

The endo-polysaccharides from JZx fermentation mainly contained low-molecular weight (MW) (1.5 kD, 61%) polysaccharides composed of D-glucose, L-rhamnose, D-mannose in a molar ratio of 2.0: 16.0: 1.0, which exhibited stronger antioxidant activities than that of CK's in vitro with stronger hydroxyl radical scavenging capacity and higher TEAC and FRAP values (Zhang et al. 2014).

2.3.11.3 Conclusion

A novel and efficient physical method of irradiation to induce mutation using compound a low power He–Ne laser and UV was employed to effectively improve the endo-polysaccharide production from *P. igniarius* mycelial fermentation. A mutant strain JZx was screened and selected after five generations. The yields of dry mycelia and endo-polysaccharides were 20.7 g $L^{-1}$and 1.4 g L^{-1}, respectively, which were increased by 40% and 57%, compared with CK. Under the low-power He–Ne laser and UV irradiation, the polysaccharide from JZx mainly comprised D-Glc, D-Man, and L-Rha with a molar ratio of 2.0: 1.0: 16.0 and a large amount of low-MW fractions (1.5 kDa, 61%). Endo-polysaccharides extracted from JZx fermentation mycelia showed stronger radical scavenging capacities and antioxidant activities than those of CK. This study might provide an alternative method for enhancing the metabolites produced in other *Phellinus*. The polysaccharide of JZx had stronger antioxidant activities, and could be explored as a novel natural antioxidant for application in functional foods and medicine.

2.3.12 Pulsed Light Processing of Food

2.3.12.1 Background

Foodborne disease is one of the most serious public health problems in the world. With the rapid development of the food supply chain, foodborne disease are spread worldwide which is harmful to human health and seriously hinders the development of the economy. WHO (2012a) estimated that in each year, about 2.2 million deaths are caused by foodborne or waterborne diarrhea. Between 2012 and 2016, the number of people affected by foodborne diseases in the United States was approximately 48 million, and 3000 people died (CDC 2012). The nine kinds of foodborne pathogens detected led to an annual economic loss of $6.5 to $35 billion (WHO 2012b). In China, 1 out of 6.5 people are suffering from a disease caused by foodborne pathogens. With the globalization of the food supply chain, the prevalence of recognized diseases has been expanding, and the number of unknown new pathogens has also been increasing. Moreover, the resistance of pathogens has been increasing continuously, and the need for the prevention and control of foodborne diseases is high.

2.3.12.2 Methods and Results

- **Pulsed Light for Rice Disinfection and Aflatoxin Degradation**

Pulsed light (PL) technology has been proven to be effective in the decontamination of food such as in vegetables, fruit, and water (Gómez-López et al. 2005; Krishnamurthy et al. 2007; Bialka and Demirci 2008). However, grains were not suitable for

PL disinfection because their rough surface produces a shadow effect. The shadow effect blocks the irradiation of the light and reduces the efficacy of PL treatment. Additionally, a longer treatment time or higher light intensity could swiftly cause the products to reach high temperature. Combined the PL irradiation and holding could achieve a simultaneous decontamination and drying effect of rough rice (Wang et al. 2016a). Freshly harvested rice with moisture content of 23.1% was inoculated by *Aspergillus flavus* and treated by PL followed by holding treatment. At an intensity of 1.08 W cm^{-1} for 21 s of PL treatment, a 0.29 log CFU g^{-1} reduction on *A. flavus* spore counts was achieved. After holding, a 5.2 log CFU g^{-1} reduction was achieved. Heating removed 3.3% of water from the rough rice. The milling quality of the rice was not affected. Therefore, the combined PL and holding treatment has good potential for use in the rice industry for simultaneous decontamination and drying (Wang et al. 2016a).

Rough rice is easily contaminated by aflatoxins B_1 (AFB_1) and B_2 (AFB_2) that are produced by *A. flavus*. AFB_1 has been classified as a Group I human carcinogen (IARC 2002). At present, few methods can effectively remove or detoxify AFB1 from contaminated rough rice; however, PL is able to degrade and detoxify aflatoxins in seconds. In this study, AFB_1 and AFB_2 were produced by *A. flavus* which was used to inoculate rough rice, and the PL treatment time of 80 s reduced AFB_1 and AFB_2 by 75.0% and 39.2%, respectively. A treatment time of 15 s reduced the same aflatoxins in rice bran by 90.3% and 86.7%, respectively. Toxicological tests showed that the mutagenic activity of AFB_1 and AFB_2 was completely eliminated and the toxicity was significantly decreased. These results show that PL technology has promising potential for decontaminating rice by disinfecting fungi, as well as degrading and detoxifying aflatoxins in rough rice and its by-products (Wang et al. 2016b).

- **Pulsed Light for Enzyme Inactivation**

Proteins have strong absorption of radiation with a wavelength of 280 nm (Hollósy 2002); therefore PL has the potential to be used in enzyme inactivation. To investigate the effects of PL on the activity and structure of horseradish peroxidase (HRP), different intensities and enzyme residual activities were measured. The results show that complete inactivation of HRP was obtained by 10 pulses of PL treatment at an intensity of 500 J per pulse. The microstructure and spectral analysis indicated that the secondary and tertiary structures of HRP were destroyed (Wang et al. 2017a, b).

2.3.12.3 Conclusion

PL treatment is a promising alternative for microbial inactivation as a high degree of inactivation efficiency can be achieved with short-time irradiation. PL technology has promising potential for the decontamination of rice by disinfecting fungi as well as degrading and detoxifying aflatoxins in rough rice and its by-products. Further research is needed to evaluate its usefulness in the decontamination of other food products. PL devices with good penetration and processing time need to be designed for commercial purposes. In addition, the suitability of the PL processing on indus-

trial scale needs to be compared with other nonthermal and conventional thermal processes.

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Chapter 3
Green Separation Technology in Food Processing: Supercritical-CO_2 Fluid Extraction

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Abstract One of the most important trends in the food industry today is the demand for "natural" foods and ingredients that are free from toxic-chemical additives. Supercritical-CO_2 fluid extraction is considered as one of the "green" and environmentally friendly separation technologies that have emerged as attractive alternatives to traditional methods for the concentration of bioactive compounds. The greatest advantage of supercritical-CO_2 fluid extraction is that it is rapid and highly selective with shorter extraction times than traditional methods. It is particularly favorable for the extraction of thermally labile bioactive substances that easily degrade when subjected to traditional extraction techniques. Supercritical-CO_2 fluid extraction technology is available in the form of a single stage batch process, and could be scaled up to a multistage semi-continuous batch coupled with a multi-separation process. With improved processing conditions and reduced cost, supercritical-CO_2 fluid extraction will become even more economical at low throughput. Extracts from natural sources are key elements in the manufacturing of health-promoting functional foods and ingredients. Thus, the development and use of "green" separation processes and technologies is likely to continue to be widely employed in the processing of bioactive components, especially for use as supplements for health-promoting foods.

Keywords Separation · Extraction · Bioactive compounds · Supercritical-CO_2 extraction · Health-promoting compounds

3.1 Introduction

Separation technology is used to recover high-value components from agricultural commodities, as an important operation for the production of food products such as oil and proteins. It is also especially important for the development of health-

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promoting food ingredients and high value-added food products such as antioxidants and flavors. Separation processes such as extraction, concentration, purification, and fractionation of bioactive components (phytochemicals) from agricultural materials are the main processes used to obtain high-value end products which may become wellness ingredients for use in functional foods and nutraceuticals. Many potential high-value products can be developed from natural resources by different separation technologies and processes. Carotenoids including lycopene, β-carotene, astaxanthin, and lutein make up a global market nearing $1 billion with a growth rate of approximately 3%. Therefore, efforts to utilize natural agricultural materials for the production of high value-added products, especially health-promoting foods and ingredients, are of great interest to the food and biotechnology industries.

Traditional extraction techniques employ large amounts of toxic organic solvents for removing targeted components from plant materials. In many countries, to address safety, health, and environmental issues, strict regulations regarding the use of organic solvents (i.e, hexane) are forcing the food industry to search for alternative processes. Furthermore, increasing consumer awareness of the presence and use of organic chemical solvents in food, and the desire to buy natural products, is encouraging the food and natural products industry to develop and commercialize "green" technology. Recently, there has been considerable interest from the scientific community, resulting from increasing industrial demands, in the research and development of extraction and separation technologies. The aim is to eliminate the use of organic chemicals, as these products increasingly being used in producing functional ingredients and natural products (e.g., nutraceuticals and supplements). Supercritical-CO_2 fluid extraction technology is one of the possible alternative methods for providing a "green" processing technique for food processing applications. The "green" separation technologies and processes provide detailed information on the equipment selections, system design, and methods for extraction and purification of multiple classes of phytochemicals from plant materials while retaining, or even improving their bioactivity and functionality.

3.2 "Green" Separation Process Design

All over the world, there is pressure for the industry to adopt new sustainable separation processes that do not require the use of environmentally damaging organic solvents. Separation is an interphase mass transfer process because it involves heat, mass, and phase transfers, as well as chemical reactions among the components of the plant materials. The engineering processes for separation systems include modeling, simulations, optimization control studies, and thermodynamic analysis. The principles of mass conservation and component transfer amounts are used to analyze and design industrial processes. Proper understanding of molecular properties and thermodynamics constitute powerful tools for the design of successful separation processes. Moreover, the design of a separation process is strongly dependent on the phase equilibrium scenario, which is highly sensitive to changes in operating

conditions. Thus, phase equilibrium engineering plays a key role in the synthesis and design of these processes. The applied knowledge necessary for system design is comprised of data banks, experimental data, phenomenological phase behavior, thermodynamic analyses, mathematical modeling procedures for phase equilibrium process calculations, mass and heat transfer analyses, the characteristics of the targeted components, and the effects of the processing conditions.

3.2.1 Technical Requirements for a Separation System

The design of a separation process depends on the separation to be performed and the properties of the materials used, as well as the targeted bioactive components. An important consideration, in determining how appropriate a separation technique and system are, is the purity requirements for the end products. In most organic solvent (toxic-chemical-free) separation systems, a combination of new techniques is necessary for system optimization.

Product design is related to reasonable separation and purification steps, economic feasibility, and raw material selection. "Green" separation processes are environmentally friendly processes that result in less air pollution and industrial waste (e.g., energy, greenhouse gas emission, and reduction of waste water production). The following issues may be involved in the consideration of a potential system designs:

(a) Knowledge of phase equilibrium, mass transfer rate, and solubility data are important for scaling up the extraction process and equipment.
(b) Information and experimental data on the effects of processing conditions on the physicochemical properties and degradation of the bioactivity of compounds are important for the design of a suitable extraction system and the procedure.
(c) Proper solvent selection is based on the solubility characteristics of the targeted compounds which should readily dissolve in the extraction solvent, ideally to achieve as a substance pure as possible.
(d) A pump for transporting the solvent is required for recycling. An additional pump may be required for co-solvent incorporation during extraction.
(e) An extractor, which will be responsible for charging a solid material into a high pressure and potentially high temperature zone; a separator, which may involve changes of pressure and temperature; and a sample collector, should be considered during the designing process of the system.
(f) The capacities of the heat exchanger and condenser should also be considered. For example, the heat capacity of water is very large, so considerable effort is necessary to remove the excess stored energy.
(g) For the choice of a suitable pump and extraction capacity, the dimensions of the extraction vessel and the optimization of the processing parameters are required.
(h) Economics and safety should always be considered and may be the determining factor in designing a separation system.

3.2.2 Food Quality and Separation Systems

The major issues related to product quality after separation are the effects of processing on the bioactivity of extracts and the nutritional value of the end products, as well as specific quality characteristics. To comply with food safety regulations, no toxic-chemical solvent residues are permitted in the end products, e.g., "green" food products; all nutrition and health regulations must be met. Some other important requirements include high stability of nutrients and bioactive components, low operating temperatures to reduce thermal effects, the exclusion of light to reduce light-induced UV irradiation effects, and the exclusion of oxygen to reduce the effects of oxidation. The final products must maintain uniformity, quality, and consistency, as well as a purity that can meet food or pharmaceutical grade requirements.

3.2.3 Scaling Up Technology for Industrial Production

Scaling up an innovative separation process for large-scale manufacturing is essential in order to carry out the separation in a reproducible and consistent manner for commercial purposes, and to avoid potential exposure to biological or chemical post-translational modifications which could result in poor product quality. Avoidance of enormous variations from process to process necessitates attention to detail at all stages of product development. When the technology in a food process is designed for industrial-scale production, an important area for consideration is the balance of capital and operating costs as the scale of the operation increases. The process of scale up also involves optimization with respect to increasing the efficiency of each stage, giving rise to increased demands on the accuracy of the online quality control. The process must be reliable for industrial-scale production of food or pharmaceutical grade ingredients that will be used in a wide variety of applications including food, nutraceuticals, pharmaceuticals, and cosmetic products.

3.3 Supercritical-CO_2 Fluid Extraction

The extraction of health-promoting components from plant materials has usually been accomplished by conventional extraction processes such as solid–liquid extractions employing methanol, ethanol, acetone, or hexane, and also through steam distillation or evaporation processes to remove solvents from the extracts. Currently, the demand for natural bioactive compounds is increasing due to their use by the functional food and pharmaceutical industries. Thus, there has been increasing interest in the use of "green" separation technology able to provide high quality and high bioactivity extracts while precluding any toxicity associated with the solvents. Some of the motivations for employing "green" technology as viable separation techniques are:

tightening government regulations on toxic-chemical solvent residues and pollution control, consumers' concern over the use of toxic-chemical solvents during processing, and increasing demand for higher quality products which traditional processing techniques cannot achieve.

One of the most important considerations in developing new extraction processes is safety. In this sense, a variety of processes such as supercritical-CO_2 fluid extraction, membrane-based separation, molecular distillation, and pressurized low-polarity water extraction, are generally recognized as "green" separation techniques, and are considered clean and safe processes which meet the requirements (Herrero et al. 2006; Chang et al. 2008). They have recently been developed and are regarded as emerging innovative separation technology that is able to meet food quality and safety requirements. These processes can be used to solve some of the problems associated with conventional organic solvent-oriented separation. Operation parameters and other factors related to the quality of the original plant material, including geographic origin, harvesting date, storage, and any pretreatment processes prior to extraction, also influence the separation operation and the final composition of the extracts obtained.

3.3.1 Principles and Properties of Supercritical Fluid Extraction

A gas or liquid is normally used as the extraction solvent for supercritical fluid extraction. When a gas or liquid is compressed and heated past its critical point, it enters the "supercritical phase"; in this state the extraction medium is called a "supercritical fluid" (SCF). The critical temperature (T_c) and pressure (P_c) at which this happens are unique to each pure substance. In the supercritical state, the SCF possesses properties of both gases and liquids. For example, the liquid-like density of an SCF provides its high solvent power whereas the gas-like viscosity and diffusivity, with zero surface tension, enhance the transport properties of the solvent during extraction. Therefore, these unique properties enable the SCF to penetrate into porous solid materials more effectively than a liquid solvent, resulting in faster mass transfer, therefore providing faster and greater extraction yields.

The extraction process can easily be adjusted by altering the pressure and temperature. However, operating pressures and temperatures above the critical point would affect the properties of the SCF such as its density, viscosity, diffusivity, heat capacity, and thermal conductivity, and would enhance the ability of the SCF to penetrate and extract the target molecules from the source material. One of the main characteristics of an SCF is the possibility of modifying its density by changing the pressure and/or temperature. Since density is directly related to solubility (Raventós et al. 2002; Shi et al. 2009a, b), by altering the extraction pressure, the solvent strength of the fluid can be modified. The power of a solvent's extracting ability increases with density at a given temperature, or with temperature at a given density.

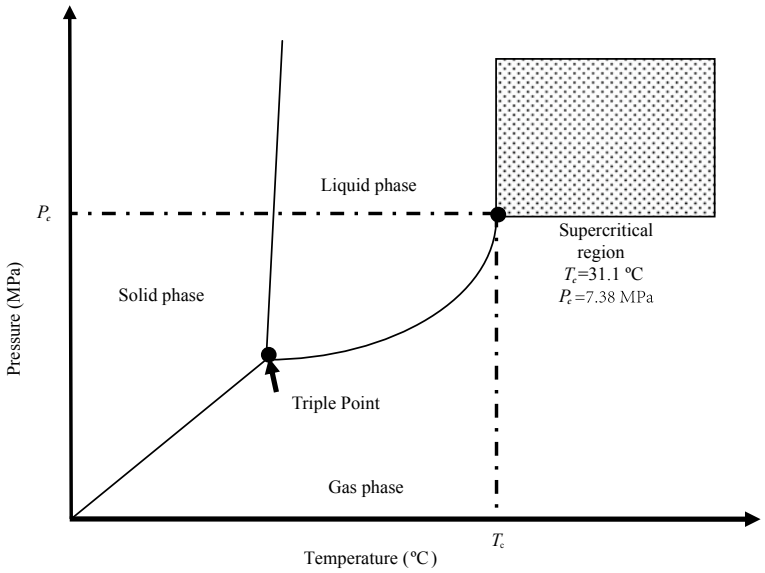

Fig. 3.1 Supercritical pressure–temperature diagram for carbon dioxide

Many solvents are candidates for SCF extraction. The most desirable SCF solvent for the extraction of natural products for food and medicine is carbon dioxide (CO_2), and this extraction process is called supercritical-CO_2 fluid extraction. The advantageous characteristics of CO_2 are that it is inert, nonflammable, noncorrosive, low cost, easily available, odorless, tasteless, and environmentally friendly, with relatively mild critical conditions of 7.38 MPa pressure (P_c) and 31.1 °C temperature (T_c) (Fig. 3.1). Its near-ambient critical temperature makes it ideal for thermolabile natural products (Mendiola et al. 2007) and because it is a gas at room temperature, once the extraction is completed, a substantial elimination of CO_2 without residues can be achieved by simply decompressing the system, yielding a solvent-free extract. However, CO_2 is not a perfect solvent for high molecular weight and polar compounds. Small amounts (ranging up to 20, molar fraction) of polar or nonpolar co-solvents called modifiers can be incorporated to increase the solubility of such compounds during supercritical-CO_2 fluid extraction.

Carbon dioxide, therefore, has favorable properties including the ease of changing selectivity by the addition of a relatively small amount of modifiers such as ethanol and other polar solvents (e.g., water). As a result, CO_2 is considered to be the most desirable SCF for extracting natural products for food and medicinal applications (Shi et al. 2007a, b, c; Kassama et al. 2008; Yi et al. 2009). Other SCFs, such as ethane, propane, butane, pentane, ethylene, ammonia, sulfur dioxide, water, and chlorodifluoromethane, are also used for supercritical fluid extraction.

In basic terms, the SCF extraction system consists of pumps for delivering the solvent and co-solvent throughout the system and for raising the pressure of the recy-

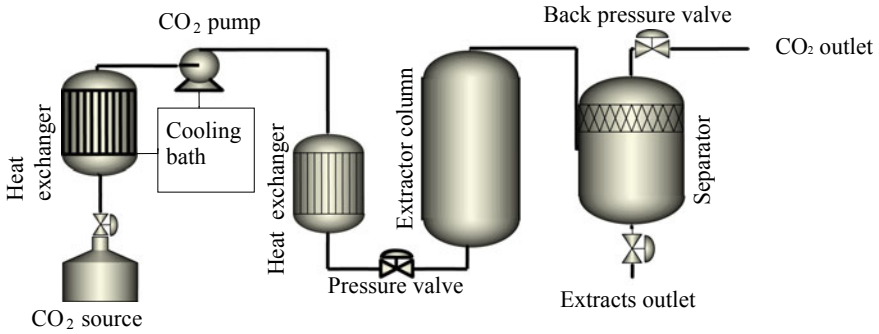

Fig. 3.2 Schematic diagram of a typical single stage supercritical fluid extraction system with CO_2

cled solvent, a high-pressure extractor, a pressure reduction valve, heat exchangers, compressors, and one or more separators in which the extract is collected and the solvent (e.g., CO_2) is depressurized and removed (Fig. 3.2). The extraction cells and the separators are commonly equipped with independent controls for temperature and pressure, thus the fractionation of the extracted compounds can be carried out by stepwise decompression. This means that different compounds can be collected in separators, depending on their differential solubility in the SCF. Additionally, it is possible to install a system to recycle the fluid employed. For batch and single stage modes, the raw materials are usually ground up and charged into a temperature controlled extractor, forming a fixed-bed (Shi and Zhou 2006). The process of SCF extraction requires intimate contact between the packed beds formed of ground solid substratum (fixed-bed of extractable material) with a supercritical-CO_2 fluid which is fed into the extractor through a high-pressure pump. During the extraction process, the solid phase comprised of the solute and the insoluble residuum (matrix) is brought into contact with the fluid phase, which is the solution of the solute in the supercritical-CO_2 fluid (solvent). The extracted material is then conveyed to a separation unit via a pressure reduction valve. At reduced temperature and pressure, the extract precipitates in the separator while the CO_2, free of extract, is recycled to the extractor.

The physicochemical properties of supercritical-CO_2 greatly influence the solvent strength and the solubility of the target compounds in the fluid. In supercritical-CO_2 fluid extraction, the physicochemical properties of supercritical-CO_2, such as the density, diffusivity, viscosity, and dielectric constant, can be controlled by varying the operating pressure, temperature, or both in combination (Tena et al. 1997). Thus, the separation process can be affected by simply changing the operating pressure and temperature to alter the solvating power of the solvent. After modifying CO_2 with a co-solvent, the extraction process can significantly enhance selectivity and separation power, and in some cases, it can even extend the solvating power to polar components.

Many challenges to the design and development of commercially viable SCF extraction processes for natural products remain. These challenges include the need for a better understanding of the phase behavior and solubility of multi-bioactive component mixtures in supercritical-CO_2 fluids. In addition, there is much need for the generation of fundamental data, including solubility, density, and interfacial tension, as well as changes in mass transfer phenomena under different operating conditions such as temperature, pressure, flow rate, and the effects of the composition and proportion of co-solvents (Lucien and Foster 2000; Marcus 2006). Solvent power is related to the density of the supercritical-CO_2 fluid, and it can be varied by changing the operating conditions, mainly temperature, pressure, and flow rate. Generally, density decreases with increasing temperature at a constant pressure, and pressure has more pronounced effects on the changes in density at a constant temperature. Shi et al. (2007a, c) observed that, with an increase in density, the solvating power of CO_2 increased lycopene extraction from tomato skins. Moreover, the region above the critical point provides the greatest density changes, and thus for even the slightest changes in pressure and temperature, the density of CO_2 will effectively change the operating variables (pressure and temperature) in this zone. In the region below the supercritical point, the density of CO_2 changes, but it does not vary substantially with the changes in pressure and temperature.

Targeted compounds have different solubilities in supercritical-CO_2 and that greatly influences the extraction efficiency and bioactivity of the extracts (Lucien and Foster 2000). The impact of solubility enhancement on selectivity has been assessed, and some opportunities for improving the selectivity of extraction have been highlighted and extensively studied (Yi et al. 2009). Generally, the elevation of the temperature leads to an increase in the solubility of the target components in supercritical-CO_2. However, this also increases concern for the stability of bioactive extracts because the bioactivity of natural extracts may degrade when subjected to high processing temperatures.

Most of the investigations into solubility have been concerned with binary systems consisting of a single solute in contact with only supercritical-CO_2. In contrast, solubility data from multi-component systems have not been well established. This is important because intermolecular interactions between components can significantly alter the selectivity of the supercritical-CO_2. For example, in some studies, the addition of a co-solvent (modifier) to supercritical-CO_2 resulted in an enhancement of the solubility of the target component (Sauceau et al. 2004). Food grade solvents such as water, lipids, and ethanol can be used as co-solvents to increase solubility and enhance the extraction yield. Furthermore, not only the composition of the co-solvent, but also the proportion of co-solvent is considered to be a factor for system and process design, and for the optimization of operating conditions, as excess co-solvents may cause either negative, or negligible and noneconomic effects. Vasapollo et al. (2004) found that the presence of vegetable oil as co-solvent improved yields and contributed to the stability of lycopene. Shi et al. (2009a) investigated the effects of ethanol, water, and canola oil modifiers on the profile of lycopene extractions, and found that extraction efficiency was improved by the addition of any of the these modifiers, and that yields increased with increasing amounts of modifier

(from 5% to 10%). However, the rates of yield increase were lower when the ethanol concentration was increased from 10% to 15%.

3.3.2 Process Systems

Changes in food processing practices and new opportunities for innovative food products have spurred interest in supercritical-CO_2 fluid extraction. There are many advantages of using SCF instead of conventional organic solvents. These include achieving higher purity extracts, absence of toxic solvent residues, single-step processing, reduced operating costs, selective fractionation, faster separation, environmental friendliness, and physiological compatibility. In addition, the oxygen free operating system prevents oxidation, and the use of low temperatures minimizes thermal degradation of sensitive materials.

Due to consumer perception of the negative impacts of chemical solvent extraction, and the increasing demands for natural products from natural sources with no toxic-chemical contamination, there is world-wide pressure for industry to adopt new sustainable processes. Under such pressure, supercritical fluid-CO_2 technology has been developed and successfully used to extract essential oils, functional fatty acids, antioxidants, and other bioactive compounds, including the extraction and fractionation of carbohydrates (Glisic et al. 2007; Montañés et al. 2008, 2009; Mitra et al. 2009; Sanchez-Vicente et al. 2009; Shi et al. 2010a). It has been found to be particularly relevant in food and pharmaceutical applications that process and handle complex, thermo-sensitive, and bioactive components, which increasingly applies to production of nutraceuticals, flavorings, and other high-value items. Examples of these applications include the purification of solid materials, separations of tocopherols and antioxidants, removal of pesticide residues from herbs, production of medicines and food products, the detoxification of shellfish, the concentration of fermented broths, fruit juices, essential oils, spices, and coffee, as well as the separation of caffeine (Perrut 2000; González et al. 2002; Kassama et al. 2008; Martinez et al. 2008; Miyawaki et al. 2008; Liu et al. 2009a, b; Herrero et al. 2010).

Supercritical-CO_2 fluid extraction is governed by four key steps: extraction, expansion, separation, and solvent conditioning. These steps are accompanied by four generic primary components: extractor (high-pressure vessel), pressure and temperature control system, separator, and pressure intensifier (pump). The major process parameters are temperature, pressure, and flow rate. Most commercial operations for supercritical-CO_2 extraction of solid materials operate using a batch system (Fig. 3.2). Once the supercritical-CO_2 and the feed reach equilibrium in the extraction vessel, achieved through the manipulation of pressure and temperature to provide the ideal operating conditions, the extraction process proceeds. The mobile phase, consisting of the supercritical-CO_2 fluid and the solubilized components, is transferred to the separator where the fluid is then reduced through decrease of the pressure of the system. The extract precipitates in the separator while the supercritical-CO_2 fluid is either released to the atmosphere or recycled back to the extractor.

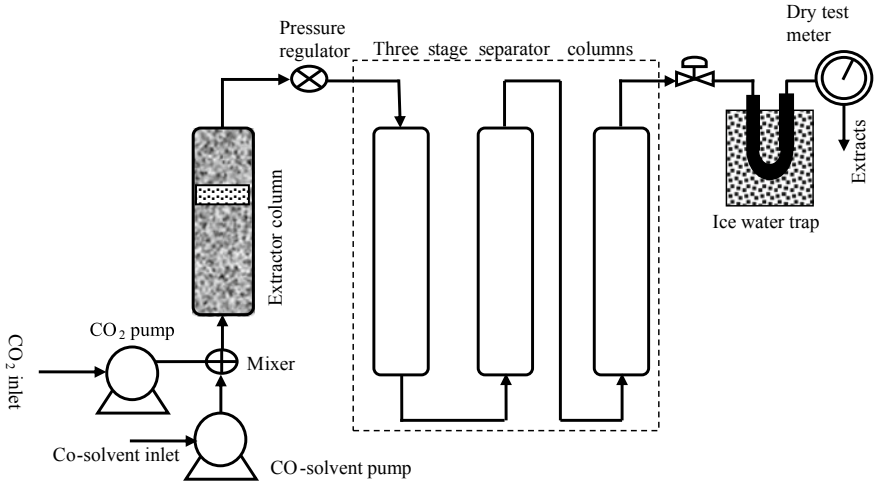

Fig. 3.3 Schematic diagram of a supercritical fluid extraction system used to fractionate bioactive components from a plant matrix using supercritical carbon dioxide

Recently, industry has focused on "fractional separation" where the natural materials are extracted under relatively severe pressure and temperature conditions to remove all of the desired components. The resulting fluid extract is then passed through a series of 2, 3, or 4 separator vessels in which the operating parameters (temperatures and pressures) are set to selectively precipitate one specific component (Fig. 3.3). This can create a range of unique fractions with new application potentials. For example, this fraction system is able to extract/fractionate a number of different carotenoids (e.g., β-carotene, lycopene), oleoresins, and colorants from tomatoes. High antioxidant activity phenolic compounds have also been extracted from rosemary leaves with supercritical-CO_2 (Chang et al. 2008; Huang et al. 2010; Shi et al. 2010b; Xiao et al. 2010). Rizvi and Bhaskar (1995) evaluated the feasibility of supercritical fluid processing of milk fat (i.e., fractionation, scale up, and economic aspects), and reported that scaling up a supercritical extraction processes was a practical approach.

In situations where highly volatile components are being extracted, a multistage configuration may have to be employed as shown in Fig. 3.4 (Kassama et al. 2008). The processes described above are semi-batch continuous processes, where the supercritical-CO_2 flows in a continuous mode while the extractable solid feed is charged into the extraction vessel in batches. In a commercial-scale processing plant, multiple extraction vessels are sequentially used to enhance the process performance and output. Semi-continuous extractor designs allow intermittent loading and unloading of solid material through lock-hopper vessels, reducing downtime and improving production efficiency. The need to improve the design to create truly continuous modes is growing. Supercritical-CO_2 fluid extraction could be cost effective under large-scale production conditions.

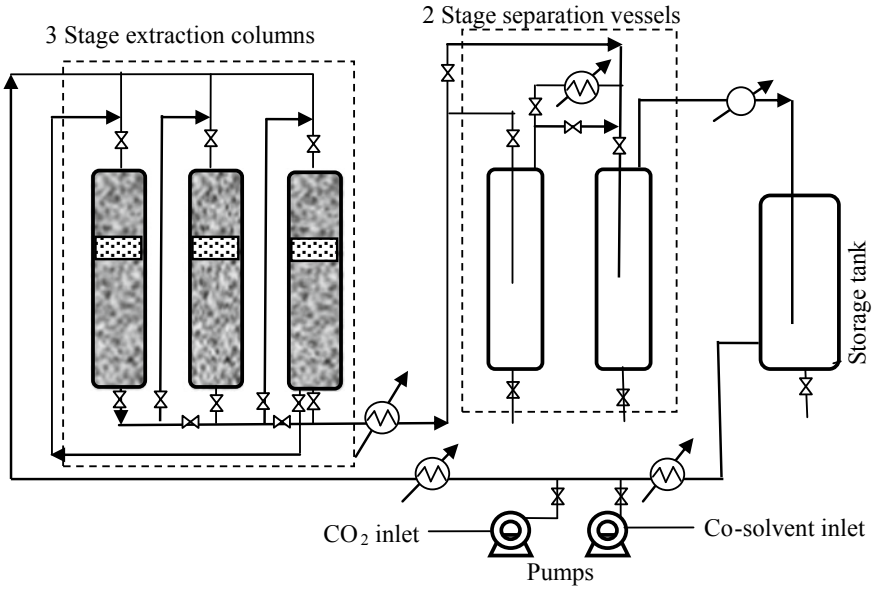

Fig. 3.4 Schematic diagram of a commercial-scale multistage supercritical fluid extraction system used to fractionate bioactive components. (The symbols "⏦" and "⧗" represent pressure valves and heat exchangers, respectively)

One of the main processing aspects that should be considered in SCF extraction is extraction optimization. The use of optimum conditions for the different variables influencing extraction could significantly enhance the recovery or extraction yield of the target compounds. With the aim of effectively optimizing these variables (extraction temperature, pressure, time, type, percentage of modifiers, sample size, etc.), different approaches have been applied. Appropriate experimental designs and statistical modeling should be used to optimize these processes as the compounds of interest will each have their own unique characteristics and are likely to require different specific temperature and pressure combinations. Understanding these specific characteristics; the physicochemical properties of the targeted bioactive compounds; the effects of the parameters influencing extraction efficiency, bioactivity, and cost; and determining the optimum parameters required to maximize the yields and bioactivity of the targeted components, can help to establish the optimal conditions for large-scale processing. One of the major advantages of superficial-CO_2 fluid extraction is that it eliminates the refining process which is otherwise required to remove undesirable compounds before consumption when conventional oil extraction techniques are used. If some valuable compounds are contaminated, they can also be lost during the refining process. By using supercritical fluid extraction, extracts can be obtained which are enriched with the particular compounds of interest, for example wheat germ oil (Eisenmenger and Dunford 2008) and rice bran oil (Soares et al. 2016).

Supercritical fluid extraction has attracted a great deal of interest in recent times as a "green" processing technology and is increasingly being used in food, natural products, and pharmaceutical applications. It is of particular interest due to its environmental benefits. Furthermore, it provides flexibility as the conditions can be optimized and various modifiers can be added to obtain selective fractionation of target compounds.

3.4 Applications in Food Industry

One of the most important trends in the food industry today is the demand for all-natural food ingredients that are free of chemical additives. Natural antioxidants for food are made from derivatives of plant byproducts. A major advancement in supercritical-CO_2 fluid extraction technology was made by its application to the decaffeination of coffee, tea, and other bioactive components (essential oils from spices) used as ingredients in food. Likewise, supercritical-CO_2 fluid extraction is used to extract flavor, fragrance, and high-value compounds used as ingredients in food, pharmaceutical, and nutraceutical products.

Large-scale supercritical-CO_2 fluid extraction has become practical for the extraction of high-value products from natural materials. The solvating power of supercritical-CO_2 fluid is sensitive to temperature and pressure changes. Thus the extraction parameters may be optimized to provide the highest possible extraction yields of the target components with maximum bioactivity (Kassama et al. 2008; Shi et al. 2009c; Yi et al. 2009). Although a temperature rise in the extraction process can increase the solubility of components in supercritical-CO_2 fluids, any negative effects on thermally labile target components should be considered. The intensity and duration of heat processing can affect the health-promoting properties of bioactive components. Therefore, ideally the extraction time and temperature should be minimized which also leads to a more economically viable process. Excessively high flow rates may reduce the contact time between the solute and the solvent and restrict the fluid flow in the sample if it becomes compacted. The optimal flow rate appears to vary with the targeted molecule and relatively high flow rates may have a negative effect on the extraction of some components. Under consistent flow rates and operating temperatures, increasing pressure can significantly improve extraction yields. Most supercritical-CO_2 applications extract natural products or bioactive compounds at pressures between 20 and 50 MPa, and at temperatures between 40 and 80 °C. Because of the poor solubility of some bioactive substances in supercritical-CO_2, food and/or pharmaceutical grade modifiers are sometimes added, in proportions between 3% and 20%, to help with extracting more polar compounds from plant materials.

Large--scale supercritical-CO_2 fluid extraction has become a reality for the extraction of high-value products from natural materials. The solvating power of supercritical-CO_2 fluids is sensitive to temperature and pressure changes; thus, the extraction parameters may be optimized to provide the highest possible extraction

yields with maximum antioxidant activity (Kassama et al. 2008; Shi et al. 2009c; Yi et al. 2009). With this innovative technology, a process could be designed to extract natural nutrients without the fear of organic solvent residues. A compendium of process parameters used for different product applications is listed in Table 3.1.

3.4.1 Extraction of Bioactive Compounds

Most separation procedures involve physical and chemical processes such as centrifugation, filtration, membrane separation, precipitation, chromatography, solvent extraction, crystallization, evaporation, molecular distillation, and supercritical-CO_2 fluid extraction. To overcome the detrimental effects of conventional extraction techniques, a rapid separation process is needed to avoid significant loss of quality or stability of the natural components. Most bioactive components used as functional food additives are used in concentrated form and consequently, appropriate extraction procedures are required when removing them from their original matrices. Some compounds are thermolabile, volatile, and prone to degradation when they are subjected to intensive heat in their concentrated form.

Supercritical extraction with CO_2 is the most viable method for food applications. Baysal et al. (2000) extracted lycopene and β-carotene from tomatoes using supercritical-CO_2 fluid. The processing conditions used were pressures of 20, 25, and 30 MPa; temperatures of 35, 45, 55, and 65 °C; resident times of 1, 2, and 3 h; and CO_2 flow rates of 2, 4, and 8 kg h^{-1}. The best conditions for lycopene extraction were 2 h at a flow rate of 4 kg h^{-1}, pressure of 30 MPa, temperature of 55 °C, and with the addition of 5% co-solvent (ethanol). They noted that, too much ethanol decreased the homogeneity of the extraction mixture, and reduced the separation efficiency.

Yi et al. (2009) investigated the effects of supercritical-CO_2 fluid extraction parameters on the antioxidant activities of lycopene extracts from tomato skins, and found that the activity of lycopene extracts differed with the yield. For each unit of lycopene extract, the antioxidant activity level was constant below 70 °C, but then gradually decreased above 70 °C due to isomerization of the lycopene which occurs as a result of the higher temperature. The ratio of all-*trans*-lycopene to the *cis*-isomers changed from 1.70 to 1.32 when the operating temperature increased from 40 to 100 °C. No significant effects of pressure or flow rate on the antioxidant activity were observed.

Shi et al. (2009a, b) found that the optimized conditions for lycopene extraction to achieve higher yields with maximum anti-oxidative properties were 70 °C and 30 MPa, with a flow rate of 1.5 mL min^{-1} using an I-L separation cell. Optimum process parameters of 56 °C and 26 MPa with an extraction time of 4 h were reported for the extraction of passion fruit seed oil with a good yield of 25.8%; yields of 89.4% and 72%, were achieved for high unsaturated fatty acids and linoleic acid, respectively (Liu et al. 2009a). Aromatic plants are mostly used as raw materials from which to extract natural antioxidants. Yépez et al. (2002) obtained high yields of odorless and flavorless extracts with high antioxidant activity from coriander (*Coriandram stivum*) under moderate conditions of 45 °C and 17.7 MPa. Ribeiro et al. (2001)

Table 3.1 Supercritical extraction process parameters for some selected bioactive components from agricultural material by supercritical-CO_2 fluid extraction

Product extracted	Raw material	Raw material Pretreatment	Component concentration (%)	Temp. (°C)	Pressure (MPa)	CO_2 flow rate (L r^{-1})	Time (h)	MC (%)	Co-solvent	Recovery (%)	Source
Wheat germ oil	Wheat germ	Milled, powder (20 μm diameter)	10.2	35–50	13–41	0.12	2	4.3–11.5		98.7	Ge et al. (2002)
Essential oil piperine	Black pepper	Dried ground	1.5 5.7	40	9–15	2.55		9		18	Perakis et al. (2005)
Essential oil	Eucaliptus leaves	Air drying		50	9	1.1	2.5	9.5		2.4	Porta et al. (1999)
Essential oils gingeroles	Ginger rhizomes	Dried ground	30	40	30	138	2	8.8		8.4	Catchpole et al. (2003)
Triglycerides carotenoid	Palm oil fiber	Oven dried		45–55	20–30	1.45	2.25	5		7	Franca and Meirele (2000)

(continued)

Table 3.1 (continued)

Product extracted	Raw material	Raw material Pretreatment	Component concentration (%)	Temp. (°C)	Pressure (MPa)	CO_2 flow rate (L r^{-1})	Time (h)	MC (%)	Co-solvent	Recovery (%)	Source
Essential oil Capsaicine alkaloids	Chili pepper	Dried ground	2	40	30		2	8.8			Catchpole et al. (2003)
Essential oil	Coriander	Dried grounds (0.4 mm)	96	50	15	3	3			0.61	Anitescu et al. (1997)
Antioxidants	Sweet Thai Tamarinds	Dried ground (mesh 40–70)		35–80	10–30	0.3	5		Ethanol		Luengthanaphol et al. (2004)
Essential oil	Grape seed	Wash dry ground	80	35–75	20–47		0.75	10	2% Ethanol	77	Lee et al. (2000)

found that high antioxidant activity in extracts from lemon balm (*Melissa officinalis* L.), was achieved by optimizing the extraction conditions at 10 MPa and 35 °C for 4 h. Hadolin et al. (2001) found that extraction conditions of 60 °C and 20 MPa produced the most concentrated vitamin E-rich oil from *Silybum marianum*, with a relatively high extraction yield. Perretti et al. (2007) demonstrated the fractionation of fish oil containing a high fraction enriched in ω-3 fatty acids and with a suitable EPA/DHA ratio. These results highlight the possibility of modifying the fatty acid ethyl ester composition of extracts by optimizing the extraction conditions in terms of pressure, temperature, and flow rate.

Tsuda et al. (1995) and Luengthanaphol et al. (2004) compared supercritical-CO_2 fluid extraction to other extraction methods, in terms of their effects on the bioactivity of the extracted compounds. The studies illustrated the superiority of antioxidants extracted with supercritical-CO_2 fluid and modifiers, although some disparity occurred which could have been caused by the varieties used. Macias-Sanchez et al. (2005) extracted carotenoids and chlorophyll from *Nannochloropsis gaditana* and achieved the highest yield at 20 MPa and 60 °C. Wang et al. (2005) also reported that the antioxidant activity of *Bupleurum kaoi* fractionated with supercritical-CO_2 fluid gave the highest yield of phenol and the strongest antioxidant capacities.

Extraction and fractionation of carbohydrates by supercritical fluid extraction have been also been recently reported. The extractions involve the use of CO_2 with a relatively low amount of polar modifier to effectively fractionate the carbohydrate extract formed by lactose and lactulose. Montañés et al. (2008) used a full factorial experimental design to evaluate the effects of extraction pressure, temperature, proportion of modifier, and flow rate. They later reported the optimum conditions for processing to be 100 bar, 100 °C, and 0.2 mL min^{-1} with 4% modifier (Montañés et al. 2009). Supercritical fluid extraction has also been widely employed for the extraction of aromatic compounds. The application of mild pressures and temperatures (100 bar and 40 °C) allowed the highest concentration of aromatic compounds to be extracted from sugar cane (Gracia et al. 2007).

3.4.2 Fractionation of Flavors and Fragrances

The extraction of flavor compounds and fragrances by supercritical-CO_2 is paramount in the food industry. Mother Nature is a splendid synthesizer of flavors and fragrances in natural products. The fact that it is cleaner and safer makes supercritical technology an ideal candidate for extracting such valuable and heat sensitive products in contrast to toxic organic solvents. The high value-added natural products are good for use in soft drinks; one example is ginger extract which gives the pungency and flavor to ginger ale (Fasoli et al. 2012).

Bhattacharjee et al. (2003) compared Likens-Nickerson extraction and supercritical-CO_2 fluid extraction methods on Basmati rice. They reported that supercritical fluid extraction was superior and that the extract of the flavor components was purer and bore the closest resemblance to the original Basmati flavor (Liken-

Nickerson method). A desired fragrance is isolated from concentrates extracted from flowers using a several-stage process. This process consists of initial solvent extraction, usually with an organic solvent (hexane), which yields an intermediate product called concrete (Reverchon and Poletto 1996). This product contains fragrances and other components such as paraffin, fatty acids, fatty acids methyl ester, *di*-, and *tri*-terpenic compounds, and pigments. The post processing of the concrete can be done using supercritical-CO_2 fluid extraction. Reverchon (1997) used single step supercritical-CO_2 fluid extraction, at a pressure of 8 MPa and temperature of 40 °C, followed by a two-stage fractional separation procedure, with the first separator at 9 MPa and −5 °C, and the second at 1.5 MPa and 10 °C. These conditions allowed highly efficient fractionation. The first stage was used to remove cuticular waxes. Under these optimum conditions the extracted volatile rose oil contained 50% 2-phenylethanol. When a co-solvent (ethanol) was mixed with supercritical-CO_2 fluid, a yield of 50%–60% was obtained (Sastry and Mukhopadhyay 1994). Jasmine fragrance extracted at 12 MPa and 40 °C gave superior results compared to other solvents. Sastry and Mukhopadhyay (1994) experienced an increase in yield from 45% to 53% with the use of co-solvents. Similarly, supercritical-CO_2 fluid has been used effectively to extract fragrances from orange, marigold, sandalwood, and vetiver.

3.4.3 Cholesterol Free Food Products

Cholesterol is an inevitable substance required for the daily maintenance of the human body. Lipoproteins are vehicles that transport cholesterol to various bodily tissues to be used, stored, or excreted. High density lipoprotein (HDL) termed "good cholesterol" transports cholesterol back to the liver, where endogenous metabolism prevents cholesterol buildup and reduces the risk of heart disease. Low-density lipoprotein (LDL) termed "bad cholesterol" causes fat buildup in the arteries, increasing the risk of coronary heart disease (atherosclerosis). The indiscriminate consumption of saturated fats in our diet may raise the total LDL above 100 mg/dL and decrease HDL below 35 mg/dL, thus increasing the risk of heart disease. The recommended daily intake of cholesterol is about 300 mg (James and Ralph 2000). The correlation between serum cholesterol level and mortality rate associated to cardiovascular disease has been reported in many studies (Griffin 1999).

Pork meat has cholesterol content about 30–450 mg per 100 g, for poultry this is 70 mg per 100 g, fish is 35–70 mg per 100 g, and beef is 65–331 mg per 100 g. One common source of cholesterol is from the consumption of fried fast-food products (French fries, onion ring, chicken nuggets, etc.). The fast-food industry uses hydrogenated fats for their deep fat frying processes because of its stability and high economic turnover. Hydrogenated fat is a potential source of *trans*-fatty acids which are taken up by food during cooking and ultimately ingested by the consumer. *Trans*-fats have been shown to increase LDL cholesterol levels and reduce HDL cholesterol levels, thus raising the risk of heart disease. Public health initiatives such as the National Cholesterol Education programs have raised consumer awareness,

resulting in increasing advocation for healthy foods with low cholesterol. Thus, the food industry is under tremendous pressure to address this consumer concern.

Supercritical-CO_2 fluid extraction has the potential to revolutionize the oil/fat industry. Many researchers (Dunford and Temelli 1995) reported the feasibility of supercritical fluid extraction of lipids from food without compromising their organoleptic quality. Chao et al. (1993) used a similar extractor configuration to the one discussed earlier with three stage separation to remove cholesterol. They applied pressure at 17, 11, and 4 MPa for each stage sequentially, and were able to achieve higher selectivity for cholesterol at the lower pressures. The results also showed that the fractions collected from the separator at 4 MPa contained cholesterol concentrations of approximately 272–433 mg per 100 g of lipid. Furthermore, Chao et al. (1993) used an operating pressure between 10 and 30 MPa, and temperature range of 30–50 °C to reduce the cholesterol level in ground beef.

Hardardottir and Kinsella (1988) also explored the removal of lipids and cholesterol from fish muscle with supercritical-CO_2 fluid. They removed between 80% and 99% of the cholesterol using fluid pressures of 14–35 MPa and temperatures of 40–50 °C. Although the authors noted limited effect on the lipid/cholesterol yield with increased extraction pressure and temperature, increasing the extraction time from 3 to 9 h significantly ($P < 0.05$) increased the yield. Yeh et al. (1991) used eight operating conditions (pressures from 10.3 to 3.8 MPa, temperatures from 40 to 55 °C, and CO_2 density from 342.3 to 723.4 g L^{-1}) to optimize their process, and observed that at 10.3 MPa and 55 °C the cholesterol level was reduced from 2867 mg per 100 g to 14.1 mg per 100 g. Supercritical-CO_2 fluid technology was used to fractionate milk fat, which is an excellent raw material with specific functionalities used in many products (Rizvi and Bhaskar 1995). Extracting cholesterol from anhydrous milk fat with supercritical-CO_2 fluid, used in conjunction with adsorbents (silica gel) to maximized yield was demonstrated by Huber et al. (1996).

3.4.4 Separation of Spices and Essential Oils

Spices have strongly flavored or aromatic components that can be used in small quantities in food as a preservative or flavoring ingredient. Chilli (*capsicium species*), ginger (*Zingiber officinalis*), and pepper (*piper nigrum* L.) are classic pungent flavorings while ginger and chili have additional nutraceutical values. These products have high economic value in their concentrated forms. The extraction of spices is usually carried out in two stages (the first stage separates the pungent oleoresins and the second stage separates the essential oil fractions. Essentials oils are typically volatile terpenes and esters). Essential oils are concentrated from pure plant extracts and have long been revered for their therapeutic applications; they are derivatives from flowers, leaves, stems, berries, rinds, resins, or roots of plants (Sanchez-Vicente et al. 2009; Liu et al. 2009a). These are very important ingredients and food additives of high value.

Catchpole et al. (2003) performed a detailed study on the extraction of spices and essential oils using supercritical CO_2, propane, and dimethylether fluids. They reported ginger to be the easiest of all spices in terms of optimized yield relative to pressure and temperature, while capsaicin in chili could be extracted at moderate pressure and temperature especially with the use of modifiers. The chili oil fraction contains fatty oil and carotenoids, and it is speculated that the fatty oil acts as modifier for the capsaicin (Peusch et al. 1997). Perakis et al. (2005) extracted black pepper oil with much duress, because its viscous characteristics meant that higher pressure and moderate to high temperatures were required. The use of supercritical propane for extracting spices was reported by Illes et al. (2000). They found propane could adequately extract fatty oils, tocopherols, and carotenoid but was inadequate for capsaicins; on the other hand, CO_2 was adequate for capsaicinoids, fatty oils, and tocopherols but not for carotenoids.

Catchpole et al. (2003) have conducted the supercritical extraction of ginger with three extraction fluids (CO_2, propane, and dimethyl ether). Propane gave the lowest yields while dimethyl ether gave the highest. They reported dimethyl ether to have mutual solubility with water. Ginger contains a high amount of volatiles and CO_2 extraction offers the advantage of dividing the extract into oleoresins and essential oil fractions by using a two-stage separation procedure with sequential pressure reduction.

Similarly, if propane or dimethyl ether is used, considerable heating is required which ultimately results in thermal degradation, and a larger energy requirement in the form of cooling, depressurization, and boiling to recover the essential oils. In the case of ginger, the oxygenated fraction was much greater than the steam distilled oils and the gingerol in the oleoresin was extracted without decomposition. Oleoresins and piperine from peppers were extracted with insignificant losses, although a longer processing time was required. Similar trends were observed for chili and pepper. The extracts contained carotenoid pigments, and those obtained with supercritical-CO_2 fluid were bright red with pink residues while those from propane and dimethyl ether were dark red. The extract obtained from chili with supercritical-CO_2 fluid was a yellow viscous pastry semisolid, while those extracted with dimethyl ether were yellow/black and liquid at room temperature with a high quantity of water diluting the essential oil and piperine content. Nguyen (1991) described the extraction of antioxidants from Labiatae herbs (rosemary, sage, oregano, and thymus) with supercritical-CO_2 fluid at pressures in the vicinity of 50 MPa and temperatures ranging from 80 to 100 °C. The extracted oleoresin was precipitated into two fractions at various levels of pressure and temperature. The first fraction consisted of a green-brown, oil-soluble, heat-stable, resin containing less than 2% essential oil and exhibited remarkable antioxidant properties. The second fraction was the essential oil containing more than 95 mL steam distilled oil per 100 grams.

The use of supercritical-CO_2 fluid for the production of essential oils or oleoresins from spices is possible while a suitable combination of pressure and temperature is selected. The oils extracted with supercritical technology were found to be more valuable than those extracted using other techniques due to their high quality in terms of chemical composition and percentage of sesquiterpene compounds. Supercritical-

CO_2 fluid extraction and hydrodistillation extraction methods were used to extract essential oil from juniper (*Juniperus communis* L.) (Pourmortazavi et al. 2004). Oils obtained by supercritical-CO_2 fluid and hydrodistillation showed significant differences ($P < 0.05$); the former was more selective and particularly efficient for the isolation of α-thoujone and limonene. Anitescu et al. (1997) did a comparative analysis of coriander oil with supercritical-CO_2 and steam distillation. They concluded that oils obtained by supercritical extraction possessed a far better aroma than either the commercial or hydrodistillation extracted oils.

3.4.5 Decaffeination of Coffee and Tea

Caffeine (1,3,7-trimethylxanthine) is a bioactive plant component commonly found in popular beverages such as teas (*Camellia sinensis*), coffees (*Coffee Arabica, canephora, liberica*) (since 1820s), and soft drinks (Ashihara and Crozier 2001). Caffeine is a secondary metabolite, a product of nucleic acid catabolism, and belongs to the group of compounds known as purine alkaloids. Excessive ingestion of caffeine may cause certain health problems such as palpitations, gastrointestinal disturbance, anxiety, tremors, increased blood pressure, dizziness, and insomnia (Ogita et al. 2002).

Caffeine provides aroma and flavor coupled with stimulant. Coffee beans contain approximately 2%–3% caffeine, while tea leaves contain approximately 5%, depending on the variety and species (Jameel 2003). Decaffeinated coffee must contain less than 0.1% caffeine by dry weight, as specified by European Economic Commission (EEC) regulations. Therefore, the decaffeination of coffee and tea presents a significant challenge to both producers and processors. The demand for decaffeinated coffee on the world market is high, it accounts for more than 20% of coffee sales in the USA, with demand growing by 50% among the adult population (Jameel 2003).

Research in genetic engineering to produce transgenic tea and coffee plants deficient in caffeine is in progress (Uefuji et al. 2003). However, the consumption of genetically modified products is still contentious globally, and supercritical-CO_2 fluid extraction technology gives the best option for combating these issues. The decaffeination of coffee and tea is the first known commercial operation using supercritical-CO_2 fluid extraction technology in the food industry.

In the past, methylene chloride was used for the decaffeination of coffee with one cycle of production lasting between 24 and 36 h while the end products usually contained toxic residues, thus posing more harm than the caffeine. Due to its suspected carcinogenic effect, the FDA placed regulations against methylene chloride. However, the decaffeination process with supercritical-CO_2 fluid can be accomplished on green coffee beans, roasted coffee beans, or tea leaves without deleterious effects on the flavor even after 10 h of processing, and many patents already exist for such processes.

The process requires charging the extraction vessel containing the coffee beans with CO_2 at a pressure of 7–22 Mpa and temperature of 31 °C. The caffeine is

dissolved in the supercritical-CO_2 fluid stream, which subsequently enters a washing tower or alternatively activated carbon scrubbers. Distillation, recrystallization, or reverse osmosis is used in some instances to entrain the caffeine. This method can strip coffee of its caffeine content (0.7%–3%) by 71%–97% (Caragay and Little 1981). The caffeine recovered is sold for medicinal purpose and for use in soft drinks. Peker et al. (1992) reported that soaking raw coffee beans in water prior to processing could enhance the rate of decaffeination.

3.4.6 Fish Oil Concentration

Fish oils are characterized by a high percentage of unsaturated straight-chain fatty acids ranging from C_{14} to C_{22} with one to six double bonds. They contain essential fatty acids (EFA) and polyunsaturated fatty acids, grouped into omega-6 and omega-3 EFAs. The main sources of omega-3 are flaxseed, walnut, marine plankton, and fish. This review focuses on omega-3 oils derived from fish. Eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) are predominant in fish oil, and have been reported to contribute to the prevention of atherosclerosis, heart attacks, depression, and cancer if consumed in sufficient quantities (Chow 2000). Fish oil derivatives in the form of omega-3 oils are in high demand as food additives. For example, Ocean Nutrition's ME-3TM Omega-3 powder is currently used in several breads. In the US Wegman's Food Markets, Rochester, New York (NY) launched breads fortified with MEG omega-3 fats, two slices of which offer 80–90 mg of omega-3 (Ohr 2005). Encapsulated omega-3 fatty acids are available for fortified bakery products.

Fish oils are processed as fatty acids, or as methyl or ethyl esters which are more stable than the free acid form (Espinosa et al. 2002). Fatty acids are highly soluble in CO_2 and as a result supercritical-CO_2 fluid extraction is a preferred method of fractionation. With this technology, it is possible to separate heat sensitive compounds (omega-3 fatty acids) and avoid toxic solvent residues in the final product. The isolation and fractionation of omega-3 PUFA (polyunsaturated fatty acid) from fish, fish oil, and esters using supercritical-CO_2 fluid have been studied by several researchers (Letisse et al. 2006; Rubio-Rodríguez et al. 2008; Amiguet et al. 2012).

Amiguet et al. (2012) extracted omega-3 PUFAs rich oil from byproducts by supercritical CO_2 extraction at 35 MPa and 40 °C, and produced 137 mg of oil per gram of dried byproducts with 7.8% ± 0.06% EPA and 8.0% ± 0.07% of DHA. Eisenbach (1984) fractionated the ethyl esters from cold fish oil using supercritical-CO_2 fluid at a pressure of 15 MPa and an extraction temperature of 50 °C. Alkio et al. (2000) produced EPA and DHA with 50% and 90% purity, respectively, from transesterified tuna oil using carbon dioxide. Temelli et al. (1995) obtained the highest yield of omega-3 fatty acids at 35 MPa and 35 °C without denaturing the protein during supercritical-CO_2 fluid extraction. A higher concentration of omega-3 was achieved with supercritical-CO_2 fluid. At 25 MPa pressure, and temperature from 40 to 80 °C, no significant effect on the yield was observed in oil extraction from krill (Yamaguchi et al. 1986). Hardardottir and Kinsella (1988) did not observe any

change from the recovery of fatty acids in rainbow trout at operating pressure ranging from 13 to 35 MPa and temperature from 40 to 50 °C.

3.5 Summary

One of the most important trends in the food industry today is the demand for "natural" foods and ingredients that are free from toxic-chemical additives. The growing interest in natural food has raised the demand for natural health-promoting products of non-synthetic origin. The demand for ultra-pure and high value-added bioactive compounds is redirecting the focus of the food and pharmaceutical industries into seeking the development "green" technologies for their products. Extracts from natural sources are key elements in the manufacturing of health-promoting functional foods and ingredients. Improving the efficacy of "green" separation processes and technologies is critical to the use of bioactive components in health-promoting functional foods and in nutritional supplements. High-value functional substances can be obtained from biological materials by various purification and separation techniques from plant materials and byproducts. The challenge in the separation processes is to meet food regulation guidelines while conducting the separation effectively and economically. Public health, environmental, and safety issues are all major concerns in the use of organic solvents in food processing. Emerging "green" processing technologies, such as supercritical-CO_2 fluid extraction, have been widely used in different fields, including the extraction of essential oils, food ingredients, natural products, pharmaceutical and cosmetic products, and by-product recovery, as well as for food toxicology and eco-toxicology studies.

Supercritical-CO_2 fluid extraction is considered to be a "green" and environmentally friendly separation technology which has emerged as an attractive alternative to traditional methods for the concentration of bioactive compounds. A supercritical-CO_2 fluid extraction process offers the unique advantage of adding value to agricultural material by extracting the bioactive compounds from agricultural raw materials and byproducts for functional food development. The separation problems encountered in the production of soluble materials have a number of aspects which influence the nature of the extraction technique chosen. One of the greatest advantages of supercritical-CO_2 fluid extraction is its rapidity, with shorter extraction times than traditional methods. Supercritical-CO_2 fluid extraction is particularly favorable for the extraction of thermally labile bioactive substances and the process can be easily controlled by adjusting the temperature and pressure.

Supercritical-CO_2 fluid extraction also offers the advantage of adding value to agricultural waste by extracting antioxidants and flavonoids, such as lycopene from tomato skin, essential oils, flavors, and fragrances, which are then used for the fortification of foods and other applications. Supercritical-CO_2 fluid extraction can be utilized to provide healthy snack foods. De-fatting and de-cholesterol treatment with supercritical-CO_2 fluid extraction has been demonstrated to be applicable to food products. Although most of the tests were conducted on dehydrated products,

research has shown successful application of supercritical-CO_2 fluid on high moisture products where extraction could be accomplished without compromising the organoleptic characteristics.

Supercritical-CO_2 fluid extraction is available in the form of single stage batch process and could be augmented to allow multistage semi-continuous batch processing coupled with multi-separation. Although significant accomplishments have been obtained in the last couple of years that should not warrant complacency. Since batch modes render supercritical-CO_2 fluid extraction technology cumbersome for certain industrial applications, the need to improve the design to allow for continuous modes is growing. Supercritical-CO_2 extraction is only cost effective for large-scale production, which makes it ideal for the decaffeination of coffee, tea, and hops. With improvements in processing conditions and reduced cost, supercritical-CO_2 fluid extraction will become increasingly economical at low throughput. Extracts from natural sources are key elements in the manufacturing of health-promoting functional foods and ingredients. Thus, the development and use of "green" separation processes and technology is likely to continue to be widely employed in the processing of bioactive components, especially for use as supplements for health-promoting foods.

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Chapter 4
Power Ultrasound for Extraction and Modification of Polysaccharides from Medicinal Fungi

Jianyong Wu

Abstract Power ultrasound (US) (low-frequency range of 20–100 kHz) has been used to enhance or facilitate a wide range of chemical, food, and biological processes (in liquid media) such as extraction, dispersion, filtration, cell disruption and homogenization, chemical and enzymatic reactions, microbial fermentation and plant biotechnology. After several decades of research on and development of the US processes, the current interest is still high and rising on new and innovative applications in many areas, such as nanotechnology, biotechnology, food processing, drug delivery, and biomedical engineering. Power US is a unique, convenient, direct, safe, and green technique, and more favorable than thermal and chemical means for the processing of food, as well as medicinal and biological products. Polysaccharides and polysaccharide-protein complexes of edible and medicinal fungi are important and attractive sources of functional food and nutraceutical products because of their health benefits and bioactivities such as antitumor, immunomodulatory, hypoglycemic, hypolipidemic, and antiviral activities. Power US may be an innovative and effective tool for the extraction of the fungal polysaccharides and for the modification of their functional properties. This review is to give a brief account of the current research trends and the most promising applications of power US in chemical, food and bioprocesses, a comprehensive outline of our recent studies on the application of power US in the extraction and modification of polysaccharides from medicinal fungi, particularly the major procedural factors, the extraction kinetics, and the effects on the molecular properties and bioactivities of polysaccharides extracted from the medicinal fungi.

Keywords Ultrasonic process · Acoustic cavitation · Medicinal fungi · Polysaccharides · Molecular properties · Bioactivities

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4.1 Introduction

Power ultrasound is regarded as a safe, clean, mild (low-temperature), and convenient tool for processing food and medicinal products without introducing hazardous chemical and biochemical reagents (as by the chemical and enzymatic processes), and for preserving heat-labile bioactive products (versus thermal processes) (Patist and Bates 2008). Power US has been exploited for a wide range of processing operations such as extraction, emulsification, filtration, homogenization and activation of chemical and biochemical reactions. Ultrasound-assisted extraction (UAE) of bioactive natural products from medicinal plants and various other sources is one of the most widely studied ultrasonic processes, and has also found applications in laboratory sample preparation and industrial production (Vinatoru 2001; Feng et al. 2008; Vilkhu et al. 2008; Chemat et al. 2017). In addition to process enhancement (direct and common effects), power US may be a useful means for the modification and improvement of the functional properties of food products such as lower viscosity, higher solubility and stronger bioactivity (Soria and Villamiel 2010; Chandrapala et al. 2012).

Edible fungi (mushrooms) are well known for their nutritive and medicinal properties, and have enormous potential for nutraceutical and therapeutic uses. Polysaccharides (PS), including polysaccharide-proteins (PSPs), is well-known bioactive constituent of edible/medicinal fungi with notable antitumor and immunomodulatory activities, and they have found commercial applications as immunotherapeutic agents and adjuvant cancer drugs (Ooi and Liu 2000; Wasser 2002; Ramberg et al. 2010). Fungal PS have also shown several other activities including antioxidant, hypoglycemic, hypolipidemic, and antiviral. PS-rich aqueous extracts and partially purified PS fractions of mushrooms and fungal mycelia are widely applied in functional foods. The multiple health benefits and increasing application of fungal PS have motivated considerable research efforts on their separation and purification, structural characterization, and structure–activity relationships (Zhang et al. 2007). Extraction is the first key step for the separation of PS from mushrooms and other materials. Most of the fungal PS for commercial and research uses are extracted from mushroom fruiting bodies and fungal mycelia, and only a small group of exopolysaccharides (EPS) from the liquid broth of mycelial fermentation.

This chapter starts with a brief description of the physical characteristics of ultrasound and acoustic cavitation as the primary cause for the effects of power US, and a brief summary of the general applications of power US in chemical, food and biochemical processes. The main focus will be put on ultrasound-assisted extraction (UAE) and power US for the partial degradation of PS from edible and medicinal fungi, to illustrate and discuss the major procedural factors, procedural kinetics and the effects of power US on the molecular properties and bioactivities of PS.

4.2 The Physics and General Applications of Ultrasound

Ultrasound refers to sound waves or mechanical vibrations at frequencies above the audible human range which is usually below 16–18 kHz (Fig. 4.1). A well-known natural phenomenon is the use of US for communication in some animals (e.g., bats and whales). Ultrasonic technology has been widely applied in material testing (flaws in mechanical structures) in engineering, in the navigation of submarines in the military, and medical imaging (fetus scanning in obstetrics). The ultrasonic devices for detection and diagnostic purposes usually apply low-intensity US waves in a high-frequency range (>0.5 MHz). The US for these applications is classified as diagnostic US which does not cause any significant effects or permanent changes in the tested object. The US useful for product processing belongs to power US in the much lower frequency range of 20–100 kHz, which is usually applied at much higher intensities to produce physical and chemical changes to the sonicated object.

Power US is a versatile tool in the laboratory and industry for various processes and operations, such as cleaning, filtration, extraction, degassing, biological cell disruption, and stimulation of chemical and biochemical reactions (Table 4.1). The use of high-intensity US to facilitate specific chemical and biochemical reactions, a field of research known as sonochemistry, has been actively going on for several decades (Mason and Lorimer 2002). Although US is a well-established field of physics and has found many applications, it still needs to find new areas of research and application. In recent years, power US has been widely explored as a safe, clean and convenient tool for processing food and medicinal products (Patist and Bates 2008; Feng et al. 2008; Chemat et al. 2011, 2017). The application of power US in food processing is aimed not only at enhancing the conventional processes, but also at modifying the properties, functions and quality attributes of food products, such as their solubility, texture, viscosity, and emulsifying property (Soria and Villamiel 2010). Power US has been experimented with for various dairy processes such as whey ultrafiltration, milk homogenization, cutting cheese blocks, and enhancing milk fermentation (Chandrapala et al. 2012). In meat processing, power US has been experimented with for curing, marinating, freezing and thawing and also for modifying the texture of meat, its water retention capability, and its color (Tao and Sun 2015).

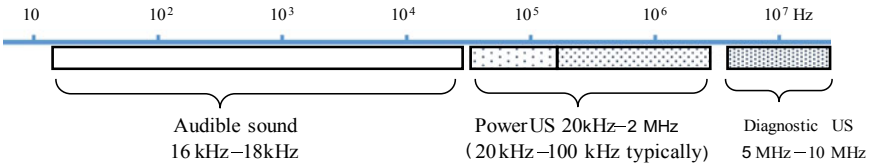


Fig. 4.1 Spectrum and classification of sound waves

Table 4.1 Established applications of power US in laboratory and industrial processes

Physical processes (sonoprocessing)	Chemical reactions (sonochemistry)
Defoaming/degassing cleaning/sterilization	Electrochemistry
Crystallization/precipitation	Chemical synthesis
Emulsification/dispersion	Heterogeneous catalysis
Homogenization/cell disruption	Polymer synthesis/degradation
Extraction/leaching	Enzymatic reactions
Filtration (ultrafiltration)	Wastewater treatment (Anaerobic sludge digestion)
Plastic soldering and cutting	

4.3 Cavitation and Consequences Induced by Power Ultrasound in Liquid Media

Cavitation is the formation and dynamic consequences of microbubbles or cavities in a liquid when it is subjected to the rapidly alternating pressure of high amplitude (Mason and Lorimer 2002; Suslick 1988). When US propagates through a liquid, the vibrating sonic waves cause the oscillation of the liquid elements, resulting in regions of compression (high pressure) and rarefaction (negative pressure) (Fig. 4.2). Cavitation occurs in the regions of rarefaction when the negative pressure overcomes the forces holding the liquid together and breaks the liquid apart. In the succeeding positive pressure cycle, the bubbles are compressed or even crushed to total collapse. The total collapse of the cavitation bubbles takes place instantaneously with a sudden release of the bubble energy, producing shock waves and causing a sharp rise in the local temperature. The high pressure and temperature arising from the cavitation process are sufficient to cause mechanical damages and chemical changes to the objects and the liquid in the region, such as the erosion of metals and formation of free radicals. Figure 4.3 shows the eroded aluminum foils at various power levels and exposure periods in an ultrasonic bath, which is also a simple method for detecting the occurrence and intensity of cavitation caused by ultrasonic irradiation in a liquid. In pure water free of bubbles, the onset of cavitation (*true cavitation*) is estimated as 1500 atm. However, the cavitation thresholds in practice are usually much lower (<20 atm) due to the presence of bubbles or nuclei of cavities (such as solid particles) in the liquid (*gaseous or pseudo-cavitation*). The onset of cavitation also depends on the frequency of ultrasound, the surface tension and the viscosity of the liquids.

The intense energy released during cavitation is sufficient to induce chemical reactions in the liquid. In water, the principal chemical reaction induced by the cavitation is the decomposition or sonolysis of water molecules into free radicals, and eventually the formation of H_2O_2 and H_2, $OH + H \rightarrow H_2O \rightarrow H_2O_2 + H_2$.

The cavitation process described above is known as transient or inertial cavitation, in which the voids or vapor-filled bubbles collapse completely during the high-pressure cycle or within a few pressure cycles. There is another type of cavitation,

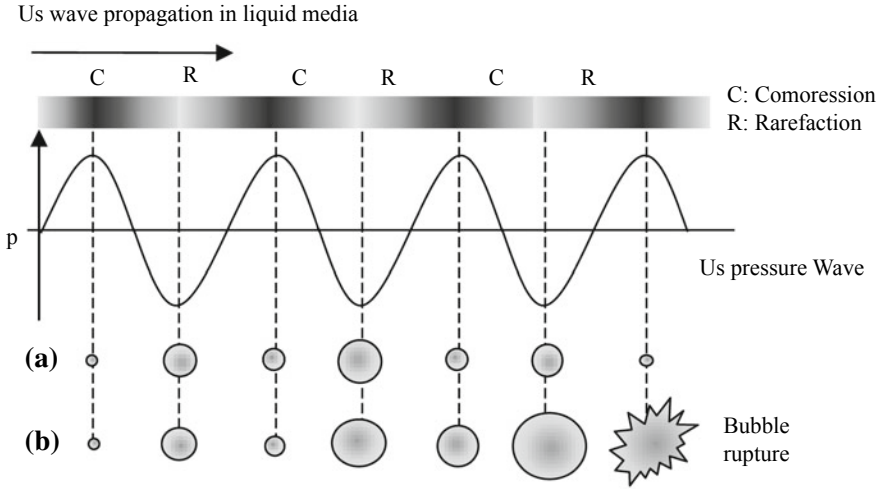


Fig. 4.2 Cavitation event and consequences induced by power US in liquid media. **a** Stable cavitation (non-collapsing): bubble expands (low p) and contracts (high p) alternately, oscillating and existing for many acoustic cycles. **b** Transient cavitation: cavity/bubble increases in size, and eventually collapses at an unstable size, creating immense local high T and p up to 5000 K and 2000 atm (1 atm = 1.01×10^5 Pa)

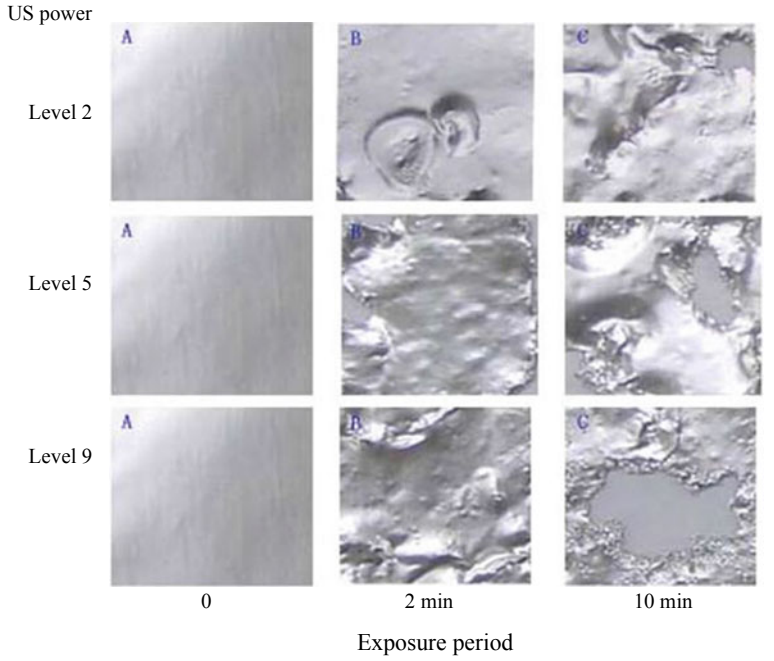


Fig. 4.3 Erosion of aluminum foils caused by acoustic cavitation at various power levels and exposure periods (in an ultrasonic bath)

in which the bubbles will not collapse but continue to oscillate and exist for many acoustic cycles, known as stable cavitation. The stable bubbles usually contain a great amount of gas as well as some liquid vapor. Transient cavitation mainly occurs at high intensities and stable cavitation at low intensities, though this also depends on other factors. Transient cavitation is a violent event and may be destructive to cells and biological molecules.

Cavitation is of primary significance in the applications and various effects of high-intensity US. Many of the chemical and biological effects of high-intensity US in liquid media are also believed to be consequences of acoustic cavitation, including free radical production, cell disruption and denaturation of biological molecules, such as proteins and enzymes. Cavitation is recognized as a chief cause of cell lysis, and may also be responsible for the sonoporation and permeabilization of animal cells. Another hydrodynamic event induced by power US in a liquid is acoustic streaming, the time-independent (steady) flow (or eddy motion) of a liquid (Suslick 1988). Acoustic streaming is a result of the attenuation or absorption of the sound energy by the fluid particles. In an ultrasound field, the fluid elements or small particles such as cells in the fluid are subjected to an "acoustic torque" which causes the fluid particles to swirl. One type of acoustic streaming occurs in a homogeneous liquid, which causes a convective flow of the liquid. Another is in the boundary layer of the liquid on the surface of a solid. An important cause of acoustic streaming is stable cavitation, the oscillation of the bubbles in a bulk liquid or near a solid surface. In a cell suspension, acoustic streaming may induce a spinning motion of the cells or the intracellular structures, and the eddy motion of the intracellular fluid. Acoustic streaming may cause shear stress and accelerate the mass transfer at the boundary layer.

4.4 Ultrasonic Devices and Power Measurements

Two types of US devices are commonly used in the laboratory to treat solid materials in liquid media, ultrasonic processors and ultrasonic cleaning baths (Fig. 4.4) (Santos et al. 2009; Chemat et al. 2011). An ultrasonic processor applies US directly into the sample liquid with a probe horn transducer which is inserted into the sample liquid (Fig. 4.4a) (direct exposure). An ultrasonic cleaning bath irradiates the sample indirectly through the liquid in the bath and the container wall of the sample solution which is immersed in the bath (indirect exposure) (Fig. 4.4b). The processor probe is more powerful and effective, but can cause contamination and local heating of the sample solution. The cleaning bath has the advantage of noninvasive exposure to keep the sample intact and for more uniform distribution of US intensity in the sample liquid.

For the probe horn, the ultrasound intensity I (in W cm^{-2}) is represented by the power per unit area of the probe tip ($=\pi r^2$, where r is the radius of the tip surface)

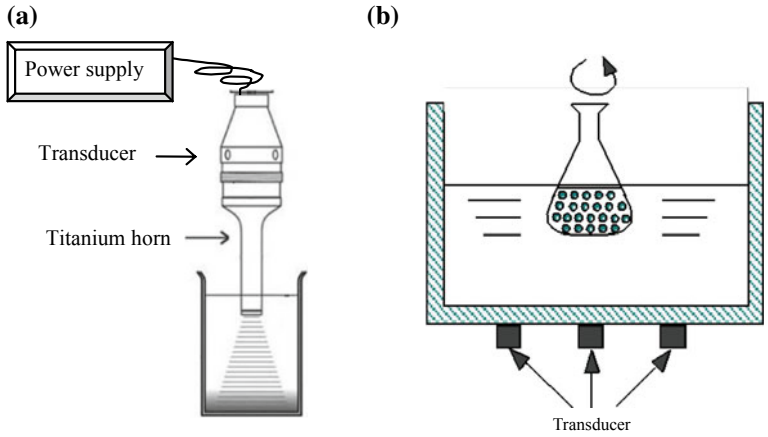


Fig. 4.4 Two common types of ultrasonic devices for laboratory uses. **a** Ultrasonic processor with a probe for direct irradiation; **b** cleaning bath for indirect irradiation

$$I = \frac{P}{\pi r^2} \tag{4.1}$$

The power level in the ultrasonic processors is usually controlled by adjusting the amplitude (%). The actual power P (in W) transferred into the sample liquid (water) at a given amplitude can be determined or calibrated by the calorimetric method according to the following equation (Mason 1990):

$$P = C_p W \left(\frac{dT}{dt}\right)_{t=0} \tag{4.2}$$

where C_p is the heat capacity and W the mass of sample liquid (e.g., 4.2 kJ kg^{-1} $°C^{-1}$ for water) receiving the US irradiation, and $(dT/dt)t = 0$ the initial rate of temperature change (T) in the sample liquid. In our previous studies (Cheung et al. 2013, 2015), we performed the calibration of two ultrasonic processors by measuring the temperature change of water contained in a well-insulated polycarbonate flask. The water was irradiated with a probe horn for 15–20 min, during which the water temperature was recorded. The actual power was roughly proportional to the amplitude shown in the processor panels (Table 4.2).

Other useful and scalable US process parameters include US power density, P/V, power per unit volume of liquid and energy density, Pt/V, total amount of US energy to the liquid over the treatment period.

Table 4.2 Actual ultrasound power and intensity transferred into the extraction liquid corresponding to the amplitude read in ultrasonic processors (by Eqs. 4.1 and 4.2)

Processor	Amplitude (%)	Probe tip diameter (mm)	Power, P (W)	Intensity, I (W cm^{-2})
VCX 130	70	12	24.5	21.7
	20	12	2.76	2.44
CTXNW-2B	20	15	77.9	44.1

Note $I = P/\pi\ r^2 = P/\pi\ (0.6)^2$; $P/V = P/90 \times 10^3$ kW m^{-3} (r = tip diameter/2 = 12 mm/2 = 0.6 cm; liquid volume of extraction $V = 90$ mL)

4.5 Ultrasound-Assisted Extraction of Natural Products

Ultrasound-assisted extraction (UAE) can be defined as the application of power or high-intensity US to enhance the extraction of solid constituents in a liquid solvent. UAE has been applied in the laboratory for a long time for the extraction and homogenization of chemical, food and biological samples with ultrasonic cleaning baths or probe horns, often as a routine sample preparation step prior to analysis and measurement (Mason and Lorimer 2002; Santos et al. 2009; Chemat et al. 2009). A widely attempted area of applications of UAE is in the separation of bioactive natural products from medicinal plants (Vinatoru 2001; Vilkhu et al. 2008). The chief advantages expected of UAE for the separation of bioactive natural products over the conventional methods include higher extraction yield and extraction rate (shorter time and higher throughput), and lower extraction temperature. The low-temperature operation of UAE is particularly desirable for preserving the properties and activities of heat-sensitive food and medicinal products which can deteriorate in the conventional hot water or hot-solvent extraction processes. Power US enhances the extraction of natural products with water and also with organic solvents such as ethanol and petroleum ether (Toma et al. 2001). In addition to enhancing the existing extraction processes, power US can make new commercial extraction processes possible and improve the properties and bioavailability of micro-nutrients and therapeutic compounds. UAE is also overall more favorable for laboratory analysis and large-scale separation of food and medicinal products than other non-conventional techniques such as microwave-assisted, pressurized, and supercritical-fluid extractions in terms of simplicity, energy efficiency, scalability, versatility, safety and environmental compatibility (Vinatoru 2001; Patist and Bates 2008; Chemat et al. 2009).

4.6 Ultrasound-Assisted Extraction of Polysaccharides from Medicinal Fungi

4.6.1 Bioactivities of Polysaccharides from Edible and Medicinal Fungi

Edible and medicinal fungi (mushrooms) have become the important sources of immunomodulating and anticancer agents. Polysaccharides (PS) are major bioactive constituents of fungi exhibiting notable antitumor and immunomodulatory activities, and other valuable medicinal properties (Ooi and Liu 2000; Moradali et al. 2007; El Enshasy and Hatti-Kaul 2013). Crude or partially purified PS extracted from mushrooms and fungal mycelia have been widely applied to functional foods and cosmetic products (Wasser 2002; Stachowiak and Regula 2012), and a few purified PS fractions such as β-glucans and PS-protein (PSP) complexes from edible and medicinal fungi have found commercial applications for immunotherapy and anticancer treatment, and as the adjuvant for chemotherapy/radiotherapy (Ramberg et al. 2010; de Silva et al. 2012). An enormous effort has been made to study the structural characteristics, molecular properties and medicinal functions of fungal PS as summarized by several reviews (Zhang et al. 2007; Yang and Zhang 2009; Wasser 2010; Stachowiak and Regula 2012; Mizuno and Nishitani 2013). In addition to the well-known anticancer β-D-glucans and PSPs, many other PS structures, including glycans and heteroglycans from fungi, have been found with antitumor and immuno activity. Most of these antitumor PSs are in a high molecular weight (MW) range in the order of 10^5 to 10^6 or higher. Most previous studies on UAE of natural products were aimed at the low-MW organic molecules such as phenolic and aromatic compounds, alkaloids, and essential oils from plants and various other sources (Toma et al. 2001; Vilkhu et al. 2008). With increasing interest in the bioactive and other useful properties of natural PS, more recent studies have been devoted to UAE of PS from plant materials, mainly herbs and agricultural plant residues (reviewed by Ebringerová and Hromádková 2010) and from edible/medicinal mushrooms such as *Lentinus edodes* (Shiitake) (Nian et al. 2004), *Trametes versicolor* (Yunzhi) (Pan et al. 2010), and *Ganoderma lucidum* (Lingzhi) (Chen et al. 2010), and from by-products of *Agaricus bisporus* production (Aguilo-Aguayo et al. 2017). These studies have shown that UAE is a viable alternative to the classical methods, such as hot water extraction (HWE) for the isolation of PS from plants and fungi with the advantages of a shorter extraction time, lower consumption of solvents and extraction temperature. However, a specific concern for UAE of PS and related biopolymers is polymer degradation at high US powers and long periods. Although power US has been utilized for controlled degradation of high-MW PS to desirable low molar masses, this effect should be minimized during the extraction of PS to preserve the structural and molecular properties.

4.6.2 Factors and Effects of Power Ultrasound on the Extraction of Polysaccharides

Ultrasound-assisted solid–liquid extraction is a complex physical process involving several classes of factors, namely ultrasonic (equipment design and operation), solid material (physical properties, particle size and morphology), liquid solvent (solid-to-solvent ratio), and the environment (temperature, agitation), which can all significantly affect the rate of UAE. Ultrasonic enhancement of extraction is mainly attributed to the disruption of cell walls, the reduction of particle size and enhanced mass transfer through solid particles by the hydrodynamic activities of cavitation bubbles (Veličović et al. 2006; Chemat et al. 2011).

In a previous study by the author's group (Cheung et al. 2012), UAE was applied to extract water soluble PSPs from three important medicinal mushrooms, *Grifola frondosa*, *Coriolus* (*Trametes*) *versicolor,* and *Lentinus edodes*. Compared with HWE, the yields of total water extract and PS attained by UAE were higher than HWE with *G. frondosa* and *L. edodes* mushrooms, but lower with *C. versicolor*. The protein contents of PSP obtained with UAE were significantly higher than those with HWE for all three mushrooms. The antioxidant activities of PSP obtained by UAE were also generally higher. Based on GPC analysis, the overall molecular weight distributions of PSPs by UAE was lower than those by HWE, suggesting that UAE was less effective for extracting high-MW PSP factions than HWE. The different yields and molecular properties of PSPs from UAE versus HWE are most probably attributed to the different extraction mechanisms (mechanical versus thermal) and conditions (low versus high temperature) of the two extraction methods. Another notable phenomenon observed in this study was a strong dependence of UAE efficiency on the morphology and aggregation of the mushroom particles (Cheung et al. 2013). As shown in Fig. 4.5a, the solid powders of *G. frondosa*, and *L. edodes* mushrooms in water were in a dispersed particulate form while that of *C. versicolor* mushroom formed very large aggregates.

Under a scanning electron microscope (SEM) (Fig. 4.5b), the C. versicolor mushroom exhibited a fiber form and the morphology was not significantly changed after UAE, while the other two mushrooms were irregular fragments before UAE and turned to more porous fragments after UAE.

4.6.3 Procedural Characteristics and Kinetic Models of UAE

The kinetic models for the UAE of natural products are usually adapted from those for conventional solid–liquid extraction or leaching, a well-known process of mass transfer. The mass transfer of the extractable substances (ES) from the solid particles to the liquid involves several intrinsic steps, but is usually viewed as two major steps, a rapid dissolution or washing of ES on the surface of the solid particles followed by a slow diffusion of ES stored in the internal sites through plant particles into the bulk

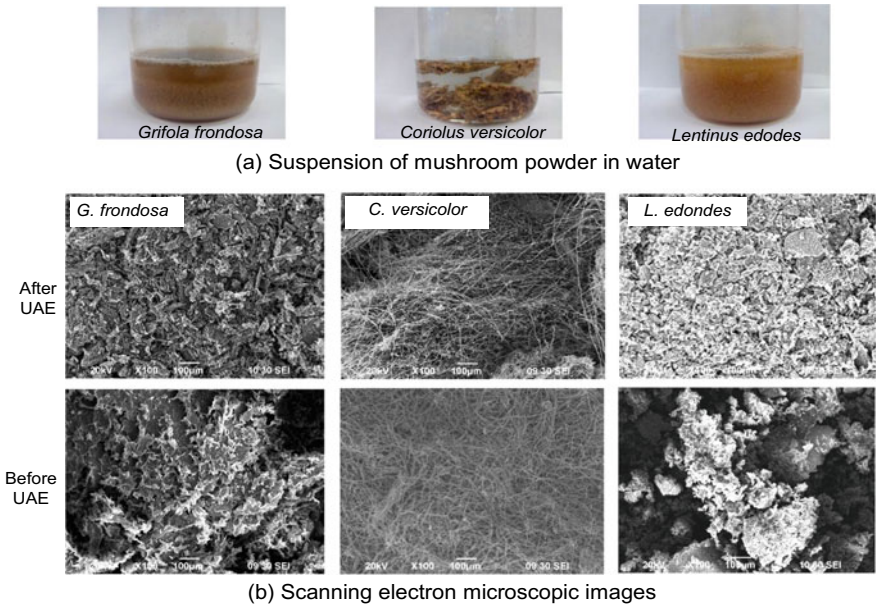


Fig. 4.5 The solid powder of three mushroom species: **a** suspension in water; **b** SEM images before and after ultrasonic extraction (adapted from Cheung et al. 2013)

liquid. Usually the first step is very fast and the second step, diffusion of ES through plant particles, is slow and the rate-limiting step of the overall extraction process. A typical trend of the change in the concentration of the ES with extraction time is shown in Fig. 4.6. Based on this mass transfer mechanism and experimental data, several simplified empirical models have been developed for describing the extraction kinetics of natural products from plant materials in various mathematical forms, such as parabolic, hyperbolic, power law and exponential (Kitanović et al. 2008). For ultrasonic extraction of ES from the aerial parts of two plant herbs, the kinetics of bioactive ES extraction was satisfactorily correlated by two theoretical models, the unsteady diffusion and the film theory and an empirical model of Ponomaryov (Veličović et al. 2006). A second-order kinetic model originally developed for conventional extraction has been shown to be satisfactory for the ultrasonic extraction of phenolic antioxidants from the dry peel of pomegranate marc with the ultrasonic processor having been operated in both continuous and pulsed modes (Pan et al. 2011). These studies showed that the rate constant k and other model parameters depended both on the US intensity (W cm^{-2}) and the plant materials.

In our previous studies (Cheung et al. 2013; Cheung and Wu 2013), UAE was applied to several important edible and medicinal fungi in fruit body and mycelial form, including Grifola frondosa (maitake), Lentinus edodes (shiitake), Cordyceps sinensis Cs-4 and Cs-HK1, and Ganoderma lucidum (Linzhi). The experimental results, yield of total water extract versus time data, were fitted to several empiri-

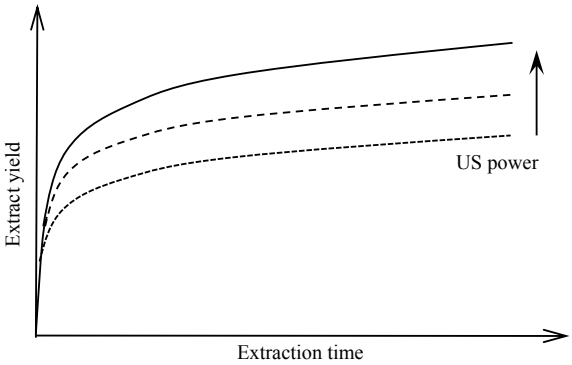


Fig. 4.6 Typical UAE time course (kinetic trend)

cal models for solid–liquid extraction by linear regression (Table 4.3). Based on the correlation coefficients R^2 values, the extraction data (yield versus time) of two mushrooms in fruit body form showed a close fit to three models, power law, Weibull's exponential and Elvich logarithmic equations, and the data of three fungi in mycelial form showed a close fit to the parabolic diffusion model. The model constants as well as the extraction rates were dependent on the experimental conditions, including US power, temperature, solid particle size, and solid-to-liquid ratio. Significant correlations were found between the extraction rate and the US power density, dy/dt versus P/V and between extract yield and energy density, y versus E/V ($= Pt/V$). The kinetic and process parameters are useful for a rational design and an efficient operation of the UAE processes. The kinetic and process parameters are useful for a rational design and an efficient operation of the UAE processes. The fit to different kinetic models with the fruit body and mycelial form is an indication of a significant dependence of the extraction kinetics on the properties of the mushroom materials.

4.7 Ultrasonic Polymer Degradation

4.7.1 Characteristics and Mechanisms of Ultrasonic Polymer Degradation

The use of high-intensity power US for degradation or depolymerization of macromolecules was an early topic in sonochemistry (Price 1990; Mason and Lorimer 2002), while it is still regarded as an unconventional means compared with the chemical, thermal, and enzymatic means that are more commonly used in industrial processes (Wasikiewicz et al. 2005; Patist and Bates 2008). Regardless of the different methods, a general feature of polymer degradation is the molecular weight-dependent kinetics with which the degradation rate or reduction of MW is usually fast in the initial period when the MW is relatively high, and slow in t h e later stage

Table 4.3 Correlation coefficients (R^2) from the linear regression fit of the UAE experimental data (total extract yield y vs. time t) to the empirical kinetic models with five different fungal species (adapted from Cheung et al. 2013; Cheung and Wu 2013)

Model	Fruit body form		Mycelial form		
	G. frondosa	*L. edodes*	Cs-HK1	Cs-4	*G. lucidum*
Parabolic diffusion $y = y_o + y_1 t^{1/2}$	0.907	0.871	0.995	0.955	0.965
Power law $y = B t^n$	0.984	0.964	0.926	0.900	0.879
Weibull $y = 1 - e^{(-t_m/D)}$	0.984	0.964	0.913	0.898	0.877
Elovich $y = E_o + E_1 \ln t$	0.984	0.963	0.906	0.885	0.868
Unsteady diffusion $y = (1 - b)\, e^{-kt}$	0.743	0.717	0.927	0.859	0.890
Peleg hyperbolic $y = C_1 t/(1 + C_2 t)$	0.710	0.767	0.534	0.483	0.422

when the MW is low. With the ultrasonic method, the degradation rate approaches zero with no further reduction of MW as the MW reaches a minimum limit, *Mlim*, which can be quite high. In a previous study from the author's group (Chen et al. 2010), the average MW was as high as 730 kDa after the high-intensity US degradation of a high molecular weight exopolysaccharide (EPS) of a medicinal fungus. Therefore, US is mainly effective and suitable for partial degradation of polymers but not for complete or extensive depolymerization. Other than the molecular size, US usually does not cause significant changes in the primary structure and functional groups of a polymer molecule. Ultrasonic depolymerization usually results in a narrowing of the MW distribution (MWD) or a decrease in the polydispersity. In contrast, enzymatic and thermal degradation often leads to MWD broadening (Tayal and Khan 2000; Xu et al. 2008). Vodeničrová et al. (2006) evaluated three methods, including microwave-heating, γ-radiation, and ultrasonication for the degradation of xyloglucan.

Compared with US, microwave-heating and γ-radiation caused more extensive reduction of the MW and notable changes in the primary structures of the polysaccharide molecule. It was concluded that US was the most convenient and effective method for partial degradation of XG samples and the preservation of the primary structures.

Several kinetic models have been established for US degradation and fitted to various polymer structures and experimental conditions (Akyüz et al. 2008). Two main modes of the chain session have been established for polymer degradation with US and other means, both random and mid-point. Most previous studies on ultrasonic depolymerization have suggested a mid-point chain scission mechanism. The following first-order kinetic model has been proposed (Price 1990; Wu et al. 2008):

$$\ln \frac{M_0 - M_{\lim}}{M_t - M_{\lim}} = kt \quad \text{or} \quad -\frac{dM_t}{dt} = k(M_t - M_{\lim}) \tag{4.3}$$

where M_0 and M_t are the number average MW at time 0 and t, respectively, and k the rate constant. A simplified first-order kinetic model which was originally derived for the low-temperature degradation of cellulose, a high-MW natural PS with a linear polymer chain, has been widely applied for hydrolysis of PS by various methods, including ultrasonic and enzymatic methods (Tayal and Khan 2000),

$$-(\mathrm{d}L/\mathrm{d}t) = kL$$

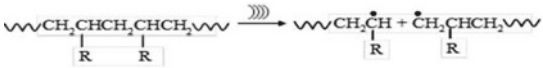
where L is the total number of hydrolysable linkages. L is proportional to DP = M/m (M = average MW of polymer and m the molar mass of the monomer). When $M/m \gg 1$, the integrated kinetic model is represented by,

$$\frac{1}{M_t} - \frac{1}{M_o} = kt/m \tag{4.4}$$

where M_0 and M_t are the average weight-average MW of PS at time zero and time t of the degradation process, respectively. However, with this model, the polymer molecular weight M_t continues to degrease but not reach a minimum limit, $M_{\lim}$. The reaction rate constant k is related to temperature by the Arrhenius equation, $k = \mathrm{A}e^{-Ea/RT}$, with which the activation energy Ea can be estimated.

In a recent study, we evaluated the effect of power US on the solution properties of a plant polysaccharide, konjac glucomannan (KGM), which is widely used as a thickening and gelling agent in liquid foods. The exposure of KGM to high-intensity US resulted in a sharp and rapid decrease in the apparent viscosity, e.g., that of 1% *w/v* KGM in water from more than 50 Pa s to a negligible level similar to that of water within 10–20 min. The reduction of intrinsic viscosity ([η]) of the KGM solution in the initial period was slower than the apparent viscosity and continued throughout the treatment period, and the overall time course followed the simplified first-order kinetic model. In a recent study (Pu et al. 2017), US degradation of six dextran standards with MW of 4.47×10^4–1.75×10^6 resulted in a reduction of the molecular weight and also of the polydispersity index, a more homologous dextran solution. The degradation kinetics or time course of molecular weight reduction was closely fitted to the first-order model Eq. 4.4.

Cavitation is regarded as the predominant cause of US-induced polymer degradation in most cases. When a polymer solution is irradiated by US, the polymer chain being caught in the rapid flow of solvent molecules and the shock waves created by the rapid oscillation and collapse of cavitation bubbles are subjected to a high shear force, and are broken if the force is high enough. The most possible reaction in US-induced polymer degradation is the production of macro-radicals, for example,



Since cavitation is the major cause of polymer degradation, the factors determining the threshold and intensity of cavitation are also the chief factors affecting the degradation. Therefore, the degradation rate usually decreases together with the viscosity and concentration of the polymer solution, and the volatility of the solvent (Price 1990; Grönroos et al. 2008).

4.7.2 Degradation of Natural PS for Improved Properties and Bioactivities

Polysaccharides (PS) from plants, fungi and microbial sources are abundant natural sources of nutraceutical and therapeutic materials. However, the natural PS are mostly in a high molecular weight (MW) range and tend to form aggregates or gels in solution, exhibiting poor solubility and high viscosity which is unfavorable for medicinal applications. A direct and effective approach for improving the solution properties is partial depolymerization. Power US irradiation is a preferred means for the controlled degradation of biopolymers, improving the properties of the solution and preserving the primary structure and functionality. Ultrasonic degradation has been exercised for partial degradation of various carbohydrate polymers such as celluloses, starch, dextrans, chitosans, and xanthans to attain lower viscosity, higher solubility and other desired properties (Kardos and Luche 2001; Gogate and Prajapat 2015). US degradation has also been employed as an experimental method for generating lower MW fragments of fungal PS to investigate the MW-dependent properties and bioactivities, e.g., scleroglucan into several MW fractions from 2.2×10^6 to 1.4×10^5 (Falch et al. 1999) and lentinan from 2.83×10^6 to 1.87×10^5 (Zhang et al. 2007). The US treatment reduced the MW and intrinsic viscosity dramatically, but preserved the basic molecular structure and antitumor activity. US degradation of apple pectin resulted in lower average MW, more uniform MW distribution, lower viscosity, but did not alter the primary structure (Zhang et al. 2007).

The first ever case for partial degradation of mushroom PS with power US was documented in 1981 by Tabata et al. (1981) on schizophyllan, a β-glucan from a Schizophyllum commune mycelial culture. The US treatment decreased the MW and intrinsic viscosity dramatically, but it preserved the basic molecular structure and antitumor activity. The degradation mainly occurred by cleaving the glycosidic bonds in the main chain, but not the side-chain linkages. The partially degraded PS, Sonifilan, has been used as an antitumor drug in Japan. A few studies have been reported by another group (Šndula et al. 1999; Machová et al. 1999) on ultrasonic depolymerization of microbial PS, retaining the immunomodulation activity. In their studies, (1-3)-β-glucans isolated from *Aspergillus. nigar* fungal mycelia and *Saccharomyces cerevisiae* yeast were carboxymethylated, and then degraded by US with a

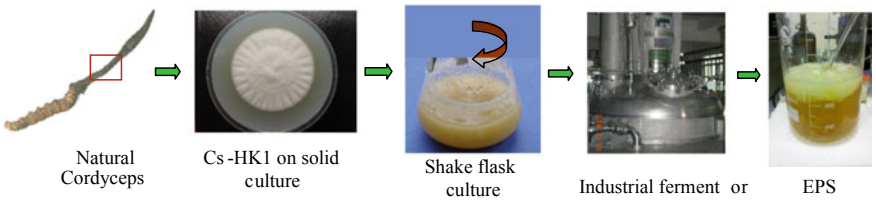


Fig. 4.7 Establishment of the Cs-HK1 mycelial culture and application to liquid fermentation for production of mycelial biomass and exopolysaccharides (EPS, isolated from mycelial fermentation broth by ethanol precipitation)

20 kHz probe horn at 100 W. Without significant activity before US degradation, the US-degraded lower MW glucans gained high antimutagenic activity in mice for both intraperitoneal and oral administration of the PS preparations. Zhou and Ma (2006) reported a study on the ultrasonic degradation of bioactive PS extracted from a red alga (*Porphyra yezoensis*) to improve the immunoactivity and water solubility.

4.7.3 Ultrasonic Degradation of the Exopolysaccharide of a Medicinal Fungus Cs-HK1

Cordyceps sinensis, generally known as the Chinese caterpillar fungus, winter worm-summer grass (Dong-chong-xia-cao) is a rare and precious medicinal fungus which has been used as a favorable tonic for hundreds of years in China (Zhou et al. 2009). Because of the very limited supply and high value of natural Chinese caterpillar fungus, mycelial fermentation has become the most viable process for mass production of the fungal materials. Cs-HK1 is a fungus isolated from a natural *Cordyceps* fruiting body in our lab and Cs-HK1 mycelial culture has been established and applied for production of mycelial biomass and exopolysaccharides (EPS) through liquid fermentation (Fig. 4.7) (Leung et al. 2006; Yan and Wu 2014). The Cs-HK1 mycelial fermentation has been successfully carried out in large-scale industrial fermentors up to 2000 L (Wu et al. 2014). In shake-flasks and various volumes of stirred fermentors, the Cs-HK1 mycelial culture usually produces 3–5 g L^{-1} of EPS over 5–6 days. The crude EPS isolated from the Cs-HK1 mycelial culture broth by ethanol precipitation consisted of high-MW PS over 10^6 Da and lower PS-protein (PSP) complexes (Leung et al. 2009; Yan et al. 2010; Wang et al. 2010; Huang et al. 2013). The high-MW EPS has a high viscosity and low solubility in water.

In a previous study, we evaluated the effects of power US on the MW and solution properties of a high-MW fraction of the EPS (MW > 1000 kDa) produced by the Cs-HK1 fungus (Wang et al. 2010). The EPS dissolved in water was irradiated with an ultrasonic probe and at an intensity of 35 W cm^{-2} or higher, the apparent viscosity as well as the intrinsic viscosity of EPS solution decreased by nearly 85% within 10 min, and the solubility of EPS in water increased by more than fourfold. In contrast, the

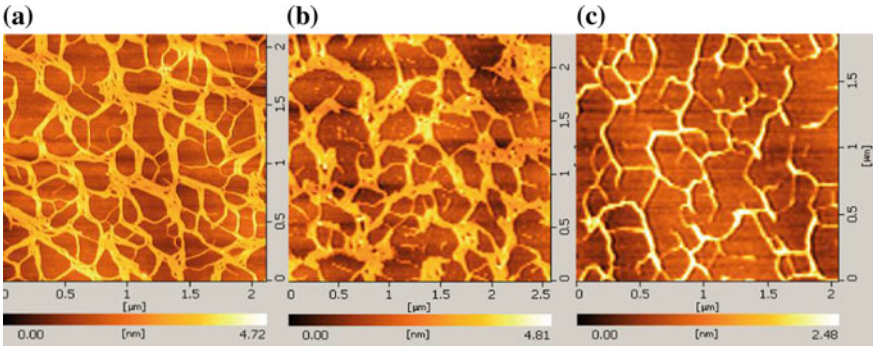


Fig. 4.8 Morphological characteristics of EPS aggregate networks in an aqueous solution (AFM images of EPS at 10 g ml^{-1} in DI water). **a** untreated; **b** sonicated at 20% amplitude for 4 min; **c** sonicated at 70% amplitude for 60 min

intrinsic viscosity of EPS was reduced by only 20% in a 1.0 M (mol L^{-1}) sulfuric acid solution at 50 °C for 9 h. The US treatment also led to a notable reduction of the maximum MW and a more uniform MW distribution. As observed under atomic force microscopy (AFM), the native EPS formed a large interwoven network of aggregates resembling a fishnet (Fig. 4.8). After the US treatment (at 35–50 W cm^{-2} for 60 min), the thick strands of the native EPS network were split into thinner and fragmented strands. The AFM imaging plus Congo-red test suggested that the native EPS network was linked with thick strands of triple or multiple helices and was split into single helices by US. The results indicate that US caused changes in the EPS higher order conformation and aggregation. As seen from the dynamic light scattering (DLS) spectra of EPS samples, the number of peaks corresponding to different aggregate sizes was four with the native EPS and was reduced to three peaks after 1 min of US treatment, and to a major peak after 60 min of US treatment corresponding to the size range of 100–1000 nm (Fig. 4.9). The DLS data indicate that the US treatment resulted in a more uniform size distribution of the EPS aggregates.

4.8 Ultrasonic Disruption of Fungal Mycelia for Effective Product Recovery

4.8.1 Liquid Fermentation for Production of Edible Fungi and Bioactive PSPs

Two major forms of edible/medicinal fungi are produced commercially, mushroom fruit body on solid, lignocellulosic substrate and fungal mycelium by liquid or solid fermentation.

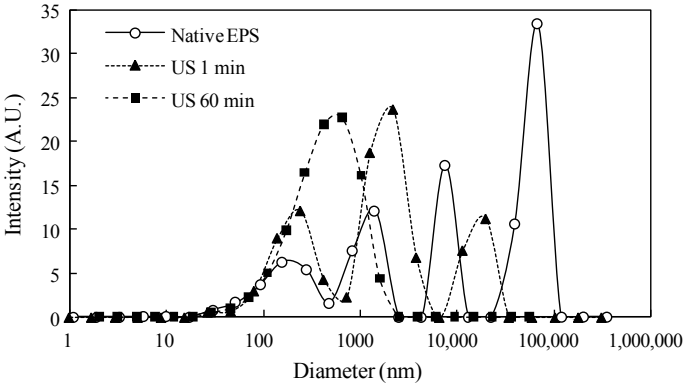


Fig. 4.9 A dynamic light scattering (DLS) spectra of native EPS and EPS sonicated for various periods of time at a given US power level. EPS dissolved in 0.1 M NaCl; DLS performed with Malvern Zetasizer Nano 3000SHA at 90° scattering angle for 10 min at 25 °C

Liquid or submerged fermentation in stirred and aerated bioreactors is the most advanced and efficient for the production of fungal mycelia and their useful metabolites, though the production cost (for equipment, operations and technical labor) is usually much higher than the traditional solid cultivation/fermentation processes. Submerged fermentation is more suitable for the production of high-quality and high-value mushroom components. Exopolysaccharides (EPS) are extracellular PS secreted by the fungal cells into the liquid medium, which are mostly combined with peptides or proteins as PSPs. Because of its potential for mass production as the industrial microbial PS, the production of EPS by liquid fermentation has been extensively experimented with several important edible/medicinal fungi in liquid (Wagner et al. 2003; Zhong and Tang 2004; Smiderle et al. 2012), though it has not found much commercial application. Therefore, the mycelial biomass is the major source of PS and other fungal products from liquid fermentation.

In a typical process for the production of PS via submerged fermentation, the mycelium biomass is separated from the broth by filtration or centrifugation and is then extracted with hot water at nearly 100 °C for a few hours. The hot water extraction process may be repeated one to two times with the solid residue for complete recovery. The water extract is concentrated by evaporation under vacuum, and is dried to yield the water extract as a mixture product of low- and high-molecular weight water soluble components. For the isolation of PS and other biopolymers, the concentrated extract solution is saturated with an organic solvent such as ethanol. The precipitate is collected as the crude PS composed mainly of PS, proteins and their complex PSPs, and it can be further purified and fractionated through multiple steps including dialysis, ion-exchange and gel-filtration chromatography, etc. These steps are also applicable for EPSs from the liquid medium.

Although power US is a common mechanical means for homogenization and extraction of biological cells and tissues, the applications are limited to small vol-

umes in the laboratory for sample preparation and analysis. To the best of our knowledge, few studies have been reported on ultrasonication of mycelial broth for product recovery from fermentation, and little is known about the characteristics of the related process and the effects of high-intensity ultrasound on the physical properties (mycelial morphology, broth rheology) of the mycelial broth and the medicinal properties of the products of fermentation. Most previous studies on ultrasonic disruption of microbial cells have been carried out on bacteria and yeast, but only a few on filamentous fungi or fungal mycelia (Idrissi et al. 1999; Taubert et al. 2000; Klimek-Ochab et al. 2011). Some of these studies have shown that mechanical methods are more effective than the chemical methods commonly used for the disintegration of uni-cellular bacteria and yeasts. A possible cause is related to the special morphological structures of fungal mycelia and mycelial aggregates which are highly resistant to the diffusion of chemical solvents (Taubert et al. 2000). Klimek-Ochab et al. (2011) showed that ultrasonication and bead milling were most favorable for the extraction of soluble proteins and for the preservation of active enzymes. The cell wall of fungi is mainly formed from covalently cross-linked chitin, glucans and glycoproteins. Chitin accounts for 10%–20% of the cell walls of some filamentous fungi, but only 1%–2% of yeast cell walls by weight. Chitin has a strong tensile strength contributing significantly to the integrity of the fungal cell wall.

4.8.2 Ultrasonic Disruption of Fungal Mycelia for Effective Product Recovery from the Highly Viscous Fermentation Broth of the Cordycepssinensis Cs-HK1 Fungus

The Cs-HK1 mycelial fermentation broth produced a viscous, pulpy slurry product containing 20–25 g/L biomass and 3–5 g/L EPS. The mycelial fermentation broth containing high-MW EPS of Cs-HK1 and other filamentous fungi is highly viscous and difficult to be processed by filtration or centrifugation for solid (biomass)–liquid (medium) separation. In a previous study, high-intensity US was employed to disrupt the Cs-HK1 mycelia to reduce the viscosity and also to release the intracellular products (Cheung et al. 2015). As shown by the microscopic images, the filamentous mycelia were disrupted into shorter fragments by US irradiation (Fig. 4.10) and the apparent viscosity of the broth decreased notably with the US treatment time (Table 4.4). The US treatment promoted a rapid release of intracellular products (as total water extract and biopolymers) into the liquid medium (Fig. 4.11). There was also a notable increase in the antioxidant activity of the total water extract with US treatment time, due probably to the continued release of bioactive products from the mycelia. The results suggest that the power US treatment of the viscous mycelial broth can greatly facilitate the product separation and recovery process and also improve the medicinal properties of the products.

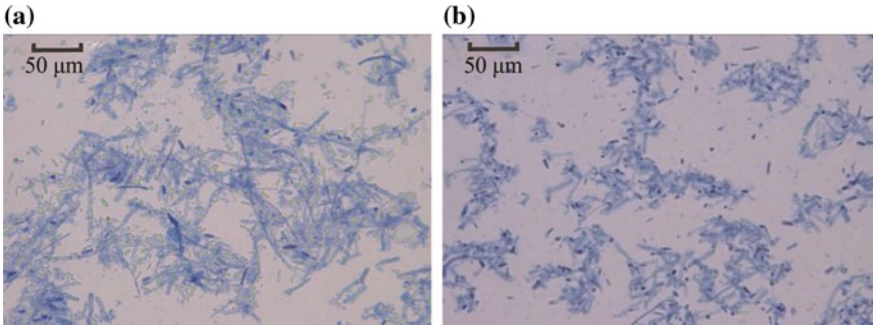


Fig. 4.10 Microscopic images of Cs-HK1 mycelia; **a** before US treatment; **b** after US treatment (at 70% amplitude for 30 min; Bar = 50 μm)

Table 4.4 Viscosity reduction of mycelial broth with US treatment (measured with a rotational viscometer at 20 °C) (data from Cheung et al. 2015)

γ (s^{-1})	Apparent viscosity (cP)		
	No US	US 3 min	US 60 min
0.1	2367	2180	407
0.2	1410	1287	267
0.5	771	735	152

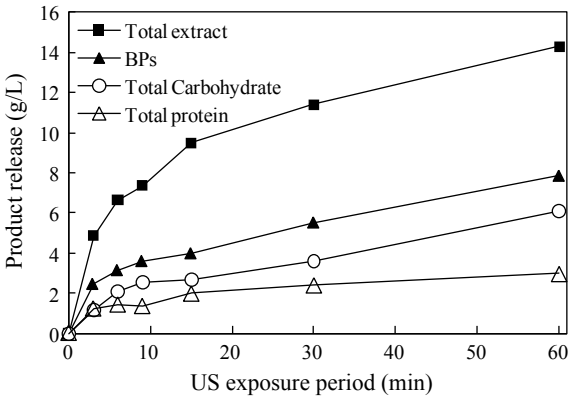


Fig. 4.11 US-induced release (concentration increase) of intracellular products from mycelial biomass (US power at 70% amplitude with a VCX750 W processor; BPs = biopolymers) (adapted from Cheung et al. 2015)

4.9 Conclusion

This chapter has mainly outlined the recent studies on the applications of power US in the extraction of polysaccharides or their protein complexes PSPs from edible and medicinal fungi and in the partial degradation of the high-MW PS for improving the properties of the solution. Following are some major findings from our previous studies:

(a) The PSPs attained by ultrasound-assisted extraction (UAE) from various mushroom materials are significantly different from those attained by hot water extraction (HWE) in chemical composition and MW range.
(b) The extraction kinetics and efficiency of UAE are strongly dependent on the microstructure and aggregation of mushroom particles.
(c) US power density P/V and energy density E/V are useful and scalable procedural parameters for design and operation.
(d) Power US is a preferred means for the partial and controlled degradation of polymers to lower MW and narrower MWD without drastic changes in the primary chemical structures, and is most suitable for the degradation of bioactive PS and for preserving the native functionalities.
(e) Power US is a versatile processing tool which is applicable in a wide range of processes and can cause physical, chemical, and biological effects. Cavitation is a chief cause for most of the physical, chemical, and biological effects of power US in various process systems. Power US is one of the most favorable means for the nonthermal processing of food and medicinal products, such as the US-assisted extraction of the useful constituents from these products. The merits of technology of power US processing include the clean, safe, simple, convenient operation and equipment, and the lower temperature condition. In addition to the improvement of procedural efficiency, power US may be applied to modify favorably the quality and functionality of the product. With the significant advances in ultrasonic technology and equipment over the last decade, large-scale applications of ultrasonic processing technology are becoming more feasible and cost-effective.

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Chapter 5
Pulsed Electric Field Assisted Extraction of Bioactive Compounds

Xin'an Zeng and Zhihong Zhang

Abstract The pulsed electric fields (PEF) technology is one of the innovative nonthermal processing methods. It can cause the permeabilization of cell membranes when dealing with short treatment time with less energy consumption, and minimize the deterioration of the quality of the food compounds. The PEF treatment has been considered as a promising method, thus arousing more and more the researchers' interest in organism extraction. The PEF treatment can enhance the extraction yield of bioactive compounds, such as polyphenols, anthocyanin, and plant oil from plant tissues and their by-products, as well as the soluble intracellular matter from microorganisms. The purpose of this chapter is to summarize the principles of extraction, and the application of the PEF on the extraction of bioactive compounds from plant tissues in recent years.

Keywords Pulsed electric fields (PEF) · Extraction · Plant tissues · Bioactive compounds

5.1 Introduction

In the last two decades, the development of PEF technology for nonthermal food processing applications has grown fast, and has been used in food processing, especially for liquid foods, such as fruit juice, vegetable juice and milk, in order to protect their rich antioxidants or nutrients (vitamins, phenolic compounds, pigments, etc.) that were easily degraded under the traditional thermal processes (Azmir et al. 2013). The pulses of PEF treatment applied in liquid foods are at a high voltage (20–80 kV cm^{-1}) in the treatment chamber between two electrodes. Due to the design of the treatment chamber, the treatment time of the PEF system was less than 1 s, and the energy loss was minimized, which was caused by the food conductivity generating heat (Moussa-Ayoub et al. 2013). Regarding the attributes of the quality of food,

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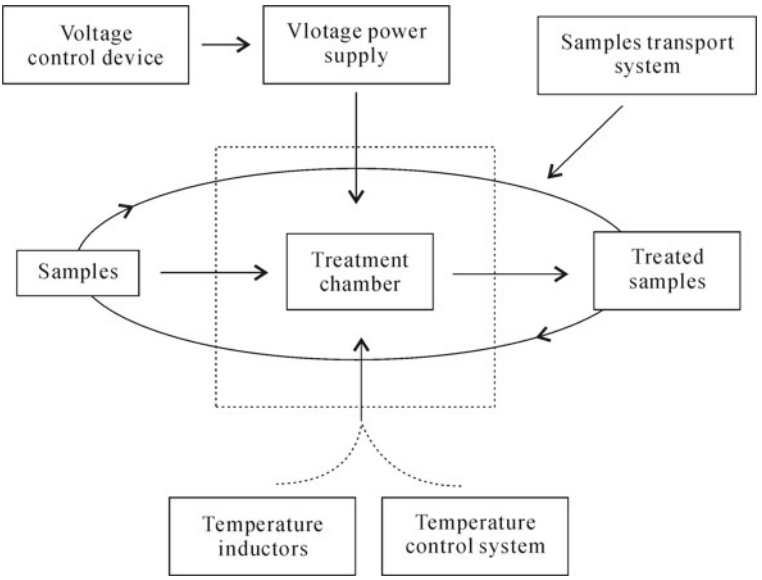

Fig. 5.1 The scheme of the PEF treatment system

the PEF technology is superior to the traditional heat treatment method on the quality of food. This is because it avoids or greatly reduces the detrimental changes in the physical properties of ingredients and sensory quality of foods, especially for those heat-sensitive nutrients, such as vitamin C, β-carotene, and anthocyanins, etc. (Janositz et al. 2011; Marsellés-Fontanet et al. 2013; Moussa-Ayoub et al. 2013; Torregrosa et al. 2005).

In general, PEF treatment system mainly contains a pulse generator, a treatment chamber, a fluid handling system, a monitoring system, and a temperature controlling system (Azmir et al. 2013; Vorobiev and Lebovka 2010), as shown in Fig. 5.1. Due to its irreversible electroporation, the PEF treatment has been proved to be a promising method with a high capability for increasing the cell membrane permeability. Traditionally, the most popular extraction methods, such as the heating treatment and organic solvent extraction, are solid–liquid extractions, which are an unsteady-state mass transfer of multi-components from the solid matrix to the solvent one. Compared with conventional methods referred to above, the PEF application in extraction can not only increase the mass transfer during the crude extraction process with high yields and a better quality of the extracts (Corrales et al. 2008; Donsì et al. 2010), but it can also be more environmentally friendly and highly efficient due to the less amount of synthetic and organic chemicals used during the reaction associated with a short operational time (Azmir et al. 2013; Singh et al. 2015). Some previous studies also reported the capacity of the PEE to increase the extraction yield during the compression of plant tissues and the microbial culture medium (Azmir et al. 2013; Goettel et al. 2013; Luengo et al. 2013; Puértolas et al. 2013).

The PEF techniques has been studied that can be applied to extract bioactive compounds from diverse kinds of crude materials. Therefore, this review focuses on the mechanism of the PEF function, the specific equipment, and the parameters in the extraction processing, as well as the efficiency of extraction in order to provide a comprehensive summarization of PEF applications in the extraction of bioactive compounds from foods and their by-products.

5.2 Mechanism of PEF Extraction

In the studies of the PEF treatment applied in microbial inactivation, several mechanisms have been proposed to explain the mechanism. Among them, the two most popular and widely accepted mechanisms are electrical breakdown and electroporation (disruption) of cell membranes (Barbosa-Canovas et al. 2000; Liu et al. 2014; Schoenbach et al. 1997; Wang et al. 2016). In the investigation of the applications of PEF exaction, the main mechanism is the electroporation theory. The cells (plant, animal or microorganism) are temporarily exposed to high voltage electric field pulses which can induce the destabilization of the lipid bilayer and proteins in the cell membranes. In addition, after being exposed to high electric fields, the plasma membranes of cells become permeable for the small molecules; this induces swelling in the cell, and eventually the rupture of the cell membrane (Fig. 5.2). When the sphere-shaped biological cell is exposed to an external electric field (E), the potential of the transmembrane will increase as a result of the charging process at the membrane interfaces. The potential differences U_m can be approximately calculated by Eq. 5.1, which is derived from solving Maxwell's equation in spherical coordinates assuming several simplifying restrictions (Hofmann and Evans 1986)

$$U_m = 1.5 \times R_{\text{cell}} \times E \times \cos(\theta) \tag{5.1}$$

where U_m is measured and the direction of the vector E, R_{cell} is the radius of the cell, θ is the angle between the sit on the cell membrane.

When the E exceeds the E_c, a critical transmembrane potential can be induced, and results in the formation of reversible or irreversible pores in the membrane. Typically, the majority of cell membranes E_c is in the range of 0.2–1.0 V (Liu et al. 2015; MahničKalamiza and Vorobiev 2014). According to Eq. 5.1, the factors which cause irreversible pores in the membrane have a close relationship to the strength of the electric field and the cell radius. The critical transmembrane potential is generated by the external electric field and decreases with the cell radius. Compared with the size of microbial cells (≈1–10 μm), cells in plant tissues have an extremely larger size (≈100 μm). Therefore, the strength of the electric field required for electro-plasmolysis in plant cells (0.5–5 kV cm^{-1}) is lower than that required for the inactivation of microorganisms (more than 10 kV cm^{-1}) (Chaturongakul and Kirawanich 2013; Chen et al. 2013; Toepfl et al. 2007).

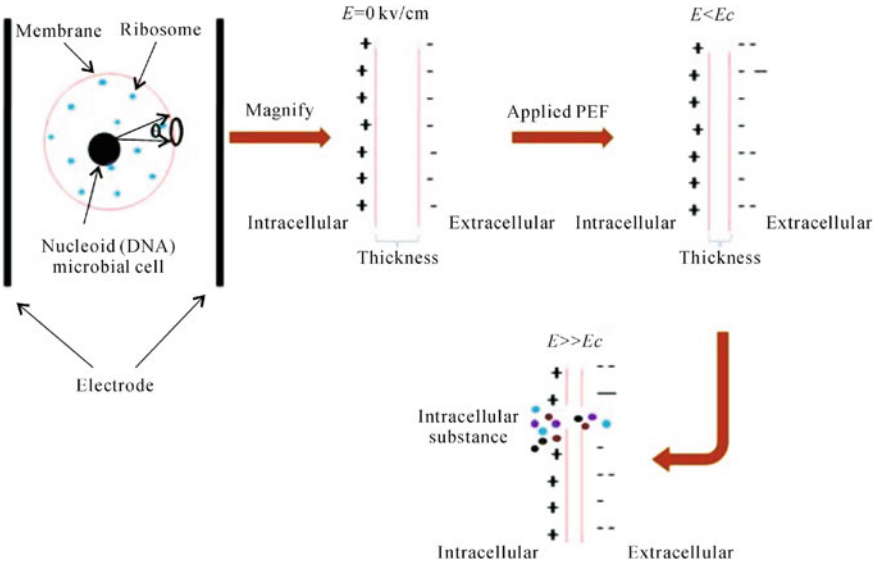

Fig. 5.2 The electroporation theory of PEF treatments applied on the cell

5.3 Effects of PEF Treatment on Extraction

The texture or microstructure of most of the food materials would be altered under the PEF intensity of 1–10 kV cm^{-1}. The effects and experimental conditions are listed in Table 5.1. Goettel et al. (2013) pointed out that the PEF treatment at 23–43 kV cm^{-1} on microalgae Auxenochlorella protothecoides with fresh water can result in cell disintegration causing the release of soluble intracellular matter into the suspensions. With the increase of inputting specific treatment energy, the efficiency of disintegration increased, whereas the PEF intensity showed little influence. As for the suspensions with a biomass content of 100 g dry weight per kg of suspension, the input energy was necessary for the cell rupture and was considered the range of 1 MJ kg^{-1} dried algae. The PEF treatment can only cause the spontaneous release of soluble components. Puértolas et al. (2013) investigated the effects of the PEF treatment on the extraction yields of anthocyanins (AEY) from the purple-fleshed potato (PFP) at different extraction times (60–480 min) and temperatures (10–40 °C) using water and ethanol (48% and 96%) as extraction solvents. The results demonstrated that the highest cell disintegration index ($Zp = 1$) was obtained at the PEF intensity of 3.4 kV cm^{-1}, treatment time of 105 μs (3 pulses of 3 μs) with the lowest specific energy (8.92 kJ kg^{-1}). The PEF treatment increased the AEY, especially at a lower extraction temperature in the water reaction system. The AEY reached 63.9 mg/100 g (fw) from the untreated samples with 96% ethanol solvent under the reaction time of 480 min at 40 °C which was similar to 65.8 mg/100 g (fw) obtained from the PEF-treated samples by using water as the extraction medium. Therefore,

the PEF can be a more environmental-friendly method to be used in the extraction of anthocyanins from the PEF treatment with a water medium instead of the solvent (ethanol) without decreasing the AEY. Boussetta et al. (2014) studied the role of the PEF treatment on the yields of polyphenols from flaxseed hulls. The research results showed that the extraction yield of polyphenols could reach 80% under the PEF treatment at 20 kV cm^{-1} for 10 ms. The lower PEF intensity applied in the extraction processing, the lower extraction efficiency was observed. In addition, the rehydration of the samples before the PEF application can improve the efficiency of the reaction. About 37% of the increased extraction efficiency was observed after its rehydration for 40 min before being subjected to the PEF treatment. The addition of ethanol, citric acid, and sodium hydroxide can also enhance the efficiency of the extraction of polyphenols. The highest amount of polyphenols was yielded with the extraction solvent mixtures containing 20% ethanol and 0.3 mol L^{-1} hydroxide sodium. The study also confirmed that the alkaline hydrolysis displayed a more effective role than the acidic hydrolysis.

From the findings of the above studies, the results showed that the effectiveness of the PEF treatment mainly depended on the PEF intensity, the specific energy input, the pulse numbers, the temperature, and the properties of the treated materials (Donsì et al. 2010; Pataro et al. 2011; Vorobiev and Lebovka 2010). Moreover, it has been shown that when compared to the extraction from the plant tissues, the microbial cells required a higher intensity of the electric field and a longer treatment time. García et al. (2005b) evaluated the PEF resistance of four Gram-positive (*Bacillus subtilis*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Staphylococcus aureus*) and four Gram-negative (*Escherichia coli*, *E. coli* O157:H7, *Salmonella* serotype Senftenberg 775 W, *Yersinia enterocolitica*) bacterial strains under the same treatment conditions. The microbial characteristics such as cell size, shape or type of the cell envelopes had no significant influence on the microbial PEF resistance while the pH of the treatment medium showed great effects on the PEF-resistant bacteria. Therefore, the PEF parameters were different and depended on the targeted products from different microbial cells, and even the same PEF treatment parameters can cause different results because of the changed external conditions which can change the efficiency of extraction, such as temperature, pH or type of solution, etc. (García et al. 2005a; Loginova et al. 2011).

A longer treatment time and a higher PEF intensity mean more energy input into the reaction system resulting in an increased temperature of the solutions. Due to this, the PEF can minimize the degradation of heat-sensitive compounds (Buckow et al. 2013; Oms-Oliu et al. 2012). Thus, compared with the conventional extraction methods, the PEF can also be considered a pretreatment process for plant materials under relatively mild processing conditions, such as low temperature and neutral pH value, and can significantly shorten the extraction time and reduce the usage of extraction solvents.

Table 5.1 The effects and experimental conditions of PEF treatments on extraction of food materials

Sample	Process conditions			Effects	References
	Field strength (kV cm^{-1})	Pulse duration (μs)	Pulse number		
Auxenochlorella protothecoides	23–43	1	10	PEF-induced cell disintegration resulted in the release of soluble intracellular matter into the suspension, but the field strength hardly had any influence	Goettel et al. (2013)
	40	8.3	500	The PEF treatments resulted in incomplete damage of yeast cells, and extraction of ~70% of ionic substances, ~1% of proteins and ~16% of nucleic acids was obtained by PEF	Liu et al. (2013)
Purple-fleshed potato (*Solanum tuberosum*)	3.4	105	35	PEF treatment increased the anthocyanin extraction yield (65.8 mg/100 g fw)	Puértolas et al. (2013)
	1	1000	2	PE + PEF allowed production of mushroom extracts with high contents of fresh-like proteins and polysaccharides, and the extracts were clear and their colloid stability	Parniakov et al. (2013)
Orange peel (*Citrus sinensis*)	1, 3, 5, 7	60	20	With increasing the electric strength, the total polyphenol extraction yield and the antioxidant activity were increased	Luengo et al. (2013)

(continued)

Table 5.1 (continued)

Sample	Process conditions			Effects	References
	Field strength (kV cm^{-1})	Pulse duration (μs)	Pulse number		
	3.4	105	35	The anthocyanin extraction yield (65.8 mg/100 g fw) increased	Parniakov et al. (2013)
Grape skin (*Vitis vinifera* var.)	5 and 10			PEF treatment at room temperature caused an increment of the colour intensity, anthocyanin content and of total polyphenolic index, and these three increased with the electric fields strength raised	López et al. (2008)
	20	10,000	1000	PEF treatment allowed the extraction of up to 80% of polyphenols	Boussetta et al. (2014)
Flaxseed hulls (*L. usitatissimum*)	20	10,000	1000	PEF treatment allowed the extraction of up to 80% of polyphenols	Boussetta et al. (2014)
	2.5	15	50	PEF treatments enhanced total anthocyanin extraction in water from red cabbage by 2.15 times with a higher proportion of nonacylated forms than the control ($P < 0.05$)	Gachovska et al. (2010)
Red grape (*Vitis vinifera* var.)	2, 5, 7	3	16	With increment of the electric field, the extraction rate of anthocyanins and total phenols was increased	Puértolas et al. (2010)

(continued)

Table 5.1 (continued)

Sample	Process conditions			Effects	References
	Field strength (kV cm^{-1})	Pulse duration (μs)	Pulse number		
Chenopodium rubrum	0–1.6	0–30	0–10	Higher loss of cell viability. More effective pigment release at increasing field strength than that with increasing number of pulses	Dörnenburg and Knorr (1993)
	16.88	18	9	The maximum yield of chondroitin sulphate is 6.92 g/L	He et al. (2014)
Fresh red beetroots (*Beta vulgaris* L.)	0–9	2–40	5–100	Treatment of 5 pulses at 7 kV/cm (2.5 kJ/kg) and 10 kg/cm^2 at room temperature permitted the extraction of 90% of the total red colouring in 35 min	López et al. (2009)
Maize germ	0.6, 7.3		120	Higher oil yield (up to 88.4%) and increased amount of phytosterols (up to 32.4%) was reached simultaneously. Oil yield increased marginal by 2.9% in comparison to untreated sample, and phytosterol increase reached 14.7%	Guderjan et al. (2005)
Chicory root (*Cichorium intybus*)	0.1–0.6	1×10^3–5×10^7	1×10^3	The PEF application for enhance of the soluble matter extraction from chicory	Loginova et al. (2010)
Citrus peels (*Citrus sinensis*)	1, 3, 5, 7	60	20	PEF increase the cell disintegration index, and 5 kV/cm treated the orange peels increased the quantity of naringin and hesperidin	Luengo et al. (2013)

(continued)

Table 5.1 (continued)

Sample	Process conditions			Effects	References
	Field strength (kV cm^{-1})	Pulse duration (μs)	Pulse number		
Fresh matured coconut (*Cocos nucifera*)	0.1–2.5	575	0–200	Treatment with HELP under suitable conditions ($E =$ 2.5 kV/cm, $n = 20$ pulses, $t = 575$ μs and frequency = 1 Hz) resulted in 20% increase in milk yield with reference to the untreated samples	Ade-Omowaye et al. (2000)

5.4 Conclusion

Due to consumers' concern of potential hazards in the extracts and health risks by using organic solvents as an extraction medium, the demands for natural products extracted from plants and other materials are high, especially for those nutrients extracted from by-products. At the same time, the growing demands for naturally extractive bioactive compounds encourage more and more researchers to focus on exploring more advanced and effective extraction techniques. As an emerging non-thermal method, the PEF technology has been applied in microbial inactivation in the food industry, which is mainly attributed to an external electric field generating a reversible or irreversible permeabilization of the cytoplasmatic membrane of eukaryote and prokaryote cells. In recent years, the PEF treatment used in the extraction of intracellular compounds has become a popular research topic. The mechanism of cell permeabilization caused by the PEF has not been extensively studied. One of the generally accepted mechanisms is the dielectric breakdown theory, which is also identified in the extraction of a theoretical basis. Based on the previous studies, it has been proved that the PEF treatment can improve the efficiency of extraction in the food materials, but the results are different when using different extraction materials. Therefore, the application of the PEF in the extraction of nutrient compounds should be carried out in the following aspects in future research: (1) The characteristics of different plant materials and extracted compounds should be taken into consideration when using the PEF technology; (2) Due to the different food materials and PEF equipment, adequate experiments should be made before the application in industry; (3) Even if the PEF technology has some advantages in the extraction processes, it is still necessary to develop an optimum processing to combine with other methods, and a suitable extraction system should also be considered.

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Chapter 6
Pulsed Electric Field Processing of Protein-Based Foods

Wei Zhao and Ruijin Yang

Abstract Pulsed electric field (PEF) technology has high potential and a promising future in food processing for the pasteurization of pumpable foods and the reduction of enzymatic activity. Compared with conventional thermal pasteurization methods, foods are less affected initially by PEF processing and maintain higher quality throughout the storage period. PEF processing may be a good alternative treatment to thermal methods in protein-based foods. This chapter focuses on microbial inactivation from PEF processing of protein-based foods, and the effects of PEF on the quality of these foods. Finally, ways are proposed to achieve food quality assurance and food safety in the PEF processing of protein-based foods.

Keywords Pulsed electric fields (PEF) · Nonthermal processing · Protein-based foods · Food quality and safety · Protein structure

6.1 Introduction

PEF processing is an emerging and very promising nonthermal technology for the pasteurization of pumpable foods. It can disinfect pumpable foods by rupturing microbial cell membranes with short, high-voltage pulses that inactivates endogenous enzymes at or near room temperature (Zhao et al. 2012). Compared with thermal pasteurization methods, PEF has less initial effect on the food and quality is maintained throughout the storage period (Zhao et al. 2012, 2014). Owing to this great potential and the feasibility for commercial use of the PEF processing as an alternative means of pasteurization, this technology has drawn considerable attention from scientists and the food industry.

Research into this emerging technology has been ongoing around the world for the last decade. Most of the research up until now has been at a laboratory, or a pilot plant scale, but both have shown promising results. In parallel with the development

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of PEF processing, advancements in high-voltage pulsed power systems mean that PEF processing can be scaled up from low-volume laboratory scale systems, into large and industrial production scale installations (Puértolas et al. 2010). Now, a successful transfer of processing conditions from lab to industrial scale has been achieved. At present, treatment is carried out using 80 kW modular system with an average power of up 240 kW and a treatment capacity of 10,000 L h^{-1} for microbial decontamination (Toepfl 2011). The energy saving with PEF processing is another important advantage over conventional thermal treatments. Owing to the technique developments available for industrial scale processing, the total treatment costs can be estimated in a range of 1–2 € per ton of material to be treated for cell disintegration, or 0.01– 0.02 € per liter of liquid media preservation (Toepfl 2011).

A large number of studies have fully demonstrated that small molecular compounds in plant-based foods, mainly aromatic compounds and health-related phytochemicals, are not significantly affected by PEF treatment (Odriozola-Serrano et al. 2013). However, there is a lack of knowledge on the effects of PEF treatment on biological molecules such as proteins. Protein-based products, including functional foods containing peptides and bioactive proteins, are potential application targets for PEF processing. This technology could effectively inactivate most endogenous enzymes in foods (van Loey et al. 2002) and similarly, it could also influence proteins. This review focuses on PEF processing of protein-based foods and the effects of PEF on the protein quality.

6.2 Applications of PEF Processing on Protein-Based Foods

PEF processing has been applied to inactivate pathogenic and spoilage microorganisms in a variety of protein-based foods including liquid egg, milk, soymilk, fruit juice, and other beverages where it has shown positive effects. PEF processing can extend the microbiological shelf life to 3–10 weeks for raw milk and liquid egg, and 5–8 weeks for fruit juice, soymilk, or milk in refrigerated conditions (Zhao et al. 2012, 2014). Salvia-Trujillo et al. (2011) evaluated the safety of PEF-processed fruit juice-whole milk, and fruit juice-skimmed milk beverages with an initial concentration of 10^7–10^8 CFU mL^{-1} of Listeria innocua. The results showed that for both beverages, PEF treatment at 35 kV cm^{-1} for 1800 μs achieved a 5 log reduction in the L. innocua population, in line with FDA recommendations. Over 56 days at 4 °C after PEF treatment there were no significant changes in pH, acidity, or soluble solid content values confirming the microbial stability of the beverages. Another study conducted by the same research group reported that PEF treatment at 35 kV cm^{-1} for 1400 μs could also reduce the population of L. innocua in a fruit juice-soy milk beverage by 5 log units. Sepulveda et al. (2009) compared the effects of thermal treatment (66 s at 85 °C) with PEF treatment (50 μs at 30 kV cm^{-1}) on the inactivation of bacteria, moulds, and yeasts, as well as a shelf life evaluation of an orange juice-milk-based beverage with initial bacterial, and yeast and mold counts of 5.99 and 5.43 log CFU mL^{-1}, respectively. The reductions in bacterial and yeast and mold

counts induced by PEF treatment were slightly more than those induced by thermal treatment (4.5 and 4.1 log CFU mL^{-1} for thermal against 4.5 and 5 log CFU mL^{-1} for PEF), whereas both the PEF and thermally processed samples remained stable during the entire 4-week storage period at 8–10 °C.

However, the conclusions of different research groups about the pasteurization of protein-based foods by PEF processing are inconsistent. This may be due to the different types of equipment applied across a wide range of operating conditions as well as the use of samples with different microbial contamination levels.

The bacterial resistance to PEF in liquid food depends on the target microorganism, PEF parameters, process temperature, and pH (Martín-Belloso and Sobrino-López 2011; Rodríguez-González et al. 2011; Salvia-Trujillo et al. 2011). It has also been found that the food composition, especially of proteins and fat, can cause resistance to the treatment and protect microorganisms against PEF (Salvia-Trujillo et al. 2011; Jaeger et al. 2009). The concentration-dependent effects of milk protein on PEF inactivation of *Lactobaciu rhamnosus* has been observed by Jaeger et al. (2009). Bermúdez-Aguirre et al. (2012) found that PEF was more effective against *Bacillus cereus* spores in skimmed milk than in whole milk, which indicated that lipids could protect microorganisms from the action of PEF. Compared with low pH foods such as fruit and vegetable juices, PEF processing is less effective at pasteurizing protein-based foods. Hermawan et al. (2004) and Jin et al. (2009) claimed that the reduction of pathogens in liquid whole eggs by exclusive PEF treatment was limited. PEF treatment at 25 kV cm^{-1} for 250 μs at a temperature of 20 °C could only inactivate 1 log of *Salmonella enteritidis* in liquid whole eggs (Hermawan et al. 2004). These results were confirmed in a study by Jin et al. (2009) where 1.3 logs of *S. typhimurium* in liquid whole egg were inactivated by the same PEF treatment conditions. Monfort et al. (2010) reported that the maximum inactivation levels of 1.9 log 10 cycles of the target *S. serovar* in the best-case scenario for PEF treatment of liquid whole egg were at 250 kJ kg^{-1} and 25 kV cm^{-1}. This level of inactivation indicated that PEF technology by itself cannot guarantee the security of liquid whole eggs based on United States Department of Agriculture (USDA) and European regulations. Moreover, recent studies have confirmed the occurrence of sub-lethally injured microorganisms after PEF treatment, indicating that electropermeabilization is not an "all-or-nothing" effect (Pina-Pérez et al. 2009; Zhao et al. 2011). The protective effects of proteins and lipids in food might favor the occurrence of sub-lethally injured microorganisms in PEF processing. In addition, protein-based foods such as liquid egg and milk generally possess a higher pH compared to vegetable and fruit juices, which is beneficial to the recovery of sub-lethally injured microbial cells during the storage period. These represent the challenge in PEF processing of protein-based foods.

The occurrence of cell damage due to PEF raises the possibility of designing combined processes that enable increased microbial lethality in protein-based foods (Monfort et al. 2010). Many studies have demonstrated that the application of PEF treatments combined with other preservation methods may enhance microbial inactivation in a synergistic manner through the inhibition of the repair process and recovery of the sub-lethally injured microorganisms. Among these additional hur-

dles to microbial recovery, combinations involving antimicrobial compounds and mild thermal treatments were of particular interest due to their broad antimicrobial effects and ease of commercial implementation (Martín-Belloso and Sobrino-López 2011). Gallo et al. (2007) demonstrated that the addition of nisin prior to PEF treatment exhibited an additive effect on the inactivation of *L. innocua* in whey. Sobrino-López et al. (2009) reported that a mixture of 1 IU mL^{-1} of nisin and 300 IU mL^{-1} of lysozyme in milk processed with PEF for 1 200 μs at 35 kV cm^{-1} extended the shelf life of milk for 7 days in refrigerated conditions. Walkling-Ribeiro et al. (2009) investigated the effects of a combined treatment of preheating (at 30–50 °C) and PEF on the native microbes in milk. They found that the maximum inactivation of 6.4 log was achieved with a combination of preheating at 50 °C for 60 s, followed by PEF treatment at 40 kV cm^{-1} for 60 μs. Jin et al. (2009) reported that the effectiveness of PEF for the inactivation of pathogenic *S. typhimurium DT104* in liquid whole egg was dependent on the treatment temperature and pH. Increasing the treatment temperature from 20 to 40 °C at neutral pH could induce an additional reduction of 0.8 log. Guerrero-Beltrán et al. (2010) reported on the feasibility of treating milk with PEF and electrically induced heat. They found that PEF processing at temperatures over 53 °C could be an effective treatment for the inactivation of *L. innocua*. The same research team (Sepulveda et al. 2009) observed that milk heated by PEF-generated heat for 10 s at 65 °C, and which received PEF treatment at 35 kV cm^{-1} for 11.5 μs, lasted for up to 24 days in refrigerated conditions. Reduction of the population of *S. enteritidis* in milk by more than 9 log was achieved using PEF treatment (25 kV cm^{-1} and 75–100 kJ kg^{-1}) followed by heat treatment (52 °C/3.5′, 55 °C/2.0′, or 60 °C/1.0′) (Monfort et al. 2012). In particular *B. cereus* spores, which have high heat-resistance, could be inactivated (3.6 log reduction) in milk by nisin (50 IU mL^{-1}) combined with PEF treatment (40 kV cm^{-1}, 360 μs, 65 °C), indicating the advantages of synergistic applications of PEF and other techniques. Recently novel combinations of PEF with other emerging physical technology such as manothermosonication and microfiltration (Walkling-Ribeiro et al. 2009) have been proposed to achieve food safety and quality. The bactericidal action resulting from the use of combined treatments allows a significant reduction in their individual intensities while maintaining microbial acceptance.

6.3 Effects of PEF Processing on the Quality of Protein-Based Foods

It has been demonstrated that PEF processing has less initial effect on the quality of plant-based foods, and that this is maintained at a higher level throughout the storage period, compared to conventional thermal methods (Odriozola-Serrano et al. 2013). Many studies have been conducted to compare the effects of PEF and thermal treatments on the quality of protein-based foods. The effects of PEF processing were compared to traditional heat pasteurization (66 °C for 4.5 min) on the quality

of liquid whole eggs (Marco-Molés et al. 2011). The results show that the color, microstructure, and lipoprotein matrix appeared to be less affected by PEF treatment than heat treatment. Additionally, compared with PEF processing, heat pasteurization had a significant impact on the water-soluble protein contents of the liquid whole egg samples (19.5%–23.6% decrease), and the mechanical properties of the egg gels (increases up to 21.3% and 14.5% in hardness and cohesiveness, respectively). Yu et al. (2009) determined the coagulation properties of PEF-treated milk compared with raw milk and thermally pasteurized milk, the results showed that PEF-treated milk exhibited better rennet ability in comparison to thermally pasteurized milk. The recent study conducted by Monfort et al. (2012) showed that PEF processing (25 kV cm^{-1} and 75–100 kJ kg^{-1}) followed by heat treatment at 52, 55, and 60 °C exhibited stronger capacity for microbial inactivation, and had less effect on the soluble protein contents of liquid whole egg than heat pasteurization at 60 and 64 °C.

From these investigations, it can be concluded that compared with thermal pasteurization, PEF processing has less effect on the quality of protein-based foods. However, enzymes, as special proteins with catalytic activities, could be inactivated by some PEF processing conditions. In the same way, PEF processing could induce changes in the structure and function of food protein components, affecting quality of the treated protein-based foods.

Many studies have looked at changes in the stability of food, and the functions of food proteins, after PEF treatment. Marco-Molés et al. (2011) reported on the changes in stability and function of liquid whole egg treated by PEF at 19 kV cm^{-1} for 30 μs, 32 kV cm^{-1} for 30 μs, and 37 kV cm^{-1} for 18 μs with a pulse width of 6 μs and pulse repetition frequency of 250 Hz. This processing applied to non-homogenized samples induced an increase in viscosity and a decrease in foaming ability. The higher the applied electric field strength, the poorer the foaming capacity. The foaming capacity of homogenized liquid whole egg decreased by approximately 50% after PEF treatment at 37 kV cm^{-1}. When high-intensity (37 kV cm^{-1}) PEF was applied, the protein granules appeared to be more deformed and degraded, and phase separation occurred in the PEF-treated samples at 48 h after treatment. The results indicated that the PEF affected the lipoprotein network, and destabilized the colloidal emulsions.

6.4 Effects of PEF Processing on the Structure of Proteins

Compared with aromatic compounds and health-related phytochemicals in foods, protein always possesses larger size and molecule weight, which causes its sensitivity to processing conditions. The response of protein to physicochemical stresses and the related effects on protein functionality are shown in Fig. 6.1. Food proteins are potentially subjected to several physicochemical stresses such as thermal, chemical, pressure, and mechanical stresses during processing. Under the physicochemical stresses, unfolding of the protein structure occurs, affecting the spatial structures. It also influences biological activity such as the catalytic activity of enzymes, and the

physical and nutritional properties of bioactive proteins, as well as thermal properties such as enthalpy and thermal stability (Budi et al. 2005). The movement of amino acid residues in the protein molecules caused by the unfolding of the protein structures could influence changes in distribution, hydrophobicity, and hydrophilicity (Kinsella et al. 1994). Some physicochemical stresses could directly affect ionization, and restrict the inherent molecular flexibility. These changes in the physicochemical properties of protein ultimately influence the water holding capacity, emulsification, and foaming properties of protein-based foods (Kinsella et al. 1994). If the applied physicochemical stresses are strong enough, protein aggregation could occur upon the interaction of the changed proteins, and soluble and insoluble protein aggregates would be formed. This greatly influences particle size, solubility, and ultimately relates to the rheological properties, stability, and texture of the food (Kinsella et al. 1994).

The application of PEF may be a potential alternative to thermal processing in the protein-based foods, because while protein-based foods are less affected by the PEF processing than by thermal pasteurization, two treatments did achieve similar microbial inactivation (Marco-Molés et al. 2011; Monfort et al. 2012). However, researchers also have studied the effects of PEF on the quality of proteins in the treated protein-based products. From a theoretical perspective, it seems reasonable that electric field stress could ionize some chemical groups and disturb the electrostatic interactions inside the polypeptide chain of the protein, and decompose the secondary structures due to the interactions between the electric field and dipole moments of peptides (Wada 1976).

Under the PEF processing, the unfolding of protein structures occurs, accompanied by changes in the secondary structure, and polarization of the dipole moments of some bonds (Liu et al. 2009; Li et al. 2007). This further affects thermal properties such as enthalpy and thermal stability (Liu et al. 2009). Subsequently, the physicochemical properties of protein, including charge distribution, hydrophobicity, and hydrophilicity (Li et al. 2007; Zhao et al. 2007), ultimately influence the water holding capacity, emulsification, and foaming properties of the protein-based food (Marco-Molés et al. 2011; Zhao et al. 2007). Protein aggregation was also observed, affecting the solubility, rheological properties, stability, and microstructure of the food (Xiang et al. 2011a, b; Yu et al. 2009; Monfort et al. 2012; Marco-Molés et al. 2011; Zhao et al. 2009). Currently, the mechanisms of PEF treatment on protein are still not totally understood. On the basis of the published studies on the action of PEF on enzymes and food component proteins (Budi et al. 2007; Castro et al. 2001; Li et al. 2007; Liu et al. 2009; Xiang et al. 2011a, b; Zhao and Yang 2008; Zhao et al. 2012; Zhong et al. 2007), a tentative model to explain the action of PEF on proteins can be proposed (Fig. 6.2). Polypeptide chains in the protein possess a strong dipole moment that has the potential to be affected by electric fields, and the local electrostatic fields and electric interactions of the polypeptide chains in the proteins could also be disrupted by an external electric field. Under PEF treatment, the movement of free electrons, ions, and other charged particles; polarization, i.e., the displacement of bound charges, electrons in atoms, atoms in molecules; the orientation of molecular dipole moments; and an increase in the dielectric constants of

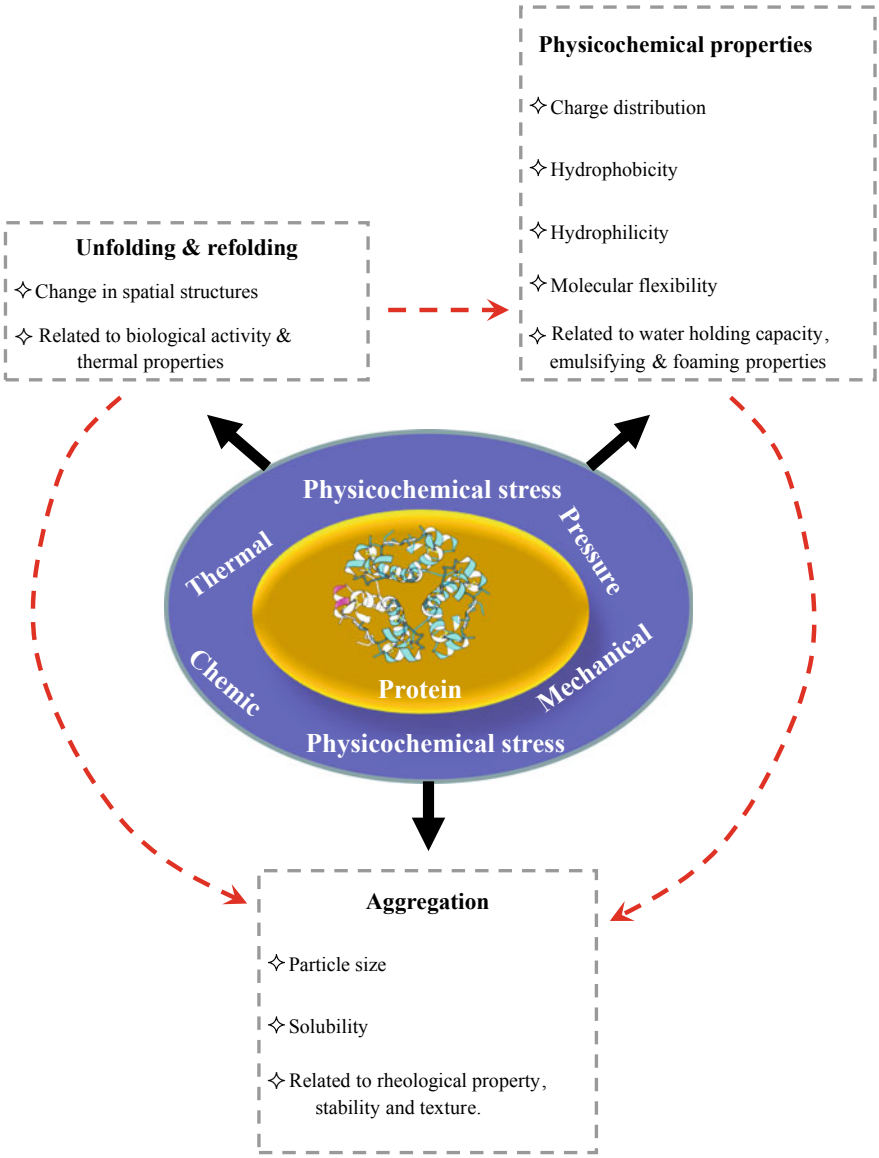

Fig. 6.1 The response of protein to physicochemical stress and the related effects on protein functionality

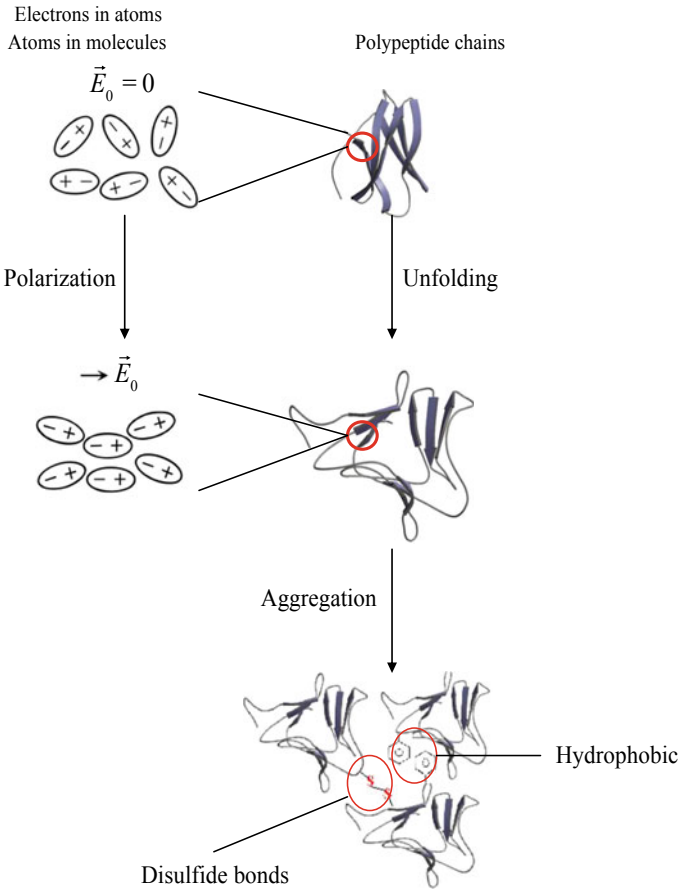

Fig. 6.2 Schematic diagram of the action of PEF on protein

molecules occur. The PEF induces an increase in the dielectric constant of proteins causing unfolding polypeptide. Afterwards, the secondary and tertiary structures of proteins become looser and less defined. Dissociation of non-covalently linked protein subunits involved in the quaternary structure may also occur. Simultaneously, more hydrophobic and thiol groups are exposed to form protein aggregation. These conformational changes are associated with the conformational change of the active site, the inhibition of the binding of substrate to protein, and the destabilization of the protein structure, which ultimately changes the protein's functions.

6.5 Future Perspectives of PEF Studies in Protein-Based Foods

Compared with fruit and vegetable juices, more severe PEF conditions are required for substantial microbial reductions in protein-based foods. This could induce changes in the qualities of the protein in the treated protein-based food. It is essential for PEF processing of protein-based foods to increase microbial inactivation while retaining heat sensitive components. Future research is required to identify a method to achieve food quality assurance and food safety in PEF-treated protein-based foods. On the other hand, the effectiveness of PEF treatment of protein-based foods can be improved by combining it with other techniques that may capitalize on the cellular damage caused by PEF. PEF can be used in food preservation as a single preservative technology or in combination with other processing methods such as the addition of antimicrobial substances, moderate heat, and pH. PEF stresses microbial cells and makes them more sensitive to other techniques. The strength of applied PEF could be reduced for the retention of quality of protein-based foods.

6.6 Summary

The developments of PEF equipment and applied technology are making PEF performance scalable, from low volume, laboratory scale systems, to large industrial production scale installations. Application of PEF processing may be a good alternative to thermal methods in the protein-based foods, but a better understanding of the actions of PEF on protein would allow industry and consumers to better understand and evaluate the potential of PEF technology as an alternative or complement to traditional methods for food preservation.

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Chapter 7
Research Progress on Power Ultrasound Technology

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Abstract In recent years, power ultrasound (PU) has attracted considerable interest in food science and technology due to its promising applications in food processing and preservation. It is well known to have significant effects on the rate of various processes in the food industry, and it is recognized as innovative technology for achieving the objective of sustainable "green" chemistry and industrialization. Using ultrasound, food processes can be completed in seconds or minutes with higher reproducibility, lower processing costs, easier operation, higher product purity, elimination of post-treatment of waste water, and lower energy requirements than traditional processes. Several processes such as extraction, degradation, sterilization, and enzyme modification have been applied efficiently in the food industry. Food processes performed under ultrasound treatment will be affected by cavitation phenomena and mass transfer processes. This chapter presents the current knowledge on the applications of ultrasound in food processing.

Keywords Ultrasound · Food processing · Cavitation · Extraction · Degradation · Sterilization · Enzyme

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7.1 Introduction

Sound waves with a frequency that exceeds the upper limit of human hearing (~20 kHz) are classified as ultrasound, and they can be divided into different frequency ranges. Until recently, most applications of ultrasound in food technology involved a nondestructive analysis that is particularly useful for quality assessments; such applications use high-frequency ultrasound (100 kHz–1 MHz) with low power (typically <1 W cm^{-2}). On the other hand, the power levels used in low frequency (20–100 kHz) applications are so high (typically in the range of 10–1000 W cm^{-2}) that they have ability to change the physical or chemical properties of food (McClements 1995). This kind of ultrasound is usually referred to as power ultrasound (PU).

In recent years, PU has attracted considerable interest in food science and technology owing to its promising effects for food processing and preservation. It is recognized as an innovate technology for achieving sustainable "green" chemistry and industrialization (Chemat et al. 2017). As an innovative food technology, PU can be applied to develop gentle but targeted processes to improve the quality and safety of processed foods, and to offer the potential to improve existing processes, as well as develop new processing options (Knorr et al. 2004).

7.2 Principles of Power Ultrasound in Food Processing

The effects of ultrasound on liquid systems are mainly related to the phenomenon of cavitation (Fig. 7.1). Ultrasound is propagated via a series of compression and rarefaction waves induced on the molecules of the medium it passes through (Mason et al. 2005). At a sufficiently high power, the rarefaction cycle may exceed the attractive forces of the liquid molecules and cavitation bubbles from gas nuclei will emerge within the liquid. These bubbles, distributed throughout the liquid, grow over the period of a few cycles to a critical size until they become unstable and violently collapse (Barbosa-Canovas and Rodriguez 2002). For food processing purposes, it is important to address the generation of heat due to ultrasound and the related implosion of cavitation bubbles that can cause rapid changes in temperature, up to 5500 °C, and pressure, up to 50 Mpa, which produces extremely high-shear energy waves and turbulence in the cavitation zone. These combined effects have a variety of applications (Knorr et al. 2004). The extent of cavitation is determined by many factors, including ultrasound intensity (W cm^{-2}), medium viscosity, surface tension, vapor pressure, nature and concentration of dissolved gases, the presence of solid particles, temperature, and pressure of the treatment (Soria and Villamiel 2010). When liquid processing is designed for large-scale systems, the ultrasonic density (W cm^{-3}) should be considered (Patist and Bates 2008).

Another phenomenon, resulting from the variations in bubble size and subsequent collapse, is the development of strong microstreaming currents, associated

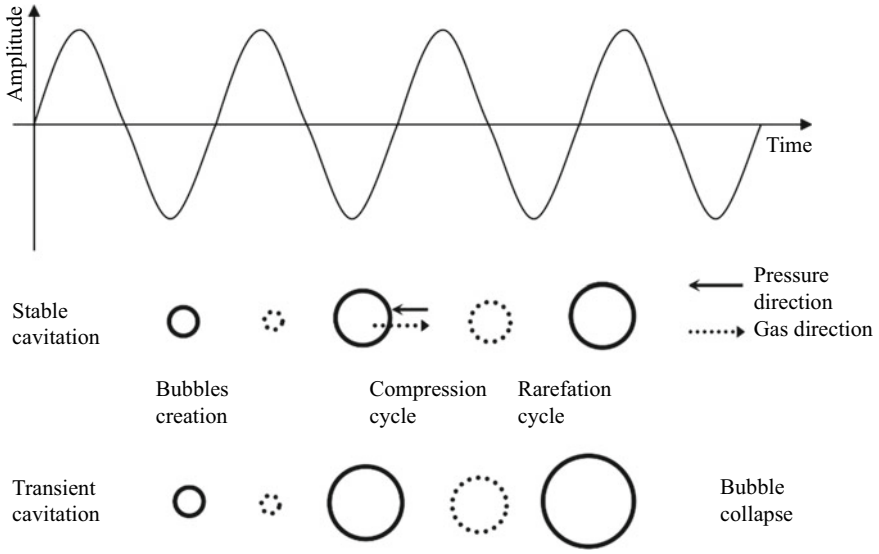

Fig. 7.1 Cavitation effects of ultrasound

with high-velocity gradients and shear stresses that alter the characteristics of the media. Moreover, part of the acoustic energy can be absorbed as heat. However, depending on the operating conditions and substrates, the temperatures are usually lower than 70 °C (Villamiel et al. 2000). Another important effect is that water molecules can be converted into highly reactive-free radicals ($H_2O \rightarrow H + OH$) that can react with other molecules (Riesz and Kondo 1992). These mechanisms may induce physical and chemical effects with potential applications in the food industry.

7.3 Applications of Power Ultrasound in Food Processing

Power ultrasound has been used as an alternative to conventional food processing operations for sonocrystallization, emulsification, defoaming, modification of the functional properties of food proteins, inactivation or enhancement of enzymatic activity for improvement of shelf life and quality, microbial inactivation, freezing, thawing, freeze drying and concentration, and drying, as well as facilitating the extraction of various foods and bioactive components (Awad et al. 2012).

7.3.1 Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) is an emerging technology that can facilitate the release of extractable compounds and enhance mass transport by disrupting plant cell walls and other cells more easily. UAE is a "green" method that avoids the use of large quantities of organic solvents and reduces the working time. Several food components such as aromas, pigments, antioxidants, and other organic and mineral compounds have been extracted efficiently from a variety of matrices (mainly animal tissues, and plant materials) (Vilkhu et al. 2008). The UAE process can be completed in a few minutes with high reproducibility, and it reduces the consumption of solvent, simplifies manipulation, and produces higher purity products compared with conventional extraction techniques such as Soxhlet extraction, maceration, and Clevenger distillation.

7.3.1.1 Mechanisms of UAE

During the UAE process, the cavitation phenomena cause high-shear forces in the media. The implosion of cavitation bubbles on the product's surface results in micro-jetting, which can generate effects such as surface peeling, erosion, and particle breakdown. Additionally, implosion of cavitation bubbles within a liquid medium leads to macro-turbulences and micro-mixing. To understand the effects of ultrasound on a vegetal matrix during UAE, experimental results show that there are several different combined mechanisms that must be understood, such as fragmentation, erosion, capillarity, detexturation, and sonoporation.

During the application of ultrasound to a liquid media containing raw materials, rapid fragmentation of the raw materials is observed. The impact of fragmentation induced by ultrasound was illustrated throughout the example of chlorophyll extraction from spinach leaves (Chemat et al. 2017). The effects were examined using an ultrasound probe (20 kHz, UIP1000 HdT, Hielscher). During the processing, the quick fragmentation of the spinach leaves happened in the first minutes of sonication, whereas the leaves did not change during conventional extraction performed by maceration. Comparing the extraction rates of chlorophyll from UAE and maceration, a linear increase was observed at the beginning of UAE, corresponding to the direct solubilization of chlorophylls. The effects were most probably due to the reductions in particle size during the application of ultrasound. Fragmentation of friable solids caused from ultrasonic cavitation has been identified by several authors (Suslick and Price 1999). The fragmentation process was resulted from interparticle collisions, and shockwaves through collapsing cavitation bubbles in the liquid. A direct consequence of the reductions in particle size was the increase in the surface area of the solid, resulting in greater mass transfer and higher extraction rates and yields.

Some studies have already noticed the erosion of raw plant materials when treated by ultrasound. For example, the UAE of Boldo leaves was studied by Petigny et al. (2013) using an ultrasonic probe (20 kHz). The results showed that the extraction

yields were enhanced by 25% with UAE treatments. From scanning electron microscope (SEM) observations on the leaf surfaces before and after UAE treatments, the results showed that leaves were not fragmented, but localized effects were observed. Boldo leaves possess trichomes on the surface of leaves, which seemed to be specifically impacted by ultrasound. This erosion enhanced the ability of water to access the leaf for further extraction. Another possible mechanism for extraction enhancement could be the implosion of cavitation bubbles on the leaf surface to induce the erosion of plant structures, and to speed up the release of target components from the extraction medium. The erosion could be used for several purposes such as cleaning or sonochemical reactions with metals (Chemat et al. 2017).

Ultrasonic capillary effect (UCE) refers to an increase in the depth and velocity by which a liquid can penetrate into canals and pores under sonication (Mason 2015). These ultrasounds induced effects were experimentally demonstrated with molten aluminum by Tzanakis et al. (2015). Although the mechanism of UCE is not fully understood, a relationship between cavitation and UCE has been established. UCE could also be one of the mechanisms that increases extraction yields. In the study by Pingret et al. (2012), the recovery of total polyphenols from apple pomaces was performed using ultrasound and the extraction kinetics improved under sonication. The impact of UCE on extraction was proposed by Vinatoru (2001). The author identified that the swelling index of several vegetable matrixes increased from 5% to 10% when using ultrasound. By increasing the swelling and rehydration of vegetable tissue, ultrasound impacts positively on the extraction, desorption, and diffusion of a solute out of the vegetable structure. UCE can directly improve the mass transfer process. The improvement in the water holding capacity of the matrix under ultrasound applied to meat brining or curing has also been investigated by several authors (Carcel et al. 2007; McDonnell et al. 2014; Ozuna et al. 2013; Siro et al. 2009; Stadnik et al. 2008). They reported the modification of meat structures after ultrasound treatment as well as an enhancement of salt and moisture transfer processing. The ultrasonic intensity is considered to be an important factor with great influence on the results (e.g., between 40 and 50 W cm^{-2} is optimal for the ultrasound-assisted brining of pork loins) (Carcel et al. 2007).

The sonoporation effect of ultrasound is well known in the field of biology, and it is applied when permeability of cell membranes is desired. Sonoporation has been used in vitro for uptake of molecules by cells, e.g., drugs, genes (reversible sonoporation), or for cell destruction (irreversible sonoporation). For these cases, high ultrasound frequencies greater than 500 kHz were applied (Karshafian et al. 2009). However, a few studies have focused on the use of low frequencies (20 kHz) for cell wall permeabilization (Miller et al. 2002), or bacteria inactivation (Ugarte-Romero et al. 2006). In the field of extractions, sonoporation can be used to create reversible or irreversible cell membrane pores, which result in the release of the cellular contents into the extractive medium. The study performed by Meullemiestre et al. (2016) on the processing of wet yeast (*Yarrowia lipolityca*) for the recovery of oil in yeast cells, used ultrasound at 20 kHz. The ultrasound-treated yeasts exhibited a high impact on the surface and perforations of the visible membranes.

In an ultrasound-treated solid–liquid mixture, shear forces were generated within the liquid and at the vicinity of the solid materials, resulting from the evolution (oscillation and collapse) of cavitation bubbles within the fluid. The streaming and acoustic microstreaming effects are of interest for mixing and emulsification applications (Veillet et al. 2010). In the case of leaves, the oil glands would explode after ultrasound irradiation. It could be hypothesized that the shear forces generated by the collapse of cavitation bubbles close to the oil glands, is what caused them rupture. Another hypothesis may be that there was a pressure build-up within the glands, or cavitation inside of the glands (Vinatoru et al. 1997).

Ultrasound extraction causes the destruction or detexturation of plant structures, which has been observed when obtaining essential oil from caraway seeds (Chemat et al. 2004). The total oil yields of conventional extraction (reflux extraction with hexane) and UAE (ultrasound probe, 20 kHz) were similar; however, a higher selectivity towards terpenes was noted for UAE. Distinguishable physical changes to the caraways seeds were noticed following the extraction process. After ultrasound, the gradual degradation of cell walls was observed: after 30 min, the cell walls were affected by varying degrees; by 60 min, the cell structures were totally broken and converted to undefined shapes. It can be assumed that this cell disruption improves the accessibility of the solvents (Chemat et al. 2004).

In summary, several mechanisms of UAE have been proposed, such as fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress, destruction, and detexturation of plant structures. However, during UAE treatments, a combination of effects is likely to occur. These effects are sequential during the extraction processes. The intense mixing effects generated by the propagation of ultrasound through a liquid medium can enhance the mass transfer rates. The mixing effects at a macroscopic scale are due to acoustic streaming, especially microstreaming occurring at a local level. The combined mixing and physical effects of ultrasound on the raw materials may have applications that can enhance the performance of UAE.

Ultrasound can facilitate extraction from dried materials using a two-stage process: (i) vegetal materials are steeped in solvents to facilitate the swelling and hydration processes; (ii) diffusion and osmotic processes drive the mass transfer of soluble constituents from the materials into the solvents. Toma et al. (2001) monitored the effects of ultrasound on crushed fennel seeds, hop strobiles, pot marigold, peganum seeds, mint leaves, lime flowers, and elecampane root in terms of the swelling index (SI) and extractive value (EV) in the ultrasonic bath (33 kHz), as measured using a microscope. The data shows that all of the sonicated tissues absorbed extra water, and the EV of many species increased with a short sonication time. This indicates that low frequency indirect sonication has a significant influence on the swelling process of dried plants. Microscopic examination of vegetal materials revealed that sonication can significantly affect the structures of vegetal tissues. Xu et al. (2014) investigated the effects of ultrasound on the yield and swelling behavior of pectin extraction from grapefruit peel (*Citrus paradisi* Macf. cv. *Changshanhuyou*). The results showed that the SI presented similar trends to the pectin yields. The results of these studies provide strong evidence to support that a mechanism of UAE is a hydration process, which occurs simultaneously with vegetal material fragmenta-

tion. Petrovic et al. (2012) analyzed the kinetics and mass transfer phenomena for different extraction processes from thyme leaves (*Thymus vulgaris* L.) using ethanol. These processes included Soxhlet extraction and ultrasound-assisted batch extraction at the laboratory scale, as well as a pilot plant batch extraction with mixing. The results show that ultrasound contributed most to the increase of mass transfer rates, increasing the rate by ten times compared to Soxhlet extraction during the period of slow extraction.

7.3.1.2 Influencing Parameters of UAE

The sonochemical effects of ultrasound in a liquid are attributed to acoustic cavitation phenomena. As ultrasound is a mechanical wave, its characteristics such as frequency, wavelength, and amplitude can affect the acoustic cavitation and extraction. In addition to the input power, the reactor design and probe shape can influence the process (Chemat et al. 2017).

- **Frequency**

Ultrasound frequency can affect the cavitation effect. The most commonly used frequencies in UAE processing are between 20 and 100 kHz. Toma et al. (2001) noticed a reduction in the physical impact on the structure of marigold petals when applying higher frequencies (500 kHz) compared to 20 kHz. Chukwumah et al. (2009) reported selective extraction of some phenolics from peanuts at frequencies of 25 kHz (higher extraction of daidzein and genistein) and 80 kHz (biochanin A and *trans*-resveratrol). However, longer extraction durations were required at 80 kHz. González-Centeno et al. (2014) evaluated three frequencies (40, 80, and 120 kHz) for the extraction of phenolics from grape pomace. Using the surface response methodology to study the influencing parameters, the authors highlighted that 40 kHz was the most effective.

As the ultrasound frequency increases, the production and intensity of cavitation in the liquid decreases. At higher frequencies, acoustic cavitation is more difficult to induce since the cavitation bubbles need a delay to be initiated during the rarefaction cycle. The cycles of compression and rarefaction might be too short to allow the growth of the cavitation bubbles. The length of the rarefaction phase (for the growth of cavitation bubbles) is inversely proportional to the ultrasonic frequency. Therefore, larger amplitudes and intensities are required to generate cavitation at high frequencies (Chemat et al. 2017).

At lower frequencies, there are fewer transient cavitation bubbles, but they have larger diameters that show the physical effects (Leong et al. 2011; Mason et al. 2011). The effects of frequency may be linked not only to the cavitation bubble size, but also to its influence on the resistance to mass transfer (Esclapez et al. 2011).

- **Intensity**

The measurement of the actual applied acoustic power in a sonochemical process has not been reported in details, although some physical methods can directly or indirectly measure the applied energy. These methods estimate the transferred energy

by measuring either chemical or physical changes in the medium when ultrasound is applied. The most common physical methods are the measurement of acoustic pressure using hydrophones or optical microscopes, the aluminum foil method, and the calorimetric method (Chivate and Pandit 1995; Margulis and Margulis 2003). Among the chemical methods, the indirect measurement of ·OH radicals formed by sonoluminescence, or chemical dosimeters have been used (Suslick et al. 2011). As an example, to calculate the power by calorimetry, the actual input power from the device is converted to heat, which is dissipated in the medium. The effective ultrasound power (P) is calculated according to Eq. 7.1 (Contamine et al. 1995; Toma et al. 2011).

$$P = m \times C_p \times \frac{dT}{dt} \tag{7.1}$$

where C_p is the heat capacity of the solvent at constant pressure (J g^{-1} C^{-1}), m is the mass of solvent (g), and dT/dt is the temperature rise per second.

The ultrasound intensity (*UI*) is expressed as the energy transmitted per second and per square meter of emitting surface (Tiwari 2015). This parameter is directly correlated to the amplitude of the transducer, and consequently to the pressure amplitude of the sound wave. As the pressure increases, the collapse of the bubbles will be more violent. To achieve the cavitation threshold, a minimum value of ultrasound intensity is required. Regarding extraction, *UI* is a relevant input value that strongly affects extraction efficiency. *UI* is calculated using the calculated power delivered to the media, as shown in Eq. 7.2 (Tiwari 2015).

$$UI = \frac{P}{S} \tag{7.2}$$

where *UI* is the ultrasonic intensity (W cm^{-2}), P is the ultrasound power (W) as calculated by Eq. (7.1), and S is the emitting surface of the transducer.

In some studies, *UI* is calculated according to Eq. 7.3 It better demonstrates that the ultrasound power transmitted into the processed system is related to volume

$$UI = \frac{P}{V} \tag{7.3}$$

where *UI* is the ultrasonic intensity (W mL^{-1}), P is the ultrasound power (W) as calculated by Eq. (7.1), and V is the volume of liquid in the system (mL).

An increase of *UI* generally results in an increase of sonochemical effects. However, it is worth noting that a high UI can lead to rapid deterioration of the ultrasonic transducer, resulting in liquid agitation instead of cavitation, and in poor transmission of the ultrasound through the liquid media. However, the amplitude should be increased when working with high viscosity liquids such as oils (Chemat et al. 2017).

The effects of *UI* were evaluated at 16.4, 20.9, and 47.6 W cm^{-2} at 20 kHz for soybean oil extraction by Li et al. (2004). The study showed increases in yields up

to 20.9 W cm^{-2}, after which no further increases were observed. A similar tendency was noted by Wang et al. (2015), whose study on the UAE of pectin at 20 kHz indicated that the UI (varied between 10.18 and 14.26 W cm^{-2}) should be subjected to an optimization, since the highest value of *UI* would not lead to the highest yields.

Xu et al. (2014) investigated effects of ultrasound and/or heating on the swelling behavior of the material, and the yield and kinetics of pectin extracted from grapefruit (*Citrus paradisi* Macf. cv. *Changshanhuyou*) peel. For ultrasound-assisted heating extraction (UAHE), the collapse of cavitation bubbles became more violent as the amplitude or power increased, since the resonant bubble size was proportional to the amplitude of the ultrasonic wave (Brotchie et al. 2009; Merouani et al. 2013). However, when the power density became higher than 0.40 W mL^{-1}, a significant decrease in pectin yields occurred ($p < 0.05$). This was probably because cavitation was reduced at high bubble volume concentrations. A cloud of cavitation bubbles produced around the probe tip could screen and reduce the energy transmission into the reaction medium; this is called the "saturation effect" (Contamine et al. 1995). An increase in interbubble impacts would increase the probability of the bubbles deformation collapse in a nonspherical manner, thus decreasing the energy efficiency of the collapse. Ultrasonic degradation of the extracted pectin would also be responsible for yield decrease because the degradation effect on pectin increases with rising ultrasound intensity (Zhang et al. 2013a).

- **Medium Parameters**

Solvent choice in UAE is driven by the solubility of the target metabolites but also by physical parameters such as the viscosity, surface tension, and vapor pressure of the solvent. These physical parameters will affect the acoustic cavitation phenomenon, and specifically, the cavitation threshold. The initiation of cavitation in a liquid requires negative pressure during the rarefaction cycle to overcome the cohesive forces between the molecules composing the liquid. The amplitude of the ultrasound should be increased when working with samples of high viscosity, because as the viscosity of the sample increases, the resistance of the sample to the movement of the ultrasonic device also increases. Therefore, a high intensity (or high amplitude) is advised in order to obtain the necessary mechanical vibrations to develop cavitation. A solvent with low vapor pressure is preferred for UAE, as the collapse of the cavitation bubble is more intense compared to solvents with high vapor pressure (Flannigan and Suslick 2010). Moreover, vapor pressure depends on the temperature of the liquid medium.

Temperature has a strong effect on the efficiency of extractions. In UAE, some authors have reported a beneficial effect of a temperature increase from 20 to 70 °C (Shirsath et al. 2012). This effect has been justified by an increase in the number of cavitation bubbles and a larger solid-solvent contact area, as well as by the enhancement of solvent diffusivity with consequent enhancement of desorption and solubility of the target compounds. However, this effect decreases when the temperature is near the solvent's boiling point. Thus, some authors have reported the beneficial effects of low temperatures (below 30 °C) (Esclapez et al. 2011; Palma and Barroso 2002; Zhang et al. 2008). It is important to optimize extraction temperatures to obtain the

highest yield of the target compounds without degradation, because this parameter can vary depending on the type of product.

The cavitation bubbles are formed from gas (vapors) dissolved in the liquid (Chemat et al. 2017). Gases dissolved in the solvents would act as nuclei for new cavitation bubbles (Leong et al. 2011). When external pressure is increased, a greater acoustic pressure is required to induce cavitation; however, once the cavitation threshold is reached under external pressure (>1 atm), a higher intensity of cavitation bubble collapse is attained than at normal pressure, hence increasing the cavitation effect (Leong et al. 2011).

The plant matrix that can be used could either be fresh (e.g., algae, yeast) or dry (e.g., herbs, oleaginous seeds). Since the extractive systems are heterogeneous complex porous media, the size of the cavitation bubbles has an effect on the efficiency of the extractions. The extraction yields may also vary due to the plant material's structure, plasticity, or compositional differences that result in different impacts from cavitation effects (Chemat et al. 2017).

- **Shape and Size of Ultrasonic Reactors**

Since ultrasound waves are reflected by solid surfaces, the shape of the reaction vessel is critical. The best choice would be a vessel with a flat bottom, such as a conical flask, in order to attain minimal reflected waves (Chemat et al. 2017). The thickness of the vessel should also be kept to the minimum to reduce attenuation. It is necessary to calculate the optimum reactor dimensions and the position of emitter in relation to the transducer to maximize the energy transferred to the medium (Sun et al. 2010). Further advances have been made by accounting for the lack of homogeneity of the pressure field in the reactor in order to optimize the process efficiency (Esclapez et al. 2010, 2011).

7.3.1.3 Applications of the UAE Process

Hammi et al. (2015) demonstrated the efficacy and absence of degradation for the UAE of antioxidants from *Zyzyphus lotus* fruit. The use of ultrasound was also proved to be a promising technology for the extraction on carotenoids from tomato byproducts (skin, seeds, and part of the pulp) (Luengo et al. 2014). Ultrasound significantly increased the extraction yield, 143% compared with conventional extraction, and did not cause any degradation of the carotenoids. Sivakumar et al. (2011) reported significant improvement (13%–100%) in the extraction yield of natural colors obtained from different plant materials. Kimbaris et al. (2006) demonstrated that the use of ultrasound for essential oil extraction from garlic reduced the degradation of thermal-sensitive molecules, compared with hydro-distillation.

Wang et al. (2016) compared the properties of pectin from grapefruit (*Citrus paradisi* Macf. cv. *Changshanhuyou*) peel extracted by ultrasound-assisted extraction (UP), and by the conventional heating extraction (CP). Results showed that UAE caused more severe degradation than heating extraction, and the degree of acetylation of UP was slightly higher than that of CP. UAE did give rise to changes to the physico-

chemical properties of UP compared with CP. The results proved that ultrasound irradiation has more significant degradation effects on the main chains, and less on the side chains, of pectin compared with thermal and acidic degradation. The UAE process with a shorter extraction time and lower temperature was supposed to protect pectin side chains from further degradation.

7.3.2 Ultrasound Degradation

7.3.2.1 Applications of Ultrasound Degradation

The physico-chemical properties of polysaccharides treated with ultrasound have been extensively investigated (Ebringerova and Hromadkova 1997; Koda et al. 2011; Wang et al. 2010). As supported by Baxter et al. (2005), the sonication of chitosan led to a decrease in the intrinsic viscosity while maintaining the acetylation degree. In a study by Cheng et al. (2010), the sonication of starch resulted in a drastic decrease in viscosity and a distinct increase in solubility. Huang et al. (2007) found that there was almost no change in the crystalline structure of ultrasound-treated corn starch granules, while the amorphous area was slightly destroyed and pores or channels were detected.

Zhang et al. (2013a) investigated the effects of ultrasound on the molecular weight and structures of the apple pectin. The results indicate that the average molecular weight of apple pectin decreased significantly after ultrasound treatment and the molecular weight of degraded products had a uniform and narrow distribution. The ultrasound intensity and temperature play an important role in the degradation reaction. A degradation kinetics model of apple pectin fitted to the second-order kinetics model from 5 to 45 °C. The degree of methylation of the apple pectin reduced when ultrasound was applied. Ultrasound treatment could not alter the primary structure of apple pectin according to the results determined by high-performance liquid chromatography (HPLC), IR, and nuclear magnetic resonance (NMR). Meanwhile, the viscosity of untreated apple pectin was 10^3 times larger than the ultrasound-treated apple pectin, which showed predominantly viscous responses ($G' < G''$) over the same frequency range. These results suggest that ultrasound can provide a viable alternative method for the modification of pectin (Zhang et al. 2013a, b, 2015).

González-Centeno (2014) studied the effects of ultrasound on the molecular weight, structure, and antioxidant potential of a fucoidan found in *Isostichopus badionotus*. The antioxidant activity assay showed that the antioxidant activity of ultrasound-treated fucoindan was slightly improved. Therefore, ultrasound is considered to have a unique effect on the degradation and modification of polysaccharides by decreasing the molecular weight while improving their bioactivities (Zhou et al. 2008).

7.3.2.2 Combined Technologies

Although recently reported studies have shown that sonochemical degradation of various polymers and organic pollutants can be achieved, sonolysis has drawbacks including energy consumption and a longer reaction time. Further, they are still not used at an industrial scale owing to economic viability (Rayaroth et al. 2016). In this case, ultrasound is often used in conjunction with conventional techniques, such as biological, UV, Fenton, ozonation, and electrochemical methods, in order to increase the effectiveness and reduce chemical or energy usage.

Yue et al. (2008) investigated the effects of ozone in combination with ultrasonic and UV irradiation on the degradation of chitosan, and reported that the use of ozone in 35, 50, and (65 ± 5) mg min^{-1} doses, and ultrasound treatment at 550 W and 40 kHz resulted in the reduction of the viscosity-average molecular weight by 31.18%, 37.03%, 41.38%, and 12.74%, respectively. The combined operation resulted in a significant reduction by 90.25%, 95.52%, and 96.82%, respectively.

Prajapat and Gogate (2015) studied the degradation of guar gum using ultrasound, UV, and ozone. The use of UV + H_2O_2 (0.1% loading) resulted in approximately 99.1% degradation in 20 min. Whereas, UV + KPS (0.1% loading), ultrasound + O_3 (100 mg h^{-1}), and UV + O_3 (100 mg h^{-1}) resulted in the reduction of intrinsic viscosity by approximately 98.3%, 99.1%, and 98.3% in 90, 30, and 35 min, respectively. In scaled up studies (7 L capacity), a maximum degradation of 98.2% was achieved in 150 min using an ultrasound bath + UV + H_2O_2 (0.03% loading). The structure of the treated guar gum was also characterized by Fourier transform infrared spectroscopy (FTIR) and it was established there were no significant changes to the chemical structure as a result of the treatment.

Zhi et al. (2017) applied a combination of ultrasound and a Fenton system (US-Fenton) to pectin degradation. This combination significantly accelerated the degradation process and also greatly improved the degradation efficiency, as demonstrated by the appearance of much smaller (5.2 kDa) products within 60 min. The ultrasound accelerated Fenton process degrades pectin by functioning as a catalyst for the generation of free radicals. The RG-I domain, the most elective portion of natural pectin, was well preserved and highly enriched.

7.3.3 Ultrasound Sterilization

Ultrasound alone is not very effective at killing bacteria in food (Piyasena et al. 2003); however, the use of ultrasound coupled with pressure and/or heat is promising. Thermosonic (heat plus sonication), manosonic (pressure plus sonication), and manothermosonic (heat and pressure plus sonication) treatments are some of the proposed methods, as they are more energy-efficient in the reduction of microbial and enzymatic activity in comparison to conventional heat treatment (Demirdoeven and Baysal 2009; Piyasena et al. 2003).

7.3.3.1 Mechanisms of Microorganism Inactivation

A frequency of 20 kHz has been used, which is available commercially, and this has proved quite satisfactory in microbial inactivation. The variable parameters are temperature, treatment time, and acoustic power (Demirdoeven and Baysal 2009), while the effects of ultrasound in liquid media depends on many variables, such as the characteristics of the treatment medium (viscosity, surface tension, vapor pressure, nature and concentration of dissolved gases, and the presence of solid particles), treatment parameters (pressure, and temperature), ultrasound generator performance (frequency, and power input), and size and geometry of the treatment vessel (Berlan and Mason 1992). The resistance spores, Gram-positive, and coccal cells to ultrasound treatment is higher than for vegetative cells, Gram-negative, and rod-shaped bacteria (Feng et al. 2008). It has also been shown that the mortality rate varies among different strains, for example, *Escherichia coli* and *Saccharomyces cerevisiae*, were reduced by more than 99% after ultrasonication, whereas *Lactobacillus acidophilus* was reduced by 72% and 84% depending on the media used (Cameron et al. 2008).

When ultrasound is applied to a liquid or slurry, it achieves both physical and chemical effects. This occurs through the formation and collapse (cavitation) of high-energy micro-bubbles (Krefting et al. 2004; Leighton 2007; Maisonhaute et al. 2002). Research has been conducted to understand the mechanism played by ultrasound on the disruption of microorganisms, which has been explained by acoustic cavitation and its physical, mechanical, and chemical effects that inactivate bacteria, and de-agglomerate bacterial clusters or flocs (Joyce et al. 2003). The cause of microbial death is mainly thinning of cell membranes, localized heating, and the production of free radicals (Butz and Tauscher 2002).

- **Physical Effects of Ultrasound**

During the ultrasound process, longitudinal waves are created where a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion (Sala Trepat 1995). These regions of pressure change cause cavitation to occur, and gas bubbles are formed in the medium. At the point where cavitation occurs the condensed molecules collide violently, creating shock waves (Piyasena et al. 2003) which create regions of very high temperature and pressure in very short periods of time; the order of temperature variation is 109 °C s^{-1} (Demirdoeven and Baysal 2009). The high-shear energy wave can travel at 570 km h^{-1} at the surface of solid boundaries. The hot zones can kill some bacteria, but they are localized and do not affect large enough areas (Piyasena et al. 2003).

- **Chemical Effects of Ultrasound**

The lethal effect of ultrasound on some microorganisms was first demonstrated by Kaloyereas (1955); thus, ultrasound has been proposed as a means of sterilization for liquid food (Pagan et al. 1999a). Most applications will use a combination of ultrasound and other preservation methods (Raso et al. 1998a, b).

Ultrasound is known to disrupt biological structures, and potentially cause cell death when applied with sufficient intensity and these bactericidal are attributed

to intracellular cavitation. That is, micromechanical shocks disrupt structural and functional components of the cells up to the point of cell lysis (Hughes and Nyborg 1962; Lopez-Malo et al. 2005; Williams et al. 1970).

Li et al. (2016) investigated the ultrasound-induced damage to *Escherichia coli* and *Staphylococcus aureus*. The results show that the damage was independent of the initial bacterial concentrations, while the mechanism of cellular damage differed according to the bacterial species. For the Gram-negative bacterium *E. coli*, ultrasound worked first on the outer membrane rather than the cytoplasmic membrane.

7.3.3.2 Combined Technologies

Research activities now focus on the combination of ultrasound with other preservation processes (e.g., heat and mild pressure) which appear to have the greatest potential for industrial applications (Butz and Tauscher 2002), such as reduction in processing times and increased efficiency at the industrial level.

- **Thermosonication**

The combination of ultrasound and heat treatment is able to reduce the operating requirements (e.g., temperature levels and processing times) while achieving a microbial inactivation similar to conventional heat treatments (Villamiel et al. 1999). Reduction of the temperature and processing time should result in improved food quality. These combined treatments have been reported to lower the maximum processing temperatures by 25%–50% (Demirdoeven and Baysal 2009).

The heat resistance of *Bacillus cereus, Bacillus licheniformis*, and thermoduric streptococci decreased following ultrasonication treatment at 20 kHz (Betts et al. 1999; Burgos et al. 1972; Garcia-Graells et al. 1998; Piyasena et al. 2003). The food properties, such as shelf life and surface color in orange juice, could be improved (Zenker et al. 2003), while organoleptic characteristics could also be enhanced (Lopez-Malo et al. 2005). The effects on microbial destruction, of the combined treatment in a continuous process, were demonstrated by the comparison of the integrated time-temperature intensity (F value) of each treatment (Demirdoeven and Baysal 2009). The continuous (24 kHz, 400 W, 120 μm) and pulsing ultrasound treatments at 60 °C over 10 min were applied to different juices (i.e., pineapple, cranberry, and grape) (Bermudez-Aguirre and Barbosa-Canovas 2012).

During thermosonication the heat contributes to the mechanical disruption of cells, making them more susceptible to cavitation (Chandrapala et al. 2012); however, some studies (Lopez-Malo et al. 1999; Raso et al. 1998b) have shown that the effectiveness of the cavitation phenomena could decrease with increasing temperatures. At high temperatures, vapor pressure is higher and the viscosity is lower, reducing the energy release during the bubble implosion (Guerrero et al. 2001). Herceg et al. (2012) applied ultrasound at different temperatures (20 and 60 °C), and reported that the maximum inactivations of *Staphylococcus aureus* were achieved when milk was treated in a thermosonication process at 20 kHz, 600 W, 120 μm, and 60 °C for 12 min. Herceg et al. (2012) also reported that the antagonistic effect of temperature

was not detected due to the presence of solid elements in the milk suspensions, which increased the cavitation phenomena. Wordon et al. (2012) reported that the synergistic effects between ultrasound and heat could also be linked to the ability of ultrasound to produce nonlethal intracellular injuries, resulting in more vulnerable cells and increasing their disruption rates from the heat treatment. Li et al. (2017) investigated the combined effects of ultrasound and mild heat on the viability of *S. aureus* in association with cell membrane integrity, and intracellular enzyme activity. The antibacterial value of thermosonication was greater than the sum of the individual treatments; when treating suspensions containing solid particles the enhancement of the sonication effect indicates that liquid food products such as milk and juice are most suitable for thermosonication (Sango et al. 2014).

- **Manosonication**

The simultaneous application of ultrasound and external hydrostatic pressure up to 600 kPa (manosonication, MS) increases the lethality of treatment substantially (Demirdoeven and Baysal 2009). This is due to an increase in free radical production (Vercet et al. 1998) and higher bubble implosion (Whillock and Harvey 1997). It was reported that the *D*-value of *Listeria monocytogenes* in a citrate phosphate buffer decreased from 5.70 to 2.50 min when the pressure was raised from 0 to 200 kPa in combination with ultrasound at 20 kHz and 90 μm (Manas et al. 2000). Another study showed a reduction in *D*-values from 1.52 to 0.28 min in *Yersinia enterocolitica* in a citrate phosphate buffer when there was an increase in pressure from 0 to 300 kPa with ultrasound at 150 μm and 20 kHz (Raso et al. 1998b). This phenomenon can be attributed to the fact that hydrostatic pressure can enhance some effects such as sonoluminescence (emission of short bursts of light from imploding cavitation bubbles in a liquid), and free radical production (Manas et al. 2000).

The following relationships were developed with respect to amplitude on the manosonication inactivation rate (Pagan et al. 1999c):

$$\log D_{ms} = \log D_0 - 0.0091 \times (A - 62) \tag{7.4}$$

where D_{ms} is the decimal reduction time (min) for each manosonication treatment, D_0 is the control decimal reduction time (min) for a manosonication treatment at an amplitude of 62 μm, and A is the ultrasonic wave amplitude (μm). With respect to pressure on the manosonication, the inactivation rate was related to pressure as

$$\log D_{ms} = \log D_0 - 0.0026 \times p + 2.2 \times 10^{-6} \times p^2 \tag{7.5}$$

where D_0 (min) is defined at an amplitude of 117 μm, 40 °C, and ambient pressure, and p is the static relative pressure (kPa).

Above this pressure, there is a decrease in effectiveness, associated with a decrease in cavitation, because ultrasound waves are unable to overcome the combined cohesive forces of pressure and the cohesive force of the liquid molecules (Condón et al. 2004).

- **Manothermosonication**

Combinations of heat, pressure, and ultrasound can be applied to achieve a higher microbial inactivation, called manothermosonication (MTS). While in most vegetative cells the lethal effect of MTS was additive, on *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus cereus*, *Bacillus sterothermophilus*, *Saccharomyces cerevisiae*, and *Aeromonas hydrophila*, a synergistic effect was observed (Pagan et al. 1999c; Raso et al. 1998b). For example, the *D*-value of tomato pectin methylesterase (PME) at 62.5 °C was reduced, from 45 min in thermal treatments to 0.85 min by MTS (Lopez et al. 1998). The effectiveness of this treatment can be related to two mechanisms acting independently, i.e., heat treatment and manosonication (Raso et al. 1998b). The lethality of ultrasound under pressure is almost not modified by an increase in temperature unless lethal temperatures are reached (MTS treatments), in which an additive lethal effect is generally attained (Alvarez et al. 2003; Pagan et al. 1999a, b).

Application of ultrasound at 117 μm, 20 kHz, ambient temperature, and low pressure can produce a *D*-value for *L. monocytogenes* of 4.30 min. The *D*-values decreased significantly when higher temperatures were applied, compared with cases where temperature and pressure were applied alone (e.g. the *D*-value for manothermosonication was 1 min, and the *D*-value without the use of ultrasound was 2.37 min, at 55 °C and 200 kPa) (Pagan et al. 1999b). Alvarez et al. (2006) found that ultrasound at 117 μm, and 20 kHz, with pressure of 175 kPa, and temperature of 35 °C reduced the *D*-value of *Salmonella Senftenberg* in McIlvaine's citrate-phosphate buffer until 1.71 min. When the temperature was raised to 67 °C, the *D*-value decreased to 0.02 min. The same synergetic effect of temperature was also described by Lee et al. (2009). The identification of the synergistic effect of temperature on microbial inactivation is a key point for optimizing this technology (Sango et al. 2014).

7.3.3.3 Applications of Ultrasound Sterilization in the Food Industry

Ultrasound is a nonthermal technology which contributes to the increase of microbial safety and prolongs shelf life, especially in food with heat-sensitive, nutritional, sensory, and functional characteristics.

- **Fruit and Vegetable Industry**

For decontamination lettuce, spinach, shredded carrot, truffles, cherry tomatoes, and strawberries, high-power ultrasound with low frequencies between 20 and 45 kHz, and short treatment times of 1–10 min were generally applied. For different applications and combinations of the parameters (such as power, frequency, temperature, and time), the microbial reduction with ultrasound varies between 0.5 and 1.98 log CFU g^{-1} (Alegria et al. 2009; Alexandre et al. 2012, 2013; Brilhante Sã José et al. 2012; Cao et al. 2010; Chen and Zhu 2011; Elizaquivel et al. 2012; Huang et al. 2006; Sagong et al. 2011; Rivera et al. 2011; Zhou et al. 2009). The single and combined effects of ultrasound with some chemicals such as organic acids, acidified sodium

chloride, ethanol, chlorine dioxide, and peracetic acid on microbial inactivation in some fruit and vegetables were extensively studied (Brilhante Sao Jose and Dantas Vanetti 2012; Huang et al. 2006; Sagong et al. 2011; Susana Rivera et al. 2011; Zhou et al. 2009).

- **Meat Industry**

High power ultrasound is a potential tool for the reduction of microorganisms in poultry (Haughton et al. 2012; Kordowska-Wiater and Stasiak 2011; Loretz et al. 2010), pork, and other meat (Birk and Knochel 2009; Morild et al. 2011). Several researchers have demonstrated that the antimicrobial effects of ultrasound are enhanced when used in combination with other decontamination/preservation techniques such as hypochlorite, mild heat, pressure, steam, or organic acid (Arroyo et al. 2011; Bilek and Turantas 2013; Piyasena et al. 2003).

Ultrasound has been used in beef, chicken, and pork, however, the combination of parameters (frequencies and treatment times between 24 and 45 kHz and 2–120 min, respectively) is applied for improving the general quality of the meat (Dickens et al. 1991; Leal-Ramos et al. 2011; Ozuna et al. 2013; Xiong et al. 2012). High power ultrasound, with low frequencies and treatment times (20 kHz–47 kHz and 2–1800 s, respectively), is generally used for antimicrobial purposes.

7.3.4 Ultrasound Applied in the Inactivation and Activation of Enzymes

7.3.4.1 Inactivation of Enzymes

- **Mechanisms**

The inactivation of enzymes by ultrasound is mainly the result of protein denaturation, either by shear forces from the formation and collapse of cavitation bubbles, or by the free radicals produced from the sonolysis of water molecules. Ultrasound makes stable cavitatin bubbles vibrate, creating shock waves which cause strong shear, and microstream in the adjacent liquid. Under these extreme conditions, sonication could cause the breakdown of hydrogen bonding and van der Waals interactions in the polypeptide chains, leading to the modification of the secondary and tertiary structure of the protein. With such changes, the biological activity of the enzyme is usually lost. The extreme localized increase in pressure and temperature also leads to homolytic water molecule cleavage, generating high-energy intermediates such as hydroxyl, and hydrogen-free radicals.

The formation of free radicals due to the sonolysis of water is another mechanism by which the inactivation of enzymes takes place. During sonication, the formation of free radicals is the most widely reported mechanism of enzyme inactivation, which can be measured by the rate of hydrogen peroxide (H_2O_2) generation. The free radicals interact with the amino acid residues of the enzymes that participate

in structural stability, substrate binding, or catalytic functions, and thus affect the enzyme activity. Barteri et al. (2004) studied the inactivation of fumarase by ultrasound. They concluded that the inactivation of the enzyme was due to the formation of disulfide-linked aggregates during sonication. The effects of free radicals on the inactivation of trypsin resulted indirectly through the strong protective effect of mannitol against ultrasound inactivation, with the presence of polypeptide fragments following sonication (Tian et al. 2004). Aggregation of laccase was observed following sonication at different frequencies (20, 50 and 500 kHz). The role of free radicals on the ultrasound inactivation of enzymes was indirectly confirmed through the effect of free radical scavenging solutes on horseradish peroxidase (Grintsevich et al. 2001), catalase (Potapovich et al. 2003), glucose-6-phosphate dehydrogenase (G-6-PDH) (Karaseva and Metelitza 2006), and urease (Tarun et al. 2003). Sonication-induced aggregation was observed in α-amylase, while no such aggregation was observed in β-amylase, which showed that the free-radical-induced oxidation of amino acid residues is dependent on the structure of the protein (Liu et al. 2003).

- **Influencing Factors**

Ultrasonic inactivation of enzymes depends on ultrasound-related parameters such as frequency and ultrasonic power, and enzyme-related factors such as enzyme type, concentration, pH of the medium, and temperature (Tarun et al. 2006). The ultrasonic inactivation of different types of enzymes has been reported.

The effect of sonication on laccase from *Trametes villosa* has been studied at different ultrasonic powers and frequencies. The inactivation kinetics for all the treatments increased in comparison with heat treatments at 50 °C. Sonication, carried out at 72 W and 150 kHz, promoted the formation of protein (laccase) aggregates, which became more evident with increasing treatment times (up to 4 h), and led to inactivation by hindering the active sites. Half-life (time required to reduce the activity to a half of the initial value) was found to decrease by up to 80%–82% when using ultrasound treatment (Basto et al. 2007b).

Grintsevich and Metelitsa (2002) investigated the inactivation kinetics of horseradish peroxidase (POD) in a 0.01 M phosphate citrate buffer (pH 5.4) by applying high-frequency (2.64 MHz) ultrasound (1 W cm^{-2}, at 35.5–55 °C for 1–2 h). At pH less than 5, the free radicals generated during ultrasonic cavitation reacted rapidly with the functionally important amino acid residues at the active sites of POD. Lopez and Burgos (1995) showed a similar pH dependence (*D*-value increased with decreasing pH) in both thermal and manothermosonication inactivations of peroxidase.

The effects of ultrasound on the activity of lactoperoxidase (LPO) were studied by Ertugay et al. (2003), using different power levels (90–360 W at 20 kHz), times (up to 120 s), and temperatures (20 and 40 °C). The higher ultrasonic amplitudes showed three times as many inactivations of LPO enzymes at 40 °C. Additionally, an increase in temperature and sonication time showed a synergistic inactivation effect. Gebicka and Gebicki (1997) studied the effect of cavitating ultrasound (22 kHz) on peroxidases, horseradish peroxidase, and lactoperoxidase; they found that the

activity of peroxidases decreased because of conformational changes upon sonolysis when the sonication time increased.

The inactivation of *Bacillus amyloliquefaciens* α-amylase type II (A 6380) by ultrasound has been studied at a frequency of 30 kHz (Kadkhodaee and Povey 2008). The sonication was carried out in a thermostated water bath over a temperature range from 20 to 80 °C. Thermosonication greatly enhanced the efficacy of enzyme inactivation (by more than 50%), and also lowered the activation energy (19.29 kJ mol^{-1} K^{-1}) compared with thermal inactivation (109 kJ mol^{-1} K^{-1}). Ultrasound strongly decreases the energy barrier required for enzyme inactivation. Manas et al. (2006) studied the inactivation of egg white lysozyme by manothermosonication (117 μm, 200 kPa, 70 °C) where an increase of the implosion intensity of bubbles undergoing cavitation occurred due to the effect of pressure on the efficacy of ultrasound.

The effects of ultrasonic power (100–500 W at 20 kHz) and duration on the function and structures of trypsin have been studied by Tian et al. (2004). The activity of trypsin decreased gradually with the increase of ultrasonic power up to 400 W, with a larger decrease from 400 to 500 W. Combined treatments (manothermosonication and thermosonication) accelerated trypsin inactivation only at low temperatures (Vercet et al. 2001). The effect of ultrasound (26.4 kHz, 26 W cm^{-2}) on the activation of a mixture of chymotrypsinogen and trypsinogen has been studied, and the results show a significant decrease in proteolytic activity at 26.4 W cm^{-2} (Ovsianko et al. 2005).

- **Applications**

Juice

Pectinmethylesterase (PME), a ubiquitous enzyme found in plants, can hydrolyze pectin resulting in a decrease of cloud stability, and a reduction of viscosity due to pectin chain degradation. Ultrasound was reported to inactivate PME in tomato juice and orange juice (O'Donnell et al. 2010) in combination with heat and/or pressure. Wu et al. (2008) reported a reduction in the *D*-value for PME inactivation at 60 and 65 °C, compared to thermal inactivation.

Raviyan et al. (2005) reported increased inactivation of PME in sonicated tomato juice for a temperature range of 50–72 °C, dependent on cavitational intensity. The reduction of PME activity in sonicated lemon juice resulted in enhanced cloud stability during storage for 18 days at 4 °C compared to thermally processed lemon juice (Knorr et al. 2004). The improved cloud stability observed during storage could be due to the mechanical damage of the PME protein structure during sonication.

Polyphenoloxidase (PPO) is a copper-containing enzyme that causes enzymatic browning in fresh fruit and vegetable products such as juices. Enzymatic browning is a problem during the processing of fruit and vegetables (Yemenicioglu and Cemeroglu 2003). Cheng et al. (2007) reported changes in PPO in sonicated (35 kHz, for 30 min) guava juice. The low power levels can induce the stimulation of enzymes, whereas the higher power levels inactivate enzymes by denaturing them.

Peroxidase (POD) is a heme-containing enzyme which can be used to evaluate the efficiency of vegetable blanching because of its relatively high thermal stability.

Thermosonication has been reported to reduce the blanching time required for inactivation of POD in watercress; for example, to obtain 90% POD inactivation at 90 °C, a thermal treatment time of 70 s is necessary compared to 5 s when combined with thermosonication treatment at the same temperature (Cruz et al. 2006). The results show an increase in POD activity during the blanching of watercress for thermosonication in a temperature range of 40–80 °C, and a decrease in enzymatic activity at a higher temperature range of 82.5–92.5 °C. De Gennaro et al. (1999) reported first order inactivation kinetics for POD during sonication.

Dairy

Applications of ultrasound in the dairy industry have been reviewed by Villamiel et al. (1999). Thermoresistant enzymes can reduce the quality and shelf life of heat-treated milk and other dairy products. The simultaneous applications of heat and ultrasound under pressure (manothermosonication) have been found to be more effective than the heat treatment alone in the inactivation of the heat-resistant protease and lipase secreted by *Pseudomonas fluorescens* (Vercet et al. 1997). Sala et al. (1995) has reported that enzyme inactivation increases with an increase in the content of solids, and decreases with an increase in enzyme concentration.

Villamiel and de Jong (2000) outlined the effect of ultrasound on native milk enzymes. No effect on milk enzymes was observed when the ultrasound was applied without thermal treatment; however, inactivation effects were reported when the sonication was carried out above 61 °C. In skimmed milk, the concentration of solids is lower than in whole milk, resulting in a reduced ultrasonic effect. However, the concentration of enzymes in skimmed milk (alkaline phosphatase, AP and γ-glutamyltranspeptidase, GGTP) is also lower than that in whole milk leading to a more pronounced effect, as these enzymes are linked to fat globules and can be liberated by the ultrasound effect to the serum phase; on the other hand, lactoperoxidase (LPO) is located in the whey. The main cause of the larger decrease in enzyme activity in whole milk compared to skimmed milk by ultrasound and heat (75.5 °C; 102.3 s) could be the higher concentration of solids in whole milk (Villamiel and de Jong 2000).

7.3.4.2 Activation of Enzymes

- **Mechanisms**

Using ultrasound treatments at appropriate frequencies and intensity levels can lead to an increase of enzyme activity due to several different effects. These effects can be subdivided into physical and biochemical effects.

Physical effects: There are a number of effects enhancing enzyme activity based on pure physical effects elaborated in the following parts.

Mass transfer and micro-mixing: Enzymatic reactions are often limited by a lack of substrate due to the structural configuration of the substrate (Cadoret et al. 2002) or restricted diffusion of the substrate to enzymes or vice versa (Francis et al. 1995).

Ultrasound waves at different frequencies can overcome this limitation by inducing fluid motion and increasing mass transfer, ensuring substrate availability at the enzyme and the removal of products from the enzyme (Sinisterra 1992). This increase in mass transfer is obtained either at lower frequencies by the generation of cavitation, or at higher frequencies through the generation of turbulent microstreams and the induction of a turbulent channel flow in narrow porous structures (Bengtsson and Laurell 2004; Francis et al. 1995). Starting in the early 1990s, a number of workgroups investigated the effect of synergistically enhancing enzyme activities for different application. Typically, systems that are involved solid–liquid or liquid–liquid interfaces were investigated. Such studies included: enzyme scouring of cotton (Basto et al. 2007a, b; Yachmenev et al. 2004), paper recycling (Xie et al. 2002), cellulose hydrolysis (Aliyu and Hepher 2000; Barton et al. 1996; Li et al. 2005), sucrose hydrolysis (Sakakibara et al. 1996), esterifications (Xiao et al. 2005), hydrolysis of esters (Lie Ken Jie and Syed-Rahmatullah 1995), hydrolysis of phenolic compounds in wastes and bleaching (Entezari et al. 2006; Entezari and Petrier 2004; Tauber et al. 2005), and biofilm removal (Oulahal-Lagsir et al. 2003).

Enzyme release: Ultrasound waves at low frequencies and high intensities can induce cell break-up leading to a discharge of the cell contents including enzymes (Farkade et al. 2006; Persike et al. 2002; Vargas et al. 2004). However, using ultrasound at lower intensities can also release enzymes from cells while causing little damage to cell membranes (Roncales et al. 1993). These applications allow the enhancement of enzyme activity by overcoming the limitations of inter-membrane transport and improving enzyme extraction from cellular material.

Immobilized enzymes: The effects of increasing the activity of immobilized enzymes by using ultrasound are described by mass transfer and micro-mixing, and increasing substrate availability by enhanced mass transfer to and through the carrier material (Ma et al. 2017). The frequencies used were in the range of 1 MHz or greater, allowing turbulent microstreams to be generated.

Pretreatment procedures leading to enhanced enzyme activity: Not only can the combined processes of enzymatic reactions and ultrasonication lead to an improvement in enzyme activity, but a substrate or immobilization carrier pretreatment with ultrasound irradiation can also significantly enhance substrate conversion. The enhancement occurs through an increase in the available substrate surface, e.g. degradation of the substrate masking substances such as lignin on cellulose (Entezari and Petrier 2005; Li et al. 2005; Wood et al. 1997), grafting of a polymeric surface on which enzymes are immobilized (Jiang and Xiang 1992; Popa et al. 1994), or the size reduction of oil droplets by means of ultrasonic emulsification (Ramachandran et al. 2006).

Biochemical effects: Ultrasound waves can also induce biochemical effects in living cells, leading to an increase of the production of certain enzymes (Wu and Ge 2004; Wu and Lin 2003). The tissue reverts to normal metabolism if there is no further ultrasonic stimulation (Wu and Lin 2002).

- **Applications**

Ultrasound activation of enzymes

Polygalacturonase (PG) is one of the most commonly used enzymes in fruit and vegetable processing. Ultrasound has the potential to enhance enzyme activity, modify the PG enzyme, and enlarge its application range. The maximum activity of PG was observed at 4.5 W mL^{-1} intensity with an ultrasound duration of 15 min, under which the enzyme activity increased by 20.98% over the control. The results of degradation kinetics and the thermodynamics of hydrolysis reactions catalyzed by PG certified that ultrasound treatment could make PG exhibit higher reaction ability. After ultrasound treatment, the value of V_{max} for the enzymatic reaction increased, whereas K_m decreased in comparison to the control. These results demonstrate that the substrate was converted into the product at a higher rate and efficiency, and that the enzyme displayed better affinity to the substrate. Ultrasound improved the temperature stability of PG and prolonged its lifetime without affecting its optimum temperature. Fluorescence spectra and far-UV CD spectra revealed that ultrasound treatment irreversibly decreased the amount of tryptophan on the PG surface, but increased the β-sheet in PG secondary conformation, possibly by the exposure of more active sites (Ma et al. 2015).

Subhedar and Gogate (2014) investigated the effect of low intensity ultrasonic irradiation on the activity of cellulase. The results show that ultrasound has a positive effect on the activity of cellulase. The maximum cellulase activity was observed at an intensity of 17.33 W cm^{-2} and ultrasonic treatment times of 30 min, under which the enzyme activity increased by approximately 25% over the untreated enzyme. A significant reduction in the thermodynamic parameters was observed after ultrasonic irradiation.

The effects of energy-gathered ultrasound on the activity, kinetics, thermodynamics, and molecular structure of alcalase were explored by Ma et al. (2011). The results show that the highest alcalase activity was achieved when the sample was treated with energy-gathered ultrasound at 80 W for 4 min, under which the enzyme activity increased by 5.8% over the control. Fluorescence and CD spectra revealed that the ultrasonic treatment had increased the number of tryptophan on the alcalase surface slightly, increased the number of α-helices by 5.2%, and reduced the number of random coils by 13.6%. The changes in enzyme conformation induced by ultrasound may lead to the increase of enzymatic activity.

A study by Jadhav and Gogate (2014) shows that lipase had a maximum activity at an ultrasound intensity of 12.22 W cm^{-2} and an optimized sonication time of 9 min. The maximum increase in the activity of the enzyme was twofold. Immobilization of the enzyme was achieved after sonication at the optimized parameters while retaining its activity, which gave retention of 47.9% in the activity of the tributyrin hydrolysis reaction. The use of ultrasound certainly provided some permanent intensification in the activity of the enzyme.

Comparison of the impact of ultrasound on free and immobilized cellulase shows that the highest activity of the free biocatalyst was achieved when the sample was treated with ultrasound at a frequency of 24 kHz and an intensity of 15 W for 10 min,

under which the enzyme activity increased by 18.17% over the control (Wang et al. 2012). The highest activity of immobilized cellulase was achieved when the sample was treated with 24 kHz ultrasound at 60 W for 10 min, under which the activity of the enzyme increased by 24.67% over the control (Wang et al. 2012).

Introduced during the immobilization process, ultrasound at an intensity of 9 W mL^{-1} for 20 min increased the immobilization yield by 92.28% more than the control. Higher V_{max} and lower K_m were obtained after ultrasound treatment, indicating the increased catalytic efficiency, and the enhanced affinity of immobilized pectinase (Ma et al. 2017). For the increase in activity of commercial lipase immobilized on beads of a macroporous acrylic resin, when low intensity ultrasound was used after being used up to four times (Batistella et al. 2012), and even up to 8 (Zheng et al. 2013) or 10 times (Liu et al. 2015).

Ultrasound-assisted enzymatic hydrolysis

Comparative studies on lipase-catalyzed hydrolysis of soy oil in solvent-free systems were carried out in a shaking ultrasound bath (ultrasonic intensity 1.64 W cm^{-2} and frequency of 28 kHz). Under the optimum conditions, the overall enzymatic hydrolysis assisted by ultrasound was two times higher than that assisted by shaking (Liu et al. 2008).

Xenobiotics, such as anabolic steroids in urine, have also been deconjugated with the help of β-glucuronidase and an ultrasound probe in only 10 min. The experimental data suggests that the reaction followed Michaelis–Menten kinetics and that ultrasound affected the initial reaction rate, which was higher, compared to the classical method of incubation at 55 °C. Also, the values of V_{max} and k_{cat} were higher for the ultrasonic assay, whilst the Michaelis–Menten constant obtained from both methods showed similar values. Deactivation of the enzyme was also observed under ultrasound treatment, which was particularly evident for experimental conditions with an excess of substrate (Galesio et al. 2012).

The production of bio-ethanol from lignocellulosic materials is currently impeded by the high cost and low efficiency of enzymatic hydrolysis and plant cellulase activity. This can be partially overcome by the introduction of low energy ultrasound (using a 50 kHz ultrasound hexagon reactor system; at 50 °C) during the enzymatic hydrolysis of corn stover and sugarcane bagasse where the enzyme efficiency was greatly improved (Yachmenev et al. 2009). Another study was carried out by using cassava chip slurry as feedstock, the reducing sugar release from the slurry samples with enzyme addition during sonication was as high as 180% of the control samples. Heat generated during sonication did not account for the increased reducing sugar release (Nitayavardhana et al. 2008). The enzymatic degradation of lignocellulose was improved through the use of ultrasonic treatment at a frequency between 2 and 200 kHz and 30–48 °C. Continuous irradiation can result in a decrease in hydrolysis compared to discontinuous application, probably due to the fact that constant mixing does not allow the cellulase to rebind to cellulose for catalysis to occur (Ingram and Wood 2001).

The ultrasonic intensification of enzymatic depolymerization of aqueous guar gum solution has been reported by Prajapat et al. (2016). The kinetic rate constant was found to increase with an increase in temperature and cellulase loading. In the presence of cellulase, the maximum extent of depolymerization of guar gum has been observed at an ultrasonic power of 60 W, and a treatment time of 30 min. The results reveal that enzymatic depolymerization of guar gum results in a polysaccharide with a low degree of polymerization, viscosity, and consistency index, without any change in the core chemical structure which could make it useful for incorporation in food products.

The factors that affect the efficiency of the enzymatic hydrolysis of cellulose with low frequency ultrasound have been considered by Szabo and Csiszar (2017). The optimal operating conditions were reached at 60% amplitude and 9 mm. The yield depended mainly on important factors such as amplitude, the presence of a reflector, distance from the horn, and the form of the substrate.

Ma et al. (2016a) have investigated the synergistic effects of ultrasound and pectinase on pectin hydrolysis. The hydrolysis rate of pectin achieved maximum value with ultrasound treatment at 4.5 W mL^{-1} intensity and a treatment time of 10 min, resulting in an increase of 32.59% over the control. The optimum temperature for the hydrolysis reaction was 50 °C. The value of V_{max} increased whereas K_m decreased in the sonoenzymolysis reaction compared with the routine enzymolysis reaction, which indicates that pectin was hydrolyzed at an elevated rate, and that the pectinase exhibited a stronger affinity to the substrate with ultrasound. The degree of methoxylation (DM) of sonoenzymolysis pectin significantly decreased, whereas the degree of acetylation (DAc) remained unchanged compared to the original and enzymolysis pectin (Ma et al. 2016b).

7.3.5 Other Applications

7.3.5.1 Emulsification

Ultrasonic emulsification is primarily driven by cavitation, wherein bubbles collapse at the oil-water interface causing disruption that result in the formation of very fine emulsions.

Ultrasonic emulsification offers several benefits over conventional methods such as use of mechanical shaking, colloid mills, high- or ultra high-pressure homogenizers, and microfluidizers. For example, the energy required to produce an emulsion by ultrasound is less than that needed for conventional methods. Also, emulsions generated by ultrasound are more stable, require minimal surfactant, and have a submicron size and an extremely narrow size distribution. Ultrasonic emulsification has attracted much interest for the homogenization of milk (Wu et al. 2001; Bosiljkov et al. 2011; Windhab et al. 2005), aroma encapsulation (Mongenot et al. 2000), and online processing of tomato sauces, fruit juices, mayonnaise, and other similar blended food products. Several parameters affect the emulsification process including

hydrostatic pressure, gas content (Behrend and Schubert 2001), pre-emulsification (Jafari et al. 2007), viscosity of the continuous phase (Behrend et al. 2000), oil:water ratio, surfactant concentration (Abismail et al. 1999; Jafari et al. 2006), position of the ultrasonic probe in relation to the liquid–liquid interface (Cucheval and Chow 2008), ultrasonic power, and exposure time (Jafari et al. 2006; 2007).

7.3.5.2 Filtration

Membrane technology is extensively used in the food and dairy industry, for water purification and treatment of liquid effluent streams (Maskooki et al. 2010). One of the critical issues in filtration is the decline in permeate flux as a result of both concentration polarization and membrane fouling. The application of ultrasound has been proven to be an effective approach for enhancing the flux in ultrafiltration or microfiltration processes, and to improve the cleaning of fouled membranes (Muthukumaran et al. 2005a, b, 2007).

7.3.5.3 Viscosity Modification

Controlling the viscosity of food systems by ultrasound is one of the most promising processes. The elucidated merits of the ultrasonic process are that: no chemicals or additives are required; it is cost effective; and there are no large changes in the chemical structure. The ultrasonic process has been confirmed to be applicable for many kinds of starches (corn, potato, tapioca, and sweet potato) and polysaccharides. Jambrak et al. (2010) used an ultrasound probe at different intensities (34, 55, 73 W cm^{-2}) and treatment times (15 and 30 min), and an ultrasound bath at an intensity of 2 W cm^{-2} with treatment times of 15 and 30 min, for corn starch suspensions. The results show that ultrasound treatment of corn starch distorted the crystalline region in the starch granules prior to a reversible hydration of the amorphous phase, which resulted in the destruction of the granular structure.

7.3.5.4 Tenderization

The effects of ultrasound on meat texture are predominantly from cavitation effects, as the samples are chilled immediately after ultrasound treatment. Ultrastructural changes in the muscle, either by physical disruption of collagen or myofibrils, or by increased enzymatic degradation of the muscle through increased availability of calcium ions for the calcium-mediated calpain system, increased the ability of ultrasound to rapidly tenderize meat (Jayasooriya et al. 2007).

7.3.5.5 Defoaming

PU in pulsed operation (1 s/1 s) has been described as an effective procedure to remove foam and dissolved oxygen (80% of foam reduction) with very low energy consumption (40 kJ L^{-1}) in super-saturated milk (Villamiel et al. 1999). Recently, a stepped-plate air-borne ultrasound defoamer was developed and commercially applied to control the excess foam produced during the filling operation of bottles and cans on high-speed canning lines, in fermenting vessels, and other reactors (Gallego-Juarez et al. 2010).

7.3.5.6 Crystallization

Sonocrystallisation is the application of ultrasound energy to control the nucleation of a crystallization process. The use of the power ultrasound provides a useful approach to produce crystals with desired properties. Sonocrystallisation facilitates process control by modulating crystal size distribution and morphology (Deora et al. 2013).

7.3.5.7 Freezing

Ultrasound has gained considerable interest in food processing and preservation due to its ability to control and modify nucleation and crystal growth (Knorr et al. 2004; Li and Sun 2002a; Sanz et al. 1999; Mason et al. 1996). Several studies have indicated the potential of using ultrasound to accelerate freezing rates and to improve the quality of frozen plant foods, such as potatoes (Li and Sun 2002b; Sun and Li 2003). Ultrasound-treated frozen potatoes exhibited a better cellular structure with fewer extracellular voids and less cell disruption/ breakage than those without acoustic treatment (Sun and Li 2003). The most important effect of power ultrasound in food freezing is due to acoustic cavitation, which not only promotes ice nucleation by micro-bubbles, but also enhances the heat and mass transfer due to the violent agitation created by the acoustic microstreaming (Zheng and Sun 2006). Cavitation bubbles arising from sonication benefit the freezing process by reducing the resistance to both heat and mass transport at the ice/liquid interface, thereby increasing the freezing rate. The random motion of the cavitation bubbles is also suspected to break down any ice dendrites as they form (Chow et al. 2004).

7.3.5.8 Thawing of Frozen Foods

More studies have been carried out to investigate the effectiveness of PU for thawing frozen foods by varying ultrasound parameters such as frequency and power (Kissam 1985; Kissam et al. 1982; Brody and Antonevich 1959). Miles et al. (1999) reported that overheating occurred near the surface of frozen foods at high intensities, as well as at high and low frequencies, due to the increase in attenuation with frequency, and

the onset of cavitation at low frequencies. They were able to overcome this problem by adjusting the frequency (500 kHz) and intensity (0.5 W cm^{-2}) for frozen beef, pork, and cod, which were thawed to a depth of 7.6 cm within about 2.5 h. In other work, a block of frozen Pacific cod was exposed to 1500 Hz acoustic energy and up to 60 W continuous input to the transducer (Kissam et al. 1982). The block thawed in 71% less time than the water-only controls, and the acoustic waves did not alter the quality of the flesh.

7.3.5.9 Fermentation

Ultrasound can influence the course of fermentation by improving mass transfer and cell permeability leading to improved process efficiency and production rates. These methods produce no degradation or chemical alterations in the fermentation media (Henning and Rautenberg 2006). The velocity of an ultrasonic wave traveling through a fermentation tank can also be used to infer the concentration of alcohol and sugars during the fermentation process (Resa et al. 2005). Studies have shown that empirical relationships can be developed between the ultrasonic parameters and the concentration of alcohol and soluble solids in wine (Winder et al. 1970) as well as the density of beer (Becker et al. 2001) while fermentation is ongoing.

7.3.5.10 Drying

Ultrasonic dehydration can be utilized at low temperatures, which prevents the degradation of the nutritional components of food at high temperatures (Kumar et al. 2014; Lechtanska et al. 2015; Schoessler et al. 2012), it can also improve color, antioxidant volume, hardness, etc. (Bantle and Eikevik 2011; Kowalski et al. 2015; Santacatalina et al. 2016). The PU improves heat and mass transfer phenomena in drying processes. It can be assumed that supplementary ultrasonic effects, such as moisture and vapor migration improvement, boundary layer reduction, evaporation and sublimation improvement, and changes in structure or properties (density, viscosity, etc.) of the material, have contributed to the drying process (Musielak et al. 2016).

7.3.5.11 Freeze Concentration

Due to the ability to initiate nucleation at lower degrees of supercooling, PU can be an effective tool for controlling the size of ice crystals in freeze concentrated products (Zheng and Sun 2006). Botsaris and Qian (1999) utilized an ultrasound-assisted freeze concentration system and used it to induce ice nucleation at low degrees of supercooling.

7.4 Concluding Remarks

The high-power (low frequency) ultrasound modifies the properties of food by inducing mechanical, physical, and chemical/biochemical changes through cavitation, which reduces reaction time and increases yield under mild conditions compared to conventional procedures. Power ultrasound is considered to be a green technology with many promising applications in the food industry. Despite the potential applications of ultrasound in food processing, there are some limits for extensive applications such as the cost of probes, portability, and the ability to modify it in industrial applications. Therefore, extensive research is necessary to integrate fully automated ultrasound systems for food production lines, reduce cost, save energy, and ensure high food quality, and safety.

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Chapter 8
Supercritical-CO_2 as a Nonthermal Alternative Technology for Food Safety

Xingqian Ye, Sophia Jun Xue and John Shi

Abstract The practical application of supercritical-CO_2 (SC-CO_2) has been proposed as an alternative nonthermal pasteurization/sterilization technique to the traditional thermal disinfection processes. In addition, the SC-CO_2 treatment can be used to effectively improve the food safety of juices, vegetables, meats, and their products without impairing the quality or affecting the flavors and the nutrient contents of foods. This review includes a summary of recent research performances of SC-CO_2 as a nonthermal disinfection technology in different areas of applications for food safety. Moreover, it addresses the limitations of the technology and its associated regulations.

Keywords Supercritical-CO_2 (SC-CO_2) · Nonthermal process · Inactivation · Pasteurization · Sterilization

8.1 Introduction

Thermal sterilization is considered as a traditional method of sterilization that has long been recognized as one of the most effective methods for inactivating microorganisms, and it has been widely applied in the food industry. However, the thermal processes are associated with some disadvantages; in fact, high temperatures destroy not only the microorganisms, but they can also damage the heat-labile nutrients, the color, and the taste of food. Consumers are increasingly more conscious of the health benefits and risks associated with food consumption. Therefore, interest is growing for a nonthermal sterilization technique in the food industry in order to ensure microbiological safety without the deterioration of the quality of the products. The development of nonthermal but effective sterilization methods is of utmost importance.

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The supercritical carbon dioxide (SC-CO_2) technology has been extensively developed and widely used for separation processes of the heat-sensitive bioactive compounds, some nutrients, flavors, and pigments of foods in the food industry because its operating temperature is just above its critical temperature (31.1 °C), and because SC-CO_2 is nontoxic, nonflammable, chemically inert, recyclable and readily available in high purity, as well as the fact that it leaves no residues upon the removal from the product (Li et al. 2009). Recently, studies have shown evidence that the SC-CO_2 treatment has high inactivation effects for a broad variety of microorganisms, from bacteria to viruses and yeast because of its high diffusivity and solvent power (Zhang et al. 2006; Garcia-Gonzalez et al. 2007, 2009; Spilimbergo and Ciola 2010; Federico et al. 2011; Ferrentino et al. 2012a; Perrut 2012; Rao et al. 2015; Huang et al. 2017). Therefore, the SC-CO_2 technology has been considered by the food industry as a viable alternative and a promising nonthermal disinfection technology to the traditional pasteurization/sterilization processes in the preservation of natural flavors and nutrients of foods.

This review presents the recent studies and published facts concerning the SC-CO_2 technique for microbial inactivation and addresses issues regarding technology, such as the mechanism of carbon dioxide bactericidal action, the potential for inactivating vegetative cells and bacterial spores, and the regulatory hurdles which need to be overcome. Moreover, the review also reflects on the opportunities and especially the current drawbacks of the SC-CO_2 technique for the food industry.

8.2 The Mechanism of the SC-CO_2 on Bacterial Inactivation

The mechanism of microbial inactivation can be explained in that SC-CO_2 promotes cell rupture, the modification of the cell membrane with extraction of the cell wall lipids and intracellular substances, and the inactivation of key enzymes for cell metabolism.

Hu et al. (2010) reported that the SC-CO_2 had the ability to extract constituents from the cells and cell membrane, modify the structure of the cell membrane and damage the proteins, especially the enzymes. Bae et al. (2009) used scanning electron microscopy (SEM) and energy-filtering transmission electron microscopy (EF-TEM) methods to investigate the morphological changes of the *Alicyclobacillus acidoterrestris* spores treated by high-pressured CO treatment at 10 MPa and 70 °C for 30 min. The results from SEM micrographs revealed that the treated spores were crushed and exhibited a high degree of holes on the surface. The EF-TEM micrographs showed an enlarged periplasmic space and a loss of cytoplasm in the treated spores. Based on these images, it was concluded that the SC-CO_2 directly affected and inactivated the *A. acidoterrestris* spores. Therefore, the mechanism of inactivation of bacterial action by the SC-CO_2 could be clearly explained as follows: the SC-CO_2 treatment modified and increased the permeability of the inner membrane of the cell. The

cell plasma membrane, which is derived from the inner membrane, became leaky and was unable to carry out proper energy metabolism. Meanwhile, the crucial proteins lost their function with the SC-CO_2 treatment, which in turn, may have induced lethal effects and inactivation. Bertoloni et al. (2006) found that different intracellular enzymes of bacterial cells (*E. coli*) were removed into the extracellular environment after a CO_2 treatment.

Garcia-Gonzalez et al. (2007) reported detailed information and described the key factors regarding the bacterial inactivation mechanism of the SC-CO_2 treatment (Fig. 8.1). The authors described a hypothetical inactivation mechanism summarized as follows: (1) solubilisation of pressurized CO_2 in the external liquid phase, (2) cell membrane modification, (3) intracellular pH (pHi) decrease, (4) key enzyme inactivation/cellular metabolism inhibition due to the lowering of the pH, (5) direct or inhibitory effects of molecular CO_2 and HCO_3 on metabolism, (6) disordering of the intracellular electrolyte balance, and (7) removal of vital constituents from cells and cell membranes. The solubilisation of CO_2 in the food system is one of the important factors of bacterial inactivation. Normally foods contain water, thus under the SC-CO_2 treatment, CO_2 can dissolve into the water to form carbonic acid (H_2CO_3), which dissociates into bicarbonate (HCO_3^-), carbonate (CO_3^-), and hydrogen (H) ionic species and follows certain equilibria of a gas and aqueous solution in the system. Under normal atmospheric conditions, the concentration of dissolved (unhydrated) carbon dioxide (aqueous CO_2) depends on the operating pressure and temperature. Water in contact with pressurized CO_2 generally becomes acidic due to the formation of carbonic acid (H_2CO_3), which frees H ions.

This lowered extracellular pH (pHex) may inhibit microbial growth, and it may also diminish the microbial resistance to inactivation because of the increased energy consumption required to maintain the pH homeostasis by the proton motive force. Therefore, the lowered pHex contributes to an increase in cell permeability in order to facilitate the penetration of CO_2 into the microbial cells. Moreover, the SC-CO_2 penetrates the cells at a much faster rate than other molecules which do not produce acidification in the solution. The SC-CO_2 can modify the cell membrane by the diffusion of CO_2 into the cellular (plasma) membrane and may accumulate into its lipophilic (phospholipid) inner layer (cytoplasmic interior). This accumulated amount of (unhydrated) CO_2 in the lipid phase may then structurally and functionally disorder the cell membrane because the loss of order of the lipid chain (a process known as "anesthesia") might increase the fluidity (and the permeability) of the membrane. Moreover, the presence of the HCO_3 ion may also act on the charged phospholipid head groups and the proteins at the surface of the membrane to alter the surface charge of the cell, thereby altering the optimal surface charge density of the membrane, and hence altering the optimal function of the membrane. On the other hand, due to the increased membrane permeability, pressurized CO_2 easily penetrates through the bacterial cell membrane and accumulates in the cytoplasmic interior of the cells, in which the relative concentrations of both aqueous CO_2 and HCO_3^- ion are controlled by the internal pH buffering as a result of the pH homeostasis in order to maintain a more or less constant cytoplasmic pHi (which is essential for optimal cell viability and cellular activity). If too much dissolved CO_2 enters the

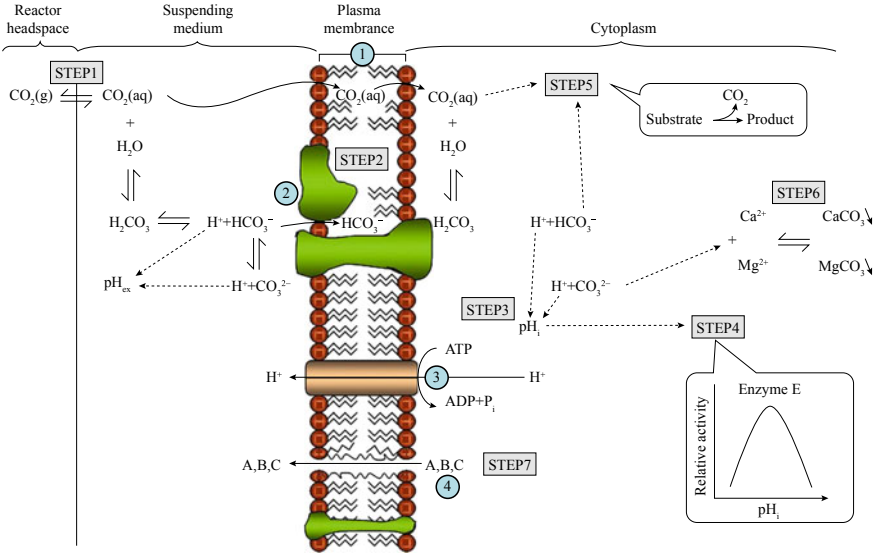

Fig. 8.1 A schematic diagram of how pressurized CO_2 may exert its lethal action on bacteria. Also shown–besides the different steps of the inactivation mechanism–are 1 a phospholipid bilayer, 2 integral membrane proteins, 3 a plasma membrane H^+-ATPase, and 4 intracellular substances (Modified from Garcia-Gonzalez et al. 2007)

cytoplasm, the cells may be unable to expel all of the resulting protons, and thus pHi may start to decrease. If pHi is lowered too much, the cell viability will be seriously impaired. Therefore, in the explanation by Garcia-Gonzalez et al. (2007), upon pressurized CO_2 exposure, microbial cells are unable to maintain favorable cytoplasmic pH homeostasis.

Thus pHi may start to decrease. If pHi is lowered too much, the cell viability will be seriously impaired. Therefore, in the explanation by Garcia-Gonzalez et al. (2007), upon pressurized CO_2 exposure, microbial cells are unable to maintain a favorable cytoplasmic pH homeostasis. The lowering of the pHi also causes key enzyme inhibition and/or inactivation because the catalytic activity of enzymes is especially sensitive to a change in pHi. Enzymes, which make up most of the proteins in the cytosol, have maximal activity at the optimum pH, and their activity declines sharply when the optimum conditions are changed. Thus, the lowering of the cytosolic pHi might cause the inhibition and/or the inactivation of key enzymes essential for metabolic and regulating processes, such as glycolysis, amino acid and peptide transportation, active transportation of ions, and proton translocation. Therefore, a loss of the biological control of the pHi of cells may be detrimental in all aspects of the intermediary metabolism and cellular function. The regulation of a metabolic pathway may occur at several levels. The reaction rate of each enzymatic reaction is not only a function of the pH, but also of the intracellular concentrations of its substrate(s), product(s), and cofactors, which are primary elements in

the regulation of the enzymatic activity. In the inactivation of bacterial cells by the SC-CO_2 treatment, the effects of HCO_3 and dissolved (unhydrated) CO_2 on carboxylation and decarboxylation reactions play an important role in the regulation of the metabolic pathway. The CO_2 plays a key role either as a biosynthetic substrate in carboxylation reactions or as a metabolic product from decarboxylation reactions. Carboxylation reactions are particularly important for the gluconeogenesis and the synthesis of certain biosynthetic precursor amino acids and nucleic acids. The accumulated CO_2 may extract vital constituents from the cells or cell membranes due to its relatively high solvating power. In this mechanism, the pressurized CO_2 first penetrates into the cells to build up the density to a critical level within the cells. After that, it removes the intracellular constituents (such as phospholipids and hydrophobic compounds) to disturb or alter the structure of the biomembrane and/or the balance of the biological system, thus promoting the inactivation of bacterial cells. Overall, it may be concluded that, although deformation and a loss of integrity of the cell membrane may be the cause of some cell death, and other mechanisms, such as key enzyme inactivation or cellular metabolism inhibition, intracellular pH decrease, the inhibitory effect of molecular CO_2 and HCO_3 on the metabolism, and disordering of the intracellular electrolyte balance, must be more important in the lethal actions of SC-CO_2 and probably act synergistically.

8.2.1 Effects of Processing Conditions on the Effectiveness of Microbial Inactivation

The supercritical carbon dioxide (SC-CO_2) technology has been recognized as a promising nonthermal alternative sterilization technique in the food industry due to its unique properties. SC-CO_2 is a single-phase carbon dioxide between the liquid phase and gas above its critical condition ($T_c = 31.1$ °C, $p_c = 73.8$ bar); above its critical point, the CO_2 has the ability to diffuse through solids like a gas, and dissolve materials like a liquid (Fig. 8.2). The liquid-like density allows a higher solvating power compared to the gaseous state. On the other hand, the gas-like mass transport properties enhance the diffusion rate when compared to the liquid state. Thus, supercritical CO_2 with these enhanced properties could be more effective than both gaseous and liquid CO_2 at penetrating into cells and extracting intracellular components and cause an increased disruption of biological systems. Besides, pressurized CO_2 has high dissolving power, high diffusivity and low viscosity, which results in the effectiveness of the inactivation of microorganisms both physically and chemically (Tomasula 2003; Gunes et al. 2005).

The phenomena affecting the microbial inactivation mechanism, such as cell rupture by pressure, modification of the cell membrane, cytoplasmatic acidification, inactivation of key enzymes for cell metabolism, and a decrease in intracellular pH are closely related to the applied pressure and temperature conditions controlling CO_2 mass transfer, as well as its solubility, and the biological activity of the micro-

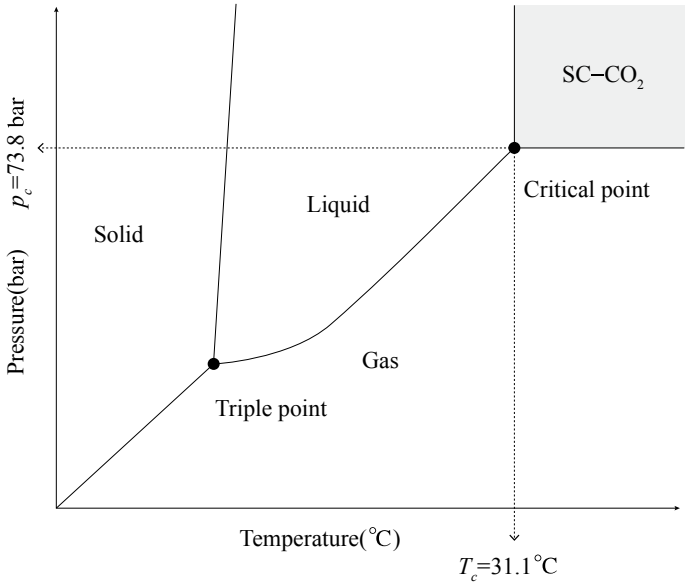

Fig. 8.2 State diagram of carbon dioxide at critical conditions

bial cells will also be affected (Spilimbergo et al. 2003; Garcia-Gonzalez et al. 2007). Time of exposure is important too, and the conditions needed for the inactivation of different microbes varies, some organisms being much more resistant than others.

Microbial inactivation is sensitive to the applied temperature. Generally, an increase in the temperature is beneficial to microbial inactivation due to the enhanced fluidity of cell membranes that could make CO_2 penetration easier and an increase of CO_2 diffusivity. However, it is better to operate the process temperature not far above the CO_2 critical value because elevating the temperature could cause the density of the solvent and its solubilization capacity to decrease quite rapidly. Thus, a higher temperature on CO_2 penetration can have a negative impact because of its inhibiting effect on CO_2 solubility. Therefore, it can be suggested that SC-CO_2 treatments should also not be performed at temperatures that are too high because they could deteriorate the quality of the food in many applications (Lucien and Foster 1999).

With respect to pressure, its increase enhances the CO_2 solubilization facilitating both extracellular and intracellular acidification (decreasing the pH), and meanwhile it improves its contact with the cells. However, the effect of CO_2 pressure is limited by the saturation of CO_2 solubility in the water content of the food matrix, the microbial cells and the microbial phospholipid bilayer (Spilimbergo and Mantoan 2005). Spilimbergo (2002) has found that above 10 MPa, the solubility of CO_2 is a weak function of pressure. An increase in pressure from 10 to 30 MPa at 55–60 °C did not appreciably influence the solubility of CO_2 in water. In the SC-CO_2 microbial inactivation process, the solubilization rate of CO_2 and its total solubility in the medium can be controlled by pressure. The higher pressure could enhance CO_2 solubilization

to facilitate both acidifications of the external medium and CO_2 penetration and the diffusion power, as well as its contact with the cells. In addition, CO_2 at higher pressures in general exhibits a higher solvating power, thus also facilitating the removal of vital constituents from the cells and the cell membranes and resulting in inactivation of bacterial cells (Lucien and Foster 1999; Garcia-Gonzalez et al. 2007).

The physical state of CO_2, either the subcritical (liquid or gaseous) or supercritical state, which can be controlled by temperature and pressure. Garcia-Gonzalez et al. (2007) stated that supercritical CO_2 was more effective in inactivating microbial cells than CO_2 under subcritical conditions. Gunes et al. (2006) studied the inactivation of *E.coli* (ATCC 4157) in diluted apple cider using three states of CO_2, and they found that the effect of temperature and pressure on inactivation of *E. coli* was largely masked by the CO_2/product ratio used in their flow through system. *E. coli* was very sensitive to dense CO_2 treatment, with a more than 6-log reduction in treatments containing 70 and 140 g/kg CO_2, irrespective of temperature and pressure. They stated that the CO_2/product ratio was the most important factor affecting the inactivation rate of *E. coli*.

Water content in the treated materials is also an important factor for the effectiveness of the SC-CO_2 microbial inactivation process. Because the solubilized CO_2 reacts with the water of treated food products to form carbonic acid (H_2CO_3) which results in cytoplasmic acidification by dissociating HCO into bicarbonate (HCO^-), carbonate (CO_2^-), and hydrogen (H^+) in bacterial cell cytoplasm. Therefore, concentrations and balance of those generated ions in the system significantly influence the inactivation rate and the efficiency (Kamihira et al. 1987; Garcia-Gonzalez et al.2007). The results of the study of the sterilization of different microorganisms with different water contents (wet cells, 65–75; dry cells, 5%–15%.) by SC-CO_2 treatment are shown in Table 8.1. Baker's yeast, *E. coli*, *Staphylococcus aureus* and conidia of *Aspergillus niger* were sterilized by treatment with SC-CO_2 at 20 Mpa and 35 °C for 120 min when the water content of each microorganism was 70%–90%. However, dry cells with a water content of 2%–10% could not be sterilized under the same conditions. Taniguchi et al. (1987) reported the same results on SC-CO_2-treated natural (aerobic) flora with no reduction to be observed on the samples with 6.8% water contents, but 1.3 and 2.3 reductions have been found on the samples containing 16.5% and 30.7% at processing conditions of 20 Mpa, 35 °C, and 120 min. Therefore, the experimental results suggested that vegetative cells with low water content were poorly inactivated and that their resistance to inactivation increased with decreasing water content. Moreover, sterilization kinetics is strongly affected by the addition of water.

The explanation of wet microbial cells is that more resistance to SC-CO_2 inactivation may be due to the direct result of an increased CO_2 solubility that enhances an increased formation and dissociation of H_2CO_3, and liberates more H^+ ions that subsequently reduce the pHex extracellular of cells of the suspending medium to lower values. In addition, water may also increase the permeability of the cell wall, hence the CO_2 may more readily diffuse through the lipoprotein barrier, and thus positively influence cell membrane modifications (Garcia-Gonzalez et al. 2007).

Table 8.1 The sterilizing effects of water contents (wet cells, 70%–90%; dry cells, 2%–10%.) on the inactivation of microbial cells are different with supercritical CO_2 at 20 MPa, 35 °C and 120 min

Microorganism	Ratio of living cells	
	Wet cells	Dry cells
Baker's yeast	5.4×10^{-7}	0.50
E. coli	7.2×10^{-6}	0.047
S. aureus	1.5×10^{-5}	0.037
A. niger (conidia)	1.2×10^{-5}	0.88
B. subtilis (endospore)	0.47	0.99
B. stearothermophilus (endospore)	1.07	0.80

8.3 The Application of SC-CO_2 Microbial Inactivation on Food Products

The microbial inactivation of food products is a crucial parameter in the safety and shelf life of food. Nonthermal pasteurization/sterilization processes have emerged as a viable alternative to those conventional processing techniques by offering safe products of excellent quality and at a very reasonable cost. Supercritical CO_2 can be considered one of the emerging technologies utilizing nonthermal microbial stress factors as the main inactivation mechanism, to approach seeking effects in order to have shorter processes and food products of very good quality. The efficacy of carbon dioxide in the supercritical state has been examined to inactivate microorganisms, both pathogenic and food-borne, including *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, yeasts, molds, and lactic acid bacteria in food products.

8.3.1 SC-CO_2 Microbial Inactivation on Vegetables and Fruits

Vegetables are valuable dietary foods that are commonly consumed raw or slightly cooked. The raw or slightly cooked vegetables retain a variety of nutritional components, such as antioxidants (polyphenols and carotenoids), vitamins, and minerals. However, they have limited food safety due to possible pathogenic contamination, which causes food spoilage, food-borne illnesses, and detrimental changes to the organoleptic quality of foods. This creates a significant problem in the food industry. Notably, spores are the most resistant form of bacteria to a variety of severe stresses, including extreme temperatures, because their structure is different and more complex when compared to other vegetative cells. Recently, studies have reported that SC-CO_2 successfully inactivated some spore species in vegetables. Ishikawa et al. (1997) reported that the SC-CO_2 treatment of *B. polymyxa*, *B. cereus*, and *B. subtilis* spores at 45, 50, and 55 °C, respectively, and 30 MPa for 60 min resulted in a 6 log cycle reduction of survival. In addition, the SC-CO_2 treatment (30 MPa, 60 °C,

30 min) of *B. polymyxa* and *B. cereus* spores also produced a 6 log cycle reduction. Thus, they conclude that the SC-CO_2 treatment under the foregoing conditions should offer higher efficiency compared to the heat treatment at 100 °C for 60 min. Spilimbergo et al. (2003) reported that the SC-CO_2 treatment can yield *B. subtilis* spore sterilization efficiency. With a treating time of 6 h at an operating pressure of 90 bar and a temperature of 60 °C, *B. subtilis* could be completely inactivated. The same conditions without the presence of supercritical fluid gave no inactivation of *B. subtilis*.

E. coli O157:H7, *L. monocytogenes*, and *S. typhimurium* are other, mostly pathogenic, organisms present in both vegetables and meats. Jung et al. (2009) investigated supercritical carbon dioxide to decontaminate *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium* in alfalfa sprouted seeds. In their experiments, the alfalfa sprouts were treated with SC-CO_2 at 10, 15, or 20 MPa and temperatures of 35, 40, or 45 °C for 5, 10, or 15 min. The results showed that treating samples with SC-CO_2 at higher pressures, temperatures, or for longer treatment times resulted in greater microbial reductions than treatments at lower pressures, temperatures, or for shorter treatment times. The SC-CO_2 treatment clearly reduced the microorganism levels in alfalfa seeds; in particular, treatment at 20 MPa and 45 °C for 15 min reduced levels of the three pathogens by >7.0 log CFU g^{-1}. However, the SC-CO treatment at high pressure and high temperature, especially treatment at 20 MPa and 40 or 45 °C, impaired the seed germination capability in some cases. Without impairing the germination capability the maximum reduction level of *E. coli* O157:H7 was 3.51 CFU g^{-1} with SC-CO treatment at 15 MPa and 35 °C for 10 min. Maximum reductions of *L. monocytogenes* and *S. typhimurium* were 2.65 and 2.48 log CFU g^{-1}, respectively, with treatment at 10 MPa and 45 °C for 5 min. Therefore, our results indicate that the SC-CO_2 treatment can be used to effectively improve alfalfa seed safety.

Spilimbergo et al. (2013) conducted a study on the effect of the pasteurization of fresh-cut carrots on the inactivation of the enumeration of the natural microbial flora (mesophilic microorganisms, total coliform bacteria, lactic acid bacteria, and yeasts and molds that are normally present in vegetables). The results of the microbial analysis showed that the inactivation kinetics of mesophilic microorganisms (a) and lactic acid bacteria (b) naturally present in fresh-cut carrots (Fig. 8.3). Increasing the temperature from 22 to 45 °C at 12 MPa induced an increase of the inactivation rate in the first 15 min of treatment at 45 °C with 3.5 log reductions for mesophilic microorganisms, but only 2.5 log reduction at 22 °C. After the first part of the treatment, no additional inactivation was noticed at 45 °C. In contrast, at lower temperatures, the rate suddenly decreased and the inactivation reached a plateau towards 3.5 log at 30 min without any further increase for longer times. For the inactivation of yeasts, molds, and total coliform bacteria, they showed that inactivation to undetectable levels (about 5 log reductions) of yeasts and molds were achieved at 22 °C in 5 and 10 min at 8 and 12 MPa, respectively. Similar results were also obtained for total coliform bacteria where inactivation to an undetectable level (5 log reductions) was reached in 5 min at 12 MPa and 10 min at 8 MPa. Experiments were also performed. Increasing the temperature to 35 °C, the same degree

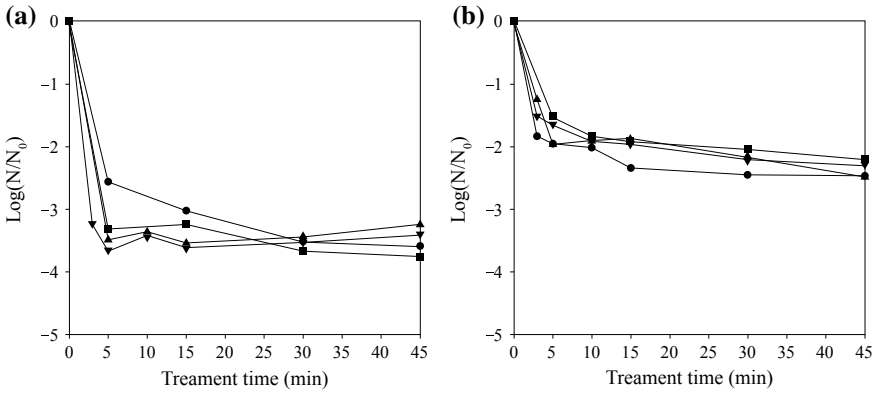

Fig. 8.3 Inactivation kinetics of mesophilic microorganisms (**a**) and lactic acid bacteria (**b**) at 120 MPa as a function of treatment time and temperature: ■ 22 °C; ● 35 °C; ▲40 °C; ▼45 °C (Modified from Spilimbergo et al. 2013)

of inactivation was achieved at 8 MPa and 35 °C for 5 min, and 12 MPa and 35 °C for 3 min both for yeasts and molds and total coliform bacteria. The study suggested that the pressure of 12 MPa seemed to be generally beneficial to increase the process efficiency compared to 8 MPa, while the temperature only slightly influenced the inactivation rate. The study also evaluated nutrients (phenol, flavonoid carotenoid, and ascorbic acid) and quality (antioxidant and texture) of treated fresh-cut carrots. The experimental results indicated that the SC-CO_2 treatment showed no detrimental effects on the polyphenolic compounds, carotenoids and the antioxidant capacity of fresh-cut carrots, while the ascorbic acid content decreased by 40%. Texture was also significantly affected, exhibiting a significant reduction up to 90% compared to the control. Zhong et al. (2008) conducted inactivation studies of *E. coli* K-12 inoculated on fresh spinach leaves. The CO_2 operated at two supercritical conditions at pressure of 7.5 and 10 MPa, temperature of 40 °C, and at a flow rate of 50 g min^{-1}. *E. coli* K-12 populations were reduced to non-detectable levels (approximately 5 log reduction) using supercritical treatment conditions at exposure times as short as 10 min. This research demonstrates that SC-CO_2 has potential as a pasteurization technology for application to leafy green vegetables.

Ferrentino et al. (2012b) evaluated the effectiveness of SC-CO_2 as a nonthermal technology for the pasteurization of fresh-cut coconuts. The quality of treated fresh-cut coconuts was also investigated. They found that the inactivation kinetics of microbiota induced 4 log CFU g^{-1} reductions of mesophilic microorganisms, lactic acid bacteria, total coliforms, and yeasts and molds on the fresh-cut coconuts with the SC-CO_2 treatment operating at 45 °C and 12 MPa for 15 min. Meanwhile, at the same SC-CO_2 treatment conditions, the hardness of coconuts was not affected by the treatment, suggesting that the treatment had great potential if applied to products with a firm texture or rigid structure. Some negative effects were observed on the microstructure of coconut tissue (Fig. 8.4) at both conditions chosen as optimal

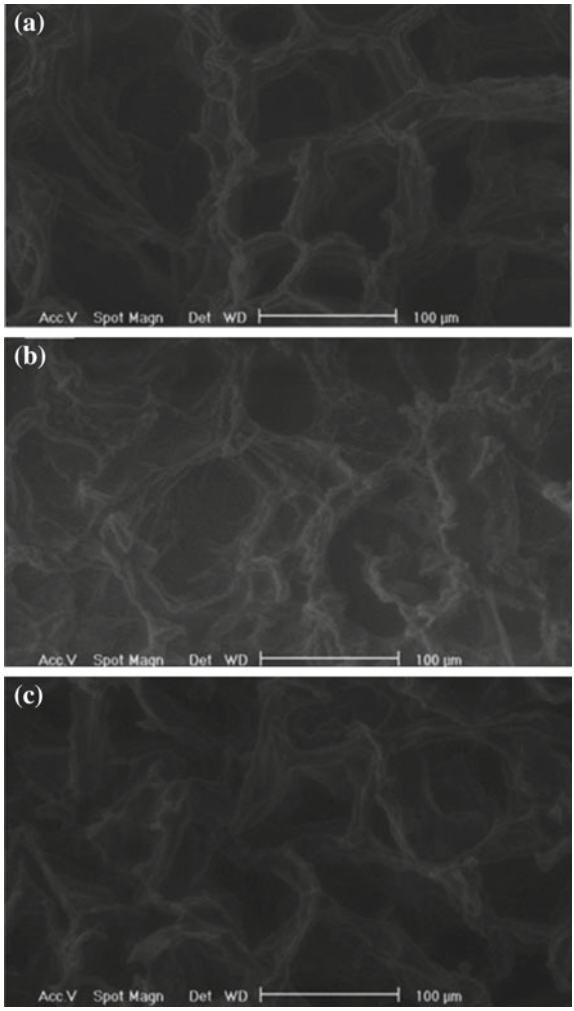

Fig. 8.4 Effects of SC-CO_2 on the microstructure of fresh-cut coconuts: **a** untreated, **b** treated at 12 MPa, 40 °C, 30 min, and **c** treated at 12 MPa, 45 °C, 15 min. Environmental scanning electron micrographs of cell tissue (Modified from Ferrentino et al. 2012a, b)

for microbial reduction. Valverde et al. (2010) achieved a total inactivation (5 log reductions) of *Saccharomyces cerevisiae* in pears at 55 °C and 30 MPa for 15 min.

8.3.2 SC-CO_2 Microbial Inactivation on Meat Products

The inactivation of microorganisms is a very important process for the meat industry. Especially microbial cross-contamination from the process environment, or improperly sanitized equipment (including knives, cutting tables, and grinders) all would cause serious impact on fresh meat and meat products. The most serious safety issues

associated with fresh meats and meat products are food-borne pathogenic bacteria, such as *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7. The contamination with pathogens can cause immediate consumer health problems, which subsequently lead to reduced economic productivity (Sofos 2008). As a nonthermal microbial inactivation process, SC-CO_2 has been considered an alternative method to control the safety of fresh meats and their products without impact on organoleptic quality and extended shelf life.

Chio et al. (2009) have evaluated the effects of the SC-CO_2 treatment for the inhibition of generic *E. coli*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 on the pork products marinated in soy sauce and hot pepper paste. They found that SC-CO_2 was more effective at destroying food-borne pathogens when it was applied to the marinated pork products. The SC-CO_2 treatment at 14 MPa and 45 °C for 40 min resulted in a greater reduction in the case of the marinated pork, and the reduction levels of *L. monocytogenes* were 2.49 and 1.92 log CFU cm^{-2} in soy sauce and hot-pepper paste-marinated pork, respectively. The results should be useful in the meat industry to help increase microbial safety and assure the microbial stability of marinades and marinated products.

Bae et al. (2010) worked on the effectiveness of decontaminating ground pork with the supercritical carbon dioxide (SC-CO_2) treatment at various conditions of temperatures at 40 and 45 °C, pressures at 100, 120, and 140 bar; and treatment times of 20, 30, and 40 min. Once the microorganisms contaminating the surfaces are embedded within the comminuted meat, they become difficult to inactivate due to the clumpy structure. The results showed that the reduction of microorganisms in ground pork ranged from 1.66 to 2.42 log CFU g^{-1} (total mesophilic plate counts, 1.66; *L. monocytogenes*, 2.42; and *S. Typhimurium*, 2.21 log CFU g^{-1}) following the SC-CO_2 treatment at 45 °C and 140 bar for 40 min.

Bae et al. (2011) examined the inhibitory effects of supercritical CO_2 (SC-CO_2) on microorganisms in fresh pork. With an SC-CO_2 treatment at 120 bar and 40 °C for 30 min, the initial mesophilic plate counts were reduced from 6.23 to 4.54 log CFU cm^{-2}, and the reduction levels of microorganisms, including nonpathogenic *E. coli*, *L. monocytoge*nes, *S. Typhimurium* and *E. coli* O157:H7 ranged from 1.99 to 2.51 log CFU cm^{-2}. The study suggested that from an economic point of view, SC-CO_2 treatment at the lower temperature of 40 °C and shortest exposure time of 30 min was more efficient compared to the treatment performed at the higher temperature or longer exposure time at the same pressure conditions, in order to avoid deterioration of food quality parameters, such as texture, water-holding capacity and color.

Huang et al. (2017) observed significant differences ($P < 0.05$) in the total bacterial count between the non-treated control samples and the treated ground pork with SC-CO_2 at 13 MPa, 35 °C for 2 h. The results suggested a significant reduction of microbial populations by the SC-CO_2 treatment with the more pronounced effect being achieved by combined treatment with SC-CO_2 and 5.0 g rosemary powder/100 g meat. However, the results from their shelf-life study indicated that when the control and treated ground pork samples were stored at 4 °C, the treatment with 13 MPa SC-CO_2 at 35 °C for 2 h could promote lipid oxidation of ground pork and result in color change during a week's period of refrigerated storage.

Wei et al. (1991) reported that a pressurized CO_2 treatment at 13.7 MPa and 35 °C for 2 h was shown to effectively kill *Salmonella* in the spiked chicken meats by 94%–98% and the chicken meats spiked with *Listeria* were only reduced by 79%–84%.

However, there is limited study on the application of SC-CO_2 for meats and meat products. It may be possible due to limited penetration and diffusion of CO_2 into the meat tissues, resulting in the reduced ability of CO_2 to damage bacterial cells. The other possibility for limited SC-CO_2 application in the meat industry is that, generally, a longer treatment time is needed to achieve substantial microbial inactivation as a result of the interactions of the food ingredients and intrinsic factors. Thus, sensory properties could be adversely affected. Therefore, it is difficult to find a compromise between microbial safety, and the attributes of physical and nutritional quality.

8.3.3 SC-CO_2 Microbial Inactivation on Fresh Fruit Juices and Milks

Fruit juices are often spoiled by microorganisms, such as yeasts (*Saccharomyces*, *Zygosaccharomyces* and *Candida* spp.), various bacilli (*Alicyclobacillus acidoterrestris* spores, *Lactobacillus plantarum*), and the heat-resistant fungus including *Byssochlamys fulva*, to produce chemicals that induce sour and off-flavors in fruit juice with important implications for the relevant industries (Gunes et al. 2006; Spilimbergo et al. 2007; Bae et al. 2009). Unpasteurized fresh fruit juices have been identified as vehicles for pathogens, such as *Escherichia coli* O157:H7, that cause food-borne illness (Gunes et al. 2005). The novel preservation method of SC-CO_2 is a promising emerging alternative technology that might be able to deliver microbiologically stable products with elongated shelf life and high sensory and nutritional characteristics of juice products.

Bae et al. (2009) investigated the lethal effect of SC-CO_2 on the inactivation of *Alicyclobacillus acidoterrestris* spores on apple juices. Complete inactivation of *Alicyclobacillus acidoterrestris* spores to undetectable levels was found when operating SC-CO_2 at 65 °C and 100 bar for 40 min and 70 °C and 80 bar for 30 min. Moreover, the treatment did not significantly affect ($P > 0.05$) the pH and °Brix of apple juice. In electron microscopic observations, the surface and internal morphological changes and extraction of intracellular materials of the treated spores were observed (Figs. 8.5 and 8.6). Porebska et al. (2017) also reported that *A. acidoterrestris* spores in apple juices were 3.9 log, of which 3.4 log were inactivated at SC-CO processing conditions of 60 MPa and 75 °C for 40 min.

Gasperi et al. (2009) studied the effects of supercritical CO_2 pasteurization on the quality of fresh apple juice. After 10 min of SC-CO treatment performed at 100 bar and 36 °C, the non-detected microorganisms were observed in the treated fresh apple juice from an initial concentration of 8.01 CFU mL^{-1}. The results confirmed the efficiency of SC-CO_2 treatment in inactivating microorganisms naturally present in

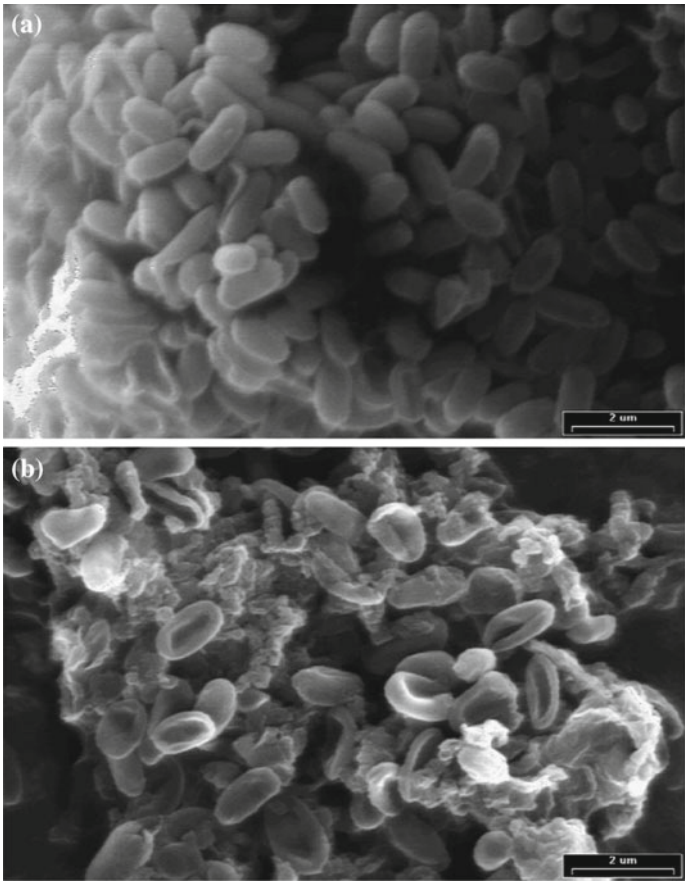

Fig. 8.5 SEM photographs of *A. acidoterrestris* spores without (**a**) and with (**b**) treatment by SC-CO_2 at 70 °C and 100 bar for 30 min (Modified from Bae et al. 2009)

fresh apple juice. Treatment at 100 bar and 36 °C for 10 min was sufficient to assure the inactivation of all the microorganisms initially present in the sample. However, SC-CO_2 treatments preserve the chemical characteristics of apple juice but induce overall volatile compound depletion in the juice headspace. In the experiments of SC-CO_2 inactivation of microflora, enzymes and some quality attributes of apple juice by Xu et al. (2011), the treatments were performed at 25 MPa and 43 °C for 2 min, and at 22 MPa and 60 °C for 3, 5 and 10 min, respectively. The results showed that the coliform bacteria were completely inactivated in all the cases. The yeasts and molds were completely inactivated and the turbidity increased at 22 MPa and 60 °C. Total aerobic bacteria were reduced by 3.72 log cycles. The enzyme of pectin methylesterase was reduced by 54.3% and polyphenol oxidase was completely inactivated after 10 min of treatment at 22 MPa and 60 °C. However, the treatment significantly influenced color and pH values.

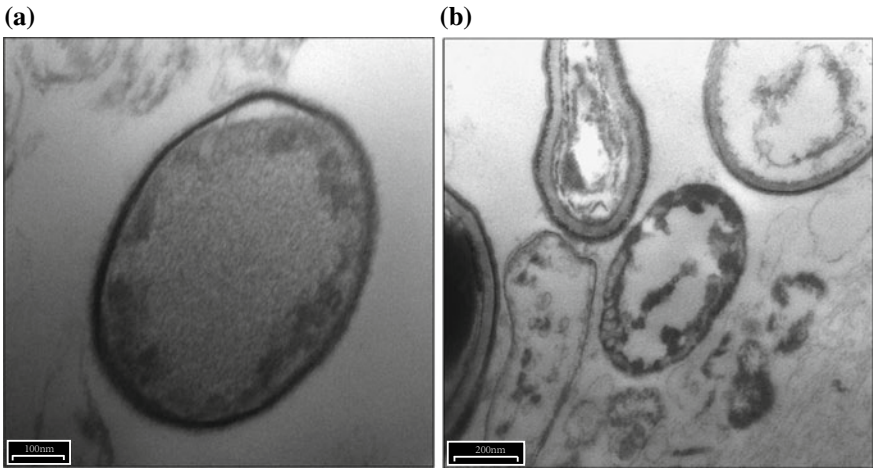

Fig. 8.6 F-TEM photographs of *A. acidoterrestris* spores without (**a**) and with (**b**) treatment by SC-CO_2 at 70 °C and 100 bar for 30 min (Modified from Bae et al. 2009)

Gunes et al. (2006) studied the inactivation of *E. coli* (ATCC 4157) in diluted apple cider using CO_2 at various supercritical conditions of 35 °C and 27.6 MPa, 5 °C and 27.6 MPa, and 45 °C and 48.3 MPa. They found that CO_2 effectively inactivated *E. coli* at all tested conditions, in which the initial population of *E. coli* in diluted cider (about 5×10^6 CFU mL^{-1}) decreased to an undetectable level.

Gunes et al. (2005) investigated SC-CO_2 inactivation of *Kloeckera apiculate*, *Candida stellate*, *and S. cerevisiae* in grape juices. The results of yeast survival showed more than a 5.2 log reduction in *K. apiculate* when treatment was carried out at a pressure of 27.6 MPa and a temperature of 35 °C for 5 min. There was a six log reduction of *C. stellate* and 5.3 log reduction of *S. cerevisiae* in treated grape juice under the same process conditions (27.6 MPa, 35 °C for 5 min). As the CO_2 in the juice concentration, temperature and pressure increased, the inactivation rate increased. CO_2 in the supercritical state was more effective in inactivating yeast than in the subcritical state. The process did not cause detectable flavor degradation.

Marszałk et al. (2015) evaluated the application of SC-CO_2 for the preservation of strawberry juice. They concluded that SC-CO_2 treated strawberry juice under selected parameters (10–60 MPa, 35–65 °C, and 10–30 min) were effective for microflora and enzyme inactivation. The biggest decrease in the total microbial count (TMC) was noticed from 4 log to 0 log CFU mL^{-1} (not detected) at pressure of 60 MPa and temperature of 65 °C for 30 min. The polyphenol oxidases were inactivated, whereas peroxidases decreased by 85%. Anthocyanins were not affected by the SC-CO_2 treatment. However, the SC-CO_2 treatment significantly hydrolyzed sucrose and ~30% losses of vitamin C, which was totally decomposed after the 4th week of storage. The study data proved that SC-CO_2 processing is a promising nonthermal alternative to pasteurization to preserve fresh cloudy strawberry juice.

Fabroni et al. (2010) used SC-CO_2 treated blood-red orange juice. The initial microbial load of freshly squeezed blood-red orange juice was 8.60×10^3 CFU mL^{-1} in Sabouraud dextrose agar (SAB), 3.82×10^3 CFU mL^{-1} in plate count agar (PCA), and 1.09×10^4 CFU mL^{-1} in orange serum agar (OSA), and after SC-CO_2 treatments, there were no culturable organisms present in the juice. After 30 days storage at 4 °C of the treated fresh squeezed blood-red orange juice, the treatment (230 bar, 36 °C ± 1 °C, 5.08 L h^{-1} juice flow rate) produced a better stabilization with a microbial load of 1.54 log CFU mL^{-1}.

Werner and Hotchkiss (2006) studied the lethal effects of SC-CO_2 on the total microbial populations and bacterial spores in raw milk. The milk pasteurized at 27.3 MPa, 35 °C for 10 min resulted in 5.36 log reduction of Native psychrotrophs and 5.02 log reduction of *Pseudomonas fluorescens.* Their studies demonstrated that greater microbial lethality can be achieved in raw milk treated with supercritical phase CO_2, and the effects of supercritical CO_2 can be enhanced by increasing pressure and temperature. Supercritical-CO_2 treatment using the flow through high-pressure CO_2 milk pilot plant processing unit can achieve bacterial reductions equal to or greater than those achieved by a range of possible pasteurization treatments. There is a CO_2 concentration threshold required for lethality. Liao et al. (2014) also reported that SC-CO at 25 MPa and 50 °C for 70 min, resulted in a 4.96 log reduction of aerobic bacteria in treated raw milk, and 2 log reduction of Coliforms and 3 log reduction of yeast and molds were found in treated raw milk under process conditions at 25 MPa and 40 °C for 50 min. Ceni et al. (2016) found 0.09 log reductions of *E. coli* in whole milk with operating SC-CO at 8 MPa and 70 °C for 1 min.

8.4 Summary

Nonthermal food processing has become a promising alternative to pasteurization or sterilization processes for the food industry due to the increased nutrient retention and minimal changes of sensory and organoleptic characteristics in processed products. Recently, the supercritical CO_2 (SC-CO_2) treatment has been considered one of the potential nonthermal processes to replace traditional thermal processes. It has been used successfully to inactivate various microorganisms and pathogenic microbes in food products, including fresh fruit juice, raw or slightly cooked vegetables, fresh meat, and meat products, with minimal and sometimes positive changes in the intrinsic quality of treated food products, as described above. The fundamental knowledge and technical concepts of SC-CO_2 microbial inactivation on food safety have been known and accepted by producers within the food industry. However, the commercialization of SC-CO_2 microbial inactivation processes is still not widespread and applied in the food industry. The optimization of processing conditions, such as pressure, temperature, treatment time, rate/density of CO_2 present in the system according to type of food and their properties (water contents, pH, and texture) and species of pathogen/microorganism remains a challenge and is still under development. Additionally, in possible exploitation at an industrial level, one

must consider not only the aim of improving microbiological safety without lowering the quality of the product, but also its economic feasibility and design, and scale-up studies are needed to integrate the technology into the existing process lines in the future.

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Chapter 9
Physical and Mechanical Pretreatment of the Raw Material for the Extraction of Health-Promoting Components

Xingqian Ye, Sophia Jun Xue and John Shi

Abstract Phytochemicals are naturally occurring plant-derived compounds and they are believed to have curative, preventative or nutritive values. They are present in many fruits and vegetables, but extracting them from these plant matrices and purifying them is quite challenging. In order to improve the yield and quality of the extracted phytochemicals, a pretreatment of the raw material is essential. Cell disruption is the most important pretreatment and this procedure can be divided into four classes: mechanical, ultrasonic, high-pulsed electronic field pulse, and nonmechanical treatments. High-pressure homogenization is the most widely used method for large-scale cell disruption. The reduction of plant material into fine particles can also be accomplished with a hammer mill or bead mill. With the bead mill, the grinding action is provided by glass beads that impact and apply shear force against the cell wall. The French Press is generally used for small operations, but it can be scaled up. The liquid cell suspension is subjected to a pressure differential causing the cell wall and membrane to burst. Ultrasonic disruption is a new method that is based on sound waves and a phenomenon known as cavitation. A major problem with sonication is the buildup of heat in the samples. Pulsed electric field treatment is a nonthermal method used to permeabilize the cell membrane of the plant material. Short pulses of high-voltage electricity can rupture the cell while maintaining the fresh physical, chemical and nutritional characteristics of the foods. Nonmechanical disruption processes include osmotic shock and freeze-thaw. The supercritical fluid treatments show great promise, especially for material of high-value health-promoting ingredients.

Keywords Bead mill · Extraction · Fresh press · Hammer mill · Homogenization · Micronization · Supercritical fluid

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9.1 Introduction

The public concern about health-related problems, the increase in the number of chronic diseases, and the detrimental effects of aging have all contributed to the rapidly growing consumer interest and awareness of phytochemicals. The naturally occurring plant-derived compounds are considered to have curative, preventative, or nutritive values. These compounds can be consumed in their natural form in foods or as isolated and purified compounds in supplements, and they represent a subcategory of a larger group of medical botanicals. Phytochemicals are present in many fruits and vegetables and other widely consumed plant materials and they have the potential for playing a significant role in the markets of functional foods and nutraceuticals.

Phytochemicals containing food ingredients not only have positive effects on health but also improve traditional food properties such as color, flavor, and texture. The use of phytochemicals in their concentrated or extracted forms is still not fully developed, and most of these active compounds are ingested directly into the food products.

Many food companies are interested in purchasing or producing/extracting phytochemicals such as lycopene, lutein, and isoflavones for inclusion in their own product lines. Incorporating or fortifying products with phytochemicals has been done to promote the sale of existing products. This trend has gained momentum due to the increased awareness of consumers, researchers, and the personnel in the food industry of the health benefits of phytochemical-based functional foods and nutraceuticals. The steadily increasing clinical evidence has supported the belief that these functional foods and nutraceuticals can provide protection against a broad range of cancers and chronic diseases. This growing evidence has raised the demand for these health-promoting compounds and the food industry has responded with new initiatives for research and product developments.

The main sources for many phytochemicals are common food commodities. However, the separation and purification of these biologically active compounds using efficient and cost-effective procedures is still a major hurdle. Among these separation techniques being developed, there are physical and chemical procedures such as centrifugation, filtration, membrane separation, precipitation, chromatography, solvent extraction, supercritical fluid extraction, crystallization, evaporation, and molecular distillation. The type of separation technology used will influence the quality of the extracted compounds and it will also determine the feasibility of the method. In the case of unstable products, a rapid separation procedure is necessary in order to avoid poor yields. For all separation and extraction procedures, a pretreatment of the raw material is an essential step if optimum yields and a high quality of the phytochemicals are to be obtained.

9.2 Raw Material Selection

In general, the phytochemicals are classified by their structure, such as carotenoids, polyphenols, sulfides, and thiols. However, in the case of phytoestrogens and protease inhibitors, their biological activity can be used as the basis of classification. Growing conditions such as temperature and rainfall pattern, soil type, and plant maturity play a significant role in establishing the phytochemical concentrations in raw plant material. Thus, it is likely that the factors are most difficult to control, such as temperature and rainfall, which may be the critical factors that determine the development of phytochemicals. Fertility practices such as soil amendments designed to deliver excess nutrients to crop plants can also have an impact on the levels of certain chemicals in plants. Although excess fertility levels can influence mineral content, there is little information as to their impact on phytochemical content or composition. Furthermore, because soil–plant interaction systems are complex, it has been difficult to assess the effects of general soil type on phytochemical contents.

To ensure consistency in a formulated product containing phytochemical extracts, it is important to specify the country of origin of each plant material used. For instance, nutmeg kernels (*Myristica fragrans*) from the East Indies have a different organoleptic profile from those from the West Indies. The East Indian nutmeg has a higher myristicin level (5% compared to 4%) and this imparts a stronger flavor. On the other hand, nutmeg from the West Indies is low in safrole (0.2% compared to 3%), which is considered desirable for safety reasons. Allowance should, therefore, be made for variations that are greater than would be anticipated from normal seasonal variations.

Although harvest maturity is one of the primary factors affecting the phytochemical contents of plants, full use of this information is not always possible. For example, most fruits and vegetables can reach their maximum vitamin contents when mature, but harvesting usually takes place at an earlier stage in order to facilitate handling and transportation. This may also be true for plant material collected for their phytochemical contents, although little information exists on this topic. One obvious procedure to improve the quality and quantity of phytochemicals is to harvest when the contents of these active compounds reach their maximum levels. However, the growth period when this maximum occurs does not usually correspond to biological ripeness. For most medicinal plants, the period of the peak accumulation of their bioactive ingredients occurs during flowering or during the vegetation period for the part of the plant in the ground. Under these conditions, the time of harvest is not the time of biological ripeness, but the time of technical or technological ripeness.

9.3 Postharvest Treatments

The first operation that is routinely performed is to wash the raw material with water, particularly, for roots such as ginger, ginseng, and echinacea. It is also essential that any stones, metals or other hard objects be removed from leaves, roots, and stems before the biomass reaches the cutting, milling or grinding circuit. Two additional operations that are frequently used are dehulling, to remove hulls from seeds, and screening, to separate the large fragments from the smaller ones. Some plant materials, such as vanilla beans, require several months of curing and maturation and are graded on the quality of this preparation. Plant material, after careful preparation by drying, curing, and aging, must be kept under proper storage conditions. For instance, peppercorns stored under damp conditions, without the free circulation of air will develop a "musty" quality, which remains in the oil even after extraction.

After harvesting, the water content of the plant parts (leaf, stalk, flower, and root) will be high. As a consequence, these plant parts are very susceptible to rapid deterioration unless appropriate procedures are used to prevent postharvest metabolic reactions. Drying is the common processing operation used to increase the storage stability of plant materials. Removing unnecessary parts before drying and reducing the raw material into smaller particles will increase the drying rates and reduce energy consumptions. It is advisable to use the maximum temperature at which the bioactive compounds are stable and the exterior parts of the plant material do not degrade. Final moisture content of 10%–14% is sufficient to allow most plant materials to be stored for long periods without deterioration. The dried material will absorb humidity during storage and therefore should be protected from high humidity by proper storage methods.

In practice, the methods of drying are divided into two categories according to the heat source (natural and artificial). The natural drying procedures are the simplest and usually involve spreading the raw material out under the sun. This procedure saves energy and equipment costs and can be done in the immediate vicinity of harvesting. Artificial drying operations include cold-air drying, hot/warm-air drying in plate-chamber driers and conveyor drying.

9.4 Matrix and Cell Characteristics

The demands for materials for food ingredients that are health-promoting, including finely ground active substances and excipients are growing. A wide variety of cell wall structures exists in nature, ranging from very fragile to very tough. The more fragile the structure, the less shearing force is required to break the cells. Mammalian cells tend to be sheared-sensitive and require only a minimal energy input for disruption. Animal tissue cells can be easily disrupted by changing the osmolality of the surrounding area or by applying a low level of ultrasonic energy. Yeast and other forms of fungi have rigid cell walls containing polysaccharides. Plant cells are in

the order of 20–100 μm in diameter. The cell wall is cellulose-based, having a high tensile strength, but low resistance to "shear" (Krishnamurthy 1999).

In the recovery of bioactive compounds from biological material, the cell disruption process is the most important pretreatment in the extraction process. The efficacy of this step directly affects the subsequent separation operations, and any losses occurring at this initial stage cannot be regained. Regardless of the extraction methods, the ability to separate the bioactive components from the raw material matrix depends on the solubilizing power of the extracting solvents, component—matrix interactions, component locations within the matrix, and the porosity of the matrix.

This can be accomplished by either wet or dry processes. Conventional dry size reductions of material powders can be accomplished by impact size reduction. Equipment commonly used falls into the category of either mechanical impact mills or fluid-energy impact mills. Examples of mechanical impact mills are hammer and screen mills, pin mills, and air-classifying mills, spiral jet mills and fluid-energy mills, fluid-energy impact mills, etc.

The physical morphology of the matrix can also have a profound influence on the efficiency of the extraction. In general, the smaller the particle size, the more rapid and complete the extraction. This effect normally results from the shorter lengths of the internal diffusion path over which the solutes must travel to reach the bulk fluid phase. The extraction of the bioactive compounds from the biological tissue is largely determined by the rate of diffusion of these compounds through the internal volume of the biological matrix. For this reason, an increase in the surface area and porosity of the matrix will generally promote more efficient and rapid extraction. Grinding the sample is generally considered an effective and practical procedure to disrupt the raw material into small particles.

9.5 Physical Disruption of the Cell Wall

Physical disruption methods for plant material can be categorized into four classes: mechanical methods (hammer mill, high-pressure homogenization, and mechanical grinding), high-pulsed electronic field pulse treatment, ultrasonics, and nonmechanical methods. These methods are most amenable to large-scale applications in the modern food and pharmaceutical industries.

High-energy input from mechanical disrupters can lead to high temperatures, and labile compounds can be destroyed by the generated heat. Therefore, some type of cooling is necessary when heat-labile materials are to be extracted. Refrigeration systems can be used to keep the operating temperature around 4–15 °C and these low operating temperatures will also increase the viscosity of the grinding solution and increase the friability of the cells, leading to more efficient disruption.

Generally, mechanical disruption is preferred for large-scale industrial extractions. Bead milling and high-pressure homogenization are typical procedures when high shear forces are required to disrupt cell wall material. The criteria by which one

selects disruption methods include the amount of shear required by the products, the specificity of the methods, the need to control temperature, the total energy input, and the capital cost.

9.5.1 Hammer Mill

The terms milling, size reduction, comminution, grinding, and pulverization are often used interchangeably. Milling is a unit operation (Fig. 9.1) where mechanical energy is applied to physically break down coarse particles to finer ones and hence, is regarded as a "top-down" approach in the production of fine particles (Rabinow 2004). The "top-down" approach has wider commercial and industrial applications. Traditionally, milling is carried out to facilitate the extraction of crude material for the ingredients of health-promoting food or to improve their bulk processing properties. Cutter mills, roller mills, pestle and mortars, and runner mills may be employed for this purpose. In these milling operations, the dried crude material for the ingredients of health-promoting food may be cut by sharp blades (cutter mill), impacted by hammers or crushed/compressed by the application of pressure (roller mill, pestle, and mortar). As a limited amount of energy is imparted, the milled particles remain relatively coarse. Technological advancements in milling equipment now enable the production of ultrafine particles of the material for the ingredients of health-promoting food down to the micron or even submicron dimensions.

The hammer mill is the most widely used grinding mill. The hammer mill consists of a series of hammers (usually four or more) hinged on a central shaft and enclosed within a rigid metal case. It produces size reduction by impact. The materials to be milled are struck by these rectangular pieces of hardened steel (ganged hammers) which rotate at a high speed inside the chamber. These radically swinging hammers (from the rotating central shaft) move at a high angular velocity causing a brittle

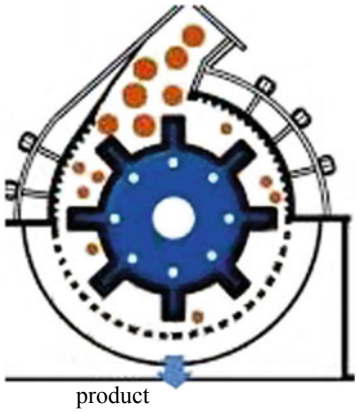

Fig. 9.1 Hammer mill

fracture of the feed material. Hammer mills produce a finished product with a size that is dependent upon openings in perforated screens or grate bars, the number, size, and type of hammers, the grinding plate setting, and the roto speed.

The reduction of the size of the material occurs by dynamic impact and by attrition and shear. The material is crushed or shattered by the repeated hammer impacts, collisions with the walls of the grinding chamber as well as particle-on-particle impacts. A screen is fitted at the bottom of the mill, which retains the coarse materials while allowing the properly sized materials to pass through as the finished product. In the feed processing process, there may be a number of ingredients that require some form of processing. These feed ingredients include coarse cereal grains, such as corn, which require particle size reduction which will improve the performance of the ingredient and increase its nutritive value. There are many ways to achieve this particle size reduction. Both hammering and rolling can achieve the desired result of producing adequately ground ingredients, but other factors also need to be looked at before choosing the suitable methods to use for grinding. Excessive size reduction can lead to wasted electrical energy, unnecessary wear on mechanical equipment and possible digestive problems in livestock and poultry.

Grinding of dry, low-fat ingredients can be accomplished with a hammer mill, although other types of material can be finely ground with a proper adjustment of the equipment. Hammer mills are impact grinders with swinging or stationary steel bars forcing the ingredients against a circular screen or a striking plate. The number of hammers on a rotating shaft, the speed of rotation, their size, arrangement, sharpness, wear pattern, and clearance at the tip relative to the screen are important in determining the grinding capacity and final appearance of the product. Heat may be of concern if the sample is held in the grinding chamber too long. Most hammer mills have a horizontal drive shaft with vertically suspended hammers. The sample material is held in the grinding chamber until its size is reduced to the diameter of screen holes or smaller. Passing through the screen, the particles are carried by gravity outside the mill, and then by air or conveyor belt to the storage bins. Oversized particles, not easily broken up, drop through the mill and maybe recycled or discarded. Thus, foreign materials, such as metal and stones, are discharged before they are forced through the screen causing damage (Hasting and Higgs 1978).

The major components of these hammer mills are shown in the picture. A delivery device is used to introduce the material to be ground into the path of the hammers. A rotor comprised of a series of machined disks mounted on the horizontal shaft performs this task. The free-swinging hammers are suspended from rods running parallel to the shaft and through the rotor disks. The hammers carry out the function of smashing the material and ingredients in order to reduce their particle size, the perforated screen, and either gravity- or air-assisted removal of the ground product. It is necessary to screen the particle size of the hammer mill to ensure particles meet a specified maximum mesh size. The hammer mill is used in pharmaceutical industries to process wet or dry granulations and disperse powder mixtures. It is also used in milling pharmaceutical raw materials, herbal medicine and sugar. The hammer mill can be used in the powdering of barks, leaves, and roots of medicinal plants, and

it can also be applied in the milling of active pharmaceutical ingredients (APIs), excipients, etc.

9.5.2 High-Pressure Homogenization

The production of nanosuspensions using high-pressure or piston-gap homogenization is a high-energy process in which the reduction in the size of the particles of health-food material is achieved by repeatedly cycling, to 200 plus cycles, with the aid of a piston, a drug suspension through a very thin gap at a high velocity, around 500 m s^{-1}, and pressure, 1000–1500 bars (Muller et al. 1998). The width of the gap, which generally falls within the range of 5–20 mm, may be adjusted according to the viscosity of the suspension and the applied pressure. Pre-micronization of the starting materials using a process like fluid-energy milling may be necessary prior to homogenization. This is to minimize clogging of the homogenization gap and to reduce milling time. When the suspension is forced through the gap at a high flow rate, the static pressure exerted on the liquid falls below the vapor pressure of the liquid at the prevailing temperature. As a result, the liquid boils and gas bubbles are formed which collapse when the liquid leaves the gap and normal pressure is resumed. The powerful cavitation forces arising from the formation and collapse of the gas bubbles, coupled with a shearing effect, bring about the nanonization of the microparticles of the health-food material (Jahnke 1998; Mohr 1987; Pandolfe 1981).

High-pressure homogenization is the most widely used method for large-scale cell disruption. A solution of cells is subjected to high shear stresses when passing through a restricted orifice under high pressure, resulting in cell disintegration. The equipment consists of a high-pressure reciprocating positive-displacement pump with one or more adjustable, restricted orifice valves. As shown in Fig. 9.2, the microparticles are forced through the gap in the micronizing zone, which creates the conditions of high turbulence and shear, combined with compression, acceleration, pressure drop, and impact, leading to the formation of the fine sizes of the materials.

All commercial models operate on the same principle and are distinguished by their capacity, the type of homogenizer valve, the pressure range, the drive mechanism, and the number of pistons. High-pressure homogenizers generate a rapidly moving liquid stream that impinges against either an immovable surface or a second high-pressure stream. The cell suspension is forced through the central hole of the valve seat, where it impinges against the valve and is forced around the valve past the impact ring into a low-pressure chamber (Bond et al. 1987; Kershavarz et al. 1987).

Hetherington et al. (1971) elucidated the effects of pressure, the number of passes, the temperature and the cell concentration, and put forward a kinetic model for the disruption of yeast cells. Various designs for the valve and valve seat (cell disrupter) and the flat valve seat (standard valve) are commonly used for microorganism disruption (Fig. 9.2). Cell disruption usually follows first-order kinetics and depends on the pressure and the number of passes through the valve assembly or orifices (Ker-

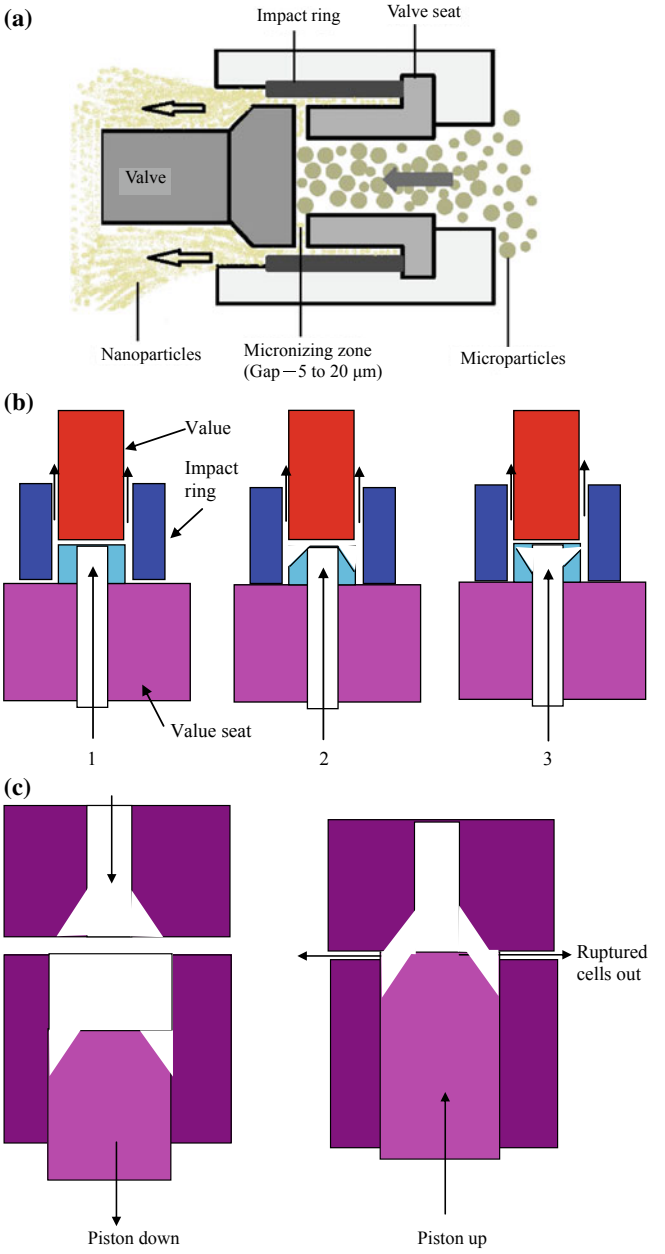

Fig. 9.2 Schematic diagram of the high-pressure homogenization process. **a** Schematic diagram of the high-pressure homogenization process. **b** Details of homogenizing valve units; ① flat-edge "Standard" unit; ② knife-edge "Cell Rupture" unit; ③ knife-edge "Cell Disruption" unit. **c** A homogenization valve. Cell suspension in liquid material. Cells are ruptured by both shear and mechanical stress

shavarz et al. 1987). As the pressure and the number of passes increase, the amount of cell breakage increases accordingly. Breakage efficiencies of 90% or higher usually require multiple passes and pressures of 35,000–140,000 kPa, with corresponding velocities of 180 to 280 m s^{-1}, are common. The amount of disruption at a given pressure also depends on the type of organism involved. Organisms with stronger walls will require higher pressure to achieve a given level of breakage. Increasing the operating pressure on high-pressure homogenizers adds considerable heat to the process stream, and some type of cooling is frequently required, especially with heat-labile products.

9.5.3 High-Speed Bead Mills

Ball milling is a popular technique for size reduction that is used for the production of microparticles, especially in research laboratories. Fundamentally, a ball mill is made up of a vessel or vial filled with balls, or rods, constructed from a variety of materials such as ceramics, agates, silicon nitride, sintered corundum, zirconia, chrome steel, CreNi steel, tungsten carbide or plastic polyamide. The material to be milled is placed inside the vessel, which is made to rotate or vibrate at a particular speed or frequency. The movement of the vessel causes the balls to cascade or move in a particular pattern, colliding with each other and with the opposing inner wall of the vessel. Size reduction of the drug particles is effected from the impact they receive from the balls as well as the attritive forces arising from the movement of the balls relative to each other. Bead mills are used in the industry for fine grinding and disruption of plant and animal cells. The design of the bead mills varies with the size and the manufacturer (Fig. 9.3). Examples of the use of bead mills have been given by Chisti and Moo-Young (1986) for the disruption of *Aspergillu* and by Currie et al. (1972) for the disruption of yeast cells. The mills consist of either a vertical or a horizontal grinding chamber containing impellers or rotating disks mounted, concentrically or off-centered, on a motor-driven shaft. The grinding action is provided by glass or plastic beads typically occupying 80%—85% of the free working volume of the chamber. Disruption can be batch-wise or continuous. The units must be equipped with a high-capacity cooling system when processing temperature-sensitive materials. Horizontal units are generally preferred for cell disruption, as the grinding action in vertical mills is reduced due to the fluidizing effects of the upward fluid flow on the beads (Kershavarz et al. 1987).

In one design of a mechanical grinder, a shaft with multiple disks spaced at regular intervals along its length turns at high speed, causing the beads to impact against the cells. Disruption of the cell results from the impact and shear force applied against the cell wall by the grinding medium. The major parameters of concern for the scaling-up of mechanical grinders are the grinding medium and the shaft speed, or the peripheral velocity. For more consistent results, the grinding medium should be uniformly sized (0.1–2 mm in diameter). The smaller bead sizes tend to be more

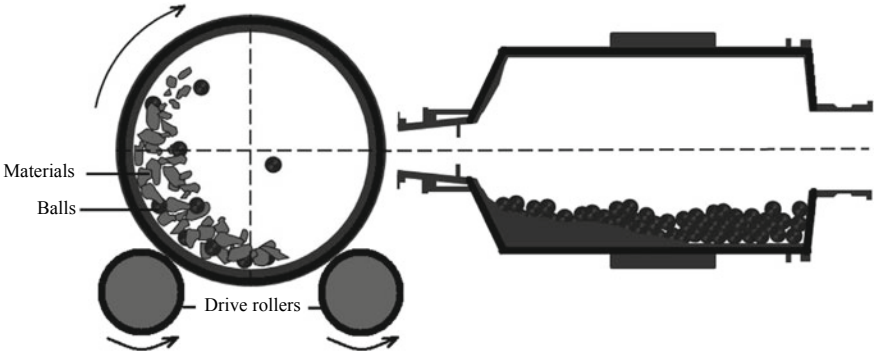

Fig. 9.3 Schematic diagram of a ball mill. The balls make up the grinding media and drive rollers help to rotate the milling chamber

disruptive because of the increased number of contact points between the beads and the cells.

The rate at which the agitator shaft rotates influences the rate at which the grinding beads impact the cells, and therefore a higher shaft speed typically leads to more rapid disruption. When scaling-up the process, the peripheral velocity is used rather than the shaft seed because the optimal shaft speed depends on the diameter of the grinding chamber, while optimal peripheral velocity is independent of vessel size. The peripheral velocity that can be achieved depends on the viscosity of the material, the type of grinding medium, and the type of agitator disk. Large-scale units typically use a slotted screen with openings small enough to prevent the passage of the grinding medium. Bead mill grinders handle flow rates up to about 10 r s^{-1}. The optimal type of cell disruption depends on the application and the scale.

9.5.4 The French Press Cell Disrupter

The French press cell disrupter (Fig. 9.4) is a shearing device that subjects liquid cell suspensions to pressures as high as 20,000 psi. (1 psi = 137.90 MPa) The sample is loaded into a chamber and the pressure is increased by using a piston until the required pressure is reached. As the piston pressure increases, the chamber pressure increases, causing the intracellular pressure of the cells to also increase. The cells are then released through a nozzle and collected. As the sample is dispensed through the nozzle, the external pressure on the cells falls rapidly to atmospheric pressure. The pressure inside the cells also drops, but at a slower rate, and different pressure causes the cell wall and membrane to burst, releasing the intracellular contents and freeing them for collection or separation. The French press is generally used for small-scale processes but can be scaled up for larger operations. As with most mechanical techniques, the energy imparted to the cell suspension becomes converted to heat

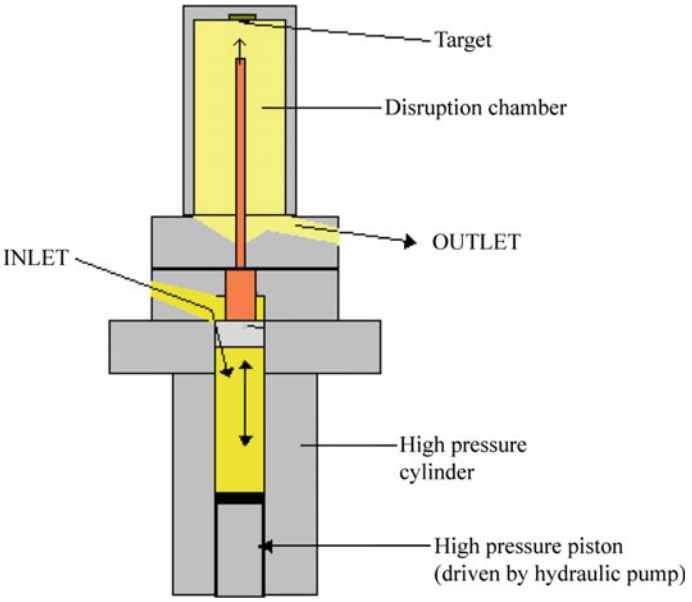

Fig. 9.4 French press cell disrupter

and therefore the system must be cooled to prevent product damage (Garcia et al. 1999). The French press can process large volumes in a short time, but its operational efficiencies make it less attractive than other large-scale disruption processes.

9.5.5 Ultrasonic Treatments

Sound is a mechanical wave created by a vibrating object (sonicator tip) and is propagated through a medium from one location to another (Fig. 9.5). In liquid media, sound waves are longitudinal, which means the particles of the medium move parallel to the direction of the wave. Longitudinal waves undergo compression, where particles in the wave are closest together, and rarefaction, where particles in the wave are farthest apart. Ultrasonic disruption occurs when sound waves with a frequency on the order of 20 kHz are converted to very rapid vibrations in a liquid, causing a phenomenon known as cavitation. The ultrasonic energy is transmitted to the liquid through a "sonicator" (or "sonifer") tip, or "horn". The very rapid vibrations of the ultrasonic device cause local low-pressure areas within the liquid. The pressure in these areas is low enough to convert the liquid to gas in the form of very small bubbles. As the local pressure changes again and begins to rise, these bubbles collapse. The collapse of these bubbles causes a shock wave to travel through the liquid, resulting in a shear force that will disrupt cells (Misonix Incorporated 2000). A major problem

with sonication is the excessive buildup of heat in the sample and potential for foaming. One way to avoid this is to use the sonicator in a pulsed mode and to let the sample cool between intervals. However, there is still the potential for heat degradation and it is also more time-consuming. The amplitude of the vibration at the end of the horn is inversely proportional to the diameter of the tip, e.g., large-diameter tips deliver less sonic energy than smaller tips do. The rapid vibration of the tip generates a large amount of heat that must be dissipated by turning the sonicator off every few minutes during use.

9.5.6 Pulsed Electric Field Treatment

The pulsed electric field (PEF) treatment is a nonthermal method to provide cell plasmolysis. As shown in Fig. 9.6, an electrical pulse consists of a burst of electrical energy. A pulse is defined as the sum of two-step functions in the time domain. The first step function is the leading edge of the pulse, and the second is the trailing edge. The duration of the pulse, or the pulse width, is the time between the two-step functions. The pulse waveform is the summation of the positive and the negative step functions separated by the duration. The most commonly used D.C. electrical waveforms are the rectangular (or square wave) and the exponential decay. A rectangular pulse is generated by gating the output of a high-voltage power supply, while an exponential decay pulse is generated by discharging an R-C circuit. Pulsed power refers to the general technology of accumulating energy compressed on a relatively long time scale (pulse charging, slow systems) with energy in time and space compressed to deliver large power pulses (pulse discharging, high-speed systems) to a desired load. These pulses may last from hundreds of picoseconds to tens of seconds (Ho and Mittal 2000).

Orientation and assembly of some high-voltage pulse generators and treatment chambers for batch processing are summarized by Ho and Mittal (2000). A typical batch treatment system is made up of a high-voltage pulse generator and a treatment chamber. Other auxiliary devices may also be used for the degassing, vacuuming, preheating, and cooling of the treatment medium. Most batch treatment chambers are made up of two parallel electrodes spaced apart to create the treatment volume. Continuous systems utilize the same operating principles, but the concept of flow dynamics has to be implemented in the design process so that the product can receive the necessary treatment conditions. Because the majority of researchers have been using small-size, batch mode operations, there are only a few studies that describe the concept of a continuous system, and there are even fewer studies that report experimental studies on them.

Ho and Mittal (1996) reviewed discoveries and theories on the mechanisms of cell electroporation using PEF. Studies on transmembrane potential (TMP) and pore dynamics remain a difficult task. Since the area of electroporation on a biomembrane is small (less than 0.1% of the total surface area) and the time sequence of electropores is in the submicrosecond range, measuring devices with subtle detec-

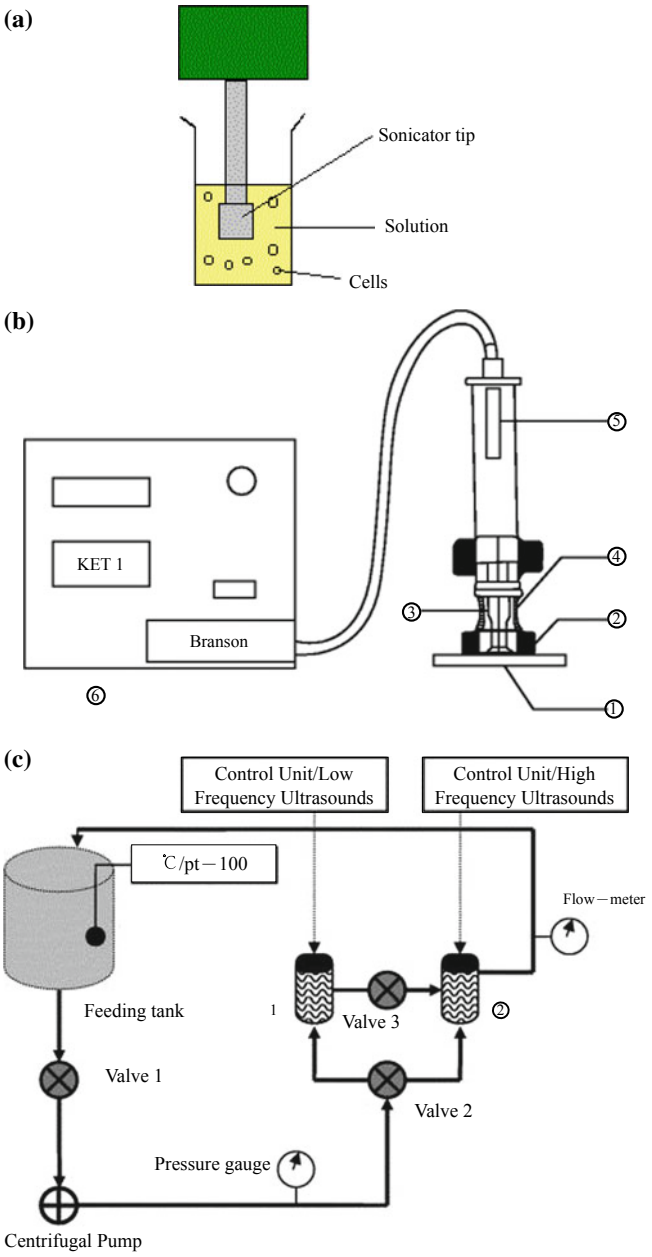

Fig. 9.5 Sonicator and application. **a** Sonicator in lab scale. **b** Schematic diagram of the experimental apparatus used for sonication: ① test surface; ② rubber join; ③ barium titanate transducer; ④ active chamber; ⑤ ultrasound generation key; ⑥ ultrasound generator. **c** Diagram showing capacity of the ultrasonication apparatus used to work in both batch and continuous conditions: ① ultrasonic ceu/low frequency ultrasounds; ② ultrasonic ceu/high frequency ultrasounds

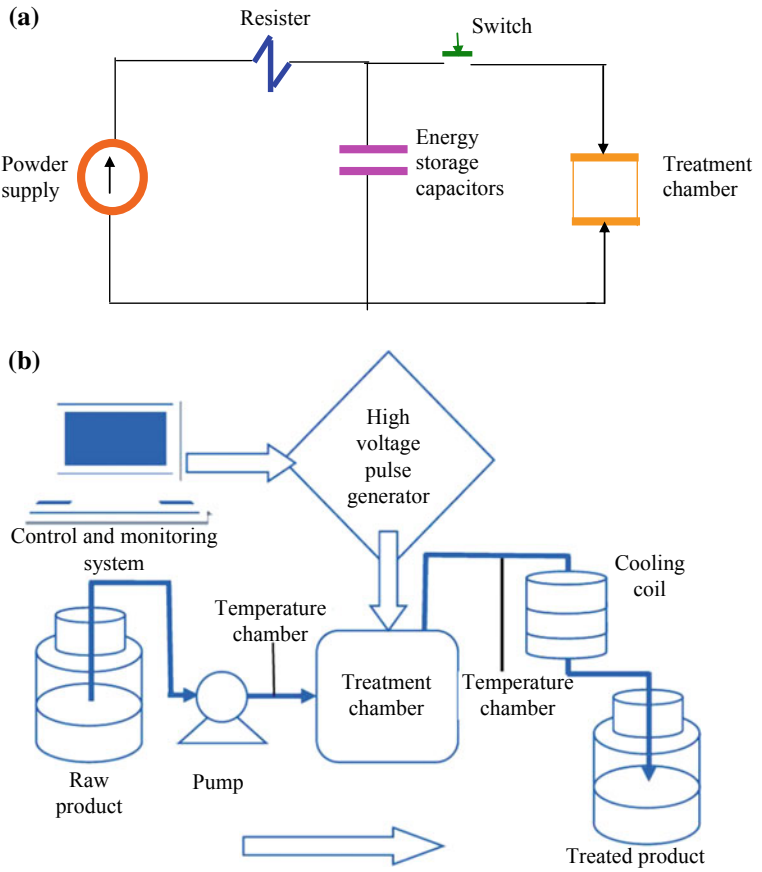

Fig. 9.6 The pulsed electronic field treatments for foods. **a** The principle of pulsed electronic field treatment; **b** schematic representation of the mechanism of high-pulsed electronic field treatment

tion and time resolution are required. While more and more studies have shown the formation sequence of electropore(s) at specific locations on various biomembranes, the widening process of the pore(s) and the subsequent membrane breakdown mechanisms remain controversial. The influence of electromechanical stress or TMP discharge and rupture seems to be a function of various factors, such as membrane properties, external medium, and the protocols of electroporators.

Electroporation is the widely accepted concept for describing the phenomenon of cell membrane discharge and breakdown under the application of short electric field pulses. It is thought that a significantly increased TMP results in membrane structure conformation, and electropores (aqueous pathways) are formed and perforated on the membrane. These pores can either be resealed or remain opened depending on the magnitude and frequency of the electric pulses.

The use of PEF to permeabilize the cell membrane of plant materials was reported initially by Coster (1965), and Scheglov (1986) used increased electrical field of 50 to 400 V cm^{-1} between 20 and 40 mm electrode gaps. This resulted in increased cell permeability of the pulp and higher juice yield upon processing. The yield of apple juice increased by up to 5%, and by 10% for blackberries and tomatoes, respectively. According to Papchenko et al. (1988), this process improved the quality of the juice as more ioche, vitamins, and micronutrients were extracted from the plant cells after the rupture of their walls. According to McLellan et al. (1991), the heated mash of apples (49 °C for 15 min, and 60 °C for another 15 min) and electroplasmolysis (240 V D.C. for 1.5 s) produced a more rapid release of juice under identical press conditions compared with enzymatic treatments. The heated mash and electroplasmolysis (EP) treatments resulted in a yield of a few percentage points higher than the control and enzymatic treatments. The EP treatment had about half the amount of suspended solids compared with the heated sample, yet almost double that of the enzyme and control samples. This indicated that cellular breakdown took place during the mash heat and EP treatments. The EP treatment delivered a product with a lighter color and that was less oxidized than the other treatments. The heated mash produced the darkest and most oxidized juice of the treatments.

Electroporation has been used to permeabilize the suspension cultures of plant cells producing secondary metabolites for product release (Knorr et al. 1994). Knorr et al. (1994) applied 1–10 pulses of 0.10–15 kV cm^{-1} electric field strength. The effectiveness of permeabilization by high-voltage electric pulses was cell-specific and depended on the location of metabolites within the cells (cytoplasm or vacuoles). This was recommended as a rapid method for the recovery of the desired iochemical from plant cells without the need for chemical or thermal treatment. The treatment of finely (1.5 mm diameter particles) or coarsely ground (3 mm diameter particles) carrots with high-voltage electric pulses (up to 2.6 kV cm^{-1}, 50 pulses) followed by expression (10 Mpa, 5 min, room temperature) resulted in a maximum juice yield of 76.1% as compared with 51.3% for the control in finely ground samples; and a yield of 70.3% as compared with 30% for the control for coarse ones (Knorr et al. 1994). Fifty of these pulses were considered optimum at 1.6 kV cm^{-1}. Carrot mash treated with a high voltage by means of high-voltage electric pulses was cell-specific and depended on the location of the metabolites within the cells (cytoplasm or vacuoles). This was recommended as a rapid method for the recovery of the desired iochemical from plant cells without the need for chemical or thermal treatment. The treatment of finely (1.5 mm diameter particles) or coarsely ground (3 mm diameter particles) carrots with high-voltage electric pulses (up to 2.6 kV cm^{-1}, 50 pulses) followed by expression (10 Mpa, 5 min, room temperature) resulted in a maximum juice yield of 76.1% as compared with 51.3% for the control in finely ground samples; and a yield of 70.3% as compared with 30% for the control for coarse ones (Knorr et al. 1994). Fifty of these pulses were considered optimum at 1.6 kV cm^{-1}. Carrot mash treated with high-voltage electric pulses resulted in lighter colored, less oxidized products with higher β-carotene values than samples pretreated with a commercial pectinase (Rohament P, 80 min at 45 °C, pH 4.5). Maximum juice yields for the enzyme-

treated juices were 68.8% for finely ground samples, and 61.8% for coarsely ground samples.

Jemain and Vorobiev (2001) investigated the impact of a moderate PEF treatment on the diffusion characteristics of apple slices. After PEF treatment (0.536 kV cm^{-1}, 1000 pulses of 100 μs duration, 8 °C temperature increase), the juice diffusion coefficient (D) was about 3.9E−10 m^2 s^{-1} at 20 °C compared to the reference value of 2.5E−10 m^2 s^{-1} for thermally denatured samples. Results indicated that PEF has desired effects on the structure and permeability of apple slices over a thermal treatment. The enhanced diffusion of soluble materials seemed to be due to a better structural reorganization after PEF treatment.

Bazhal and Vorobiev (2000) reported an increase in the juice extraction from fruits by cell membrane permeabilization using PEF. Geulen et al. (1994) observed that carrot juice extraction increased from 62% to 75% using PEF. Eshtianghi and Knorr (2000) noted that the juice yield from grapes, apples and black currants increased using PEF. The PEF treatment significantly improved quality indicators (acidity, soluble dry matter, and coloring matter) in fruit juices. The PEF effect was dependent on electrical field strength and the number of pulses affecting cell disintegration (Eshtianghi and Knorr 2002). Sugar beet extraction can be performed using the PEF treatment at low temperatures followed by pressure extraction to obtain a maximum juice yield. The PEF treatment also reduced energy and time requirements for extraction. The pulp obtained from PEF-treated samples contained more dry matter (30%) than the pulp from the conventional thermal process (15% dry matter) at 2 MPa. A uniform and tight packing of the raw material between electrodes may improve the effectiveness of the PEF treatment. Large amounts of extra-particle highly conductive liquid increased electrical energy losses due to a large current flow (Bazhal et al. 2001). The combination of pressing (3 bars, 5.4E+3 to 1E+4 s) and PEF treatment (0.10–0.52 kV cm^{-1}, 50 pulses of 100 μs width) provided optimum apple juice yield compared to untreated samples (Bazhal et al. 2001).

Ade-Omowaye et al. (2001) reported an extraction yield of about 91% and a good quality juice from paprika mash after the PEF treatment (1.7 kV cm^{-1}, 30 pulses, 0.5 kJ kg^{-1} energy input) followed by pressing at 10 MPa for 4 min. Juice from PEF-treated mash had more redness in color, a higher amount of β-carotene and vitamin C contents when compared to enzyme-treated or untreated juice samples. β-carotene has been reported to reduce the risk of skin cancer, increase immune response and protect against liver damage (Chen et al. 1996).

In cell walls and membranes, protein channels, pores, and intercellular spaces exist. The closing and opening of many channels made up of proteins are dependent on a transmembrane voltage difference (Tsong 1990; Neumann et al. 1989). When a pulsed electric field is applied, many voltage-sensitive protein channels may open before the transmembrane voltage difference reaches the breakdown value of the lipid bilayer. High-pulsed electronic field treatment involves applying repeated short pulses of high-voltage electricity to a fluid food flowing between two electrodes (Fig. 9.6). The process ruptures the cell membrane and cell wall. Treatment is carried out at an inlet ambient or refrigerated temperature for less than one second, and energy

lost due to the heating of foods is thus minimized. The treated material retains its "fresh" physical, chemical, and nutritional characteristics.

9.5.7 Some Other Physical Cell Disruption Processes

Some physical cell disruption techniques, such as osmotic shock and freeze-thaw, can also be used. In each case, the cell membrane may either be totally disrupted or made partially permeable to allow the bioactive components to escape from the cell matrix.

Osmotic shock is the sudden introduction of cells into a solution of low osmolarity as shown in Fig. 9.7, such as the transfer from 25% sucrose solution into pure water. If the change is rapid, ions from inside the cell are not able to migrate through the cell wall before the osmotic pressure differential causes the rupture of the cell. Under conditions of high concentrations of either salt, substrates or any solute in the supernatant, water is drawn out of the cells through osmosis. This also inhibits the transportation of substrates and cofactors into the cell thus "shocking" the cell. Alternatively, at low concentrations of solutes, water enters the cell in large amounts causing it to swell and either burst or undergoe apoptosis. This technique is highly efficient but is not well-suited for large-scale processes where large amounts of fluids are involved.

Freeze-thaw is another procedure that can be used to rupture cells. Slow freezing rates will promote large ice crystal growth inside the cells and these large crystals are responsible for cell disruption. The disrupted cell paste is gently warmed to prevent the loss of labile compounds. This method is slow and inefficient and has a high cost, and as a result has not gained widespread commercial acceptance.

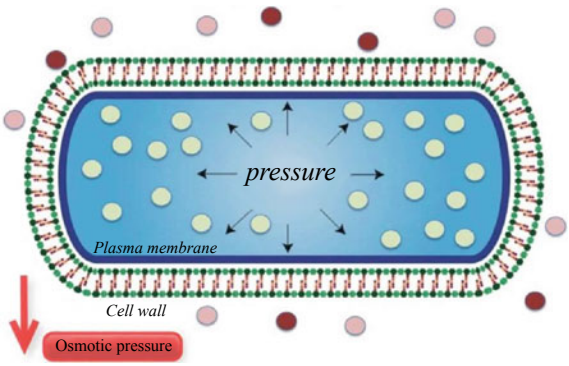

Fig. 9.7 The principle of osmotic shock to break the cell membrane

9.6 The Development of Innovative Technology

Micronization is a term used to describe size reduction where the resulting particle size is less than 10 microns. Micronization size reduction involves the acceleration of particles so that grinding occurs by particle-to-particle impact or impact against a solid surface. Fluid-energy mills are used for micronization because of the high impact velocities that are possible as a result of particle acceleration in a fast gas stream. Particle velocities in a jet mill are in the range of 300–500 m s^{-1}, compared to 50–150 m s^{-1} in a mechanical impact mill. In fact, the generic term has been used to describe various types of spiral jet mills or "pancake mills". There are many and varied reasons that manufacturers choose to grind pharmaceutical powders. Among these are increased surface areas, improved bioavailability, and increased activity. Dry powder inhalants and injectable compounds benefit from finer and more defined particle size distributions. Reproducible steep particle size distribution, those with a minimum of fine particles and strict control of oversized particles, combined with improved methods to measure particle size distributions has a change in micronizing techniques. The spiral jet mill is gradually yielding to the next generation of higher technology fluidized-bed jet mill which combines high-energy micronization with an integral forced vortex air classifier. This combination allows greater control of the maximum product particle size and usually a reduction in generated fines. In pharmaceutical products, the particle size and components may affect processing and bioavailability.

As an alternative to traditional techniques, various supercritical fluid-based processes have been proposed (Fig. 9.8). A novel technique, the rapid expansion of supercritical solutions (RESS), has recently been developed for the micronization of particles. In the RESS process, material is immersed in a supercritical fluid, resulting in a solute-laden supercritical phase. By reduction of the pressure across an expansion device, fine particles with a narrow size distribution can be obtained. One advantage of micronization by the RESS process is the ability to produce a solvent-free product without the need for additional solvents or surfactants. Low critical-temperature solvents such as CO_2 (T_c, 31.1 °C) can be used for the thermally labile materials without the risk of degradation. In recent years, micronization processes, using a supercritical fluid have been extensively studied.

9.7 Summary

Before the extraction and purification of biologically active compounds from plant material can begin, the plant cells must be disrupted, and the crude phytochemicals can be extracted. The choice of the disruption technique depends on the strength or toughness of the cellular material, the desired purity, and stability of the target compounds, and the time of disruption. Mechanical procedures that use high shear forces to rupture the cell include homogenization, ultrasonic cavitation, mechani-

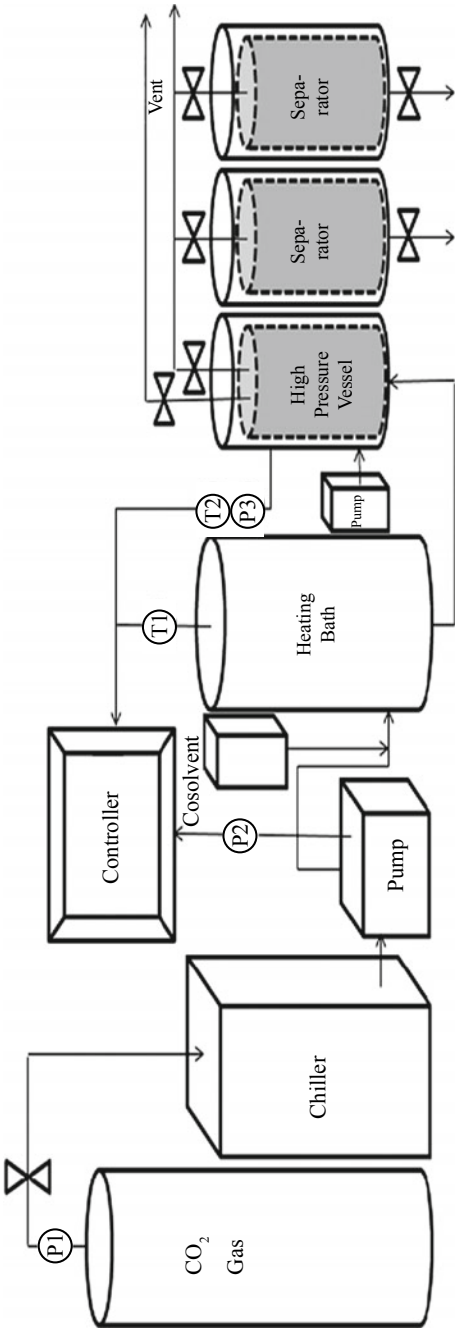

Fig. 9.8 Supercritical fluid-based processes to develop micronization of particles

cal grinding, the high-pressure French press, and bead mill. On a laboratory scale, these procedures are effective, but problems can arise when scale-up procedures are attempted. A combination of mechanical treatments, such as a French press or sonication, coupled with some chemicals or enzymatic pretreatments could lead to a more complete extraction. The more cell wall barriers can be disrupted without destroying the target compounds, the greater the yield and purity of the extracted phytochemicals. Some innovative technologies such as micronization, supercritical fluid treatments show a great promise, especially for the high-value material for the ingredients of health-promoting food.

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Chapter 10
Infrared Heating for Improved Drying Efficiency, Food Safety, and Quality of Rice

Ragab Khir, Chandrasekar Venkitasamy and Zhongli Pan

Abstract Infrared radiation (IR) heating has a promising potential to achieve high drying rate, energy saving, effective disinfestation, and disinfection of rough rice. It can also inactivate lipase and extend the storage lives of both rough and brown rice. Moreover, IR can effectively stabilize the rice bran and improve its utilization without affecting the quality of rice bran oil. Consequently, through the recent investigations about IR heating on rice, it has proven the feasibility of simultaneous drying, disinfestation, disinfection, and stabilization. This chapter summarizes the drying characteristics, milling quality, sensory quality, effectiveness of disinfestation and disinfection, rice bran stabilization, and storage stability of rough rice under IR heating. The outcomes of recent studies on rice drying by using IR heating have clearly revealed that a high heating rate, fast drying, good quality, and improved food safety can be achieved. IR drying provides a potential to store brown rice instead of rough rice to retain all benefits with reduced costs. Additionally, IR drying could effectively maintain the stability of physicochemical characteristics of rice during storage. It has been confirmed that IR heating followed by natural cooling should be an effective approach for designing IR rough rice dryers.

Keywords Infrared · Rice · Drying · Disinfestation · Disinfection · Stabilization · Storage · Quality

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10.1 Introduction

Rice (*Oryza sativa* L.) is one of the leading food crops in the world and the staple food for more than half of the world's population. It also plays a vital role in the world economy for several reasons. Rice is one of the most profitable field crops for growers and is considered as one of the essential export crops in producing countries. It has an important socioeconomic impact because of the large number of labors employed in the rice production sector. Currently, there are two main challenges in rice production: the importance of increasing production to provide for the growing population and the improvement of rice processing to maintain its quality while minimizing losses to meet the market requirements. Therefore, there is continued need to increase rice production but this necessity is hindered by the lack of availability of land, water, labor, chemicals, and technological means. Meanwhile, it is also important to enhance quality and safety, and reduce losses occurred during rice harvest, handling, and storage.

Rice drying plays a crucial role in the economy of the rice industry. The rough rice is normally dried using the conventional heated air method. However, this drying method is a slow process because heated air at a relatively low temperature must be used to preserve the rice milling quality. Additionally, it is difficult to quickly and uniformly heat the rice to a high temperature during convective drying because the rice is not in a thin layer and the temperatures of the rice kernels are limited to the wet bulb temperature of the drying air if no secondary heat sources are taken into account. Moreover, due to the relatively low air temperature, the convective drying process is not able to kill the insects in infested rough rice and inactivate the lipase enzyme responsible for lipid degradation during the rice storage period (Pan et al. 2008).

IR heating offers many advantages over the conventional drying method. It has shown a promising potential as an efficient processing method with high-quality finished food products, including dried fruit, nuts, and grains (Li and Pan 2013; Khir et al. 2011). IR drying is fundamentally different from convective drying because the material is dried directly by absorption of IR energy rather than transfer of heat from the air (Ginzburg and Grochowski 1969). Even though the penetration is limited, IR can significantly improve the heat transfer rate of food products, as well as the heat delivered into the products when compared to convective heating. Since IR heating does not depend on the medium, the temperature of rice grains is not limited by the wet bulb temperature of surrounding air and a high heating rate can be achieved. Therefore, IR drying demonstrated the high drying rate, good milling quality, effective disinfestation and disinfection of rough rice, and effective stabilization of rice bran with high processing efficiency and low energy consumption. The research also showed that IR drying provided a potential to store brown rice instead of rough rice (Pan et al. 2008, 2011; Khir et al. 2011, 2014; Ding et al. 2015a, b, 2016; Wang et al. 2014).

This chapter elaborately discusses the technical feasibility of using IR heating as an effective technology for rice drying achieving multiple goals in a single step.

Drying characteristics and quality of rough rice under IR heating, and appropriate conditions of IR heating, tempering, and cooling treatments to achieve high drying efficiency, quality, and effective disinfestation, disinfection, and stabilization of rough rice have been included in this chapter as well.

10.2 Rice Drying

10.2.1 Importance

Rough rice drying is a critical post-harvest handling process and has a direct effect on rice quality, subsequent handling processes, and commercial value of rice crop (Khir et al. 2011). Rough rice is normally harvested at a moisture content higher than the required moisture content of 12%–14% (wet basis) for safe storage (Pan et al. 2008). The drying is an important post-harvest operation in prolonging the storage life of rice by slowing down respiration and preventing deterioration due to molds and insect attack. Therefore, rough rice needs to be dried as soon as possible for improved storability and reduced handling costs and losses.

Annual loss of rice grains from harvesting to consumption is estimated to be 10%–25% (Carl 1980). The magnitude of these losses varies from country to country. These losses are significantly high in developing countries because of unfavorable climates which cause deterioration of stored grains and also because of lack of knowledge and proper facilities for drying and storage. Therefore, great efforts are being made to improve drying and storage facilities, especially in developing countries.

Proper drying and storage practices could likely result in 10%–20% increase in rice availability in some developing countries and the increased rice supply could be crucial in nourishing hungry people in these countries (Carl 1980). The reduction in post-harvest losses depends on the efficiency of the drying process. Thus, proper drying permits longer storage of grains without deterioration of quality, a better quality product for farmers' consumption and for sale, the continuous supply of the product throughout the year, the ability to take advantage of higher prices after harvesting season, early harvest which reduces field damage and shatter loss, the maintenance of viability, the ability of farmers to use and sell better quality seeds, and a better use of land and labor (Bala 1997).

10.2.2 Moisture Content

Moisture content (MC) is one of the most important factors affecting storage and subsequent handling processes, as well as the rice quality. The MC at which rice is harvested directly affects its total yield and quality. Therefore, the drying process is needed to reduce moisture content to a safe level suitable for storage, preserving grain

quality, and preventing rice product from physical and chemical changes. Lowering MC to acceptable levels involves heat and mass transfer sub processes which are fairly well established. However, changing the MC of rice, like most hygroscopic materials, causes corresponding changes in many physical properties, the summation of which can cause hygroscopic stress within kernels. If localized stresses reach levels greater than the kernel material's strength, fissures can form due to material failure (Cnossen et al. 2000, 2003). Thus, the challenge associated with rice drying is not only to reduce MC, but also to reduce it without compromising the physical integrity of the kernel. Additionally, drying should be accomplished without imposing chemical damage that would degrade rice functionality (Elaine 2004).

If rice with high MC is not dried within a given period, respiration processes could cause reduction in quality in terms of discoloration, functionality, and sensory properties. Thus, drying in a timely fashion is critical to maintaining quality at its highest possible level at the start of post-harvest operations. It is also important because other processing operations, like rice milling, depend on the quality of the dried product (Henderson and Perry 1976).

10.2.3 Drying Methods

Typical rice drying methods include natural (sun) drying, ambient air drying, and heated air drying. The natural drying is the traditional method widely used by farmers in developing countries. It is performed before or after threshing by spreading freshly harvested rough rice over the ground or plastic mat to dry under direct sun radiation. Most investigations indicate that natural drying has a high grain crack percentage and losses. Considerable losses (10%–25%) occurred during sun drying in the field for various reasons, such as rodents, birds, spoilage, and contamination (Helmy et al. 1995). During natural rice drying, the crack percentage was 10.6%. This high percentage is attributed to a long drying period and sorption and adsorption of moisture during the drying period. The rice grain breakage percentage was 15.7% for rice dried with natural drying and then milled with an abrasive type milling machine (Abdelmotaleb et al. 2001).

Drying by blowing ambient air at local site conditions is considered a simple method. It is performed by blowing ambient air with fans under different flow rates and local conditions through the rice storage location. But it is dependent on weather conditions and needs more energy to be conducted. Hindy et al. (2001) mentioned that drying by ambient air in early harvest time increased the mold growth rapidly and increased dry matter losses. Therefore, the drying process in this case needs higher air flow rates and a huge fan size to carry the task. They also mentioned that rice harvested late needs longer drying time because of lower temperatures of ambient air at harvest time.

Heated air drying is the simplest, most common and economical industrial process as far as the drying of grains is concerned. However, it is a slow and energy intensive process, which has negative impact on the economics of rice production (Donald

et al. 1992). During heated air drying, only relatively low temperatures of heated air are used to avoid damage in the milling quality. When rough rice is dried with heated air, drying occurs through convection and conduction. The heated air warms the outer layer of the rice grain first and causes the moisture to evaporate from the grain surface into the drying air. As the moisture is removed from the outer layers of the grain, moisture and temperature gradients are established within the grain. These gradients cause stresses in the grain, resulting in rice grain breakage during the milling process (Ban 1971; Kunze and Choudhury 1972; Kunze 1979).

The current rice drying practice normally uses multiple drying passes by removing a relatively small amount of moisture (2%–3%) in each pass and exposing the rice to a relatively low heated air temperature of up to 54 °C for 15 to 20 min to minimize the moisture gradient generated during drying (Kunze and Calderwood 1985). After each drying pass, the heated rice is tempered by keeping the rice from 4 to 24 h to allow the moisture inside the rice grain to become equilibrated before it is further dried. However, it was also reported that the reduction of head rice was influenced by the amount of moisture removed within a time interval, rather than by the temperature of the drying air, which indicated that a certain amount of moisture can be quickly removed with high temperature without significantly lowering the head rice yield (Stipe et al. 1972).

It is important to develop a drying method that can shorten the drying time with reduced energy consumption and maintain rice milling quality. Based on rice's thermomechanical properties, such as expansion ratio and specific volume of starch varying with moisture content and temperature, a glass transition hypothesis has been proposed and investigated for rice drying (Perdon et al. 2000; Siebenmorgan et al. 2004). It has been reported that rough rice could be dried with high air temperature (60 °C) at a rubbery state or above glass transition temperature to remove a large amount of moisture in a single pass without reducing the head rice yield (Cnossen et al. 2000, 2003). However, in commercial practice it is difficult to quickly and uniformly heat the rice to a high temperature with convective drying because rice is not in a single or thin layer and the temperatures of the rice grains are limited to the wet bulb temperature of the drying air if no secondary heat sources are used (Parrouffe et al. 1992). IR heating may provide a solution for achieving fast and relatively uniform heating, resulting in quick moisture removal with reduced moisture gradient in rice grains and improved milling quality.

10.3 Infrared Heating of Food Products

Heating and drying of food products with IR energy have been found to be distinctly different from the other conventional drying methods and have many positive attributes. Consequently, a considerable amount of research has been carried out to investigate the possibility of using infrared energy for food product drying. IR heating has the following advantages: (1) high energy efficiency and improved product quality; (2) radiation penetrates directly into the product without heating the sur-

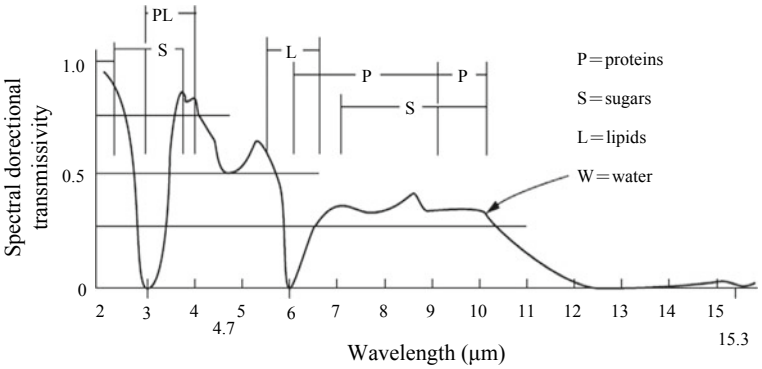

Fig. 10.1 Spectral directional transmissivity of infrared in various food components

roundings; (3) uniform heating of the product; (4) leveling of the moisture profiles in the product and low product deterioration; (5) ease of control; (6) IR sources are inexpensive and have a long service life and low maintenance cost; (7) occupies little space and may easily be adapted to conventional dryers (Amaratunga et al. 2005; Pan and Atungulu 2010).

10.3.1 Fundamentals

The origin of infrared is thermal, and its application in processing results in a direct thermal effect. In general, the substances absorb IR energy most efficiently through the changes in molecular vibrational state, which can lead to radiative heating. When radiant electromagnetic energy impinges upon a food material surface, it may induce changes in the electronic, vibrational, and rotational states of atoms and molecules. Water and organic compounds, such as proteins and starches, which are the main components of food, absorb IR energy at wavelengths greater than 2.5 μm. The key absorption ranges of food components are as visualized in Fig. 10.1 (Sandu 1986). Water shows strong absorption phenomena over the entire IR spectrum with stronger absorption bands situated at 3–4 μm. When the water component of food materials absorbs IR energy, its molecular vibrational state changes and radiative heating occurs. Then, the water vapor (molecular weight 18) moves from the internal layers of the evaporation zone towards the body surface where it evaporates to the surrounding medium, which is a gas of a large molecular weight (about 29) (Ginzburg and Grochowski 1969; Sandu 1986).

IR impinges on the material surface and penetrates it when a material is exposed to IR. The increased molecular vibration due to absorption of radiation generates heat in the material both at the surface and inner layers simultaneously, which increases the heating rate. The rapid heating of the material increases the rate of moisture

movement towards the surface and results in increased mass transfer (Sakai and Hanzawa 1994; Hebbar et al. 2004).

The absorption of IR by the product being dried depends upon wavelength and intensity of radiation, moisture content, and temperature and surface characteristics of the product. The action of IR in materials of plant origin (vegetables, fruits, and grains), water, and fat, contributes to changes in the body structure and the directional disposition (the orientation) of the molecular chains. An analogical hypothesis of the rearrangement of molecules in the surface layers of the body is explained the formation of a specific structure enabling an intense preheating of the body (Ginzburg and Grochowski 1969). Moreover, heating by IR has its own specific features connected not only with the penetration of radiation into the material, but also with a deeper effect on the molecular structure of the material. When the frequency of the incident radiation is close to the value of the frequency of the natural oscillations of the atoms (the resonance), the amplitude of the imparted vibrations of the atoms increased. At the same time, the coefficient of the energy absorption also increased (Hall 1962).

10.3.2 Applications in Food Processing

Considerable amounts of research have been carried out to investigate the possibility of using infrared energy in food processing. IR drying has been investigated as a potential method for obtaining high-quality dried foodstuffs, including fruits, vegetables, and grains (Pan et al. 2008, 2011). Also, the heating and drying of food products with IR energy has been found to be distinctly different from the other conventional drying and offers many advantages, such as high heating rate and energy efficiency, over conventional drying methods under similar drying conditions (Bilowicka 1960; Ginzburg and Grochowski 1969; Masamure et al. 1998; Afzal and Abe 1997; Abe and Afzal 1998; Hallstrom et al. 1988; Dostie et al. 1989; Sandu 1986).

IR has also been studied for the drying of cashew kernels (Hebbar and Rostagi 2001), herbs (Paakkonen et al. 1999), barley (Afzal et al. 1999), potato (Abe and Afzal 1998), shrimp (Fu and Lien 1998), rough rice (Afzal and Abe 1997), and onion (Ito and Han 1995). Person and Sorenson (1962) investigated the effects of various factors, like exposure time and intensity of radiation, on drying rate while drying alfalfa hay with IR heating. Nowak and Lewicki (2004) reported that there was a reduction in drying time for apple slices by 50% when heating with IR compared to convective drying done with equivalent parameters. Reduction in color change has been achieved in potatoes and pineapples with infrared intermit heating as well as osmotic pretreatment (Tan et al. 2001). Experimental tests have shown that IR technology is well-suited for the extraction of high-potency vitamins from herbal sources (Chua and Chou 2003).

The combination of convection and IR has appeared as one of the potential additions to the traditional drying methods for improving drying efficiency. Afzal et al. (1999) found that within temperature ranges of 40 to 70 °C, a combined hot air-FIR drying process of barley reduced required total energy consumption compared to

convection drying alone at 40, 55 and 70 °C, respectively. Combined convection and IR has shown to be a promising drying technique (Hasatani et al. 1988; Afzal and Abe 1997). Combined intermittent infrared and continuous convective heating of a thick porous material reduced the drying time by 2–2.5 times compared to that of the convective drying (Dostie et al. 1989).

10.4 Infrared Drying of Rice

Nowadays, it is important to find a method to dry rice with high efficiency and quality to meet the increase in rice production due to higher yielding varieties, more fertilizers, and faster harvesting capabilities (Khir et al. 2011, 2014). IR heating could provide a high heating rate, rapid moisture removal, effective disinfestation and disinfection, good quality, and storage stability for rice (Pan et al. 2008, 2011). Infrared radiation drying is fundamentally different from convection drying (Bal et al. 1970). IR energy is transferred from the heating element to the product surface without heating the surrounding air. The radiation impinges upon the exposed material and penetrates it and then converts to sensible heat (Ginzburg and Grochowski 1969). The penetration could provide more uniform heating and reduce the moisture gradient in the rice grain during heating and drying. Also, since IR does not heat up the medium, the temperature of rice grain is not limited by the wet bulb temperature of surrounding air and a high temperature of rice grain can be achieved in a short time for quick moisture removal (Pan et al. 2008, 2011). In addition, after the IR heating period, displacement of moisture from the rice grain core towards its surface can be achieved by tempering to reduce the moisture gradient in the rice grain. In general, IR heating offers many advantages over conventional rice drying such as: high drying rate, reduced drying time, high energy efficiency, high-quality finished products, uniform temperature in the product during drying process, and a reduced necessity for air flow across the product (Sharma et al. 2005).

Pan et al. (2008) reported that high rice drying temperatures can be achieved with a relatively short heating time by using a catalytic IR emitter with a single layer of rough rice. The moisture removal during heating increased with an increase in rice temperature. It took only 60 s to achieve a rice temperature of about 60 °C and removal of 1.7% and 1.8% points MC during IR heating alone for the low and high MC rice, respectively. Additionally, Pan et al. (2011) found that a high heating rate and rapid moisture removal for drying of freshly harvested rice can be achieved with a relatively short heating time by using a catalytic IR emitter with different drying bed thicknesses. When the drying bed thicknesses were single layer, 5, and 10 mm, only 60, 90, and 120 s were required to achieve about 60 °C rice temperature, and this heating resulted in 1.3, 1.4, and 1.3 percentage points of moisture removal for the low MC rice and 1.5, 1.6, and 1.5 percentage points of moisture removal for the high MC rice, during heating alone (Figs. 10.2 and 10.3).

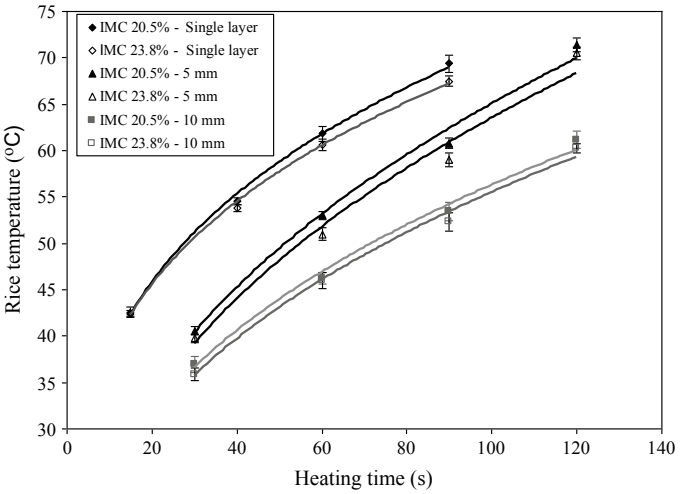

Fig. 10.2 Relationship between rice temperatures and heating time at drying bed thickness and initial moisture contents (From Pan et al. (2011). Used with permission)

10.4.1 Tempering Treatment

The tempering process after the rapid IR heating and moisture removal is essential to achieve high rice milling quality and to improve the amount of moisture removal during cooling. When a large amount of moisture is removed during IR heating, tempering is very important to re-establish the moisture equilibrium in rice kernels. Moreover, based on the glass transition phenomenon, the temperature and moisture at the rice surface are lowered first, and the starch reaches the glassy state during cooling. At the same time, the center temperature and moisture of the rice kernels are still relatively high, and the starch remains in the rubbery state. The differences in thermomechanical properties of starch at different stages would generate stress and fissures, resulting in breakage during milling and lower rice milling quality. Therefore, tempering and natural cooling would be very important for high temperature rice drying. Since tempering and natural cooling effectively preserve rice quality, controlled slow cooling could be accomplished by low rates of airflow through a bin of rice (Pan et al. 2008, 2011; Khir et al. 2011, 2014).

Pan et al. (2008) studied the effect of the tempering treatment and natural cooling on moisture removal of freshly harvested medium grain rice (M202) samples with low (20.6%) and high (25.0%) moisture content heated as a single layer using a catalytic IR emitter. They found that tempering treatment resulted in 0.2%–0.5% points higher MC removal than non-tempering, which showed that the tempering treatment significantly improved the moisture removal during cooling compared to non-tempered samples at $P < 0.05$ level. This was due to the reduced moisture gradient in the rice grain and more moisture near the grain surface after tempering.

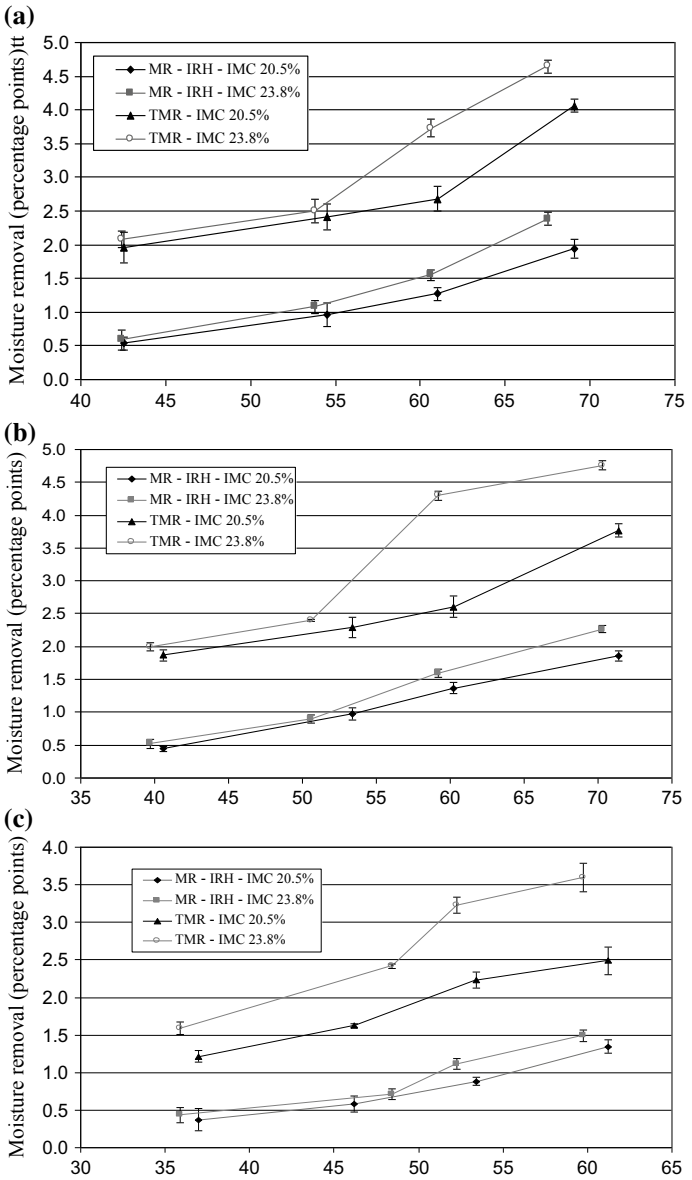

Fig. 10.3 Moisture removals of rice with initial moisture contents (MCs) of 20.5% and 23.8% caused by infrared heating and cooling after tempering treatment with drying bed thicknesses of single layer (**a**), 5 mm (**b**), and 10 mm (**c**) under radiation intensity of 5348 W m^{-2} (MR: Moisture removal; IRH: infrared heating; TMR: total moisture removal) (From Pan et al. (2011). Used with permission)

The results also indicated that tempering is even more important for high MC rice than low MC rice in order to have high MC removal during cooling.

10.4.2 Infrared Heating Followed by Natural Cooling

Since infrared can be used to quickly heat rough rice with single or thin layer to a relatively high temperature, it is possible to use the sensible heat from the heated rice to remove more moisture during cooling, which could make the overall IR rough rice drying more energy efficient. Because the tempering process after infrared heating may improve the rice quality and moisture removal during the cooling period, it further reduces energy consumption due to no additional heating during cooling.

Pan et al. (2011) reported that the trend of total moisture removal at different temperatures with different tempering and cooling treatments was more or less parallel to the moisture removal caused by heating only (Fig. 10.3). The highest total MC removals from the rice grain were 1.7%–4.4% and 2.2%–4.8% points for low and high MC rice samples, respectively, which were achieved with tempering and forced air cooling among the treatments. But the lowest total MC removals generally occurred for rice experiencing no tempering nor a natural cooling treatment. For rice treated with tempering and natural cooling, the total moisture removals were 1.4%, 2.4%, 3.2%, and 4.3% points for the high MC rice and 1.3%, 2.0%, 2.7% and 3.8% points for the low MC rice over the tested temperature range. The moisture removals were the second highest among the treatments when the temperatures were above 55 °C. These numbers indicated that 2.7%–3.2% points of moisture were removed with 1 min heating followed by tempering and natural cooling. The drying rates were much higher than the 2%–3% point moisture removal with 15–20 min heating of the current conventional heated air drying.

The natural cooling following the tempering treatment can be used to remove a significant amount of moisture and achieve high rice quality without additional energy input. Pan et al. (2008, 2011) mentioned that the recommended conditions for drying of freshly harvested rice are 60 °C rice temperature followed by tempering and natural cooling at a drying bed thickness of up to 10 mm. For total moisture removals, the moisture removed due to sensible heat during cooling was a significant portion. For example, 37% and 44% of total moisture removals occurred during cooling when the low and high MC rice samples, respectively, were heated for 60 s (to about 60 °C), followed by tempering and natural cooling. Because no additional heating energy is needed during the cooling, the high moisture removal could further improve the energy efficiency of the IR drying process.

10.5 Milling Quality

Unlike most cereal grains, rice is primarily milled when purchased. Intact milled kernels are the preferred form for consumers, and broken kernels have a low market value. Therefore, the milling quality is of utmost importance. The most important rice milling quality indicators are total rice yield (TRY), head rice yield (HRY), and whiteness index (WI). However, the primary milling quality index is head rice yield (HRY). HRY is the mass percentage of rough rice that remains as head rice after milling. Head rice is also defined as milled rice kernels that are three-fourths of the original kernel length after complete milling (USDA 1979). Minimizing HRY reduction during drying is of primary interest to the rice industry. Improper drying can produce kernel fissures which structurally weaken the kernel and make it more susceptible to breakage during subsequent hulling and whitening processes. Improper moisture and temperature changes in the kernel accrued with the current drying methods are major sources of rice fissures, lowering the HRY (Kunze 1979; Chen 1997; Chen et al. 1997; Cnossen and Siebenmorgen 2000). Fissures in the kernels are the greatest concern in the rice milling industry. Therefore, minimizing rice fissuring, and thereby improving HRY is a pressing need in the rice industry.

Infrared drying has a promising potential in improving the milling quality of rough rice. Pan et al. (2008, 2011) and Khir et al. (2011) found that high moisture removal and high milling quality of rough rice could be achieved by IR heating followed by tempering and natural cooling. They reported that the low MC (20.5% w.b.) and high MC (23.8% w.b.) rice samples dried by using IR with tempering and natural cooling had significantly higher TRY and HRY than the control when the rice samples were heated with IR to about 60 °C in single layer, 5 mm, and 10 mm drying bed thicknesses, respectively (Tables 10.1 and 10.2). The reason that the high temperature of IR heating did not damage the rice quality could be due to the relatively uniform heating in the rice kernel resulting from the IR penetration, leading a lower moisture gradient compared to conventional heated air drying. It was also reported that the rice milling quality might not be compromised with a relatively large amount of moisture removal in a single drying pass with a high drying rate if the rice could be heated quickly and uniformly to minimize the moisture gradient. When a large amount of moisture is removed during IR heating, tempering is very important to re-establish the moisture equilibrium in the rice kernels and relax the strains inside a rice kernel induced by internal stresses developed during the heating process.

10.6 Sensory Quality

Sensory quality of rice is important to all segments of the rice industry, particularly processors and consumers. Any drying treatment which adversely affects this quality would be highly undesirable. Hence, sensory quality must be considered in evaluating drying treatments. It has also been reported that cooked rice texture could be affected

Table 10.1 Quality of milled rice dried under different conditions with initial moisture content of 20.5%. From Pan et al. (2011). Used with permission

Heating time (s)	Rice temperature (°C)	Total moisture removal (%)	DBT and control[a]	Milled rice quality[b]		
				TRY	HRY	WI
			Control	68.61 a	64.11 a	41.90 a
15	42.6	2.0	Single layer	68.39 ab	64.45 a	41.50 a
30	40.6	1.9	5 mm	68.11 bc	62.67 b	41.80 a
30	37.0	1.2	10 mm	67.78 cd	62.84 b	41.60 a
			Control	68.61 a	64.11 a	41.90 a
40	54.5	2.4	Single layer	68.68 a	64.71 b	41.67 a
60	53.4	2.3	5 mm	68.38 a	62.91 c	41.80 a
60	46.2	1.6	10 mm	68.42 a	63.97 a	41.60 a
			Control	68.61 a	64.11 a	41.90 a
60	61.0	2.7	Single layer	69.26 b	65.63 b	41.60 a
90	60.2	2.6	5 mm	69.49 bc	65.05 b	42.06 a
90	53.4	2.2	10 mm	68.82 ab	65.40 b	41.60 a
			Control	68.61 a	64.11 a	41.90 a
90	69.1	4.1	Single layer	68.51 a	63.52 a	41.80 a
120	71.4	3.8	5 mm	67.91 b	62.77 b	42.00 a
120	61.2	2.5	10 mm	69.20 c	65.17 c	41.70 a

[a] *DBT*: drying bed thickness; Control: ambient air drying
[b] *TRY*: total rice yield; *HRY*: head rice yield; *WI*: whiteness index. Values from the control, single layer, 5 mm and 10 mm in each category followed by different letters are significantly different at $P < 0.05$

by the drying conditions and the final moisture content (Nguyen et al. 2001). David and Webb (1971) studied the influence of drying conditions on rice taste. They found that taste quality did not vary until temperatures exceed 50 °C.

IR drying treatments during which rice attained maximum temperatures up to 60 °C, followed by tempering and natural cooling, appeared to have no adverse effects on sensory quality. Moreover, it is important to note that these IR drying conditions maintain high milling quality of rough rice (Pan et al. 2008). Pan et al. (2011) reported that no significant differences in flavor were observed between the control and IR-dried samples. In a comparison of texture, stickiness to lips was significantly higher in control rice than in that of low IMC dried with IR. Significantly lower cohesiveness of mass was exhibited by rice samples with high IMC dried by using IR when compared to the control. Additionally, they stated that no other textural attributes differed significantly between the control and IR-dried rice samples (Table 10.3). Also, Champagne et al. (1998) reported no effect of high temperature conditions

Table 10.2 Quality of milled rice under different drying conditions with initial moisture content of 23.8%. From Pan et al. (2011). Used with permission

Heating time (s)	Rice temperature (°C)	Total moisture removal (%)	DBT and control[a]	Milled rice quality[b]		
				TRY	HRY	WI
			Control	67.90 a	63.40 a	41.80 a
15	42.4	2.1	Single layer	68.12 a	61.55 b	41.50 a
30	39.7	2.0	5 mm	68.03 a	61.50 b	41.50 a
30	35.9	1.6	10 mm	67.70 a	60.32 c	41.50 a
			Control	67.90 a	63.40 a	41.80 a
40	53.8	2.5	Single layer	68.42 b	62.18 b	41.40 a
60	50.6	2.4	5 mm	68.26 b	62.25 b	41.80 a
60	48.4	2.4	10 mm	68.24 b	61.53 b	41.50 a
			Control	67.90 a	63.40 ad	41.80 a
60	60.6	3.7	Single layer	68.98 bc	63.95 abc	41.60 a
90	59.1	4.2	5 mm	69.33 b	64.36 c	41.70 a
90	52.3	3.2	10 mm	68.80 c	63.06 d	41.60 a
			Control	67.90 a	63.40 a	41.80 a
90	67.5	4.6	Single layer	68.39 b	62.19 b	41.80 a
120	70.3	4.8	5 mm	67.92 a	60.85 c	41.80 a
120	60.3	3.6	10 mm	68.96 c	63.30 a	41.70 a

[a] *DBT*: drying bed thickness; Control: ambient air drying
[b] *TRY*: total rice yield; *HRY*: head rice yield; *WI*: whiteness index. Values from the control, single layer, 5 mm and 10 mm in each category followed by different letters are significantly different at $P < 0.05$

on the instrumental texture characteristics of the cooked rice, with the exception of cohesiveness, which was found to be lower in rice dried at a lower temperature than in rice dried at a higher temperature.

10.7 Storage Stability

There is a need in the rice industry to prevent the degradation in chemical and physical attributes and maintain the functionality and quality of rice during drying and storage operations. Undesirable changes in the physiochemical properties of rough rice can occur during drying and storage processes. The gelatinization properties of rice depend on the temperature of the drying air (Fan and Marks 1999). During storage, numerous changes occur in the physicochemical properties of rice, including color, thermal properties, and pasting properties (Tananuwong and Lertsiri 2010).

Table 10.3 Comparison of sensory flavor and texture attributes of IR-dried rice and control. From Pan et al. (2011). Used with permission

Flavor attributes	Initial MC 20%		Texture attributes	Initial MC 20%		Initial MC 25.1%	
	Control	Treated		Control	Treated	Control	Treated
Sewer animal	1.0 a	0.9 a	Initial starchy coating	2.2 a	2.2 a	2.1 a	1.9 a
Floral	0.0 a	0.0 a	Slickness	6.9 a	7.3 a	7.2 a	7.9 a
Grain/starchy	3.4 a	3.5 a	Roughness	5.6 a	5.4 a	5.1 a	5.0 a
Hay-like musty	0.6 a	0.5 a	Stickiness to lips	10.2 a	9.5 b	9.0 bc	8.7 c
Popcorn	0.3 a	0.5 a	Stickiness btwn grains	5.6 a	5.2 a	5.8 a	5.0 a
Corn	0.8 a	1.0 a	Springiness	4.0 a	4.0 a	4.3 a	4.0 a
Alfalfa	0.0 a	0.3 a	Hardness	5.3 a	5.4 a	5.6 a	5.9 a
Dairy	0.9 a	0.5 a	Cohesiveness	5.8 a	5.7 a	5.7 a	6.0 a
Sweet aromatic	0.4 a	0.4 a	Uniformity of bite	6.9 a	7.5 a	7.3 a	7.2 a
Water-like metallic	0.8 a	1.1 a	Cohesiveness of mass	5.8 a	6.0 ac	6.8 b	6.3 c
Sweet taste	1.3 a	1.2 a	Moisture absorption	5.3 b	5.2 b	5.4 ab	5.2 b
Sour	0.3 a	0.3 a	Residuals	4.7 a	4.8 a	4.7 a	4.8 a
Astringent	1.0 a	1.2 a	Tooth pack	4.1 a	4.0 a	4.0 a	4.0 a

Values in each row followed by the same letter indicated no significant difference at $P < 0.05$

Additionally, when the bran layer is exposed to the external environment, enzymatic activity may increase and cause the acceleration of lipid degradation during the storage period. These changes could affect the appearance and the cooking and eating quality of rice (Kaminski et al. 2013). Thus, a proper rice drying method is needed to assure improved stability in physicochemical properties and cooking quality of stored rice.

IR heating holds a promising solution in achieving efficient drying and simultaneously in improving the storage stability of rough and brown rice. IR drying provides a possibility to store brown rice instead of rough rice with extended shelf life and reduced cost. Moreover, IR heating can be used as an effective approach to achieve stabilized rice bran and extent its shelf life after milling without additional stabilization process. Ding et al. (2015a) found that the improvement in rough and brown rice stability during storage could be achieved through drying rough rice by using IR heating to a temperature of 60 °C followed by tempering for 4 h and natural cooling.

In another study, Ding et al. (2016) reported that IR drying had positive effects on the stability of rice color and properties in microstructure, gelatinization, pasting, cooking, and texture. They also mentioned that the physicochemical properties and cooking quality of the rice dried with IR were better maintained during storage. The

IR heating and tempering treatments showed a promising potential to achieve an effective stabilization for rice bran during storage after milling. Additionally, Wang et al. (2017) reported that the IR heating of rough rice to 60 °C followed by tempering treatment for 4 or 5 h resulted in significant reduction in lipase activity, particularly for rice with high initial MC. The storage time of stabilized rice bran with FFA concentration less than 10% could be extended to 38 days under two-pass drying compared to 7 days for the control. Based on the previous findings, IR heating can achieve simultaneous drying and effective stabilization of rice bran and provide more effective utilization of rice bran without affecting the quality of rice bran oil.

10.8 Disinfestation

Insect infestation of rice is a dire problem in the rice industry. Infestation leads to a series of problems such as weight loss, reduced quality, and even food safety issues. Moreover, infestation can lead to huge financial losses for the rice growers and processers. The elimination of insect contamination in rice is important need in the industry. One common method to control insects is to use chemical pesticides. However, these methods have led to serious environmental damage and hazards to people's health. Another problem associated with the use of chemical pesticides is that they must be applied at levels no higher than those required for good agricultural practice, which requires highly trained and educated personnel (Pan et al. 2008).

It is important to notice that rough rice could be infested with insects before and during harvesting. It is ideal to kill all insects during drying without needing any additional disinfestation treatments for extending rice storage life. The convective drying process is normally not able to kill the insects if the rough rice is infested due to the relatively low temperature (Donald et al. 1992). Therefore, alternative environmentally friendly disinfestation methods are needed to replace the typical chemical disinfestation methods (Pan et al. 2008; Wang et al. 2014).

IR heating has shown a promising potential to perform simultaneous drying and disinfestation for rough rice (Pan et al. 2008). Ginzburg and Grochowski (1969) found that 50 s treatment with IR was sufficient to destroy not only the insects (grain beetles), but also their eggs. The destruction occurs through the overheating of the harmful insects, which are colored dark-brown, and therefore absorb infrared radiation to a greater extent than the grain. Tilton and Schroeder (1963) exposed three species of insects commonly found in rice to IR and achieved complete mortality with rice temperatures ranging from 65 and 70 °C. Pan et al. (2008) found that simultaneous drying and disinfestation with high rice milling quality can be achieved by using a catalytic IR emitter to heat rough rice to 60 °C, followed by tempering.

10.9 Disinfection

Rice is susceptible to fungal infection during growing, harvesting, handling, and storage. Recently, there have been a number of reports from various countries on the occurrence of fungal contamination in rice with high levels of aflatoxin (Hussaini et al. 2011; Almeida et al. 2012; Elena et al. 2013; Ok et al. 2014; Lai et al. 2015). Mycotoxins accumulated in rice are proven to pose a potential threat to human health and decrease the quality and market value of rice grain. Fungal infection leads to grain discoloration, chemical and nutritional changes like odor, toxin contamination, and loss in viability. Fungal deterioration reduces the milling grade of the rice, resulting in economic loss to producers. Additionally, changes in color and losses in viability and dry matter can result in total rejection of rice for human consumption (Salunkhe et al. 1985). Therefore, effective disinfection processes must be used to eliminate the risk of mold growth on rice.

Chemical methods, including chlorinedioxide, calcium hypochlorite, and ozone have been widely used for the disinfection of rough rice (Andrews 1996; Oyebanji et al. 2009; Salunkhe et al. 1985). However, chemical methods may not be able to meet disinfection requirements defined by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF 1999) for a 5 log reduction in the level of pathogens on seeds. Also, when liquid chemicals are used, extra energy may be needed for the subsequent drying of the treated rice, and the prolonged chemical residual existence on rice and the compounds discharged to water and air are potentially hazardous to the environment, animals, and humans (Beuchat 1998).

Additionally, drying to below the critical moisture content for the growth of fungi is the most effective and widely used method to control microbial growth on rice. Convective heated-air drying is the common drying process used in the rice industry, during which rice grains are exposed to relatively low air temperatures (about 43 °C) to avoid lowering the rice milling quality (Kunze and Calderwood 1985). However, this drying temperature is below the temperature needed to achieve effective disinfection of rice grains.

IR heating, as a nonchemical and emerging technology with high efficiency, has a promising potential to be used as an effective disinfection method for rough rice. Wang et al. (2014) found that high heating and drying rates and corresponding effective disinfection of rough rice were achieved by using IR heating followed by tempering. They also mentioned that the MC significantly affected the disinfection of rough rice. The fresh rice with a higher MC was easier to be disinfected than rice with a lower MC.

In the same study reported by Wang et al. (2014), stored or dried rice could be effectively disinfected by rewetting the surface to increase the relative humidity (RH) and applying IR heating followed by tempering. After IR heating, the RH of the tempering environment had a positive effect on the disinfection effectiveness. They confirmed that the recommended conditions of simultaneous disinfection and drying for fresh rice were rice temperature of 60° by IR heating, tempering for 120 min, and natural cooling. For the storage rice or dried rice, the recommended conditions

for disinfection and drying involved only 20 min of tempering. It was concluded that IR heating followed by tempering and natural cooling can be used as an effective method in achieving a simultaneous disinfection and drying of fresh and storage rice.

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