Abdel Moneim Elhadi Sulieman
Abdalbasit Adam Mariod *Editors*

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This book is dedicated to our two beloved families

Preface

Fermented foods have been an important part of the diet in numerous societies; later, fermentation has been related with numerous medical advantages. Along these lines, the fermentation procedure and the resulting fermented products have recently attracted scientific interest. Likewise, microorganisms involved in the fermentation process have recently been associated with many health benefits, and so these microorganisms have become another focus of consideration.

This book will discuss various aspects concerning fermented foods of Africa by providing extensive knowledge about chemistry and bioactive compounds of food products which will help inducers to prepare recipes. Moreover, it will provide knowledge about the nutritional value and minor constituents of these food products, which will help end users, for example, manufacturers, prepare high-quality products. The information can be incorporated into our food supply at an industrial and cost-effective scale.

This book describes how fermented foods have high nutritional values and concentrates on composition, bioactive compounds, nutrient composition, safety, and application of biotechnology in upgrading certain African fermented food products.

The book presents 37 chapters on various fermented foods of Africa. The first nine chapters of the book deal with the origin, history, diversity, significance, properties, and *advantages of* African fermented foods, fermenting microorganisms*,* probiotic fermented foods and health promotion, molecular techniques for microbial community profiling of fermented foods, production of industrial enzymes, and bioactive components of fermented foods. Chapter 10 describes the transcriptomic analysis of bakery products. Chapters 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 and 36 cover various fermented food product contents from different African countries, investigating the nutritional values, bioactive components, phytochemical properties, conventional, and medicinal uses.

This book was written by experts from the different countries around the world to ensure that it will be of significance to the industry, medicine, food scientists, and pharmaceutical industries. Moreover, this book should interest scholarly researchers who require a good source of uses and a decent arrangement of references.

Hail, Saudi Arabia — Abdel Moneim Elhadi Sulieman
Ghibaish, Sudan — Abdelbasit Adam Mariod

Contents

Abbreviations

DNA	deoxyribonucleic acid
CDNA	complementary DNA
RNA	ribonucleic acid
rRNA	ribosomal RNA
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
FCM	fluorescence correlation microscopy
VNC	viable but nonculturable
LAB	lactic acid bacteria
ISO	International Organization for Standardization
IFPRI	International Food Policy Research Institute
FAO	Food and Agriculture Organization
SDS	Sudanese Standard
EC	European Commission
WHO	World Health Organization
g/l	gram per liter
%	percentage
me/L	milliequivalent per milliliter
me/Kg	milliequivalent per kilogram
° C	Celsius degree
Kcal	kilocalorie
mg	milligram
g	Gram
GDP	gross domestic product
LAB	lactic acid bacteria
ACE	angiotensin converting enzyme

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Chapter 1
Introduction: Origin, History and Diversity of African Fermented Foods

Abdel Moneim Elhadi Sulieman

1.1 The Origin and History of Food Fermentation

Fermentation is a natural process by which microorganisms such as yeast and bacteria convert carbohydrates - such as starch and sugar - into alcohol or acids. Alcohol or acids act as a natural preservative and give fermented foods a distinct coating and acidity. The key is to get the right balance. Given the history of humankind itself, it is difficult to trace the origins of fermentation. However, historians have followed the signs of fermentation in preparing foods and drinks dating back to 7000 BC.

The history of fermented food dates back to most of its food through hunting, to an organized and semi-stable life, so it has to change the way it obtains and maintains food valid for a long period of time, which requires it to use it to make use of these different methods. Which mainly depend on reducing or reducing the moisture content of food by exposing it to the sun's rays, which leads to limiting the growth of microorganisms and consequently keeping them from corruption. It is also utilized as a fermentation method, and this depends upon microbial growth and fermented products under conditions that give the resulting food the flavor. The food is non-toxic, of high nutritional value and easily digestible, which causes the person to accept those changes that have occurred due to the food because of the growth of microorganisms and people can. It is to control the fermentation conditions and make them suitable for making these changes. These methods are still used mainly in food preservation, among other methods used in preserving food in the most common to this day, but the fermentation process is the most widespread all over the world.

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1.2 Domestication of Fermenting Microbes

Domestication alludes to counterfeit determination and reproducing of wild species to get developed variations that flourish in man-made specialties and meet human or modern prerequisites. A few genotypic and phenotypic marks of domestication have been described in crops, animals and pets. However, domestication isn't extraordinary to plants and animal.

People in the past started to grasp the association with microorganisms in view of its advantages, to be specific: digestible food preserved for a long period of time, was less inclined to be harmful (until the turn of the last century) or basically tasted superior to unfermented foods.

Gradually strategies emerged among various cultures over the world globe for working with their nearby microorganism. Early dairy farmers learned, for instance, that by fermenting milk they could store dairy items for any longer than they would in its crude state, cheese was conceived.

Different cultures discovered fermentation created products with a scope of health conservation benefits like kimchi, tempeh, miso or even fish. The core fixing differed by whatever the neighborhood culture needed to hand - just as whatever nearby organisms jumped at the chance to eat. Microorganisms have been helping us to get nutrition from an assortment of substances that would be hurtful or difficult for us to metabolize, which is the reason fermented grains and dairy can be endured more effectively than their unfermented reciprocals.

Starting in the seventeenth century, two researchers who were members of the Royal Society in England and Holland discovered microorganisms. German researcher Cohn discovered there were various types of bacteria in the nineteenth century. His partner Robert Koch kept on investigating the role of bacteria play in causing human diseases until the mid twentieth century. Finally Louis Pasteur, a nineteenth century French biologist, is known as the "father of microbiology"for his spearheading work revealing the role microbes play in fermentation as just as less wanted results like infection and food spoilage. His invention, pasteurization, is as yet utilized today to kill microbes that could cause disease or cause food and drink to spoil (https://eatcultured.com/blogs/our-awesome-blog/fermentation-a-history).

1.3 Definition of Fermentation

Campbell-Platt (1987) has characterized fermented foods as those food sources which have been exposed to the activity of small scale life forms or catalysts with the goal that attractive biochemical changes cause huge alteration to the food. However, to the microbiologist, the expression "fermentation" portrays a type of energy yielding microbial metabolism in which a natural substance, generally a starch, is a natural sugar goes about as the electron acceptor (Adams 1990).

Fermentation alludes to the metabolic procedure by which natural particles (regularly glucose) are changed over into acids, gases, or alcohol without oxygen or any electron transport chain. Fermentation pathways recover the coenzyme nicotinamide adenine dinucleotide (NAD+), which is utilized in glycolysis to discharge energy as adenosine triphosphate (ATP). Fermentation just yields a net of 2 ATP for each glucose atom (through glycolysis), while oxygen consuming breath yields upwards of 32 particles of ATP for each glucose particle with the guide of the electron transport chain (BD Editors 2019).

The study of fermentation and its viable uses is named zymology and started in 1856 when French scientist Louis Pasteur exhibited that fermentation was brought about by yeast. Fermentation happens in particular kinds of microorganisms and growths that require a without oxygen condition to live, in facultative anaerobes, for example, yeast, and furthermore in muscle cells when oxygen is hard to come by (as in exhausting activity). The procedures of aging are important to the food and drink enterprises, with the change of sugars into ethanol used to create mixed refreshments, the arrival of CO_2 by yeast utilized in the raising of bread, and with the creation of natural acids to save and flavor vegetables and dairy products.

There are numerous kinds of fermentation that are differentiated by the end products, the most commonly utilized by humans are ethanol fermentation and lactic acid fermentation.

1.4 Early Fermentation

Fermentation is probably the most seasoned type of food preparing known today. A significant number of humankind's preferred food and refreshments are results of fermentation, regardless of whether naturally or prompted, for example, beer, wine, bread, sausages, and various sauces and marinades. There are a few distinct sorts of fermentations that can happen in food and liquids: alcoholic fermentation, acetic acid fermentation, and lactic acid fermentation.

Alcoholic fermentation is perhaps the most notable of the three sorts, its byproducts having been delighted in by human civilization for millennia.

Acetic acid fermentation is the procedure that starts where alcoholic fermentation finishes.. The most widely recognized consequence of this fermentation procedure is vinegar.

Lactic acid fermentation is believed to be the most the oldest fermentation technique, with fermented milk products being found in almost every culture around the world, and proof of their utilization returning back thousands of years.

1.5 Role of Fermented Food in Human Life

The main function of fermentation is to convert NADH back into the coenzyme NAD^+ so that it can be utiliozed again for glycolysis. During fermentation, an organic electron acceptor (such as pyruvate or acetaldehyde) reacts with NADH to form NAD^+, discharging products, for example, carbon dioxide and ethanol (ethanol fermentation) or lactate (lactic acid fermentation) in the process.

Some of the important roles of fermented food in human life can be summarized in economic, nutritional, and health terms:

- Adding flavor and food to food as a result of the fermentation process
- fermentation food taste process and flavor desired for Aviate gain Coordination produce important vehicles as a result of activity of micro-organisms that grow food, as are converting some vehicles food in the food to taste compounds and flavor, such as acetylcholine counting this product a many compounds producing flavor in fermented products. Aldehyde and De Acetyl
- Improving the quality of the texture during the fermentation process: 2 What is used in describing foods after the texture is an important and desirable characteristic that is used in food evaluation, which is considered to be one of the desirable qualities in the soy-made soybean soy. The fermentation of soybeans through the development of some fungi (by some Western factories) where these fungi turn these cereals into a food product for use in soups or with other dishes as a substitute for meat, and some people of East Asia grow Sudanese or coconut kernels (which are the byproducts of vegetable oil plants) after special treatment, where the breakfast converts the kesbah into a food product that is used as a meat substitute and added after slicing it to the soup.
- Fermentation saves food from corruption: 3 Food preservation from corruption is one of the most important benefits of the fermentation process. Anaerobic conditions by converting food compounds such as sugars into different organic acids depending on the type of organism, and this then becomes inappropriate for the growth of other unwanted microorganisms to reduce the acidity and acidity of the body. This way, food can be stored for a long period of time.

Food fermentation includes lactic fermentation, cell fermentation, and alcoholic fermentation. Lactic fermentation is the conversion of sugars in the foodstuff to lactic acid mainly with the formation of some of the acids in the other parts of the acid. Added to it.

Milk (milk) and its derivatives are preserved for a long period of time and can be used at different times after being converted to raw milk, for example) yogurt (method of fermentation method) and as milk is soured with milk for a long time. A high nutritional value that combines the nutritional value of yogurt (yogurt) with the nutritional value of wheat.

This process is called preserving vegetables such as calzone, carrots, cucumbers and other vegetables in food preservation, and as sometimes it is preserved foods by pickling (pickles), where these vegetables are treated as a special treatment. Minutes

approx. 2.5% food and add a saline solution of undesirable concentration and permeation to the microorganisms that are desirable for growth, and this converts the sugars present in the foodstuff under anaerobic conditions to the acidic acid control, to the acidic acid control, and to the acidic acidic acid control system. It becomes inappropriate for the growth of microorganisms In this process, two main factors inhibit the growth of unwanted organisms and some pathogenic organisms, the first factor is the salt that does not allow the growth of unwanted and unwanted and unwanted organisms. Lactic acid, acetic, biotic, etc.

Cellular fermentation is an alcohol conversion that results from anaerobic fermentation. This type of fermentation is carried out under its antenna conditions by specialized micro-organisms. It converts the alcohol formed from the anaerobic fermentation process to acetic acid which is the main component among the other compounds formed. It is added in suitable concentrations to the food subject to the pickling process, or it is added as a spice to the foodstuffs in many countries of the world.

– Biological enrichment of fermented food: 4 in fermented food compared to non-fermented food, especially for starchy food. It is noticeable that the percentage of protein increases by% on the basis of the dry weight of fermented food. These improve the characteristics of the taste, flavor, and aroma of fermented starchy foods and become easier to digest than non-fermented foods, and this increases the consumption of this type of food. The increase in the protein ratio is not limited to increasing the content of free amino acids, such as lysine and sulfur amino acids, especially in the fermentation process. Rather, it also leads to fermented legumes, and the fermented food increases the concentration of some of the food. It has been found that the consumption of peeled rice in some countries leads to the possibility of some diseases due to the content of this species.

However, when these people feed on fermented peeled rice, they do not show very low levels of B1 from food from satisfactory symptoms because the microorganisms responsible for fermentation increase the content of this type of food from the non-vitamin Liamine. The microorganisms responsible for this fermentation process also increase the content of (riboflavin) in some foods as well as vitamin Niacin, and it can sometimes increase to seven B2 in the fermented food compared to the fermented food that is not in the food.

– Fermentation reduces the proportion of toxins in the raw materials that are subject to fermentation. 5. Formed on raw materials from grains and others, especially when these materials are stored in “mycotoxin”. Mycotoxins are conditions that allow the growth of the fungi that form in them. Human health, as some are chronic failures of humans when ingested, as they cause diseases of which have a high degree of toxicity, while others cause diseases in reducing the appearance of such diseases, an important role of the kidneys and some of the troubles in the liver. And break it during the fermentation process.

The procedures and measures followed in the fermentation process, such as marinating, cooking, and others lead to the breaking and breaking of some toxins. It was

found in Neurospora fermentation that some people in Indonesia use some breakfast of 50% sex with Aflatoxin and some grains to produce fermented food substitutes for meat, it reduces the flavor of the flesh. If another type of fungus is used, it is related to sex% because the presence of these fungi along with some other microorganisms that do the fermentation process break down 70 toxins with an amount and break down the toxins and convert them into non-toxic substances. It has harmful effects on health and human life. It is well known that toxins can be disposed of in a number of ways, including physical properties that include heat and ultraviolet radiation, and other chemicals, which depend on use to destroy toxins, and to get rid of them in addition to substances that are analogous or oxidizing. Food requires sophisticated and expensive devices, as it has an impact on being very expensive, and for these reasons combined, the vital way to get rid of toxins and remove them is the best of these methods, because the thick, dirty, thick, living creatures The acidity of the medium is altered as a result of the production of large quantities of acids, particularly lactic acid, or the production of representative products by the fermenting microorganisms and, finally, by the antagonism that reduces the antagonism that reduces the antagonism that reduces the antagonism that reduces the antagonism that reduces the antagonism that reduces the antagonism that reduces the antagonization between the antagonists that control the antagonization that reduces.

It is well known that some fermented foods are consumed without cooking such as dairy products and dairy products, as well as pickled vegetables, and that some require a little required to cook soybeans five to six hours, while it takes only five to five hours. For example, this is of great importance, especially in families who spend long hours collecting and preparing materials for cooking when they are fermented, such as branches, tree leaves, and others. Fermented foods are of great importance and essential as they are foods of high nutritional value secured by the nutritional needs of proteins, vitamins and mineral salts that are appropriate to the income of the ordinary consumer in addition to being consumed with non-consumptive products.

– The fermentation process changes the composition of the elements in the raw materials: 7 The foodstuffs from which the fermented food is made have many changes, whether in the composition of the nutrients present in the raw materials or in the presence of the nutrient elements that are important in the feed. It occurs to the raw material whose chemical compounds in fermentation will not affect its content before fermentation, and a clearer example of fermentation is what happens to milk after fermentation, as it is noticed that some of the milk components increase in the proportion of lactic acid and lactic acid. Lactic acid leads to an increase in the percentage of lactic acid as well as glucose and glucose sugar in addition to increasing the percentage of some other sugars that in turn are converted to organic acids by the microorganisms that do the fermentation process in the fermentation process, between the fermentation process and the fermentation process, between the fermentation process and the fermentation process, between the fermentation process and the fermentation process, between the fermentation process and the fermentation process in the fermentation process.

Before fermentation, this has many benefits especially for people who suffer from 5% and 3% for the consumer. On the contrary, its very presence is lactose intolerance, as the presence of a small percentage Lactose intoxication is beneficial in large proportions, causing irritation of the mucous membrane of the intestine, which leads to spasms in the abdomen and severe diarrhea. The percentage of free peptides and free amino acids increases during the fermentation process due to the breakdown of the proteins present in the milk that performs important inside the stomach and intestine. As microorganisms play a role, this makes fermented brown products easier to digest in the stomach emptying process, which is faster when ingesting fermented milk compared to non-fermented milk. Among the ingredients that are increased in fermented milk, free fatty acids are observed, due to the breakdown of fat in milk and this leads to an improvement in the degree of fermentation of fermented milk and the appearance of new compounds that give flavor, taste, flavor, and flavor. Probiotics, biotech and others. Finally, the fermentation process, as it shows acids in the level of vitamins, some of which are reduced due to the consumption of microorganisms during their growth. While an increase is observed for some of them, such as folic acid, niacin, butane, and others.

1.6 Fermented Foods and Probiotics

Fermented foods are very rich in probiotics. And all studies now indicate the significance of a balance in the intestine between benign intestinal flora and harmful bacteria. And that this balance greatly influences our physical, mental and psychological health. It is rich in live enzymes, greatly improving digestion and excretion, strengthening the immune system, improving mood and reducing depression, increasing the absorption of nutrients from food such as vitamins and minerals especially vitamins B complex.

These benign bacteria play a big role in the body's immunity, as more than 60% of the immune cells lie directly behind the intestinal wall. Bacteria also maintain the integrity of the intestinal lining and thus prevent leakage of substances that stimulate the immune system, furthermore assist in the production of antibodies against germs. It is able to rid the body of toxins and even the heavy metals that enter it. It is a very important source of nutrients such as K2, which helps protect the body from plaque deposits and heart disease.

> People has been started realizing now the great role that these bacteria play outside the digestive tract. It has become clear that their safety is closely related to the safety of other organs *in* the body, especially the nervous system and mental health, which made scientists classify them as a new organ that we did not know about in the body, such as the heart, kidneys and the liver which has the same importance.

1.7 Distribution of African Fermented Foods

A wide variety of crude materials are customarily fermented in various districts regions of Africa. Accordingly, fermented foods with different attributes are delivered and they have been classified in groups, for example, fermented non-alcoholic cereals, starchy root crops, animal proteins, vegetable proteins and alcoholic beverages.

1.7.1 Fermented Cereal Products

Fermented foods play a significant role in the diet of people in Africa, where a wide variety of raw materials are fermented. People in Africa usually ferment mainly cereal-based foods such as sorghum, millet and maize; roots such as cassava; fruits; vegetables, though less widely; and, to a lesser extent, meat and fish (Maria Diaz et al. 2019).

In Africa, the significant cereals of importance are maize, sorghum, and millets which are utilized to create a various amount of fermented products. An intensive survey on these African fermented cereal products and their microbial ecology can be found elsewhere (Molly et al. 2017; Blandino et al. 2003; Franz et al. 2014; Guyot 2010; Guyot et al. 2012; Hammes et al. 2005). These food products are utilized as weaning food for infants, children and adults (Lei and Jakobsen 2004; Kalui et al. 2008). Furthermore, wide scope of cereal-based fermented foods and related procedures is a declaration to cultural diversity to the capacity of people to discover approaches to create foods in various settings. The advantageous impacts are the preservation of foods and the expansion in their organoleptic qualities in view of the creation of lactic acid and other metabolites synthesized by lactic acid bacteria (Guyot 2012).

Cereals represent a significant stable food in Africa. Nutritional specialists have focused on cereal based foods from maize, sorghum and millet sources. These crops contain big quantities of carbohydrates with therapeutic properties, for example, beta glucan from barley or oat based products control cardio vascular illness in people have been accounted for (Beck et al. 2010; Shimzu et al. 2008; Karmally et al. 2005; Keogh et al. 2003). Duchonova et al. (2013) proposed that the various gainful impacts of cereal can be exploited in various manners thus structure of novel cereal foods fixings can be focused at a particular population. In addition, cereals are good fermentable substrates for the development of probiotic microorganisms (Kochova et al. 2011; Charalampopoulos et al. 2008).

Table 1.1 Cereal fermented foods and beverages, sources and the organisms involved

Source	Product	Fermenting Microorganism
Maize/ sorghum/millet	Ogi	Lactobacillus plantarum, S. cerevisiae, Candida mycoderma, Corynebacterium, L. plantarum,
Maize/ sorghum	Fura	L. plantarum, Pedicoccus, Leuconostoc, Streptococus, Enterococcus, S. ccereviciae Pichia anomala, Candida species
Maize/ sorghum/millet	Agidi	Pediococccus acidolactic L. plantarum, Lactobacillus acidophilus, Leuconostoc, Streptococcus, Bacillus, Lactobacillus fermentum
Maize/ sorghum/millet	Kunun-Zaki	Lactobacillus leichimani, Escherichia coli, Streptococcus species
Sorghum/ millet	Burukutu	Acetobacter spp., Candida spp. Leuconostoc mesenteroides S. cerevisiae, S.chaveleri
Sorghum/ millet	Pito	Acetobacter spp., Candida species, L. mesenteroides, S. cerevisiae S. chaveleri

Source: Ome and Michael (2015)

1.7.1.1 Microbiology of Cereal Fermentation

LAB are the most widely recognized microbe which ferment cereals. LAB for fermentation in Africa is mainstream because due to their role of preservation, improved nutritional value, detoxification, creation of flavour and smell. Four genera, which are most dominating, are Lactobacillus, Lactococcus, Leuconostoc and Pedicoccus (Salovaara 2004). Other organisms are Corynebacterium, Saccharomyces cerevisiae and Streptococcus (Table 1.1). In addition, mould species such as Aspergillus, Penicillum, Fusarium and Cladosporium may be involved.

The organisms are usually from the environment or from the previous reserved batch (backslopping). This inoculation and subsequent metabolic activities bring about expanding acidity of the medium along these lines prompting to the elimination of non-lactic acid microorganisms. Subsequent microbial succession inactivates or executes a few organisms while others proceed with the fermentation. Enduring lactic acid bacteria in the fermentation form a synergy with some yeasts. The fermentation is spontaneous as a result of competitive microbial activities. Consequently, a few strains are best adjusted with quick development thereby dominating others at specific phases of fermentation.

1.7.2 Fermented Vegetables Products

Consumer trend is towards fresh, highly nutritional, Food and beverages enhanced with flavor, and rich ready-to-eat or drink (Endrizzi et al. 2006). Trends in microbiology in food include initiator use cultures with possess variable benefits (for example, probiotics activity, decrease of harmful compounds, creation of vitamins) (Bevilacqua et al. 2012).

Table 1.2 Different classification of fermented foods

Yokotsuka (1982)	Campbell-Platt (1987)	Odunfa (1988)	Kuboye (1985)	Sudanese (Dirar 1993)
Alcoholic beverages (yeast)	Beverages	Starchy roots	Cassava-based	Kissar – Staples
Vinegars (Acetobacter)	Cereal products	Cereals	Cereals	Milhat – Sauces and relishes for the staples
Milk products (lactobacilli)	Dairy products	Alcoholic beverages Proteins	Legumes	Marayiss – Beers and other alcoholic drinks
Pickles (lactobacilli)	Fish products	Vegetable	Beverages	Akil-munasabat – Food for special occasions
Fish or meat (enzymes and lactobacilli)	Fruit and vegetable products	Animal protein		
Plant protein (moulds, with or without lactobacilli and yeasts)	Legumes			
	Meat products			

Adapted from Dirar (1993)

Fermentation of vegetables at home is done without any additives and without exposing the vegetables to heat (cooking temperature), which eliminates most of the vitamins and enzymes in the vegetables. And fermentation of vegetables at home not only preserves vegetables, but also provides many benefits. It enhances vitamins and enzymes in vegetables, and through the influence of microorganisms in fermented vegetables on the lining of the digestive system, it improves the absorption of nutrients from fermented food and other foods that humans eat (Table 1.2).

The primary retail fermented vegetable products produced throughout the world include cucumber pickles, olives, pickles and fermented cabbage.

Fermented vegetables are identified with a few cultural aspects of people. Because of their overall nutritional properties, these products fermented vegetable foods and beverages when assessed, they comprised around 33% of the food sources every day expended around the world. They have also indicated as an appropriate transporter for probiotics. What's more, they contain an enormous scope of prebiotic compounds that can stimulate the development of beneficial bacteria. Accordingly, exploitation of the axis 'fermented foods – human health' based on plant fermentations is a promising and possible technique for what's to come. Essential comprehension of the relationship between food, valuable microbes, and mankind health can be critical to enhance food quality and to forestall (Panel et al. 2016).

Fermented vegetable products have regular qualities of high acidity and low pH that typically make them safe and microbiologically stable up and down their timeframe of realistic usability. However, endurance of certain acid-resistant pathogenic

bacteria can happen when they are not appropriately prepared or dealt with. Various additives, for example, acidifiers (e.g., acetic, lactic, malic, citric, and other acids), additives (e.g., sorbates, benzoates, sulfites, in some cases), and others can be added to pickled products to improve their safety and keeping quality (Eduardo et al. 2016).

1.7.3 Fermented Dairy Products

Customary systems have dominated milk creation in Africa for a long time and still flexibly impressive amounts of milk today. These systems represent above 90% of dairy ruminant population in Sub Saharan Africa (Olaloku and Debre 1992). Inactive farmers live in similar homes throughout the entire year while transitory and transhumant farmers move in search for better fields. In transhumant systems, milk surplus is imparted to neighbors or exchanged in barter, yet is infrequently sold with the exception of by family units living close.

The total milk production in Africa was 46,907,955 millions of tonnes (FAOSTAT 2016). Larger part of which 74.05% is from cows, trailed by goat (8.74%), buffalo (6.23%), camel (5.76%) and sheep (5.23%) milk. The top six African milk delivering countries in terms of milk volume are Sudan (4,391,000 tonnes), Egypt (5,598,477 tonnes), Kenya (4,925,692 tonnes), Ethiopia (3,699,373 tonnes), South Africa (3,337,018 tonnes) and Algeria (4,241,414 tonnes): these six countries produce about 50% of African milk (FAOSTAT 2016). Only approximately 15% of the total milk produced is processed to standard products (cheese, yoghurt, butter, etc.).

Over 70% of absolute milk creation experiences casual markets or is expended on the homestead (Ndambi et al. 2007). In some countries, a few endeavors are being made so as to valorise the neighborhood dairy products. For instance, in South Africa a Slow Food Presidium has been built up to advance excellent South African Raw Milk Cheeses, such as ficks burger, ganzvlei vastrap, karoo crumble and huguenot, produced by small-scale local farmers utilizing just crude milk, adopting environmentally and welfare well disposed strategies and respecting neighborhood culture and traditions (Silvana et al. 2018).

1.8 Conclusion

Fermented foods are considered a favorite among some people, as they prefer to eat them alongside main dishes, due to their delicious and appetizing taste, as well as their high content of nutrients resulting from the fermentation process. Many fermented foods are produced in Africa, most of these foods are produced at home level, have a short shelf life, and safety risks increase due to uncontrolled natural fermentation. Moreover, the consumption of these traditional foods decreases due to the preparation. Raw materials for fermentation include vegetables, fruits, dairy, meat and cereals. These nutrients are naturally fermented by the microbes prevalent

in the processing environments of these fermented foods. The preparation of these foods may be improved by using bacterial strains from their natural sources and using them for controlled fermentation processes.

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Chapter 2
Starter Cultures: Nature, Types, Characteristics, Preparation and Maintenance

Abdel Moneim Elhadi Sulieman

2.1 Introduction

Starter cultures have many definitions. They are bacterial or fungal strains either pure or mixed, utilized to begin a fermentation process. Starter culture implies chosen strains of food-grade microorganisms of known and stable metabolic exercises and that is utilized to create fermented foods of attractive appearance, body, texture and flavour. Another definition, starter culture implies the microorganisms that are chosen dependent on their capacity to create lactic acid for curd creation and a low pH to forestall deterioration, produce metabolites that give alluring flavours: or produce enzymes that ripen the dairy product (Marta et al. 2019).

The fermentation starter or starter culture is the preparation that is utilized to start the fermentation process in in various production lines, from food plants and sustainable power source plants to biotechnology and drugs. It is a microbial culture, which actually performs fermentation. Starter arrangements help the start of the fermentation process in preparation of different foods and fermented beverages. Various bacterial and other microbial strains have been utilized either in single or in mix for creating the ideal impact in the finished product. Starter cultures are bacterial or fungal strains either pure or mixed, used to start a fermentation process.

Starter cultures which are considered as GRAS (Generally Regarded As Safe) by the US Food and Drug Administration (FDA), are able to can hinder the development of unwanted microbiota, specifically pathogenic and spoilage microorganisms (Holzapfel et al. 2003; Young and O'sullivan 2011; Fraqueza et al. 2016).

Starter cultures are derived from experimentally produced, non-specific species to obtain the final product with predictable quality and quantity characteristics. Indentation farm production can be divided into two parts. After fermentation, the

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bacteria should be treated and separated from the fermentation solution, including cultured microorganisms and the rest of the nutrient solution. First, the bacteria are separated from the liquid and concentrated: there are nozzle separators and self-cleaning tablets sterilized by steam for this step of the process (Bourdichon et al. 2012).

The concentrated culture is then transferred to a lyophilized (dehydrated) dryer and dried. Finally, the cultures are filled under tight conditions and stored at low temperatures. Fermented foods and drinks have for some time been produced without utilization of starter cultures. Conventional strategies for creation incorporate backslopping, or utilizing a limited quantity of the completed explicitly safeguarded item to inoculate a new batch, utilization of microbes discovered normally on the product, and the utilization of unique compartments that take into account endurance of the starter culture microbes inside breaks and pores, the utilization of microbes discovered normally on the item, and the utilization of unique compartments that take into account the endurance of the starter culture microorganisms inside breaks and pores.

The customary techniques take into consideration the improvement of individual assortments of fermented foods and refreshments, and they are as yet drilled today for little to mid-scale creation facilities, just as in less developed countries and in natively-type products. Customery techniques, however, are inclined to slow or bombed fermentations, defilement, and conflicting quality. Conversely, present day huge scope industrial production of fermented foods and refreshments requests steady item quality and predictable production schedules just as rigid quality control to guarantee food safety Marta et al. 2019).

2.2 Types of Starter Cultures

The beneficial microorganisms are used in most of the different fields of the food industry and also as a bio-enhanced food supplement. Examples include the dairy, meat and bakery industries, in the production of fermented vegetables, in the production of wine and beer, and in the manufacture of animal foods. Starter farms are cultured from experimentally produced species, indeterminate to achieve predictable reproducible quantity and quality of product.

Starters are classified under various categories according to composition of microflora, growth temperature, type of products, flavour production and type of fermentation. These different cultures are utilized in the fermentation of milk, meat, wine, fruit, vegetables and cereals. To keep up their strength, viability and appropriateness, they are prepared, packaged, bundled, frozen or freeze-dried (Surono and Hosono 2011).

2.2.1 Classification Based on Fermenting Microorganism

On the basis of fermenting microorganism, starter cultures can be classified into the following groups**:**

1. Bacterial starters: as is the case in most types such as those utilized in the production and manufacture of cheese, ripe buttermilk and cooked butter.
2. Mold starter such as Penicillium mold utilized in the production of Comfort and Camembert cheese.
3. Starters that are often not used in manufacturing of cheese, but used in the production of dairy products such as fermented milk products like Kefir and Kummis.

2.2.2 Classification of Starter Culture Based on External Shape of the Organism

The classification of starter cultures can be based on the external shape of the organism, where the example of bacterial primers is divided into:

1. Rod bacteria such as Lactobacillus bulgaricus. Ssp. delbrueckii
2. Coccus bacteria starter culture, such as a bacteria: Leuconostoc lactis ssp. lactis biovar diacetylactis, Leuconostoc lactis ssp. Lactis biovar mesenteroids.
3. There is a third type that falls between the two types are coccus and bacillus, such as Lactococcus lactis ssp. cremoris ssp. Lactis.

2.2.3 Classification of Starter Culture Based on Growth Temperature

Starter cultures can be divided depending on the optimum growth temperature of the organism into:

1. Mesophilic starters: they prefer growing at a temperature of 20–30 ° C, such as starters used for manufacture of cheddar and Gouda cheese.
2. Thermophilic starters prefer to grow at 40–45 ° C, such as starters used for manufacture of Italian and Swiss cheeses.

2.2.4 Classification of Starter Cultures Based on Flavour Production

The natural microflora in milk is either ineffective, unmanageable and unpredictable (predicted fermentation processes), or has been completely destroyed by heat treatments (exposure to heat). Looking at milk, the starter culture can provide certain properties by controlling and predicting further the fermentation process such as flavor compounds.

The starters are classified according to their ability for flavour production into four groups:

B (L) type: The flavour producer is Leuconostocs.
D type: *L. lactis* subsp. *lactis biovar diacetylactis*
BD (LD) type: Blend of both of the above cultures
N or O type: Devoid of flavour producing organism

2.2.5 Based on the Fermentation Type

It is also possible to adopt the products of biological activities and the ability of the starter culture to produce acids, as they are divided into

1. Homofermentative organisms that produces mainly lactic acid (more than 99%) and within this type are all production acid starters, such as *Lactococcus lactis* subsp. *lactis*
2. Heterofementative organisms that have the ability to produce lactic acid and other compounds such as significant amounts of acetic acid or formic or flavour compounds. And within this group all the bacteria responsible for producing flavors in a cheese or mature butter as well as having a moderate ability to produce acid. Heterofermentative starter examples is *Leuconostoc dextranicum* (Fig. 2.1).

2.2.6 Types of Starter Cultures Used in Industrial Fermentation

The microorganisms used in industrial fermentation can be grown in shallow, batch, or submersible culture or in continuous culture (Surono and Hosono 2011; Marta et al. 2019) as follows:

1. **Static (surface) Cultures**

In this type, the microbe grows on the surface of the nutrient medium in the liquid, forming a surface growth, and we must maintain the surface growth of the

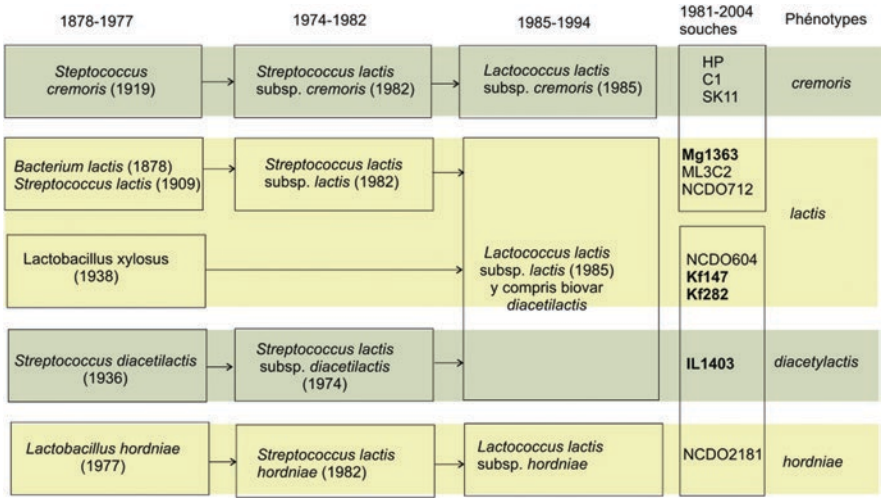

Fig. 2.1 Natural diversity and adaptative response of *Lactococcus lactis* (Francais 2006)

microbe, because if it sinks the microbe will perish and the fermentation process will stop.

2. **Submerged Cultures**

In this type of starter culture, the microbe grows on every part of the food medium, provided that air is delivered to all parts of the medium throughout the fermentation period, and this is done by circular agitation (vibrating cultures). The submerged farm method has the advantage of saving large areas, it is less expensive and gives more productivity in a shorter period.

3. **Batch Cultures**

The batch development system is considered a closed system for the development of the microbe over a limited amount of the food environment, where the fermentation process is repeated, and we note that the production in this case is in batches. This method is characterized by low costs, but the disadvantage is that production rates are related to the growth rates of the microbe. The advantage of a batch culture is that the fermenter can be used for different reactions with each separate use.

4. **Development in Incremental Batches**

This method began to be used at the beginning of the twentieth century, in order to control the growth of microbes through the use of multiple carbon sources, and this method is now widely used to produce antibiotics, vitamins and enzymes using bacteria and fungi.

5. **Shallow Cultures**

This type of fermentation uses trays that are temperature controlled by incubation in sterile rooms with constant temperatures.

6. **Continuous cultures**

This method has been used for a long time and it is also widely used in research laboratories to study the nature of growth of microorganisms or organisms used in industry, their metabolism and the materials produced from them. It depends on prolonging the log phase by adding one of the food ingredients, as well as withdrawing an amount of volume equal to the volume of the previous addition from the environment. So that the volume of the environment in the fermentation vessel remains constant with the stability of the growth rate, which causes the stability of the production rate of the final product, and this case is called the steady state. A disadvantage of continuous culture is that there is a higher risk of contamination due to the constant adjustments.

Continuous fermentation is feasible only when the inoculated cells are genetically stable (Tables 2.1 and 2.2; Fig. 2.2).

2.3 Starter Culture Preservation

An ideal starter cultures produced which must posses the following characteristics:

- It must contain the most extreme number of viable cells.
- It must be devoid from any contaminants, for example coliforms or yeasts and moulds.
- It must be dynamic under preparing conditions in the dairy and consequently upkeep of the intermediate and other cultures is extremely important.

The impact of preservation on proteolytic activity of starter culture isn't all around recorded. It has small impact and it may be the case that starter strain variety in viability during storage has the significant effect on flavour development in maturing cheese (Broome 2007).

The methods of preservation include: Liquid starter, dried culture, frozen culture and Starter concentrate (http://dairy-technology.blogspot.com).

1. **Liquid starter**:

This is the most popular and widely used form in which starter culture are handled in the dairy. Starters are normally stored in small amounts; however, a sale-up

Table 2.1 Starter cultures used for milk fermentations

Mesophilic	Thermophilic
Lactococcus lactis subsp. lactis	Lactobacillus delbrueckii subsp. bulgaricus
Lc. lactis subsp. cremoris	Streptococcus thermophilus
Lc. lactis subsp. lactis biovar diacetylactis	Lb. helveticus
Lactobacillus kefir	Lb. acidophilus
Lb. casei	Lb. paracasei subsp. paracasei
Leuconostoc spp.	Bifidobacterium spp.

Source: Surono and Hosono (2011)

Table 2.2 Lactic starter cultures used in food fermentation

Species	Subspecies	Type of starter	Main uses
Lactococcus	Lc.lactis subsp. lactis	Mesophilic	Cheeses, butter, butter milk
	Lactococcus lactis subsp. Lactis biovar. diacetylactis	Mesophilic	Gouda, edam, butter milk
	Lactococcus lactis subsp. cremoris	Mesophilic	Some cheeses, butter
Streptococcus	Streptococcus Thermophiles	Thermophilic	Yoghurt and many hard and semi-hard cheeses
Lactobacillus	Lb. acidophilus	Probiotic culture	Cheese and yoghurt
	Lb. delbrueckii subsp. lactis	Thermophilic	Fermented milk, high cook cheese
	Lb. helveticus	Thermophilic	Fermented milk, hard and semi-hard- cook cheeses
	Lactobacillus casei	Probiotic adjunct culture	Cheese ripening
	Lb. plantarum	Probiotic adjunct culture	Fermented milk (robe)
	Lb. rhamnosus	Probiotic adjunct culture	Cheeses
Leuconostoc	Leuconostoc lactis	Mesophilic	Fermented milk (Robe)

Source: Gemechu (2015), Sulieman (2017)

Fig. 2.2 Swiss cheese

system of propagation is required to meet the required volume for any production line. The working stock cultures are preserved in autoclaved reconstituted antibiotic-free skim milk powder with daily or weekly sub-culturing.

2. **Dried culture:**

Preservation by drying procedure is an elective technique for culture maintenance. The development of such procedures tries to defeat the work engaged with

keeping up liquid stock-cultures. It likewise encourages the dispatch of the dried cultures by post without any loss in activity. Many techniques are followed in drying of starter cultures:

(i) **Vacuum drying**:

The process of vacuum drying was the n typical practice, which includes mixing a fluid culture with lactose and afterward neutralizing the excess acid with calcium carbonate. The mix is somewhat concentrated by partition the whey, so yielding granules, which are dried under vacuum. The dried starters contain just 1–2% viable bacteria, and may require a few sub-culturing before returning maximum activity.

(ii) **Spray drying:**

Higher survival rates can be acquired. This system has not been utilized commercially. This technique is utilized when drying the bacterial cultures that produce bacteriocins as well as those used as starters in (dairy and cheese), because spray drying consumes less energy when removing water and maintains a higher versatility and efficiency compared to freeze-drying, given that the bacterial cultures are not exposed to freezing damage.

(iii) Freeze-dried cultures:

This technique improves the survival rates of the dried cultures and good results have been acquired compared to spray-dried starters. Theses cultures can destroy the bacterial cell-membrane, however, the destruction can be decreased by addition of certain compounds. Starter culture maintained by this technique are fundamentally utilized as inoculants for the propagation of mother cultures. There are many factors that affect the viability of lyophilized bacterial cultures, starting with the type of bacteria and the conditions for their development, as well as the method of cultivation, and that the reduction in the viability and effectiveness of these microorganisms can occur during the preparation of these cells for the process of lyophilization, and even the use of the final product after freezing for manufacture, so it is difficult Determine the exact cause of the decrease. The type of bacteria preserved is a factor in resisting damage from freezing and lyophilization, as Gram-positive cells are more resistant than negative cells such as E.col. cells (Mustafa 2020).

3. **Frozen starters**

Liquid starters cultures can be preserved by freezing at −20 to −40 °C for a few months. The frozen starters are dispatched to a dairy whenever needed, as a direct inoculants for bulk starters.

The prolonged storage of frozen culture at −40 °C can lead to deterioration of its activity, and can inhibit certain lactobacilli, but the utilization of a medium containing 10% skim milk, 5% sucrose, fresh cream, 0.9% NaCl or 1% gelatin can improve the survival rates. Presently, there a far and wide interest in the preservation of cheese-starter cultures by freezing in liquid nitrogen and in the utilization of concentrated frozen starter cultures for direct inoculation into the tank in the production of cheese (Accolas and Auclair 1967; Baumann, and Reinbold 1966). Effectively

such cultures are in commercial use in the United States for the setting of mass inoculum and for direct tank inoculation for long-set cheese manufacture. Viable counts were performed when freezing in liquid nitrogen and after storage in liquid nitrogen. There was no lessening in viable count or loss in activity of the cultures because of freezing and storage.

4. **Concentration of cells**

Various systems are utilized in the concentration of microbial cell biomass including:

(i) mechanical means: which cause some physical damage.
(ii) continuous neutralization of the growth medium at pH 6.
(iii) diffusion culture technique (removal of lactate from growth medium.

2.4 Preparation of Starter Cultures

Propagation of starter culture is a basic stage for manufacture of products acceptable quality. It is produced from experimentally produced species, indeterminate to achieve predictable reproducible quantity and quality of product (Marta et al. 2019).

For preparation, the microorganisms will be carefully chosen and added to raw material (uch as milk) to begin fermentation process. They are majorly lactic acid bacteria (though other bacteria types also apply), yeasts, and/or moulds.

Chosen of reasonable starter culture relies upon many factors, of which:

1. Production conditions
2. Accessibility of various forms of the starter to be utilized.
3. Knowledge about the starter to be utilized.

Proper starter must be selected carefully, since it has greater influence on the overall product quality.

2.4.1 Preparation of Mother Culture

Preparation of mother culture is a significant step in the preparation of bulk starter (Sulieman 2017). The mother cultures are preserved in narrow neck poly-ethylene bottle which are cleaned with a detergent, sterilized over steam and filled with 0.1% hypochlorite solution, the seals and caps are also autoclaved and held in the hypochlorite solution.

This process has advantage that contamination of starter at any one stage does not entail contamination at the next stage.

2.4.2 Bulk Starter Culture Preparation

The bulk milk for starter inoculation must be blended to decrease the impacts of contaminants that might be present in milk from a single farm. Blending also improves the quality of the bulk. Used vessels are steam sterilized and filled with bulk raw milk. The milk utilized for producing the starter must possess the following characteristics: it must be from first grade, liberated from inhibitors/antibiotic, ready to shape a smooth and homogenous coagulum, clean flavour and smell, liberated from microbes that produce antimicrobial substances. Satisfactory quantities of minerals, particularly manganese and vitamins, relatively high solid non-fat (SNF) content and the milk ought to be with moderately low free fatty acids content (Nikola et al. 2020).

2.4.3 Continuous Starter Production

This method is more efficient and advantageous. Many cheese plants today might utilize about 500–1000 gallons of starters for cheese manufacture using 100.000 gallons of milk. This type of culture needs numerous equipment's and the maintenance for disinfection and sterilization, which are expensive. A genuine hindrance of this technique is that if the culture is assaulted by a phage, the entire mass and all equipment's are contaminated, so control of purity and activity is principal Nikola et al. 2020).

2.4.4 Preparation of Master Culture

For preparation of master cultures, litmus milk recently disinfected in glass bottles is filled into polyethylene tubes. The method of inoculation is the same as for mother culture, however, milk is sterilized or pasteurized. Such cultures may be preserved indefinitely with cautious checking and testing after 3 months interval.

2.5 Uses of Starter Culture in Food Industry

The production of cheese, yogurt, fermented milk, fruits, vegetables, and sausages happens via utilization of starter cultures that are consistent, reliable, and safe. The cultures furnish the food products with a huge number of properties. Acidification of the food matrix is an essential property in countless food fermentations. Fermentation action regularly will be utilized to characterize packaging size and the unit of activity, while different attributes differentiate a culture from the scope of

other accessible starter cultures. Starter cultures are monetarily accessible in liquid, frozen, or lyophilized form from several companies serving provincial or global markets (Hansen 2014; Sulieman 2017) (Figs. 2.3 and 2.4).

2.6 Factors Causing Inhibition of Starter Cultures

Dynamic and reliable starter cultures must contain the highest possible number of viable microbial cells, be exceptionally dynamic under production conditions, and be devoid of contaminants. The fermentation process of any cultured food product depends totally upon the purity and activity of the starter culture. Likewise, the growth medium or food substrate must not contain inhibitory agents, for example, antibiotics and bacteriophage (Surono and Hosono 2011).

Numerous variables can cause an inhibition and/or decrease in starter culture activity and can result in deterioration of fermented products quality reaching the consumers, and financial loss to the producers. Therefore, factors inhibiting starter culture include:

1. **Antibiotics:**

Antibiotics that inhibit bacteria limit bacterial growth by interfering with the bacteria's protein production center, DNA reproduction, or other properties. These antibiotics inhibit the growth and reproduction of bacteria without killing them. The

Fig. 2.3 Fermented Vietnamese sausage. (Source: https://commons.wikimedia.org/)

Fig. 2.4 Sour dough bread. (Source: https://commons.wikimedia.org/)

process of killing bacteria is by means of bacteria killing agents. The agents that inhibit the bacteria must work with the immune system to get rid of the organisms present in the body. Sometimes there is no difference between inhibiting and killing agents; A high concentration of some inhibitory agents is fatal, and also low concentrations of some inhibitory agents are inhibitory (Adetunji 2011).

Some residues of antibiotics may persist in milk after treatment of dairy cows against mastitis disease. Starter culture are susceptible to very low concentrations of antibiotics, which can result in their inhibition. Moreover, the inhibitory levels of streptomycin, chloramphenicol and tetracycline seem rather high, and this apparent resistance could be attributed to culture strain variation, variation in the commercial preparations of antibiotics utilized. Likewise, it is accounted for that stress factors related with the production and storage of food may influence the adjustments in the antibiotic resistance profile of these microorganisms. Research indicates that the acquisition of antibiotic resistance in starter cultures is not a critical issue, however it represents a worry that can't be ignored (Urszula et al. 2020).

2. **Additives**

Food additives presently utilized in the dairy industry could altogether influence the development and practicality of lactic acid starters (e.g., S. thermophilus, L. delbrueckii ssp. Bulgaricus, and Lactococcus lactis) and probiotic bacteria (e.g., Lactobacillus acidophilus, L. casei, Lactobacillus paracasei, Lactobacillus rhamnosus, and bifidobacteria) utilized for fermented items (Fernandes et al. 2007, Vinderola et al. 2002). These added substances incorporate salts (NaCl and KCl), sugars (sucrose and lactose), sweeteners (acesulfame and aspartame), aroma compounds (diacetyl, acetaldehyde, and acetoin), characteristic colorings for fermented milks (red, yellow and orange colorings), seasoning agents (strawberry, vanilla, peach, and banana essences), enhancing–coloring agents (strawberry, vanilla, and peach), nisin (a polypeptide-type antibiotic produced by L. lactis which is active against spore-forming bacteria and could be utilized as a natural preservative in

addition to lactic acid), natamycin and lysozyme, and carbon dioxide as gassing agent (Korbekandi et al. 2011; Decourcelle et al. 2004).

It has been shown that probiotics are more lenient to dairy added substances than lactic acid starter bacteria (Decourcelle et al. 2004). Sodium chloride up to a certain concentration (e.g., 0.5%, but not above 1%) may exhibit stimulatory consequences for development and activity of the probiotics in fermented milks (Mortazavian et al. 2010; Mortazavian et al. 2008). Essential oils may be added to fermented milks as essences can antagonistically influence the practicality of the probiotics. Mint and ziziphora essences at a concentration of 0.2% can prompt a critical decrease in viable counts of L. acidophilus LA-5 and Bifidobacterium lactis BB-12 during storage time (Korbekandi).

It has been discovered that carbonation into milk during fermentation decreases the incubation time altogether by AT (L. acidophilus and S. thermophilus) and ABT cultures (L. acidophilus, bifidobacteria and S. thermophilus). The explanation could be stimulatory impact of this gas on starter bacteria just as its effect on the acidity of milk (Vinderola et al. 2000). Lactic acid bacteria are moderately lenient to carbon dioxide and their acidification rate is not considerably affected by this gas. Low amounts of carbon dioxide delivered by S. thermophilus have a stimulatory impact on development and activity of L. delbrueckii ssp. bulgaricus [Østlie et al. 2003).

3. **Bacteriophage:**

Phages are viruses that can attack the bacterial cells with its tail extending from its body, and the DNA is transferred to the bacteria, hence destroy starter culture, and the result is the failure of lactic acid production**.** Based on sensitivity to phages starter culture are classified into 3 main groups: Phage insensitive, Phage carriers (slight reduction in the rate of lactic acid being produced and Phage sensitive (the bacteria undergo complete lyses). Lactic streptococci and lactobacilli are the most vulnerable organisms of the starter culture attacked by phage (Fig. 2.5).

The safeguard measures, which can be followed to diminish the effect of phages, include:

- Application of aseptic technique for preparation of starter culture.
- Heat treatment of mass starter milk
- Utilization of phage resistant strains in the dairy production
- Effective filtration of air in the starter preparation room
- Properly sterilizing of the equipment's
- Location of starter room far away from production room.
- Plant personnel must not be permitted to go into into starter handling room.
- Preparation of starter culture in phage inhibitory medium.
- Development of phage resistant strains
- Use of mixed strain starters.

4. **Detergents and disinfectants residues:**

Detergents and disinfectants include just a segment of chemical contaminants in milk. Other chemical contaminants incorporate anti-microbials and sulfonamides, pesticides, herbicides, fungicides, dioxins and mycotoxins. Chemical contaminants

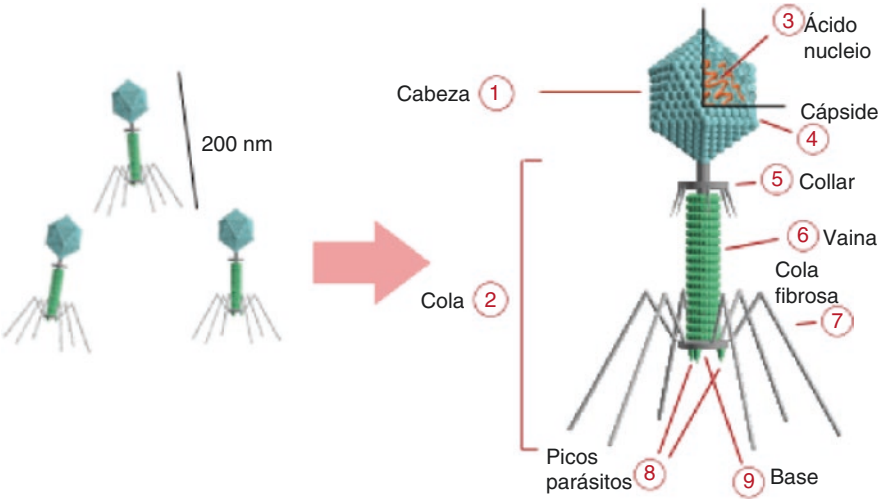

Fig. 2.5 Structure of a bacteriophage. (Source: https://commons.wikimedia.org/)

have the possibility to make toxicological harm consumers. They can be the contributory components in a few illnesses, for example allergic reactions (Joana et al. 2013; Adetunji 2011; Gomes and Demoly 2005), cancer, heart disease, Alzheimer's disease and Parkinsonism (Khaniki 1999; Riedl and Casillas 2003). Buildups of chlorine compounds in milk are not hazardous in this regard due to their fast deterioration, however different sanitizers, for example, quaternary ammonium compounds are rather stable in milk. A few detergents and disinfectants as well as antibiotics and sulfonamides, can cause genuine danger in manufacturing cheese and cultured milk products items because of decreased starter activity (Jepsen 1962; Jones 1999).

Cleaning and sanitization dairy equipment is fundamental to guarantee the sterile nature of milk. Infrequently, a few some farmers go through washing fluids and sanitizers for home use, particularly when wiping methods are completed physically. Deposits of cleansers and sanitizers in milk may meddle with the reaction of microbial inhibitor tests utilized for screening screening antibiotics in milk. Disinfectants utilized for cleaning purposes for dairy equipment might include alkaline detergents, iodophore, quaternary ammonium compounds and ampholytes), these can influence the starter activity (Romero et al. 2017).

To guarantee the quality and wellbeing of milk, it is important that acceptable cleaning and disinfectant rehearses are applied. Along these lines, avoiding the presence of residues in milking equipment.

5. **Miscellaneous inhibitors:**

Natural antibodies such as (lactenins/agglutinins) are present in milk and can inhibit the growth of starter culture. These antibodies are heat sensitive, so the heat treatment of bulk starter milk can destroy them. Also late lactation milk and spring milk have some effect on starter culture activity. Other inhibitors in milk could be

due to environmental pollution, such as insecticides which can inhibit the starter organisms, volatile and non-volatile compounds (fatty acids, formic acid, formaldehyde, acetonitrite, chloroform, ether… etc.) in concentration up to 100 ppm inhibit growth of the *Streptococcus sp.* and *Leuconostoc cremoris* .

2.7 Conclusions

Chosen starter cultures give a useful asset to driving the fermentation of food products, permitting wanted quality and safety targets to be reached. Their utilization in food fermentation brings about increasing speed of fermentation, an improvement of safety (by lessening undesirable microorganisms), and a better quality of the end product. The choice of a starter ought to be done with regards to its application, since usefulness will rely upon the sort of food, the technology applied, the ripening time, and the ingredients and raw materials utilized. The manufacture of fermented foods could be improved by using bacterial and/or fungal strains isolated from their natural sources, together with optimizing the environmental factors, nutritional requirements as well as controlled fermentation processes.

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Chapter 3
Properties and Advantages of Food Fermentation

Abdalbasit Adam Mariod and A. A. Massad Fatima

3.1 Introduction

At the beginning of its use, the word "fermentation" referred to chemical reactions in which gas was formed on the surface of the growth medium, resulting in a quantity of air bubbles. Fermentation is derived from the Latin verb (fervere) "boiling" The term was first used in the late fourteenth century in chemistry, and was widely circulated. However, it was not used in the scientific community until the beginning of the fifteenth century, and the term was later greatly expanded to include all chemical reactions associated with oxidation and reduction by microbial biology or microbial enzymes in which organic compounds are either gifting or receiving hydrogen ions. Hydrogen ion-receiving compounds will accumulate into fermentation products (Peter et al. 2016). Fermentation, is the process of extracting energy from redox reactions of chemical compounds, including carbohydrates, and by using an intrinsic electron acceptor, which is often an organic compound. In contrast, in respiration, electrons are given away to an external electron acceptor, and like oxygen, by the electron transport chain (Prescott et al. 2004). Here the fermentation process plays an important role in an atmosphere of anaerobic conditions, as there is no oxidative phosphorylation to maintain the production of ATP by the degradation process. Pyruvate is also represented by many different compounds during the fermentation process. Where the lactic fermentation process expresses

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the production of lactic acid from pyruvate; Whereas, the alcoholic fermentation process expresses the conversion of pyruvate to ethanol and carbon dioxide; However, the heterogeneous lactic fermentation process is the production of lactic acid in addition to other acids and alcohols. The fermentation process need not be carried out or carried out in an anaerobic environment. For example, even with abundant oxygen, yeast cells greatly prefer fermentation over oxidative phosphorylation (Dickinson 1999).

Fermentation is caused by microbes such as bacteria, molds and yeast. For example, we find that fungi or mold work on a mixture of sugar with mineral salts to produce penicillin. Yeast breaks down the sugar from the beans soaked in water into ethyl alcohol gas and carbon dioxide when making beer. Also, the sugar in grape juice decomposes in the same way as when making wine. Fermentation is also considered essential in the production of bread, cheese and curd. But it may be harmful in some cases, such as when fermented milk becomes spoiled. Fermented products beneficial to humans are made in large quantities. Although different types of materials are produced by the fermentation process, the basic processes involved in this remain the same. First, large stainless steel tanks are filled with an aqueous solution of nutrients. This solution is sterilized with steam to kill unwanted germs, and then specific microbes are added to the solution, to ferment the foodstuffs within a few days. The fermentation supervisors control the temperature and acidity of the materials inside the tanks. Finally, the tanks are filtered from the liquid, and the desired products are separated from the rest of the mixture, either by extraction or filtration, or by some other means. In most cases, the desired products constitute only about 5% of the mixture present in the tanks, so the purification process is often considered a very complex process (Akbari et al. 2021).

3.2 Types of Fermentation

The most important types of fermentation:

First: Alcoholic fermentation: This fermentation is the basis for:

1. The manufacture of beer and wine, if alcohol can be produced from juices of some fruits such as grapes or barley extracts.
2. It is the first stage of vinegar industry.

Sugar is converted to alcohol by fermentation induced by the yeast of the genus Saccharomyces under, an aerobic conditions, the selection of the species belonging to the previous species depends on the type of feedstock used in manufacturing industry.

When grain is used as a feedstock, the *S. cervisiae* type is used, when using apple juice, *S. moli* is used. Conditions for the type of yeast are the following: It is characterized by its high efficiency in converting sugar into different concentrations of alcohol, and has fixed qualities besides it should be easy to settle after using the fermentation process, which facilitates the separation process (Wang et al. 2019).

3.3 Important Notes in Alcoholic Fermentation

1. The sugar solution is used at a concentration of 10–18% and it is preferable that it be 12% because the concentration is more than that. It inhibits the growth of the wine, and this leads to the sugar remaining without fermentation, while the decrease in concentration leads to economical decline of manufacturing.
2. The aerobic conditions must be because the aerobic conditions cause a decrease in the formed alcohol.
3. Continuously adjusting the temperature during the fermentation process, it was found that one gram of sugar leads to the release of an amount 120 kcal of temperature, which leads to inhibition of yeast and increase the activity of lactic acid bacteria, the temperature can be controlled by using cooling devices or by spraying water on the outer surface of the tank.
4. Increasing the percentage of alcohol formed at the end of fermentation. CS2 or one of its salts is added to the ratio of 125 ppm before adding starter to help complete the fermentation process and prevent the growth of fungi, yeasts, acetic and lactic bacteria, and accelerate the growth of desired yeasts.

3.4 Alcoholic Fermentation Steps

1. they form very fast and take 3–6 days when most of the sugary material is wandered to alcohol and a second, carbon oxide, the conditions associated with this fermentation are characterized by the absence of contamination by non-microorganisms desirable.
2. formation is slow compared to the first step, taking between 2 and 3 weeks, which increases the risk of occurrence. Contamination with microorganism, such as acetic acid bacteria and lactic bacteria, leading to slow fermentation, and thus measures should be taken to prevent pollution such as ventilation of the solution, strengthening of the wine and increasing temperature, especially cold months and optimal yeast growth temperature 8.23–7.26 m.

3.5 Acetic Acid Fermentation

They are under aerobic conditions and the alcohol produced is oxidized through alcohol fermentation into acetic acid. Acetobecter, called the mother of vinegar, should be selected. By their ability to oxidize alcohol into acetic acid and exclude species that have the potential to cause oxidation as their presence leads to a decrease in the amount of vinegar that is theoretically supposed to be obtained, they arrive. The proportion of acetic acid from vinegar produced is 5%, thus inhibiting the growth of corrosive microorganism (Karekar et al. 2020).

3.6 The Importance of Fermented Foods to the Human Body

1. Bacteria are of great importance in food, which include the vital supports, which are found in dairy and many types of cheese, as well as fermented foods. Bacteria are involved in many food industries, and they have a great benefit as bacteria are used in some of the preservatives to preserve food. For a longer period.
2. Dairy products that are fermented through bacteria have great importance for the human body, such as fermented cheese, which is very useful for regulating the work of the digestive system, and it increases the body's immunity significantly, and fermented food prevents cancer and is beneficial to human health, and another example Yogurt contains beneficial bacteria, and these bacteria are the main factors behind fermenting milk and turning it into yogurt.
3. Eating fermented foods, including yogurt, leads to the provision of the body with the enzyme lactase, which works to digest the sugar lactose, which is less in humans after childhood, and many studies have shown that eating yogurt leads to the production of beneficial bacteria in the intestine that work To fix cancer cells in the intestine and prevent their activity.
4. Fermented foods also reduce harmful enzymes in the human body that increase the risk of developing cancerous tumors, in addition to the beneficial yogurt microbes working to reduce the side effects resulting from taking antibiotics, and fermented foods also reduce harmful cholesterol in the blood.
5. Food fermented by beneficial bacteria reduces the incidence of allergies and works to prevent various types of allergies, and the introduction of beneficial bacteria into the human intestine reduces the incidence of eczema in the skin, asthma and many other allergic diseases.
6. The beneficial bacteria also work to resist diseases and work to expel toxins from the body and strengthen the body, so fermented foods have a great role in preserving the human body (Farooqui 2021).

3.7 Fermented Foods Are the Elixir of Life and Protection from Disease

The process of fermentation that takes place on these foods gives it a great value and benefit, and through medical studies it has been confirmed that these foods are the most suitable for everyone so that everyone can live a better life. Many doctors call fermented food the elixir of life because it gives a great capacity. To protect the human body from diseases, among which are dangerous diseases such as cancer, as we mentioned. With the great scientific development that we have become coexisting with, it has become easy to find a lot of fermented foods, which contain live bacteria, and among those foods is fermented and cooked oat bran, these foods are fast foods that resemble yogurt.

3.8 Advantages of Natural Fermentation

The nutrients in fiber, starches, salts and minerals in naturally fermented bread are easier to digest because the beneficial bacteria have processed them during the fermentation of the dough. Wheat sensitivity and intolerance to wheat disappear when you completely abstain from eating bread fermented with commercial yeast, and instead eat naturally fermented bread. There are two methods of fermentation of bread: commercial yeast used by bakeries and also used in homes, and natural yeast. The two types differ greatly in their effect on the wheat flour, the taste of the bread, and the nutritional value of the bread. Commercial baking yeast is one type of organism that quickly emits large amounts of gas without altering the starches and fibers in the dough. As for natural yeast, it contains two types of living organisms that coexist, namely: live yeast and beneficial bacteria, which work to change the starches and fibers in the dough to make it easier to digest and the bread to become more resistant to rot and drought. The natural yeast that works gradually (the fermentation process takes an average of 8 h) has many and very necessary benefits for health that may not be suitable for bread without it, namely: Nutrients such as fibers, carbohydrates, salts and minerals in naturally fermented bread are easier to digest because the beneficial bacteria have processed them during the fermentation of the dough, and the fermentation process itself results in an enzymatic activity that improves the integrated composition of the nutrients in the bread. The natural yeast causes armies of beneficial bacteria to multiply inside the dough, like an explosion. And when you eat naturally fermented bread, the beneficial bacteria multiply in the intestine increases, which improves digestion, prevents fermentation and reduces constipation (Al-Ansi et al. 2021).

Natural fermentation treats phytic acid and phytates, which are difficult to digest and present in the bran of whole bread, and transforms them into vitamins and phosphatic acids, that is, into magnesium, calcium and iron that are useful and easy to absorb and assimilate. The acid / alkaline environment resulting from the digestion of fermented bread with commercial yeast ranges between 5.9 and 6.5, which means that it is acidic and is difficult for the body to digest. Naturally fermented bread has an acid /alkaline digestion medium of 4.8, which is the closest to equivalence. Ailments such as wheat allergy and intolerance to wheat disappear when you completely stop consuming commercial yeast fermented bread and instead eat naturally fermented bread. Wheat gluten and its fermentable starches become easier to digest and absorb after a long fermentation process. Recent research has proven that the human immune system can stop the growth of cancer cells without killing them, and one of the most important ways to strengthen immunity is to eat naturally fermented foods. People are accustomed to eating commercial yeast fermented bread, bread that improvers make it look crisp and delicious, and it actually converts in the intestine into hard-to-digest and lifeless lumps. People today desperately need to be aware of the harms of fermented bread with processed commercial yeasts, and to raise awareness of the need to return to natural fermentation, even if it takes a longer time, as a person does not have more value than his health (Maicas 2020).

3.9 Conclusion

Today nutrition experts around the world have worked to encourage people to eat fermented foods due to the great benefit that accrues to the human body as a result of consuming a large amount of those fermented foods, and it protects it from many diseases as well as increases the efficiency of the internal organs of the body and improves them and works to improve the functions of The liver reduces fats and cholesterol in the body.

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Chapter 4
Significance of African Fermented Foods in Nutrition and Food Science

Suzy Munir Salama and Abdalbasit Adam Mariod

4.1 Introduction

Food fermentation is a kind of food preservation technology that is practiced traditionally in the past in many African countries and still being used by indigenous people and most communities of the developing countries. It is performed by the fermenting action of micro-organisms especially yeast fungus and bacteria of lactic acid (Mokoena et al. 2016). In developing countries, fermented food and beaverages are considered one of the main dietary stuffs that are consumed due to their preferred taste, quality and food digestibility (Nout and Motarjemi 1997). There are various strategies for fementing food practiced by Africans including alcoholic, non-alcoholic, alkaline and amino acid fermentation based on plants, milk, insects and meat (Dirar 1993; Oyewole 1997; Steinkraus 1997). However, fermentation of plant and animal-based foods differ in the contents of their end products. Plant-based food products are rich in their contents carbohydrates and sugars which are essential substrates for completing microbial fermentation process, while animal-based food products have their characteristic of less carbohydrates and more protein content (Kewuyemi et al. 2020). The present chapter explains the significance of African fermented foods focusing on some important types of food as traditionally and commonly used in different parts of Africa and their beneficial effect in food science. Figure 4.1 shows the most important African fermented food sources.

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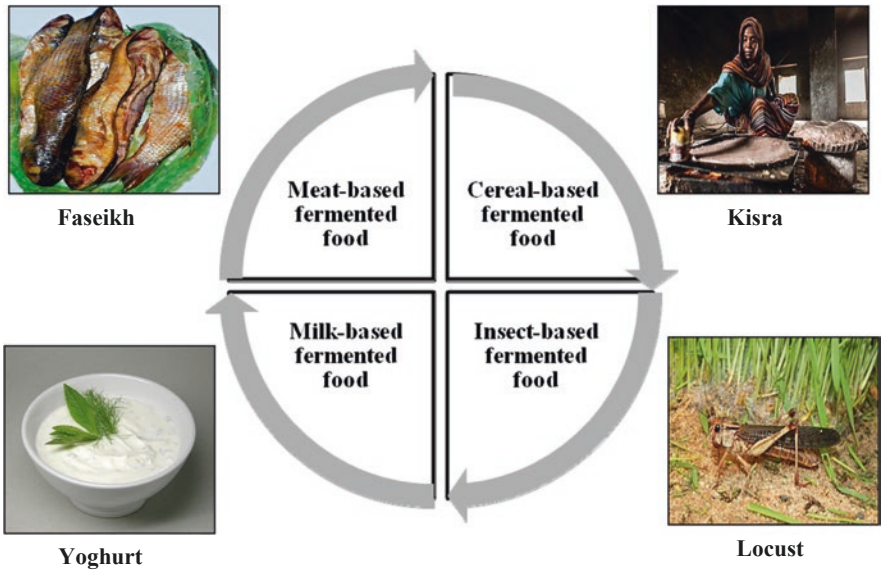

Fig. 4.1 The most important fermented foods in Africa

4.2 Cereal-Based Fermented Foods

In Africa, Cereals like oat, barley, corn and sorghum/millet are considered as primary human food stuff due to their high nutritive contents for children and adults (Achi and Ukwuru 2015). Additionally, adout 70% of the total energy intake depends on cereals in developing countries (Descalzo et al. 2018). Cereals are very good substrates for fermentation achieved by therapid growth of probiotic bacteria and micro-organisms (Kedia et al. 2007; Kocková et al. 2011). Additionally, ceral-based fermented foods from millet (*Peninsetumamericanum*), maize (*Zeamaize*) and sorghum (*Sorghum bicolor*) is traditionally and widely practiced for preserving food and improving the organoleptic properties of food by the action of lactic acid bacteria. Further, fermentation of cereals removes mycotoxins, reduces anti-nutrional factors and improves nutrional profiles of the produced food (Achi and Ukwuru 2015). Nutritionist reported that bacterial fermentation of cereals reduced the percentage of aflatoxins in African cereal-based beaverages 1000 folds (Wacoo et al. 2019). Fermentation of cereals positively elevates health-promoting biologically active compounds such as polyphenols through the break down of cereal's cell wall and the release of active polyphenols increasing their antioxidant acivity that protects against many ailments (Adebo and Gabriela Medina-Meza 2020). Morover, the probiotic activity of micro-organisms during fermentation of food modulates gut microbiota and improves the immune response of the body reducing the risk of occurrence of hypertension, hypercholesterolemia, inflammatory bowl disease, diabetes and cancer (Chileshe et al. 2020).

4.2.1 Sorghum and Millet

Sorghum and millet-based fermented foods such as *kisra*, *hulu-mur*, *merica* and *nasha* are very common in many African countries especially in Sudan, Nigeria and Tanzania as main food products for indigenous people (Adebo 2020). Sorghum contains highly nutritive components such as proteins, polysaccharides, vitamin B, iron and high fiber contents. Also it contains biologically active compounds such as anthocyanines and polyphenols of high antioxidant activity (Adebo 2020). As mentioned in the previous section, microbial fermentation of sorghum leads to cell wall rupturing of sorghum grains and releasing high percentage of the phytochemical constituents especially phenolic compounds that play key role as anti-cancer, gastrointestinal health potential, anti-dibetic andcardioprotectivecandidates (Taylor and Duodu 2015).

4.2.2 Maize

Nutrtionists reported that maize contains valuable nutrients of carbohydrates, proteins, fats, vitamins as vitamins B and C, minerals as manganese and phosphorus in addition to folate and fiber. In addition, maize is rich in phytochemical compounds *viz*phenols, carotenoids and polysterols (Saeed and Saeed 2020). Based on a recent study on the effect of fermentation on the nutritional and the phytochemical composition of maize, it was found that maize *Lactobacillus* fermentation of maize increased its constituents of vitamins B, C and E, folate, riboflavin and carotenoids compared to unfermented samples (Chaves-López et al. 2020). Additionally, fermentation increased the soluble phenolic compounds content of maize (Gabaza et al. 2018). In a study, Scientists found that the famous maize-based African fermented foods, *Ogi* and *Omidun*could significantly protect the rats from experimentally-induced colitis *via*prevetion of depletion of colonic antioxidant enzymes (Haruna et al. 2019). The maize-based non-alcoholic fermented beverage, *Munkoyo* from Zambia was found to have anti-microbial and anti-allergic activities, and suppresses diarrhea (Chileshe et al. 2020).

4.2.3 Barley

Barley is another important African cereal regards its nutrional value and biochemical composition. It is significantly rich in the dietary fiber, β-glucan and contains relative percentage of phosphorous and potassium minerals. In addition, it contains healthy unsaturated fatty acids and bioactive phenolic compounds that play key role in protecting against diseases (Lahouar et al. 2017). Fermentation of barley seeds with *Lactobacillusplantarum* was recently found to reduce obesity in rats induced

by high-fat diet and type-2 diabetes (Gu et al. 2021). In another clinical study, fermented barley diet reported considerable improvement in the complications resulted from metabolic syndrome through improvement of insulin sensitivity, plasma level of lipids and significant decline in the blood glucose level (Pan et al. 2020). The South African barley-based fermented food *Boza* showed increased contents of vitamins, minerals and fibers due to the fermenting action of *Lactobacillusplantarum* and reported improvement in the gastrointestinal health in addition to lowering cholesterol level in the blood and stimulating the immune response of the body (Ignat et al. 2020).

4.3 Insect-Based Fermented Foods

Indigenous Africans started practicing insect-based fermented food products as one of the strategies as food security in the African continent. At the nutritional level, edible insects are considered better sorce of proteins up to more than 70%, fats >50% and energy >600 kcal (Kewuyemi et al. 2020). Based on entomophagy study (Kelemu et al. 2015), there are more than 450 edible insect species are limited to different regins of Africa such as the caterpillar *Cirinaforda*, termites (*Macrotermes*spp.), crickets (*Gryllus*spp.), the grasshopper *Raspoliadifferens,* the locust *Normadacrisseptemfasciata,* beetles (*Orystes*spp.) and bees (*Apes* spp.) (Kewuyemi et al. 2020). Indigenous Africans practiced fermentation strategies of edible insects to suppress the growth of pathogenic micro-organisms as per the reports of different studies (Klunder et al. 2012). As mentioned earlie in the introduction section of this chapter, edible insects are poor in carbohydrate content and rich in protein content which is susceptible for microbial actions. Unlike plant-based foods, edible insects require pre-treatment strategies before fermentation process such as salting, smoking, boiling and drying. These pre-processes are important to remove the exoskeleton of insects and expose the vital nutrients required for the action of micro-organisms during fermentation (Borremans et al. 2018). Researchers focused on insect-based fermented food products for their nutritive characteristics. The sauces produced from the grasshopper *Locustamigratoria*was found to have very good flavour due to their high glutamate and aspartate content (Mouritsen et al. 2017). The fermented powder produced from mulberry silkworm larvae *Bombyxmori*showed high fatty acid content and *in vitro* anti-cancer activity in human liver cell line (Cho et al. 2019). The fermented paste prepared from the yellow mealworm larvae *Tenebriomolitor*reported anti-bacterial activity (Borremans et al. 2018).

4.4 Milk-Based Fermented Foods

Historically, milk and diary productssuch as youghurt, cheeses and fermented products are well known in the diets urban and rural countires of the African continent for their cheapest product and crucial role in the growth of infants as well as adults' requirements of essential nutrients (Dirar 1993; Owusu-Kwarteng et al. 2020). Ordinarily, milk and diary products are rich in their nutrional value as they contain high concentration of vitamins, minerals, proteins, micronutrients and high-energ fats (Wuehler et al. 2011). Milk and its products represent ideal substrates for fermentation by various species of micro-organisms (Quigley et al. 2013). Therefore, according to Food and Agriculture Oranization (FAO) and World Health Organization (WHO), Africans started to be aware about food safety hazards accompanying milk production, processing, packaging and transportation (FAO 2003). The traditional youghurt*Nunu*is a common fermented milk product achieved by *Lactobacillus* bacteria in Ghana (Owusu-Kwarteng et al. 2017). Indigenous people of Ghana believe that *Nunu*promotes health and protects against some ailments like diarrhea and constipation (Akabanda et al. 2014). *Mabisi*is a common milk-based fermented beverage from Zambia that is rich in its nutritive composition for children under 5 years and provides healthy metabolism for the gut microbiota (Chileshe 2019). The traditional milk-based fermented diary product *ergo* is the most traditional and nutritional food supplement that is served natural or spiced for pregnant, lactating women and babies in Ethiopia (Berhe et al. 2017). In addition, studies found that the Ethiopian *ergo* reported anti-microbial activity (Amenu 2013).

4.5 Meat-Based Fermented Foods

Fermentation of meat and fish is considered one of the best ways for their preservation. In addition, fermentation increases the nutritional value of meat and fish. Fermentation process depends mainly on the enzymatic activities of muscle and intestinal tissues as well as the metabolic activity of micro-organisms (Xu et al. 2020). Salting is one of the oldest method of meat and fish preservation, and still being used worldwide. Salting process is performed either by using amount of salt 20% more than the total weight of the fish or less amount of salt about 8% of the total body weight. The traditional salted fermented fish, Hout-Kasef is one of the popular meat-based fermented food in Jazzan region in Saudi Arabia (Gassem 2019). In Senegal, Guedj fish is one of the common local fish that is processed indigenous people though salting, fermentation and finally sun-drying (Diop et al. 2019). *Faseikh* and *muluha* are traditional salted and fermented food in Sudan and Egypt. Apart from the microbial ativity of *faseikh* and *muluha*, they are consumed by local people during ceratain events for their characteristic meat taste (Nasr-Allah and Zakar 2018). A recent study reported that the lactic acid bacteria isolated from

the traditional fermented fish had probiotic activity and could inhibit gram positive and gram negative bacteria (Amarantini et al. 2019).

4.6 Conclusion

Collectively the information gathered in the present chapter reveals that fermentation process is a method that is practiced by ancient Africans to secure food demands in that big continent and improve the nutritional value of cereals, insects, diary products, meat and fish. Rural and urban communities of Africa are still practicing fermentation of different type of food products in house or industries for their increased contents of the essential nutrients as well as their health promoting activities in children and adults. Nonetheless, fermentation process of food products requires control to avoid the poinsoning risks when consumed by human.

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Chapter 5
Microorganisms Involved in Spontaneous Fermentation and their Health Risk

Abdel Moneim Elhadi Sulieman

5.1 Introduction

Fermentation, the process of extracting energy from oxidation-reduction reactions of chemical compounds, including carbohydrates, and by using an intrinsic electron acceptor, which is often an organic compound. Through fermentation, the food becomes more nutritious, more digestible, safer for the consumer, and has a better flavor. Fermentation is also a highly efficient conservation process and a relatively lower energy cost compared to other conservation methods (https://ar.wikipedia.org/wiki/).

Consuming fermented food sources has been accounted for to bring improvements in a scope of health parameters. These beneficial outcomes can be applied by a blend of the live microorganisms that the fermented foods contain, as well as the bioactive components delivered into the food sources as by-products of the fermentation process. In numerous examples, and especially in dairy fermented foods, the microorganisms associated with in the fermentation process belong to the lactic acid group of bacteria (LAB) (Harsh et al. 2020).

The fermentation process plays an important role under anaerobic conditions, as there is no oxidative phosphorylation to maintain the production of ATP by the degradation process. Pyruvate is also represented by many different compounds during the fermentation process. Where the lactic fermentation process expresses the production of lactic acid from pyruvate; whereas, the alcoholic fermentation process expresses the conversion of pyruvate to ethanol and carbon dioxide; however, the heterogeneous lactic fermentation process is the production of lactic acid (lactic) in addition to other acids and alcohols. The fermentation process need not be carried

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out or carried out in an anaerobic environment. For example, even with abundant oxygen, yeast cells largely prefer fermentation to oxidative phosphorylation.

Fermented foods and beverages accompanied and likely encouraged the progress from hunter-gatherer communities to sessile farming communities in the Neolithic revolution about 14,000 years ago (Arranz-Otaegui et al. 2018; Hayden et al. 2013). They have remained staples of human diets for quite a long time and are an undeniably famous food classification. However, their developing prominence in the previous 20 years has prompted various misunderstandings and questions.

Fermented food is a source of probiotics, which are beneficial bacteria, which forms a protective lining inside the intestine to protect the body from microbes that cause diseases such as Salmonella and Ecoli bacteria, and since 80% of the immune defenses are found in the digestive system, eating more fermented substances enhances whole body functions.

Fermented foods are foods that contain an amount of beneficial bacteria, as these bacteria are used in the manufacture of the product, such as using bacteria to convert milk into curd. It is worth noting that these bacteria are an important addition to the nutritional value of the product due to its effect on beneficial bacteria in the intestine and its multiple benefits. Including a few servings of fermented foods in your diet every week can have a huge impact on gut health, weight management, blood sugar levels, and more. Nevertheless, it can be harmful in some cases, such as when fermented milk becomes spoiled (https://ar.wikipedia.org/wiki/).

Fermentation processes in foods regularly lead to changes in nutritional and biochemical quality comparative with the beginning fixings. Fermented foods contain complex environments comprising of enzymes from raw ingredients that interact with the fermenting microorganisms' metabolic activities. Fermenting microorganisms give a remarkable methodology towards food stability via physical and biochemical changes in fermented foods. These fermented foods can profit consumers contrasted to simple foods in terms of antioxidants, production of peptides, organoleptic and probiotic properties, and antimicrobial activity. It likewise helps in the levels of anti-nutrients and toxins level (Ranjana et al. 2020).

Microorganisms important in food fermentation might be included as pure or blended cultures or, sometimes, the desired microorganisms might be available in adequate numbers in the original raw materials (Dirar 1992). Fermentation is powerful technique for food conservation. The high encompassing temperatures of the tropics heighten the requirement for low-cost preservation strategies, for example, drying and traditional fermentations. In fermentations, microbes preserve foods as aftereffect of serious development, products of their metabolism, for example, organic acids, and bacteriocin production (Stiles 1996).

The term industrial fermentation refers to the intended utilization of the microorganisms such, with the aim of making products beneficial to humanity. Where fermented products represent many applications, including food processing as well as in general industry. The use of microbiology and procedure innovation brought about enormous upgrades in the nature of the aged food items. The quality enhancements have been incredible to the point that today all huge creation of matured food is mechanical.

Fermentation can essentially be practiced by spontaneous fermentation, back-slopping or by expansion of starter culture. The application of microbiology and process technology brought about enormous improvements in the nature of the fermented food products. The quality enhancements have been incredible to the point that today all significant production of fermented food is industrial.

In back-slopping, a small portion of a previously successful fermentation is utilized to inoculate new substrate was utilized to create starter cultures for future fermentations. However, procedures tumbled from favor in the nineteenth century simultaneously with the ascent in favor in the nineteenth century simultaneously with the ascent in and governmental legislative guidelines concerning sanitation (Whittington et al. 2019; Josephsen and Jespersen 2004).

Industrial fermentations are the biological changes responsible for the transformation of carbohydrates and similar substances under aerobic or aerobic conditions by the action of the micro-organisms, which contain enzymes and organic acids as inhibitors or fatal to some microorganisms, as these substances give the taste and smell. The color and texture are special to make the product different from the raw material.

It has been realized that fermented foods are more nutritious than their unfermented partners (Hasan et al. 2014). The expanded dietary benefit in fermented foods sources is because of the fermenting microorganisms present in them. Microorganisms are both catabolic and anabolic, break down complex compounds, and synthesize complex vitamins and other growth factors (Kennedy 2016).

5.2 Stages of Microbial Growth

When an organism is placed within a specific growth medium, the medium is fertilized with that specific bone. Here, we note that the growth of the inoculator does not happen at once, by merging, but it takes some time for this to happen. It is the period of time required to adapt, which is called the lag phase. Next to this lag phase is the phase of the organism's growth rate increasing steadily, for a specified period - this period is called the log period or the exponential period. Whereas, after a certain period of exponential phase, the rate of growth begins to slow down, due to a continuous decrease in nutrient concentrations and /or a continuous increase in (aggregate) concentrations of toxic substances. This phase, at which the rate of growth slows down, is called the deceleration phase. After the slowdown has passed, growth stops and the microbial culture enters a static phase, or a stable or stable phase. The biomass remains constant, except when specific, combined chemicals on the culture analyze the cells (chemical analysis). And if other microbes do not pollute the culture, the chemical structure remains unchanged. In addition, the mutation and alteration of the farm organelles may represent a source of pollution as well, which is called internal pollution.

The study of the bacterial growth curve is useful in dealing with bacteria and knowing their activity and different growth stages, as this is useful in:

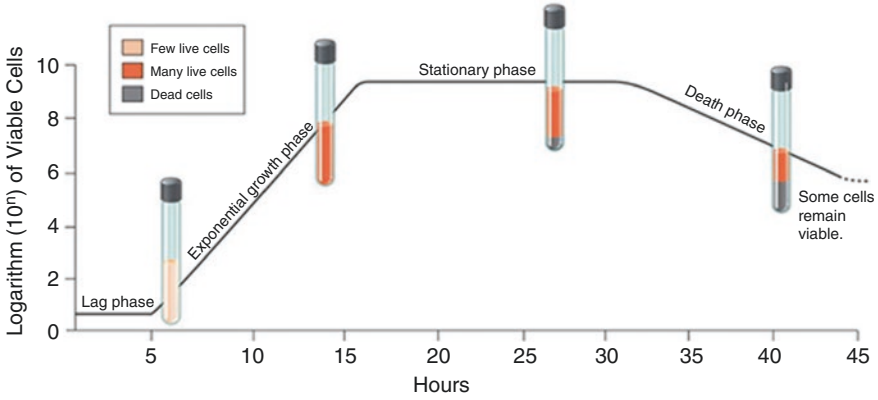

Fig. 5.1 Microbial growth curve

1. Areas of resistance and/or control
2. Pathological injuries
3. Microbial food spoilage and poisoning.

The general active phase is the most sensitive phase and is likely to die from heat or chemicals.

The number of bacteria cells can be calculated at any of the different stages of growth using the following equation:

Ns = (Ni) 2n
Ns = the total number of cells at a point on the curve.
Ni = the initial number of bacterial cells.
n = number of generations
2n = the number of cells per generation (Fig. 5.1).

5.3 Microflora of African Fermented Foods

Fermentation is caused by microbes such as bacteria, mold, and yeast. For example, fungi or yeasts are found on a mixture of sugar with mineral salts to produce penicillin. Yeast breaks down the sugar from the beans soaked in water into ethyl alcohol and carbon dioxide gas when making beer. The sugar in grape juice also decomposes in the same way as when making wine. Fermentation is also considered essential in the production of bread, cheese and curd. But it can be harmful in some cases, such as when fermented milk becomes spoiled. Fermented products that are beneficial for humans are made in large quantities. Although different types of materials are produced by the fermentation process.

Traditional fermented foods assume a significant function in the diet of various communities around the globe. Africa is maybe the landmass with the most

extravagant assortment of fermented foods, where fermentation actually assumes a significant function in fighting food deterioration, foodborne illnesses and represents a significant postharvest value addition. In fact, fermentation is yet a generally locally established home-based process utilized all through the continent (Maria Diaz et al. 2019).

Many scientists are investigating the curative and preventive properties of consuming a fermented food that contains beneficial microbes. Studies and experiments confirmed that eating such a type of food gives the body, in addition to pathological microbial resistance, the longevity without aging and strength to the point that some of them believe that eating these bacteria enable a person to live in a healthy state characterized by a high degree of activity and vitality. Or the person lives without showing symptoms of aging.

A wide assortment of crude materials are traditionally fermented in various districts of Africa. Subsequently, fermented foods with various qualities are delivered and they have been classified in groups, for example, fermented non-alcoholic cereals (mostly created from sorghum, millet and maize), starchy root crops (fundamentally delivered from cassava), animal proteins (mainly dairy products), vegetable proteins (created from legumes and oilseeds) and alcoholic beverages (delivered from cereals, sap, honey or fruits, among other materials). Fermented products have been depicted to give health benefits, such as protection against gastrointestinal disorders, prevention of hypertension and heart disease or protection from diabetes and osteoporosis. Furthermore, traditional African fermented foods contain live microorganisms that can produce health-promoting compounds, such as antimicrobials, essential nutrients or molecules with antioxidant activity, and can go about as probiotic strains (Franz et al. 2014; Tamang et al. 2016).

Lactic acid bacteria (LAB) are the most ordinarily utilized microorganisms for protection of foods. Their significance is related fundamentally with their safe metabolic activity while developing in foods using accessible sugar for the creation of organic acids and different metabolites. Their normal occurrence in foods and feeds combined with their enduring use adds to their common acceptance as GRAS (Generally Recognized As Safe) for human consumption (Aguirre and Collins 1993). In an industrial scale a specific characterized starter culture, which has been created under controlled conditions, is of first inclination with the goal that the characteristics of the completed item could be reliably kept up for quite a while. Also, current strategies for quality innovation makes workable for the microbiologists to plan and create starter cultures with explicit characteristics.

Numerous microbiological studies manage identification of organisms isolated from different fermented foods. Lactic acid bacteria isolated from tomatoes that were naturally fermented under partial anaerobic conditions were found to be Leuconostoc mesenteroides, Lactobacillus brevis and Streptococcus sp. (Beltrán-Edeza and Hernández-Sánchez 1989).

In Sudan, Badi (1987) stated that Kasha fermentation was both acidic and alcoholic, she reported that the organisms involved members of the genera *Lactobacillus* and *Streptococcus* among bacteria and *Candida* and *Saccharomyces* among the yeasts. El Mahdi (1985) found lactic bacteria, proteolysis bacteria, yeast and molds

present in counts of the order of billions of cells per ml of sour Ajin. Mohammed (1991) found that when Dabar sorghum flour in Sudan was fermented utilizing an inoculums from a previous batch of fermented dough, at the steady temperature of 30 °C, the microbial population was dominated by lactic acid bacteria, with yeast and mold counts staying low. The most prevelant microbial species that was *Pediococcuc confuses Lactobacillus confuses*, *L. brevis* and *Enterococcus francium*. The authors found that the yeasts *Candida intermedia* and *Debaryomyces hansenii* could be important in the fermentation of sorghum for making of *Kisra*. Hamad (1995) found that 99% of the bacteria isolated from different fermenting dough's were *Lactobacilli* namely, *Lactobacillus fermented*, *Lactobacillus refuter* and *Lactobacillus malodorous*.

Information about the microbial environment of natural food fermentations can be utilized to recognize biomarkers to evaluate the nature of fermented foods and would help in the design of optimum starter cultures. Overwhelming bacterial groups present in African fermented foods have been broadly analyzed utilizing culture-dependant methods however these techniques present a few constraints, for example, not having option to recognize not able to identify microorganisms in low numbers in complex environments with predominant populations (Cocolin et al. 2013). As an alternative, culture-independent methods, especially amplicon sequencing, are increasingly being used to study the bacterial populations of fermented foods, although to date, few studies have focused on African foods.

5.4 Important Microorganisms Dominating Fermented Foods

5.4.1 Lactic Acid Bacteria

Lactic acid bacteria are widely found in many nutrient-rich media such as milk, meat, drink, and vegetables, in addition to their presence in soil, lakes and the digestive system of animals and humans. Fermentation of the sugar has defined as that process results in production of lactic acid, which many bacteria carry out, has been around for a long time, and man has applied it. It contains numerous food products, and it plays an essential and important role in the fermentation industry, and dairy products. Tserovska (2002) studied bacteria have been extensively used for production a good starter culture, and to obtain fermentation products that have stable characteristics and are resistant to bacteriophage have been used throughout history in food production and preservation.

Many studies have shown the health effects of many strains of acid bacteria milk on humans, and these studies have tried to find out how these bacteria work in digestive system, and many benefits have been found, the most important of which is improving the digestion of lactose and treating diarrheal disorders (Gillilard 1990; Drouault and Cortheir 2001).

LAB-driven fermentations regularly yield by-products with bioactivity and a different scope of wellbeing advancing impacts, including protection against infectious agents, immunomodulatory impacts, hostile to allergenic impacts, anti-obesity impacts, hostile to oxidant impacts, enhancing the bioavailability of vitamins/minerals, anti-anxiety impacts, among others (Oguntoyinbo and Narbad 2015; Zhao et al. 2015; Linares et al. 2017).

The lactic acid bacteria implicated in Africa fermented foods comprise of species that belong to the genera Lactobacillus, Lactococcus, Leuconostoc and Pediococcus (Oyewole 1997). These microorganisms vary in their composition from one product to the other. As a result of changes in conditions, a great number of fermented milk types been developed (Sulieman et al. 2006). Variables include heat treatment of the milk, fermentation temperature, inoculum percentage and the concentrating of the milk. According to these conditions, different types of lactic acid bacteria become predominant e.g., producing various flavour components. Most types contain two to four types of bacteria.

Lactic acid bacteria produce additives and flavor compounds that have an important role in food, so called functional foods, and it increases the nutritional and health value of these products (Holzapfel et al. 2001; Rinkinen (2003). They also produce several antimicrobials of great importance in food preservation (Axelsson 2004). In addition, lactic acid precursors play an important role in giving the correct texture of fermented dairy products (Fryer and Rossi 2004).

LAB are the most important in dairy products in terms of their vital activity and in terms of their percentage of total flora, as they ferment lactose, producing a high percentage of lactic acid. Moreover, they produce flavoring substances, and they are Gram-positive bacteria, aerobic – anaerobic. They do not produce the enzyme catalase (this enzyme breaks down oxygenated water into water and oxygen). It is worth noting that lactic acid bacteria ferment sugars under anaerobic conditions.

LAB belong to the family Lactobacillaceae and are divided morphologically into two groups:

1. **Streptococcaceae bacteria**

It is a single or double bacteria or found in short chains, and it is one of the most important species related to it that is important in the dairy and cheese industry. The impotant genera and species of this group include: the genera: Strptococcus such as *Str.Lactis, Str.diacetylactis*:, *Str.cremoris, Str.thermophilu.* The genera Leucenostoc such as *Leu.dextranicum, Leu.citrovorum* (Fig. 5.2).

2. **Lactobacillus bacteria**

Lactobacillus bacteria is an important genera of LAB that convert lactose sugar and a number of other sugars into lactic acid, hence its name (Lactic acid bacteria) and the genus Lactobacillus spreads in many places, including the normal flora of humans. They are found in the mouth, intestine and vagina and have an important influence in maintaining the normal bacterial balance of these parts, while some researchers believe that these bacteria have an effect on the development of tooth

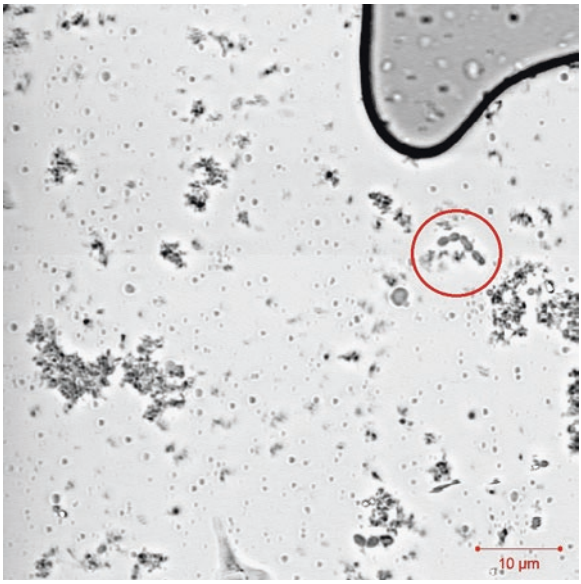

Fig. 5.2 Streptococcus thermophiles colonies. (Source: https://commons.wikimedia.org/)

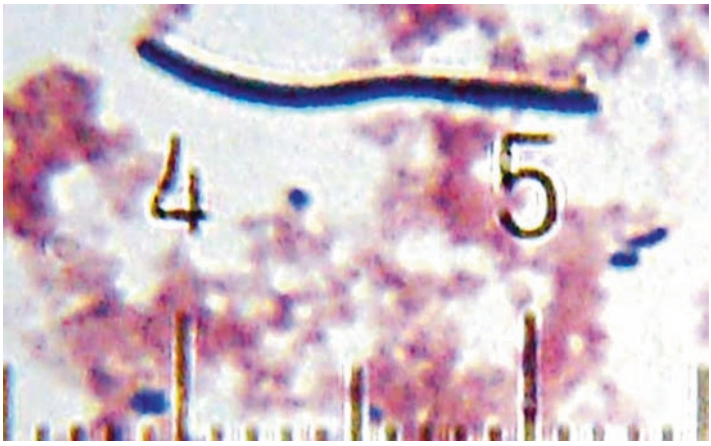

Fig. 5.3 Lactobacillus delbrueckii subspecies bulgaricus from a sample of Activia® brand yogurt. (Source: https://commons.wikimedia.org/)

decay, and are found in the gastrointestinal tract of many animals, and they are spread in nature (Fig. 5.3).

The important genera and species include:

(a) Thermobactrium:

Among the most important types of it:

Lactobacillus Lactis: Lactobacillus acidophilus: Lactobacillus bulgaricus Lactobacillus helveticus

(b) Streptobacterium, such as Lactobacillus plantarum, Lactobacillus Casei
(c) Batabacterium, one of the most important species to which it belongs: Lactobacillus brevis

Lactobacillus spp. can produce specific anti-microbial substances, which have been observed to inhibit the growth of some pathogenic microorganisms (Saad et al. 2001; Yost et al. 2002). These beneficial bacteria are most effective during periods or disease or stress and following antibiotic treatment (Table 5.1).

Table 5.1 Micro-organisms commonly found in fermenting fruit and vegetables

Organism	Type	Reaction
Acetobacter genus A. aceti A. pasteurianus A. peroxydans	Aerobic rods	Oxidise organic compounds (alcohol) to organic acids (acetic acid). Important in vinegar production
Streptococcaceae family	Gram positive cocci	
Streptococcus genus S. faecalis S. bovis S. thermophilus		Homofermentative. Most common in dairy fermentations, but S. Faecalis is common in vegetable products. Tolerate salt and can grow in high pH media.
Leuconostoc genus L. mesenteroides L. dextranicum L. paramesenteroides L. oenos	Gram positive cocci	Heterofermentative. Produce lactic acid, plus acetic acid, ethanol and carbon dioxide from glucose. Small bacteria, therefore have an important role in initiating fermentations.
Pediococcus genus P. cerevisiae P. acidilactici P. pentosaceus		Saprophytic organisms found in fermenting vegetables, mashes, beer and wort. Produce inactive lactic acid.
Lactobacillaceae Family	Gram positive rods. Nonmotile	Metabolise sugars to lactic acid, acetic acid, ethyl alcohol and carbon dioxide.
Lactobacillus genus		The genus is split into two types – Homo- and hetero-fermenters. Saprophytic organisms. Produce greater amounts of acid than the cocci
Homofermentative Lactobacillus spp. L. delbrueckii L. leichmannii L. plantarum L. lactis L. acidophilus		Produce only lactic acid. L. plantarum important in fruit and vegetable fermentation. Tolerates high salt concentration.
Heterofermentative Spp. L. brevis L. fermentum L. buchneri		Produce lactic acid (50%) plus acetic acid (25%), ethyl alcohol and carbon dioxide (25%). L. brevis is the most common. Widely distributed in plants and animals.
Yeasts	Saccharomyces Cerevisiae S. pombe Many aerobic, some anaerobes	S. cerevisiae can shift its metabolism from a fermentative to an oxidative pathway, depending on oxygen availability. Most yeasts produce alcohol and carbon dioxide from sugars.
Debaromyces Zygosaccharomyces rouxii Candida species Geotrichum candidum		Zygosaccharomyces rouxii Candida species Geotrichum candidum Tolerant of high salt concentrations tolerates high salt concentration and low aw

5.4.2 Yeasts

The occurrence of yeasts in indigenous African fermented food and beverage products has been studied for a scope of end products. Nonetheless, far less investigations have inspected have examined the yeast dynamics during the fermentations. The yeast species overwhelming indigenous fermented food and drinks are those that can able to adapt to the changing intrinsic conditions brought about by physicochemical changes, because of microbial activity (Navarrete-Bolaños 2012).

Species diversity is also affected by various extrinsic factors associated to the technological processing stages incorporating fermentation length and temperature, water quantity added, raw materials utilized, stirring, pasteurization as well as level of hygiene and sanitation (Jespersen 2003; Achi and Ukwuru 2015). Consequently, a complete comprehension, connecting intrinsic and extrinsic factors to microbial assorted variety and progressions is of outmost significance for overhauling indigenous sub-Saharan African fermented food and drinks.

A few functional properties of yeasts have been accounted for the preparing of indigenous sub-Saharan African fermented food and drinks. These incorporate fermentation of carbohydrates, flavor compound development, stimulation of LAB, degradation of cyanogenic glycosides, creation of tissue-degrading enzymes, binding and/or degradation of mycotoxins as well as probiotic properties (Pernille et al. 2019; Omemu et al. 2007; Padonou et al. 2010; Achi and Ukwuru 2015; Tamang et al. 2016). Nonetheless, in the majority of the contemplated indigenous sub-Saharan African fermented food and beverages, the functional properties of recognized yeasts have not yet been widely explained.

Genera of yeasts reported from fermented foods, alcoholic beverages and non-food blended amylolytic starters are Brettanomyces, Candida, Cryptococcus, Debaryomyces, Dekkera, Galactomyces, Geotrichum, Hansenula, Hanseniaspora, Hyphopichia, Issatchenkia, Kazachstania, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Rhodosporidium, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Sporobolomyces, Torulaspora, Torulopsis, Trichosporon, Yarrowia, and Zygosaccharomyces (Watanabe et al. 2008, Tamang and Fleet 2009; Kurtzman et al. 2001; Lv et al. 2013) (Table 5.2).

5.4.3 Yeast Interactions with LAB

Yeasts and LAB frequently exist together during spontaneous fermentations. While yeasts can develop in a moderately basic medium, LAB are more meticulous and require more supplements as, e.g., amino acids and vitamins (Viljoen 2006; Ponomarova et al. 2017). The cooperations among yeasts and LAB can be both synergistic and antagonistic, however are frequently of shared advantage. Mutualistic interactions between yeasts and LAB have been depicted for ogi, a non/low-alcoholic cereal-based beverage.

Table 5.2 Yeast species frequently found in dairy products

Identification according to Kreger-van Rij (1984)	Some equivalent names in earliest Literature
Debaryomyces hansenii	*D.subglobosus: Torulaspora hansenii*
Candida famata	*Torulopsis candida: T. famata*
Kluyveromyces marxianus	*Kluy.bulgaricus:Saccharomyces lactis: S.fragilis*
Candida Kefyr	*C.Pseudotropicalis:Torulopsis Kefyr: Torula cremoris.*
Candida stellata	*Torulopsis stellata*
Saccharmyces (Yarrovia) Lipolytica	Candida lipolytica
Candida holmii	*Torulopsis holmii*
Saccharomyces exigubs	
Pichia membranaefaciens	*Candida krusei*
Pichia fermentans	*Candida krusei*
Rhodotorula glutinis	
Rhodotorula rubra	

Source: Kreger-van-Rij (1984)

The yeasts will benefit by a diminishing in pH by the acidification encouraged by the activity of the LAB, noteworthy higher development of *Lactobacillus plantarum* has been demonstrated when co-cultured with either *S. cerevisiae* or particularly *P. kudriavzevii*, demonstrating that these yeast species give development factors for the LAB (Omemu et al. 2007), undoubtedly amino acids (Ponomarova et al. 2017).

Mutualistic associations have similarly been accounted for between species of LAB and yeasts originating from indigenous African fermented milk products (Sulieman et al. 2013; Pernille et al. 2019). At the point when co-culturing Lactococcus lactis subsp. lactis biovar diacetylactis with K. marxianus in milk, the viability of L. lactis subsp. lactis biovar diacetylactis was improved, while K. marxianus could benefit from galactose, arising from lactose degradation by L. lactis subsp. lactis biovar diacetylactis (Gadaga et al. 2001). Moreover, Lactobacillus paracasei subsp. paracasei arrived at fundamentally higher final counts when co-cultured in milk with especially K. marxianus, S. cerevisiae or Naumovozyma dairenensis (f. Saccharomyces dairenensis).

5.5 Functional Properties of Microorganims in Fermented Foods

The fermenting microorganism have numerous functional properties will be discussed in details in another chapter. These functional properties include production of antimicrobial compounds, probiotic microorganisms, antioxidant activity, production of vaccines, production of enzymes improvement of the immune system and degradation of anti- nutritive compounds.

(Xiangna et al. 2018; Grasson 2002; Asmahan 2010; Perdigon et al. 2001; Steinkrause 1995; MacDonald et al. 2012).

5.6 Conclusions

Fermented foods are prepared by the action of micro-organsims under controlled conditions, which bring about a physical change in these foods and cause a change in the biochemistry and the forms of the nutrients that make up these foods. These foods may be prepared from grains, legumes, roots, vegetables, fruits, edible parts of any plant, fish, milk or meat. Through fermentation, the food becomes more nutritious, more digestible, safer for the consumer, and has a better flavor. Fermentation is also a highly efficient conservation process and a relatively lower energy cost compared to other conservation method.

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Chapter 6
Probiotic Fermented Foods and Health Promotion

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6.1 Introduction

Fermentation is one of the oldest biotechnological processes for the development and production of desirable food products with improved organoleptic properties, increased palatability and shelf-life of the product (Ray and Joshi 2014). Food fermentation leads to inhibition of pathogenic or spoilage organisms by intermediate product developed during fermentation process, thus improving the shelf-life of fresh food product. The process of lacto fermentation occurs in food through natural bacteria or yeasts leading to production of lactic acid. Meanwhile, in the course of lactic acid fermentation, several metabolites such as such as lactic acid, carbon dioxide, ethanol, hydrogen peroxide, acetic acid, antimicrobial peptides (bacteriocin) gets produced from LAB, causing synergistically suppression as well as growth of spoilage and pathogenic microorganisms (Di Cagno et al. 2013). Furthermore along with preservation, fermentation also offers various characteristics such as flavor, texture, aroma, and nutritional enrichment into food (Ray and Joshi 2014). Bread is one of the classical examples of this innovative technique, where the principle objective of dough fermentation was to produce the distinctive organoleptic

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properties and textures in bread (Terefe 2016). Hundreds of dissimilar fermented foods are not known outside the instinctive area in which they have been fermented, produced and developed since long time ago. In addition, a huge list of fermented products are available showing that, how the diversity derives from the heterogeneity of traditional fermented food around the world based on cultural preferences, geographical areas as well as application of staple food products. Besides the huge list of fermented foods, these are some of the most common one such as beer, wine, cider, cheese, yoghurt, soya-based fermented food, beans, fish, meat and probiotics (Bell et al. 2017).

Currently, probiotics has been seen as one of the nutraceutical therapeutic approaches for the prevention and management of several chronic diseases including digestive and immune health. In addition, probiotics are also recommended as effective therapeutic interventions by health professionals including nutritionists and dieticians. Tissier was the first scientist, who identify gut microbiota from healthy breast-fed infants dominated by rods with a bifid shape bacteria (bifidobacteria), which were absent in infant formula-fed suffering from diarrhea, and led the idea how bifidobacteria played an important role in maintaining health (Kechagia et al. 2013). In 1965, Lilly and Stillwell was the first who introduced the term "probiotics" and described it as microbial derived factors that stimulate the growth of other organisms. Furthermore, Roy Fuller in 1989, stressed on the necessity of probiotics and presented the notion that probiotics have a beneficial effect on the host (Guarner et al. 2012). Initially probiotics were used for the improvement of human as well as animal health by virtue of intestinal microbiota modulation. Currently, several strains of probiotics likewise *Bifidobacteria* and *Lactobacilli* are available for human consumption for the treatment of gastrointestinal (GI) infections. Some of the positive effects of probiotic consumption include improvement of intestinal health by the regulation of microbiota, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, stimulation and development of the immune system and reducing the possibility of other ailments (Nagpal et al. 2012). Probiotics are described as 'live microorganisms' administered in acceptable amount conferring health benefits to the host' (FAO/WHO 2002). Additionally, probiotics have also been described as live microbial feed supplements that positively affect the host by improving its GI microbial balance, or in other words it can also be described as live microbial cultures ingested for health benefits beyond providing basic nutritional value (Nagpal et al. 2012; Agerholm-Larsen et al. 2000; Adnan et al. 2017; Alshammari et al. 2019). To understand the food fermentation, we have put forward some basic definitions such as probiotics, prebiotics, synbiotics and fermentation. **Probiotics;** Live microorganisms, which confers health benefits on the host GI tract when ingested in adequate amounts. **Prebiotics;** Fermented ingredients in particular, resulting into a specific change in the composition or activity of the intestinal microbiota, leading the benefits upon host health. **Synbiotics**; Any product which contains both probiotics and prebiotics strains are described as synbiotic products. **Fermentation;** A process by which a microorganism transforms food into other desirable food products, usually through the production of lactic acid, ethanol, and other metabolic end-product (Guarner

et al. 2012). In recent times, traditional fermented foods have drawn the attention of the scientific communities mainly because of its several health benefits including the probiotic organism with its health promoting effects. The market for fermented food products are growing continuously and it requires further implementation and diversification of available products. In this chapter, we bring comprehensive details of fermented food, specifically probiotics and its health benefits including mechanism of action, safety and efficacy.

6.2 Food Fermentation and Lactic Acid Bacteria (LAB)

Fermented products and their microbial and functional characteristics have been widely studied (Rhee et al. 2011). It contain microorganisms, which is generally regarded as safe (GRAS) with several characteristics of producing beneficial metabolites such as ethanol, carbon dioxide, organic acids, fatty acids and bacteriocins (Mathur et al. 2020). Moreover, fermentation of food substrate usually involves application of microorganism like LAB and yeast (Mathur et al. 2020). LAB is a non-pathogenic, Gram positive, fermentative bacteria that are linked with the production of lactic acid from carbohydrates, making them useful for food fermentation. LAB fermentative ability is been well-known for providing enrichment of nutrients, improvement in organoleptic properties, improvement in food safety as well as provides health benefits to the host. Foods fermented along with LAB, plays a significant part in serving the world's population (Rakhmanova et al. 2018). Therefore, to serve the world population, several genus of LAB have been identified for food fermentation as well as for probiotic preparation purposes such as *Lactobacillus, Lactococcus* and *Streptococcus*. Specifically, selected species are *Lactobacillus acidophilus, Lactobacillus lactis, Lactobacillus bulgaricus, Lactobacillus helveticus, Lactobacillus casei, Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus salivarius, Enterococcus faecalis*, *Bifidobacterium* spp. (Sugiharto 2016).

Apart from dairy fermented foods, fermented vegetables, cereals and meat also contains LAB, which indicates its potential industrial application in food preservation as well as in dairy fermentation (yoghurt, butter milk, cheese and kefir). Based on metabolism of glucose, LAB is classified as homo-fermentative (Embden-Meyerhof-Parnas pathway) and hetero-fermentative bacteria (pentose monophosphate pathway) leading to production of lactic acid and carbon dioxide, while pentose monophosphate pathway produces lactic acid, acetic acid, ethanol and carbon dioxide. In addition, LAB also helps to improve the quality and shelf- life of fermented food by producing several secondary metabolites such as bacteriocins, exopolysaccharides and enzymes (De Melo Pereira et al. 2020; Ibrahim et al. 2018). Therefore, LAB is considered as a very important microorganism for preserving as well as producing a wide range of fermented foods such as cucumbers (pickles), fermented milks (yogurts and cheeses), protein-rich vegetables, protein meat substitutes (*tempe*), pastes produced by fermentation of cereals and legumes (Japanese

miso, Chinese soy sauce), fermented cereal-fish-shrimp mixtures (Philippine *balao balao* and *burong dalag*), fermented cereal yogurt (Nigerian *ogi*, Kenyan *uji*), fermented meats (e.g., salami) and fermented milk-wheat mixtures (Egyptian *kishk*, Greek *trahanas*) (Steinkraus 1992). LAB contains a substantial amount of human gut flora which has been seen to possess the positive effect on intestinal tract (Gawai and Prajapati 2017). In addition, fermentation derived from LAB is considered to produce by-products with several bioactivity or health promoting effects such as anti-allergic, antioxidant, anti-obesity, immunomodulatory, anti-anxiety, as well as increases the bioavailability of minerals or vitamins (Mathur et al. 2020; Rakhmanova et al. 2018; Ashraf et al. 2021).

6.3 Selection of Probiotics and Starter Culture in Food Fermentation

Currently, large-scale fermentations with several advancements have emerged as the need of the hour, as well as selection of microorganism/starter culture for production of different food products has always been challenging. Therefore several microorganisms has been used based upon the food product fermentation such as *Lactobacillus* species in fermented milks, vegetables, and meats, *Saccharomyces cerevisiae* for alcoholic and beverage industry, as well as molds in the production of soy-based products such as tempeh, miso and shoyu. These microbial strains are used for fermentation processes and hence called as starter cultures (De Melo Pereira et al. 2020). Therefore, starter cultures are defined as any microbial culture which helps to initiate and promote the process of fermentation in food products. On the other hand, starters could also be utilized for standardization of fermented product, as well as it also reduces the food ripening/maturation time. Since, food safety of fermented food may get exposed to microbiological contaminations likewise food-borne pathogens (*Listeria* spp., *Salmonella* spp., etc) (Laranjo et al. 2019). Safety of fermented food products may be exposed by microbiological, namely food-borne pathogens (*Salmonella spp., Clostridium perfringens, Staphylococcus aureus, Listeria* spp., etc) (Laranjo et al. 2019).

The selection of right starter culture for food fermentation is very important to ensure the overall process of fermentation, improving the food safety, stability as well as organoleptic properties of the end product. Several fermented dairy products are produced with the help of LAB like curd, cheese, fermented milk, and these products are consumed around the world. Before a probiotic is used in the interest of human health, there are several criteria's that must be fulfilled to get the desired fermentation properties, hence that it can be produced and added to the food products without losing its survival and function, or creating an unpleasant taste; it must survive when passing through the GI tract, and must reach live to the site and should be able to function in the intestinal environment (Adnan and Pramaningtyas 2020; Saad et al. 2013). Several characteristics should be taken into consideration while

Fig. 6.1 Several factors associated for the selection of safe and appropriate probiotic

choosing a safe and appropriate probiotic as shown in Fig. 6.1 i.e., (Ray and Joshi 2014) safe strains, species and genera of probiotics (Di Cagno et al. 2013) viability and bioactivity during the process and storage (Terefe 2016) GI survival and resistance to gastric acid and bile acids (Bell et al. 2017) stimulating the selection of beneficial bacteria and suppressing harmful bacteria (through the production of antimicrobial compounds and competitive elimination) (Kechagia et al. 2013) antagonistic activity against pathogens such as *Clostridium difficile*, *Salmonella*, *Helicobacter pylori* and *Listeria monocytogenes* (Guarner et al. 2012) adhesion to the intestinal epithelium (Nagpal et al. 2012) anti-carcinogenic properties and anti-mutagenic (Agerholm-Larsen et al. 2000) modification and improvement of the immune system (Peivasteh Roudsari et al. 2019). In addition, there are various other factors that should be considered for the selection of starter culture for any food fermentation processes.

6.4 Probiotic and Its Mechanism of Action

The current definition of probiotics by Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) is "live microorganisms which when administered in adequate amounts confer a health benefits to the host". The word probiotic is been derived from Greek meaning "for life." Hence, probiotic is considered very useful for several therapeutic purposes, due to its ability to adhere to the intestinal mucosa, ability to colonize the intestinal tract, bile salt and gastric acid stability (Khalighi et al. 2016). Additionally, probiotic *Lactobacillus paracasei* A221 has been reported to improve the bioavailability and functionality of kaempferol glucoside in kale by its glucosidase activity (Shimojo et al. 2018). Bergillos-Meca reported that, bacterial strain *Lactobacillus fermentum* D3 has the ability to increase the bioavailability of Zn, Ca and P in *in-vitro* fermented goat milk (Bergillos-Meca et al. 2013). Furthermore, several studies also pointed out the probiotic therapeutic effects; however its mode of action is still poorly understood. In this section we have elaborated the possible mechanism of action of probiotics.

6.4.1 Mechanism of Action

The exact mechanisms of action (MOA) by which probiotics show its beneficial effect have not been well described. However, there are various proposed mechanisms that explain its favorable effects as shown in Fig. 6.2. The probiotic organism influences the host cell in many ways. Different strains of probiotic effect in various ways like by impact on intestinal luminal environment, mucosal immune system, epithelial and mucosal barrier function. Probiotic additionally affect directly or indirectly on the host cells or food components. Furthermore, efficacy of probiotics depends on its metabolic properties of the specific strains and the molecular structure (DNA or peptidoglycan) present on the surface of the microorganism. It also affects the monocytes/macrophages, dendritic cells, epithelial cells, T cells and B cells of the host cell (Nagpal et al. 2012; Cencic and Chingwaru 2010). In other words, probiotics also act by competitively binding to the adhesion sites by virtue of that it fights for cellular attachments.

It's been also proposed that probiotic bacteria inhibit pathogens by producing antagonistic components and competes for competition for nutrients (particularly iron in marine microbes), nutritional benefits such as improving feed digestibility and utilization, immune-stimulatory functions, and alteration of the enzymatic activity of pathogens (Seerengeraj 2018). Another possible MOA for probiotics is alteration in microbial flora through the synthesis of antimicrobial compounds (Rolfe 2000). Because, different types of bacteria (*Lactobacilli* and *Bifidobacteria*) produces antimicrobial compounds including bacteriocin. Bacteriocins are described as "compounds produced by bacteria that have a biologically active protein moiety and a bactericidal action". Furthermore, probiotics are reported to increase

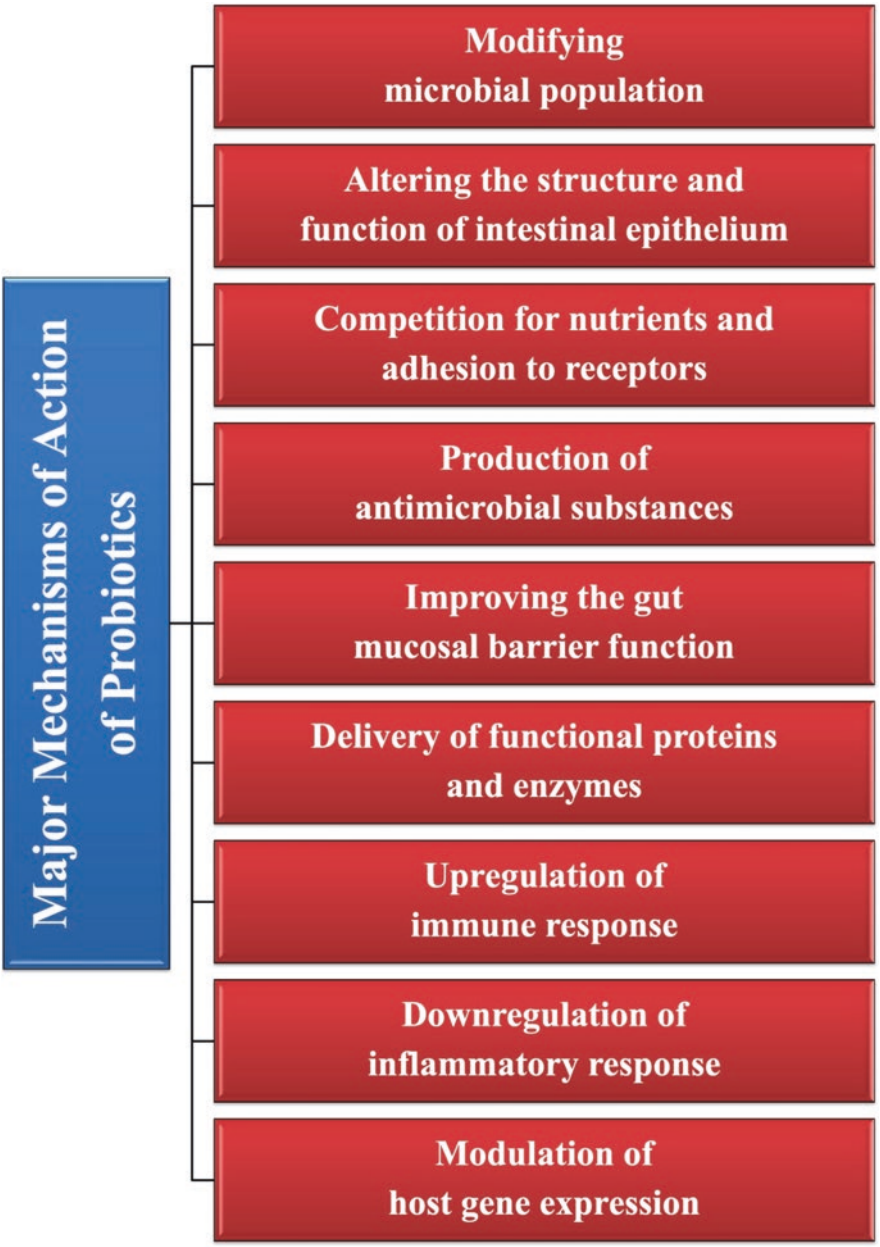

Fig. 6.2 Possible and major mechanism of action of probiotics

immunoglobulin-A (IgA) secretion, enhanced phagocytic activity of macrophages leads to increased immune response. Meanwhile, increase in IgA secretion causes decrease in the numbers of pathogenic microorganism as well as improves the condition of microflora in the intestinal tract. Therefore some of the research studies suggest that probiotics could not only fight intestinal or urogenital infections, but it could also help in inflammatory diseases, in allergic responses, even as an adjuvant in vaccination (Khalighi et al. 2016).

6.5 Bio-preservation Techniques Using Probiotic Organisms for Food Safety

Food safety and regulatory standards implementation in food production system is very crucial to ensure safe food production. Recently, development of noble chemical additives, new food pathogens and its source for causing food-borne illnesses has raised the concerns in food production system. In addition, extensive use of food preservative chemicals, antibiotic in food production system has forced the scientific communities to search more natural ways of food preservation (Akbar et al. 2016). Food preservation is a major hurdle in modern day food technology. Therefore, to present consumer a fresh, ready to eat, highly nutritious, minimally processed food to avoid contamination will improve the food preservation. Use of microorganisms and their natural by-products for bio-preservation has been commonly practiced throughout the history of mankind (Kamarudheen et al. 2014). The term bio-preservation states that preservation of food, extension of shelf-life as well as its food safety can be achieved by using living microbes and their metabolites.

Of note, the process of bio-preservation can be done by two different ways such as the inoculation of food matrix with target microorganisms, and the other one by using microbial metabolites in purified form, in particular bacteriocins (Gaggia et al. 2011). Moreover, the whole food fermentation depends on the selection of microorganism to control spoilage and make pathogen inactive. Therefore, a special interest has been shifted to a very important microorganisms i.e., LABs and their metabolites. LABs has been reported to show antimicrobial properties (like organic acids, carbon dioxide, diacetyl (2,3-butanedione), anti-fungal peptides, hydrogen peroxide and bacteriocins) and offer a unique texture and flavor to the food products (Singh 2018; Oluk and Karaca 2018). Currently these techniques are used in various dairy milk products as well as other food products. Various food product such as chicken meat, beef meat, ham, iceberg lettuce, golden delicious apples, non-fermented pickles, cold smoked salmon and cooked and fresh peeled shrimp shelf life is been improved using various microorganisms such as *E. faecium* PCD71, *L. fermentum* ACA-DC179, *L. sakei* 4808, *L. curvatus* CRL 705, *L. sakei* 10A, *Pseudomonas putida* LTH 5878, *L. mesemteroides* CM 135, CM160, PM249, *L. curvatus* LR 55, *C. divergens* V41, *L. casei* T3, *L. palntarum* Pe2, *C. piscicola* Sal3, *Leuconostoc gelidum* EU 2247 (Gaggia et al. 2011). They are non-pathogenic,

bile tolerant and salt tolerant (Kamarudheen et al. 2014). Sea foods shelf-life and safety can be increased with the help of natural or controlled microbiota and/or their antimicrobial compounds (Nath et al. 2014). Therefore, bio-preservation offers the potential to improve the shelf-life and food safety suggesting a promising approach towards preserving food by natural means.

6.6 Beneficial Health Effects of Probiotics

Fermented foods comprising of beneficial bacteria have been for hundreds of years in our food are considered as probiotics. Recently, probiotics has been studied for both *in vitro* and *in vivo* and found to be clinically effective for the treatment of various diseases. Several scientific evidences has showed the significant role of probiotics in the prevention and management of different health problems such as diarrheal diseases, liver diseases, allergies, hypertension, inflammatory and immune response, detoxifying carcinogens and many more. Researches on probiotics for human consumption are yet rare, further *in vivo* studies are needed to determine the type of probiotic strains, health conditions, dosages, as well as to demonstrate their safety and limitation.

6.6.1 Prevention and Management of Diarrheal Diseases

Diarrheal disease is the second main cause of death in under-five aged children. It is also the main cause of malnutrition in this group and it kills around 525,000 children each year. Globally, the total number of cases of childhood diarrheal disease every year is about 1.7 billion (https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease, 2017). In adults also, acute diarrhea is a familiar problem, its most popular etiology is viral gastroenteritis. Increases in travel, comorbidities and food-borne illnesses leads to more bacteria-related cases of acute diarrhea (Barr and Smith 2014). Several studies have been carried out to test the efficacy of probiotics in the prevention and management of the diarrheal diseases. These studies have reported the role of probiotics in the prevention and management of diarrheal diseases (Plaza-Díaz et al. 2018; Hempel et al. 2012; Goldenberg et al. 2017). The prevention and management of infective diarrhea, the remedy of frequent diarrhea caused by *Clostridium difficile*, in addition to the control of the diarrhea associated with antibiotics are the areas of interest of these researchers. Hospitalized children administered with probiotics, such as *Bifidobacteria* significantly prevent these children from diarrhea. One study showed that *Saccharomyces boulardii* (*S. boulardii*) decreases the duration, frequency, and hospitalization period of diarrhea, therefore, decreasing the treatment costs. In different clinical studies, *S. boulardii* was found to be active for the prophylactic as well as in the treatment of diarrhea, mainly antibiotic-associated diarrhea (Wada et al. 2010; Dash et al. 2017). The decline in

duration and frequency of acute diarrhea within 24 h was also reported in Cochrane review of meta-analysis (https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease, 2017; Shah et al. 2012).

Some probiotic strains, like *Lactobacillus rhamnosus GG (L. rhamnosus)*, *Lactobacillus reuteri (L. reuteri)*, and *S. boulardii*, looks like potent therapeutic agents for the improvement of the situation of acute diarrhea in children, when used therapeutically (Isolauri et al. 2002). *S. boulardii* was found to be an appropriate and new addition in the treatment of acute diarrhea in children. It reduces the frequency of stool, duration of illness and the number of episodes of diarrhea in a very short time (Billoo et al. 2006). Gill and Guarner (2004) reported that different strains of probiotics such as *L. rhamnosus*, *L. acidophilus*, *L. bulgaricus* and the yeast *S. boulardii* can decrease the incidence of antibiotic-related diarrhea in children as well as in adults. Beside, preventative use of probiotics was also reported to prevent hospitalized children from nosocomial diarrhea, which is the main dilemma in pediatric hospitals globally (Gill and Guarner 2004). Isolauri et al. (2002) showed in a controlled clinical research that probiotics like *L. rhamnosus*, *L. reuteri*, *L. casei* Shirota and *B. lactis* Bb12 can reduce the span of acute rotavirus diarrhea (Isolauri et al. 2002). Sur et al. (2011) have reported no difference in rehabilitation rates for adenovirus, rotavirus, norovirus, or astrovirus for children receiving *L. casei* Shirota compared with a control cohort (Sur et al. 2011). Dinleyici et al. (2011) in his study on children with *Blastocystis hominis* reported increased rate of clinical recovery and vanishing of cysts from the stool with probiotics.

Several MOA by which probiotics mediate their anti-diarrhea effect have been suggested; such as improvement of the epithelial barrier, increase in intestinal mucosa adhesion, and accompanying inhibition of pathogen adhesion, pathogenic microorganisms competitive inhibition, production of anti-microbial substances, and the immune system modulation (Bermudez-Brito et al. 2012; Dinleyici et al. 2011). Moreover, prevention of childhood diarrhea infection and antibiotic-associated diarrhea can be improved by using probiotics. It has been reported in many clinical studies that, probiotics can improve microbial imbalance and therefore constrain the generation of pathogens like *C. difficile* (Goldenberg et al. 2017; Urbańska et al. 2016). Generally, probiotics MOA in children and adults include colonization and normalization of perturbed intestinal bacterial communities, changes in the enzymatic activities linked with the carcinogens metabolism, as well as other toxic materials and synthesis of volatile fatty acids, which has a role in the conservation of energy homeostasis and organization of functionality in peripheral tissues. Additionally, probiotics also help in mucin synthesis and regulates the immune system as well as gut-associated lymphoid tissue activity (Plaza-Diaz et al. 2019).

6.6.2 Prevention and Management of Liver Diseases

Many types of liver diseases were reported globally, including diseases caused by viruses like Hepatitis A, B and C, in addition to the diseases caused by drugs and alcohol consumption such as the fatty liver and liver cirrhosis (Talwani et al. 2011). Cesaro et al. (2011) and Frazier et al. (2011) suggested that treatment and prevention of chronic liver diseases can be done by using probiotics. They reported that probiotics can prevent bacterial translocation and epithelial invasion, in addition to the fact that probiotics can inhibit bacterial mucosal adherence and synthesizing of antimicrobial peptides, at the same time reducing inflammation and stimulation of host immunity (Cesaro et al. 2011; Frazier et al. 2011). Liver disease complications could be decreased by modifying the microbiota either quantitatively or qualitatively (Eslamparast et al. 2013). Chávez-Tapia et al. (2015) reported that probiotics can modulate alterations in the gut microbiota, intestinal permeability, immune and inflammatory responses, and therefore, they can treat hepatic problems (Chavez-Tapia et al. 2015). Ranadheera et al. (2014) reported that probiotics have a big role in many liver diseases, mainly in some functional characteristics like adhesion and pro-inflammatory response (Ranadheera et al. 2014). Hong et al. (2015) showed that in a fatty diet model, *L. rhamnosus* and *L. acidophilus* moderately reduces the intrahepatic lymphocytes and TNF-α expression, also reverses irregular and decadent microvilli due to alcohol risk (Hong et al. 2015).

Different types of probiotics have a great effect on intestinal bacterial flora composition, which can show distinguished results and improvements on the metabolic health of individuals (Al-Muzafar and Amin 2017). Changed in gut flora is progressively known to play a significant important role in fatty liver disease (non-alcoholic) and is an important component of alcoholic liver disease (Kirpich and McClain 2012). In a clinical investigation involving alcoholic cirrhosis patients, who received *L. casei* Shirota three times a day for 28 days were reported to iterate neutrophil phagocytic capacity in cirrhosis, maybe due to alteration in TLR4 expression IL-10 ooze (Stadlbauer et al. 2008). Kirpich, and McClain (2012) suggested that the gut-liver axis has an important role in the pathogenesis of liver problems. Patients suffering from alcoholic cirrhosis, when received LAB showed a significant boost in liver function, by decrease in liver enzyme ALT (alanine aminotransferase) level and in tumor necrosis factor (TNF) level (Loguercio et al. 2002). When *B. bifidum* and *L. plantarum* 8PA3 were administered orally for a short period to patients with alcohol-induced liver injury, results showed that there was a significant recovery in the bowel flora in the injured liver patients, compared to the patients who received standard therapy alone (Kirpich et al. 2008).

Al-muzafar and Amin (2017) reported that a mixture of probiotic strains has a better advantage over the host health rather than a probiotic with single-strain. This mixture was reported to have an efficient effect for the management of non-alcoholic fatty liver disease via improving the leptin levels, liver function test, inflammatory markers, lipid profiles, and resist in hormone levels (Al-Muzafar and Amin 2017). They concluded that probiotics are a potential therapeutic agent that can control

hepato-steatosis and associated diseases. Chen et al. (2007) reported that, in patients with hepatitis B and C, increasing the numbers of *Bifidobacteria* and *Lactobacillus* will lead to a reduction in endotoxemia and avoids the growth of pathogens (Chen et al. 2007). The main cause of liver disease is the abnormality in the gut flora. Therefore, a well-healthy GI tract prevents a high percentage of liver diseases (Imani Fooladi et al. 2013). Comparing probiotic administration with antibiotic treatment and surgery, it is safe, with no serious side effects, and low cost. Probiotics decreases the patho-physiological signs and ameliorate many types of liver diseases (Imani Fooladi et al. 2013).

6.6.3 Modulation of Inflammatory and Immune Response

Inflammation is the protective response of tissue to a noxious stimulus, resulting in both the elimination of harmful stimuli and the starting of the healing action (Pajarinen et al. 2019). Acute inflammation is noticed by capillary dilatation, leukocytic relocation and infiltration to the local area. This leads to the clinical manifestations of redness, heat, pain and loss of function (Loi et al. 2016). One of the most important mechanisms of probiotics on human health is their modulation of the immune system (Yan and Polk 2011). Gut microbiome alteration due to the consumption of probiotics, boosts the immune system and suppress inflammatory pathways. Multiple sclerosis, an inflammatory and autoimmune neurological problem that leads to demyelination can be healed due to this improvement in the immune system by probiotics (Morshedi et al. 2019). A decrease in gut dysbiosis and gut leaky after treatment with probiotics could reduce the production of inflammatory biomarkers and weaken extreme immune system stimulation (Hosseinifard et al. 2019; Fung et al. 2017). Kwon et al. (2013) and Lavasani et al. (2010) showed that probiotics can increase the differentiation of T cells toward Th2 and also increases the synthesis of Th2 cytokines like IL-10 and IL-4 (Kwon et al. 2013; Lavasani et al. 2010). Lescheid (2014) reported that probiotics have a powerful effect in the treatment of inflammatory disorders.

Some types of probiotic strains can mend hyper permeable epithelial barriers and thus indirectly adjust inflammation by locking potentially remarkable provenances of stimuli of chronic inflammation, including LPS. They are also important inducers of other substances that have prevalent roles in modulating inflammation, including butyrate and antimicrobial peptides. Additionally, specific probiotic strains bind to nucleotide-binding oligomerization domain receptors to directly affect the activity of many important signaling pathways, affecting inflammation via boosting production of more regulatory or suppressive cytokines like IL-10 and transforming growth factor-beta (TGF-β) (Lescheid 2014). Accordingly, administration of a combination of probiotic strains will modulate multiple signaling pathways, which could have a deep combined effect on the prevention and treatment of inflammation in a short time (Lescheid 2014). Probiotics have been reported to modulate central immune responses within the GI tract via modulating signaling pathways. The MOA of the

probiotic are usually strain-specific and can have different responses within varied host cells (Llewellyn and Foey 2017). Probiotics such as *L. paracasei* CNCM I-1518 as well as *L. casei* CRL 431 were reported to stick through the Toll-like receptors and mediate immune stimulation into gastro intestinal epithelial cells (Galdeano and Perdigón 2004).

Fermentation processes are well-known for its ability to enhance the nutritional food quality by the enhancement of food nutrients. Therefore, probiotic fermented milk gets more attention recently. Consumption of probiotic fermented milk causes surges in the phagocytic and antimicrobial activity of spleen and peritoneal macrophages (Maldonado Galdeano et al. 2011). Appealingly, the cytokine produced by probiotics on immune cells stimulates the systemic immune response, with an increment in particular antibody production (Maldonado Galdeano et al. 2011). These antibodies were reported to have a significant role in lowering the infectious bacteria spreading to the liver and spleen after contaminating with *Salmonella* Typhimurium (Núñez et al. 2013). The probiotic bacteria spend around 72 h in the intestinal lumen, which is sufficient to stimulate modifications in the gut immune cells, developing the number of macrophages and dendritic cells of the lamina propria, and boosting their functionality, reflected in cytokines production (Maldonado Galdeano et al. 2011). Malnutrition leads to a substantial reduction in the defense mechanisms and increasing the susceptibility of the host to infections. Administration of probiotics will participate in restoring the thymus histology and stimulates the adaptive immune response (Núñez et al. 2013). As probiotic is been noticed to have a beneficial role in vaccine response (Zimmermann and Curtis 2018), the immunomodulatory effect of probiotic may have the possibility to help in COVID-19 infection. The ability of probiotics to regulate the gut microflora may in succession to modulate the immune system in a way that could be useful in COVID-19 (Adnan and Pramaningtyas 2020).

6.6.4 Prevention and Management of Allergies

Allergic diseases and it's prevalence in children has seen a surge apparently in the last few decades. Changing the intestinal microflora of an individual is been considered as a potential source of treatment and prevention in case of allergic disorders. Probiotics can change the microflora of the host; therefore, they may either prevent or improve allergies (Savilahti 2011). Using functional food enriched with *Lactobacilli* and *Bifidobacteria* have shown successful modulation in allergic rhinitis, food-related allergies and atopic problems (Prakash et al. 2014). Probiotics may have a positive effect for eczema prevention, and the World Allergy Organization (WAO) recommendations suggest using probiotics in lactating women, during pregnancy and in children with high risk of allergy (Zuccotti et al. 2015). Zuccotti et al. (2015) in a meta-analysis review reported that treatment with probiotics led to a marked reduction in the relative risk for eczema, compared with placebo and that

the effect was most stated when a mixture of probiotic strains was used (Zuccotti et al. 2015).

One of the main reasons for developing allergy and their correlated diseases is exposure to environmental microbes (Tang 2005). An alteration of the Th1/Th2 cytokine balance, which leads to initiation of Th2 cytokines and the release of IL-4, IL-5 and IL-13 as well as IgE production, is the main cause of allergic disorders (Michail 2009). Administration of probiotics extremely changes the gut ecology by encouraging a change in the local microbiota and cytokine production (Winkler et al. 2007), and by virtue of that it positively affect allergic disorders. Few studies available that addressed the effectiveness of probiotic administration in the treatment or prevention of allergic conditions such as asthma, allergic rhinitis and food allergy, but these studies have not been conclusive yet and led to contradictory conclusions (Michail 2009; Giovannini et al. 2007; Hol et al. 2008). The oxymoron results and the effectiveness of probiotics in the prevention and management of allergy could be due to the large strains of heterogeneity, therapy duration, as well as the amount dose usage. Also, the results of these studies bring the authors to conclude that probiotics may have a beneficial effect in the above-mentioned allergic conditions, but the current clinical data are still not adequate to allow in clinical practices.

6.6.5 Prevention and Management of Hypertension

Hypertension or high blood pressure (BP) is a medical situation in which the BP in the arteries is persistently elevated. It is a high-risk factor for cardiovascular diseases, which is a leading non-communicable health problem worldwide. Probiotics have been reported to have an anti-hypertensive effect. Many studies carried out in spontaneously hypertensive rats showed that biologically active peptides that were obtained from fermented milk, could lower in hypertensive subjects (Miguel et al. 2006). Qi et al. (2020) in a meta-analysis study showed that probiotic consumption significantly reduced either systolic blood pressure (SBP) or diastolic blood pressure (DBP), but the SBP reduction was greater than DBP compared with the control groups, and suggest the use of probiotics as an anti-hypertensive agent (Qi et al. 2020). In a systematic review study, Khalesi et al. (2014) observed that when probiotics are consumed by the subjects, there is a significant change in SBP and DBP compared with control groups (Khalesi et al. 2014). When multiple strains of probiotics were used, there will be a higher reduction for both SBP and DBP compared to consumption of a single strain. Barrett et al. (2012) and Hernández-Ledesma et al. (2004) reported that particular probiotic strains like *Lactobacilli* and *Bifidobacteria* can produce short chain fatty acids (SCFAs), conjugated linoleic acid (CLA), γ-amino butyric acid (GABA), and angiotensin converting enzymes (ACE) inhibitory peptides, which were reported to have a hypotensive effect (Barrett et al. 2014; Hernández-Ledesma et al. 2004). Santisteban et al. (2016) suggested a new

mechanism for the pathophysiology of hypertension involving a brain-gut-bone marrow triangular interaction (Santisteban et al. 2016).

The probiotics ability to lower the BP is been mainly consider due to the production of bioactive peptides during the fermentation processes, like the angiotensin-converting enzyme (ACE) inhibitory peptides (Robles-Vera et al. 2017). ACE-inhibitor tripeptides have been reported to reduce the progress of hypertension in hypertensive rats (Sipola et al. 2001). In another study, Hayakawa et al. (2004) revealed that incorporation of *L. casei* Shirota and *L. lactis* YIT 2027 strains in milk have shown to improve the mean SBP and DBP in mildly hypertensive patients (Hayakawa et al. 2004). Consumption of probiotics and probiotic fermented foods can improve the overall health status of individuals and reduces risk of developing cardiovascular diseases. Management of hypertension by consumption of probiotics is cross-linked with many different mechanisms, like improving lipid profile, bile acid deconjugation, and body mass index management. An enhancement in the absorption of nutrients, phytoestrogens and a lowering in plasma glucose levels may also influence the probiotic effect in hypertension regulation (Khalesi et al. 2014). Karbach et al. (2016) revealed that there is no difference in BP between germ-free and traditionally raised mice, which is consistent with previous study showing no effect in BP after a significant decrease in fecal microbial biomass caused by antibiotic treatment (Pluznick et al. 2013; Karbach et al. 2016). As it has been shown that probiotics and their fermented products can effectively lower inflammation and hypercholesterolemia and eventually reduces BP, this fact can support their administration in reducing the risk of cardiovascular diseases. Probiotics could be a cheap and reliable source of anti-hypertensive agents.

6.6.6 Prevention and Management of Metabolic Disorder (Diabetes)

Currently, diabetes has become an epidemic around the world affecting nearly 382 million people. According to the published data, it is assumed that each year around 1.3 million people die because of diabetes. As per International Diabetes Federation (IDF), it is projected that almost 629 million of world population will become diabetic by 2045 (Ashraf et al. 2020a). The intestinal microbiota exhibits crucial role for the non-digestible substrate fermentation such as endogenous intestinal mucus and dietary fibers. The intestinal microbiota is the most extensive and 2–10 folds higher than the number of cells that make our body and mostly found in small intestine and colon (Rad et al. 2017). Intestinal microbiota involved in a variety of aspects related to health as well as in curing the diseases (Lallès 2016). Diseases consist of varied metabolic disorders such as glucose intolerance, insulin resistance, diabetes obesity and metabolic syndrome. These microorganisms reveal an essential function in the permeability of GI mucosa and immune system, which are the significant factors in type 2 diabetes mellitus (T2DM). It was revealed that

T2DM is linked with intestinal microbiota dysbiosis (Ri et al. 2015; Wu et al. 2010). Intestinal microbiota dysbiosis may help in fat synthesis, development of adipose tissue, energy extraction from diet and leads to metabolic syndromes (Marchesi et al. 2016; Cani and Delzenne 2009; Blandino et al. 2016). Microbiota of intestinal tract also raises the adiposity, dysfunction of β-cell, systemic inflammation, metabolic endotoxemia, oxidative stress (Yoo and Kim 2016; Sun et al. 2020). Currently, modulation of intestinal microbiota performs great role in the prevention and treatment of dysbiosis allied with metabolic disorders (Druart et al. 2014).

In one study, it has been revealed that *L. rhamnosus* GG reduces gluconeogenesis in the liver and augments the insulin sensitizing hormone (Kim et al. 2013; Kim et al. 2014). Another study revealed about hypoglycemic and anti-diabetic effects of *Lactobacillus* spp. (Andrade-Velásquez et al. 2020). In an animal study of *Lactobacillus* spp. it was observed that, they reduce pro-inflammatory genes expression in mice and expresses beneficial effects in diabetic rats. Overall, numerous studies have been confirmed that probiotics and probiotics derived foods exhibit significant results in diabetes (Park et al. 2013). It has been observed that modulation of intestinal microbiota via probiotics is helpful to improve insulin-resistance (Rad et al. 2017; Cani and Delzenne 2009). Nevertheless, the efficiency of probiotics varies on different species. After clinical trials on T2DM patients, it has been reported that using fermented milk containing *B. lactis* Bb12 and *L. acidophilus* LA5 controls the reduction of anti-inflammatory cytokines and improves the glycemic control (Tonucci et al. 2017). It has also been found that probiotics decreases the blood sugar levels and insulin resistance through the improvement of inflammation. It has been reported that yoghurt with *Bifidobacterium* and *L. acidophilus* strain La-5 remarkably reduces the TNF- α and HbA1c (Mohamadshahi et al. 2014).

Probiotics are able to improve intestinal barrier function and decreases the microorganism's transmission along with their derivatives and leads to reduction in associated pro-inflammatory cytokines released through Toll-like receptor-4 signaling (Guha and Mackman 2001). Improvement in the antioxidant enzymes activities includes catalase, superoxide dismutase and glutathione peroxidase has been exhibited in other similar study of probiotics. Several strains of probiotic LAB have played antioxidant actions by several potential mechanisms that include scavenging of reactive oxygen species (ROS), chelation of metal ions, auto-oxidation inhibition and enzyme inhibition (Parle and Malik 2014). Gestational diabetes mellitus (GDM) rate is increasing across the world with other associated consequences including, overweight, obesity, preeclampsia etc. It produces acute and chronic complications to mothers as well as offspring (Petry 2020; Chu et al. 2007; Nijs and Benhalima 2020). Current therapy to control GDM is better for short term complication than long term complication. Probiotics contain the capability to avert GDM by altering metabolism (Barrett et al. 2014). Diet can influence the composition of microbiota along with gene expression with changing host metabolism. Modifying the microbiome inside the gut exhibits numerous effects. For instance, influence of inflammatory pathways, glucose and lipid metabolism and altering the nutrients absorption (Rad et al. 2017; Bäckhed et al. 2004). Clinical trials conducted on the normal

weight pregnant women with the probiotic supplements and reported that GDM rate decreased from 34% to 13%. Probiotics supplement given to the patient has *L. rhamnosus* GG and *B. lactis* Bb12. It was found that blood glucose and HbA1 levels improved than control group those depend on healthy diets without any probiotics (Luoto et al. 2010).

6.6.7 Prevention and Management of Cardiovascular Diseases

Heart diseases (cardiovascular disorders) have remained the leading cause of deaths at the global level for the last 20 years. However, rate of deaths is now escalating than ever before. Heart diseases death rate rose nearly over two million since 2000, to almost nine million in 2019. 16% of world deaths are linked with heart diseases (WHO 2020). Cardiovascular disorders may be preventable up to 90% through avoiding the recognized risk factors. Presently measured practices to avert heart disorders includes, decrease in the intake of saturate fats, stop smoking, keep up the healthy diet, moderate work out and reduce body fat contents if obese or over weight (Habib et al. 2019; Ahmad 2019; McNeal et al. 2010). Probiotics and fermented foods have been employed as a health promoting agents for many years (Ahmad et al. 2013a; Ahmad et al. 2013b). Probiotic bacteria produce acids that control the production of cholesterol due to absorption of the fiber from intestine. Propionic acid exhibits remarkable result by reducing the cholesterol production by liver. Liver bile acids are broken down by probiotics, bile acids help the body in the digestion of fats and these bile acids are produced by the liver. The recycling of bile acids is done by liver to make use again and again. Break down of bile acids by liver decreases the concentration of bile acids and to maintain the regular production of bile acids, more cholesterol is required. Consequently, cholesterol is utilized by the liver to prepare bile acids and eventually cholesterol concentration decreases in the body. It has also been reported that probiotics get nourishment through cholesterol metabolism. Thus, owing to various functions of probiotics, these could be the best food substitutes to control various diseases linked with cholesterol such as peripheral vascular disease, stroke and other coronary heart diseases. Also, it could be the better replacement of pharmaceutical products (Peirotén et al. 2020; Saini and Saini 2009).

Probiotics exhibits the potency to maintain the regulatory T cells in the immune system. Innate and adaptive immune actions exhibit crucial role in the progress of many cardiac disorders, and probiotics proved to possess potent immunomodulatory actions in various studies. Dendritic cell's key role is to process the antigen material along with transfer to the cell surface of T cells of the immune system. They are capable to distinguish diverse microbial strains by pattern-recognition receptors expression that distinguishes the pathogen-associated molecular patterns. Probiotics bacteria encourages the dendritic cell pattern of maturation by releasing of little quantity of IL-12 and TNF-α with elevated IL-10 levels and restrain

pro-inflammatory cells generation. *Bifidobacteria* remain in the intestines and they play significant role for the health by decreasing CD80 and CD40 expression and induces an upregulation of IL-10 emission. Consequently, anti-inflammatory and immunomodulatory actions produced by increasing the IL10-production (Abdolalipour et al. 2020; Saini et al. 2010; Madsen 2006). Raised low-density lipoprotein cholesterol (LDL-C) is a key threat for coronary heart diseases (CHD), and to overcome it, the main target is lipid lowering therapy (Virani et al. 2021; DiRienzo 2014; Mourikis et al. 2020). It is the need of the hour to find the LDL-C reducing agents. Recently, several probiotic strains such as *E. faecium* and *L. reuteri* have been searched with capability to decrease the LDL-C, in addition to other CHD risk factors (DiRienzo 2014). Several animal studies have suggested that probiotic strain have beneficial habits as presented in Table 6.1.

Table 6.1 Animal studies of probiotic strains on obesity and associated disorders that leads to cardiovascular diseases

Type of probiotic	Duration	Results	References
Lactobacillus paracasei CNCM I-4270	12 week with high fat diets	↑Infiltration in adipose tissue ↓Weight gain ↑ Glucose insulin homeostasis ↓Liver steatosis	Wang et al. (2015)
Lactobacillus plantarum ky1032 and *Lactobacillus curvatus* HY7601	10 week with high fat diets	↓ Fat accumulation and body weight gain, ↓ Leptin, plasma insulin, and cholesterol ↓ IL6, IL1β and TNFα in adipose tissue ↓ Fatty acid oxidation genes in the liver	Park et al. (2013)
Bifidobacterium adolescentis	12 weeks parallel high fat diets	↑Insulin sensitivity ↓ Visceral fat accumulation and body weight gain	Chen et al. (2012)
Lactocbacillus rhamnosus GG	4 weeks	↑Insulin sensitivity ↑ Glucose tolerance ↑Insulin stimulated Akt phosphorylation ↓ Skeletal muscle endoplasmic reticulum stress	Park et al. (2015)
Bifidobacteria adolescentis	12 week with high fat diets	↓ Steatosis and liver inflammation ↓ Body weight gain ↓ Development of ROS	Reichold et al. (2014)

6.6.8 *Detoxification of Cancer-Causing Carcinogens*

Globally, cancer is considered as the second-leading cause of death, and despite of the several advancements in recent drug discovery, there is still lot of research needed to counter adverse effects of drugs with the help of naturally derived medicines. Cancer represents broad categories of malignancies showing the key characteristic of uncontrolled proliferation, aided by various functional and regulatory changes, which causes spread of tumor cell throughout the body (Elkhalifa et al. 2021; Ashraf et al. 2020b; Ahmad et al. 2021). Meanwhile, several studies have demonstrated that there is a direct link between incidence of cancers and dietary habits. Since, intake of processed food has exposed human being to various ranges of toxic chemicals such as polycyclic aromatic hydrocarbons, heavy metals, acrylamide, mycotoxins, cyanotoxins, nitrosamines, phthalic acid esters, heterocyclic aromatic amines and polycyclic aromatic hydrocarbons which has been well-documented for producing mutagenic as well as carcinogenic effects (Khorshidian et al. 2016; Shoukat 2020). In addition, various other risk factors which causes cancer includes genetic factors as well as immune system of the individual's body. Currently, several reports suggest that intestinal microbiome shows very significant role in sustaining homeostasis in human body. Goldin and Gorbach were the first who conducted research studies to see the association between food and colon cancer, thereby they found the food enriched with LAB was able to reduce the colon cancer by 40% compared to control 70%. Probiotics have been found to modulate cancer cell's proliferation and apoptosis in both *in vitro* and *in vivo* studies. Furthermore, probiotic has gained substantial medical significance because of the several health benefits (Śliżewska et al. 2021).

Probiotic bacteria is been responsible for detection and detoxification of potential carcinogens, which causes cell proliferation or cell death leads and known as signaling molecules in the immune system (Górska et al. 2019). Among probiotic strains LAB are the most popular with various application in food and pharmaceutical industries. Shoukat (2020) reported that various probiotic strains including *Bifidobacteria*, LABs help in prevention colon cancer by reducing or detoxifying carcinogenic compounds. Probiotics including *Bifidobacteria* and LABs have been found to detoxify most of the carcinogens and *Bifidobacteria* have shown a significant non-toxic and non-pathogenic, and *in vivo* functional properties (Khorshidian et al. 2016; Śliżewska et al. 2021; Górska et al. 2019). Several studies suggest that *L. rhamnosus* GG, *L. pentosus*, *L. fermentum*, *L. acidophilus*, *L. helveticus*, *L. casei*, *L. lactis*; *Bifidobacterium*: lactis, adolescentis *Pediococcus pentosaceus*, *Bacillus subtilis* strains have a significant anti-proliferative effect on various human colon cancer cell lines (HGC-27, SW480, Caco-2, DLD-1, SW1116, HT-29 and HCT116), in addition it was also found to reduce the level of level of IL–8 (Altonsy et al. 2010; Borowicki et al. 2011; Orlando et al. 2012; Russo et al. 2007; Sadeghi-Aliabadi et al. 2014; Lopez et al. 2008). In addition, *L. casei* LBC80R, *L. acidophilus* CL1285, *Bifidobacterium longum* HY8001, *L. acidophilus* SNUL and *L. casei* YIT9029 has been reported to causes apoptosis or suppressed proliferation of

human colorectal cells (LS513 & SNUC2A) as well as gastric carcinoma cells (SNU1) (Baldwin et al. 2010; Lee et al. 2004). Drug used for cancer treatment such as 5–fluorouracil causes diarrhea in cancer patient as one its side effect. *Bacillus polyfermenticus,* a probiotic strain has been found to lower the cell colony formation in human colonic epithelial cells (NMC460) (Śliżewska et al. 2021). Therefore based upon the potential application of probiotic, a novel probiotic based therapy could become an alternative approach to more invasive as well as costly treatment such as chemotherapy.

6.7 Regulation, Safety and Efficacy

Recently, probiotic market has grown throughout the world, both for probiotic food or probiotic as therapeutically supplementation is been in used to enhance health benefits. Regulations for the use of probiotic around the world vary, in other words probiotic is not much regulated until unless it has not make any specific claim related to the health benefits. Of note, probiotics are regulated mainly based upon the characteristics of food supplements and regulations are monitored on the legality of any claims, rather than its efficacy, safety and quality. Additionally, probiotic properties are usually strain-specific and in general safety and efficacy are majorly related to specific formulations, and should not be generalized to specific probiotic products (de Simone 2019). LAB is considered as one of the very well-known strains used for probiotic preparations and it has safe use for human food consumption since long time back. However, ingestion of probiotic for human consumption can't be guaranteed of zero risk factor. LAB safety in fermentation application is quite clear, but several other probiotic strains used in various food materials fermentation impact on final products such as its safety, health benefits and its sensory attribute (Mortazavian 2012; Gawai and Prajapati 2017).

Recently, various strains of probiotics such as *Lactobacilli* and *Bifidobacterium* currently used by severally internationally renowned food manufacturers and starter culture suppliers for productions of safe probiotics and its related food products (Tamime et al. 2007; Sanders 2003). According to previous studies, it is suggested that *Lactobacilli* and *Bifidobacterium* does not possess any risk factors with its usage as oral consumption by healthy individuals. The Joint FAO/WHO committee proposed a framework containing information regarding strain identification and functional characterization, followed by safety assessment. According to Joint FAO/WHO guideline, the minimum tests required to characterize safety are parameters for probiotic strains are as followed (Ray and Joshi 2014) To check the antibiotic resistance patterns, (Di Cagno et al. 2013) Identify the metabolic activities of strains, (Terefe 2016) Identify the toxin produced from fermentation or during the fermentation, (Bell et al. 2017) Does the strains has the hemolytic capability? (Kechagia et al. 2013) To check whether the probiotic strains have any infectivity in immunocompromised animals, (Guarner et al. 2012) Identify the side effects of probiotic strain in human model and (Nagpal et al. 2012) To perform the

epidemiological surveillance of adverse incidents among the consumers (Gaggia et al. 2011; Mohamadshahi et al. 2014; Mishra et al. 2018).

Furthermore, several efficacy studies for probiotics have been performed to establish the product in recent times. A meta-analysis study of 11 probiotic species against the eight major GI diseases and revealed that probiotic species was highly effective in the treatment of antibiotic-associated diarrhea, infectious diarrhea, *Helicobacter pylori* eradication, *C. difficile* infection and pouchitis. At the same time these 11 probiotic strains showed poor efficacy against the traveler's diarrhea and necrotizing enterocolitis (Khalighi et al. 2016). Therefore, several observations made regarding the probiotic products and it clearly suggests that it help consumers with immunocompromised system or vulnerable people, such as the elderly, children and people with immune deficiencies (Peivasteh Roudsari et al. 2019). Few examples such as usage of probiotic product for children in day care unit under the randomized trial shows reduction in the respiratory infections as well as reduces the severity of illness (Hatakka et al. 2001). Therefore, the success of probiotic has fascinated genetic engineers to improve and produce genetically modified strains of probiotics for further applications. Currently, many probiotic products is been on the market since long time with no major safety issues and it can act differently in two consumers, hence the safety questions of probiotic product or active ingredient are very specific to the individual (Mishra et al. 2018). Meanwhile, usage of the probiotic strains should be completely non-pathogenic and should not cause any harm to the host (Peivasteh Roudsari et al. 2019).

6.8 Future Perspectives and Global Demands

The global market for probiotic supplements are continually expanding day by day. Rising awareness among the consumers regarding the prevention and management of several chronic diseases has led to an increase in demand of natural and safe health benefiting product such as probiotic. Various commercial products of probiotics are available around the world such as Aciforce (Biohorma® Natherland), Actimel (Danone® France), Activia (Danone®), Hellus (Tallinna Piimatoostuse AS® Estonian), Yakult (Yakult® Japan), which is predicted to upsurge the market share (Mishra et al. 2018). Currently, one area is being targeted for investigation of probiotic behavior via whole genome sequencing technology. This, amongst other features, will boost the functional aspects of probiotic LAB, and data will provide a good base for further LAB genetic manipulation (Zommiti et al. 2020). In addition to strains from the genera *Lactobacillus* and *Bifidobacterium*, strains from other genera, such as *Propionibacterium* and *Lactococcus*, commonly found in fermented foods, are likely to receive more attention. Strains from "new" probiotic genera are also likely to emerge, such as butyrate-producing *Roseburia* and *Clostridium*, or strains from the anti-inflammatory species *Faecalibacterium prausnitzii*. Furthermore, genetically modified probiotics should be anticipated and they may even target diseases for which there is currently no cure. Similarly, one could

anticipate that more complex mixtures might be more efficacious, as they could provide a multitude of functions to microbiota in disarray. Furthermore, published report indicated that, the global probiotics market size was valued at USD 48.88 billion in 2019 and is projected to reach USD 94.48 billion by 2027, exhibiting a CAGR of 7.9 during the forecast period (https://www.fortunebusinessinsights.com/industry-reports/probiotics-market-100083). Furthermore in Canada, foods and beverages fortified with probiotics are expected to grow at a CAGR of 6.6% by 2022. Moreover, Europe is the second largest food and beverage probiotic market. Brazil leads the probiotic market with an estimated share of 52% in 2016 and a forecast CAGR of about 11% between 2017 and 2022. In Asia, China leads the probiotic market with an estimated 35.4% market share (https://bc30probiotic.com/wp-content/uploads/2019/11/GanedenBC30-Global-Probiotic-And-Digestive-Markets-Infographic.pdf).

6.9 Conclusion

Despite the public perception of benefits provided by probiotics, the evidence to conclusively link probiotic strains to improved characteristics of health or disease is lacking. This is due to the lack of large-scale research trials and insufficient understanding regarding probiotic interactions within the human system. This has driven scientific researches with the aspirations to uncover probiotic strains that provide conclusive evidence of improvements in health and disease outcomes. More in-depth research into individual probiotic strains, combined with the application of multiple advanced measurement techniques will provide a future direction for probiotic research and in turn, aims to provide useful data to translate into routine healthcare practice. Currently, probiotics has been classified for human health application and WHO has approved probiotics usage in human beings and these are generally recognized as safe. Therefore based upon our review, we can conclude that, probiotic can be considered for various health benefits, however its clinical safety in human beings are not very clearly studied. Further clinical studies are needed to see the effect on host and food and its adverse effects in high-risk consumers.

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Chapter 7
Molecular Techniques for Microbial Community Profiling of Fermented Foods

Nawaf I. Alshammari

Abbreviations

CDNA	complementary DNA
DNA	deoxyribonucleic acid
FCM	fluorescence correlation microscopy
LAB	lactic acid bacteria
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
RNA	ribonucleic acid
rRNA	ribosomal RNA
VNC	viable but nonculturable

7.1 Introduction

New developments such as increased consumer comprehension has compelled food processors to look into the microorganisms used in their processing to meet the demands of the consumers. Pathogenic microorganisms in food are observed, investigated and identified to ensure production of healthy and safe foods. Traditionally, bacteria causing food borne diseases were identified through analysis of cultures (Ferris et al. 2004). However, some bacterial cells are not culturable and advanced techniques have been developed to widen the range of bacteria that can be detected using molecular methods. Fermentation is a term used the biochemical processes carried out by organisms during their development, growth, reproduction or death. In relation to technologies used where the knowledge of fermentation is applied, organisms are used to manufacture food, alcoholic beverages, pharmaceuticals

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amongst other products on a large-scale basis. For fermentation to occur, it is important that organisms are cultured under favorable conditions and necessities such as vitamins, trace elements, salts and nitrogen. This type of metabolism produces end products of higher commercial value and that are utilized by human beings (Zheng et al. 2011). Such by products include; enzymes, proteins, hormones, cheese, vinegar, ethanol, cider, beer and wine. Fermentation process takes place in a fermenter or a bioreactor which can be used to carry out any other biochemical reaction. Studies have shown that there has been strong links between bread baking and beer brewing in history and around the fourteenth century, brewers' yeast was discovered to leaven bread. Brewers' yeast and sourdough cultures were the only leavening agents used for making bread up and until the ninetieth century when commercial yeast was invented.

Quality techniques like aerobic plate count is useful in identifying and counting bacteria present in a preprocessed food sample. The second technique comprises compound such as bacteriocin, growth nutrients which particularly hinder growth of selected microorganisms. The third method contains indicators fluorogenic or chromogenic stratum which distinguishes different types of bacteria by use of chemical reactions. The separation and sterilization of microorganism cultures enable phenotypic analysis and culture storage (Xia et al. 2020). Phenotypic methods include bio typing which focuses on the conditions and requirements for growth of bacteria, phage typing which looks into structural differences on the surface of bacteria and serotyping. Nonetheless, genotypic methods have been developed to improve on phenotypic methods. Polymerase chain reaction is formed by DNA specific portion amplification and ribosomal RNA (rRNA) gene is a suitable PCR since it consists adequate disparity amidst species & stains and commonly dispensed amid bacteria in DNA sequence. Several PCR structures have been evolved for distinction of various species which belong to one genus and varied pathogenic bacteria. Restriction enzymes have plasmids which not only use DNA but also involve other cellular properties such as metabolic activity to identification of bacteria. Pulsed-field gel electrophoresis allows large DNA fragments to become smaller and lead them towards the electron field using consecutive varying electric fields while amplified fragment length polymorphism technique is founded on the amplification of fragments from restricted digestion of the entire genomes.

Of all food types, fermented foods were among the first to be consumed by humans. These types of food are preferred for their nutritional value and also for their cultural importance amongst people of different backgrounds (Zhong et al. 2007). Microorganisms and enzymes were discovered about a hundred and fifty and two hundred years ago, were technically used by alchemists and philosophers. Biologists had little knowledge about the use of microbiota in fermentation processes. Since then, the use of molecular techniques in examining fermentation of food has become common. The changing chemical and physical state of food micro environments affects the biochemical activities, survival and growth of fermented food. Most of ecological cell activities that occur in situ occur in solid phase. Methods of microbial characterization, identification and enumeration are used to monitor

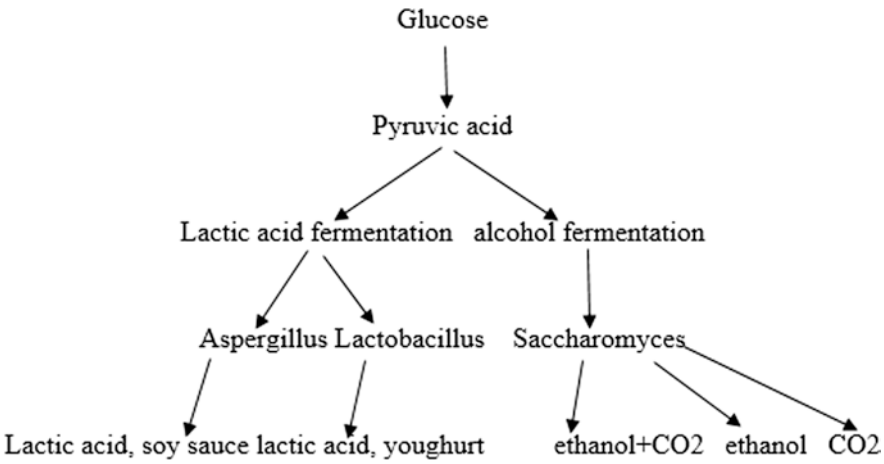


Fig. 7.1 Molecular techniques used to study microbial ecology of fermentation process

strains responsible for fermentation. There is a lot of diversity in ecosystems and molecular techniques that are culture oriented are used to study microbial communities. These studies could help to understand how microbiological processes in ripening and food processing that could improve safety when handling pathogenic bacteria. Below is a chart summarizing the fermentation process (Fig. 7.1).

7.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was first invented in 1953 by Watson and Crick when they discovered that DNA replication can be used to produce complementary DNA (cDNA) strands. This process can be used to amplify nucleic acid. Targeted areas of a bacterial gene can be amplified in an arbitrary sequence and can be a sequence of an oligonucleotide. It is easy for an oligonucleotide (a small sequence of nucleotides) to be detected. Various aspects of bacterial pathogens DNA can be tested, such as, multicopy ribosomal RNA (rRNA), cellular metabolites, toxins and virulence factors. Basic structure of DNA contains a deoxyribose sugar, a phosphate and either one of the four nucleotides. These nucleotides form complementary strands to combine with other nucleotides (Deishing and Thompson 2004). PCR is used to amplify a segment of DNA under the basis of DNA replication and can be used to screen for GMO (genetically modified organisms) in various types of food. The strand to be amplified is known as a template strand and while PCR can work the same way as gel electrophoresis, PCR can be developed further to discriminate bacteria, PCR steps was shown in (Fig. 7.2). Products can further be detected and various strains and species detected on micro wells and membranes. rRNA has shown limitation in terms of the number of strains that it can detect. These types of

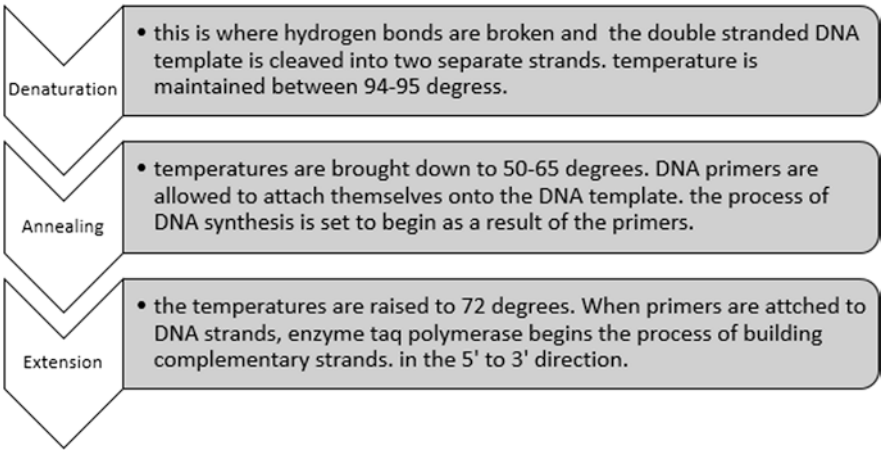


Fig. 7.2 Polymerase chain reaction steps

bacteria include uncultured as well as cultured organisms from food samples, clinical and environmental samples.

There are various methods in which PCR can be done. For example, multiplex PCR can use several primers of multiple samples of viable bacteria for the purposes of quantification and differentiation (Settanni and Corsetti 2007). Conventional PCR which utilizes the concept of reverse transcriptase is usually unable to detect dead cells and therefore, only viable cells can be detected using this method. Conventional PCR can also be used to detect VNC Cells since they cannot be detected using culture methods. The use of double stranded DNA dyes requires that one product is used for every reaction that is undertaken. It is important to note that many factors that play a part in the PCR process are difficult to regulate. These could be the equipment used, temperature, microbiological and chemical hygiene, and humidity (Stringer and Hall 2007). In addition, PCR detects the potential of an organism to produce toxins but does not detect the presence of toxic DNA in food. In addition, Enzyme of restriction, also known as endonuclease restriction, a protein developed through bacteria that cuts DNA at specific locations along the molecule (Fig. 7.3).

Apart from the fact that restriction enzymes are of different types, another benefit of using endonucleases is that the actual DNA sequence of an organism does not have to be known for a plasmid to be investigated. These enzymes split DNA at recognized sites to create a fingerprint (a fragment of DNA). Various fingerprints can be compared against one another to establish their identity. Even though plasmids do not encode housekeeping or survival genes, they encode other cell properties such as their metabolic activities, bacteriocin and antibiotic resistance. In addition, to plasmids being unstable, various strains of bacteria might lack plasmids and therefore the method of DNA fingerprinting becomes unreliable in some cases (Zhao et al. 2015). When a whole chromosome is used, there is a large number of fragments obtained and this might prove difficult to analyze. When cutting

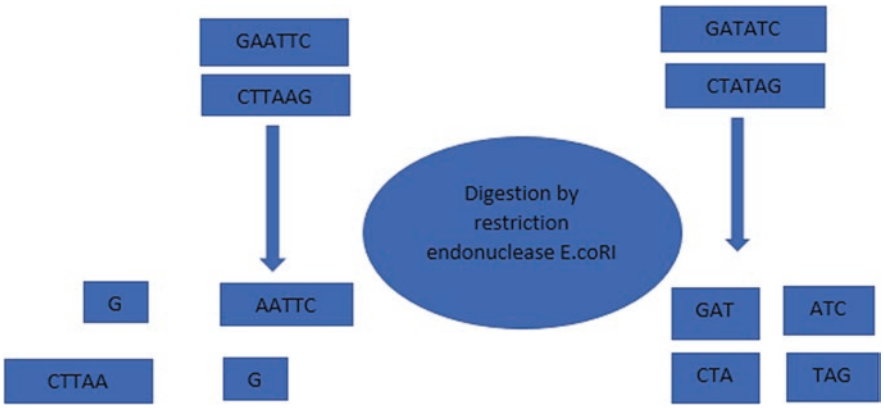


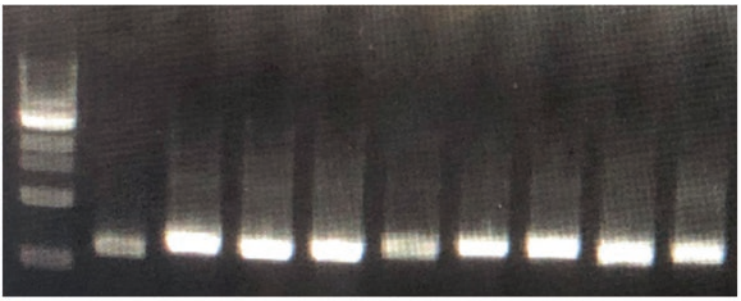
Fig. 7.3 Restriction endonuclease

techniques of low frequency are used, the number of fragments may reduce and the analysis becomes simpler.

7.3 Gel Electrophoresis

Figure 7.4 presented Gel electrophoresis, which is used to separate chromosomal DNA according to their sizes. These molecules of RNA, DNA and other proteins move through a gel filled matrix when an electric current is running through it. The ends of the gel are negatively and positively charged so molecules move from one end to the other, with the smaller molecules moving much quicker than the bigger molecules. Cantor and Schwartz were the first to replicate a karyotype of yeast *Saccharomyces cerevisiae* through gel electrophoresis in 1984. Since then, developments have been made to improve on resolutions of fragments on the screen (Taylor et al. 2005). Separation of molecules on the agarose gel is dependent on the size of DNA and independent from the sieving process. Separation of molecules also depends on different factors such as the voltage of electric current, concentration of gel, concentration of interpolating dye used, for example, ethidium bromide and buffer strength. Sometimes, the electric current may have to be increased to have a better separation and view of fragments. The analyzed fingerprints can be compared against bacterial fingerprints and their identity be established. Isolation of large DNA molecules can be done using pulse field gel electrophoresis (PFGE) which helps to produce a fingerprint of an isolate of a bacteria sample.

Bacterial isolates can be form contaminated food, sick people or food production areas (Sheikha 2021). Some of the benefits of PFGE is that it is has the ability to discriminate and is superior in differentiating strains and species and therefore used in epidemiological studies. Some of the bacterial strains that can be detected using PFGE include *Bacillus thuringiensis, B cereus, B. anthracis, S aureus, L*



A

B

Fig. 7.4 A and B. DNA separation of gel electrophoresis process

monocytogenes, C jejuni, and *Campylobacter coli.* Bacterial DNA isolation can be modified to improve enzyme digestion and lysis. Methods such as multilocus electrophoresis, amplified length polymorphism have proved inefficient when it comes to identification of these bacteria.

7.4 Amplified Fragment Length Polymorphism

This technique utilizes the knowledge of amplification of cleaved fragments that have been digested by restriction enzymes. The major steps involved in AFLP include digestion, ligation, amplification and electrophoresis. Two enzyme restrictions are responsible for digestion of DNA, producing two fragments with sticky ends (Melles et al. 2007). Ligation using adapters takes place and PCR templates are created. Matching fragments are then amplified, resulting in thirty-five to forty fragments. Some of the amplified fragments are strain specific while others are species specific. Detection of DNA is indicated by the absence or presence of DNA

bands. This method is highly polymorphic and reproduces highly compared to restriction fragment length polymorphism.

7.4.1 Restriction Fragment Length Polymorphism

RFLP 1

This technique of gene identification looks into the variations of homologous DNA and differentiates between species, populations, and individuals. The use of restriction enzyme sites can help to illustrate the differences between DNA. Using RFLP, restriction enzymes digest fragments and using gel electrophoresis, they are separated based on their size. The fragments are then passed through a membrane in a procedure known as Southern Blot. The actual length of the fragment is determined through hybridization to a DNA probe which is labelled. Although the emergence of modern and inexpensive methods of DNA fingerprinting has made this method obsolete, RFLP was an important tool for paternity testing, localization of diseases and genetic disorders and genome mapping (Drancourt et al. 2000).

When there is variation between fragments of homologous DNA or variation between individuals, then RFLP is said to have occurred. All fragments can be used in other genetic examinations and are considered as alleles regardless of whether they contain a coding region or not. When southern blot hybridization and restriction digestion are used together for the probe of rRNA, this is known as ribotyping. Ribotyping can be used to detect a wide variety of bacteria in fermentation than any other probe and it is more species and strain specific. In bacteria, ribosomal operons are arranged into 16S, 23S and 5S rRNA. This technique has proved successful in identifying strains like *Pseudomonas aeruginosa* and *Legionella spp*. These two species are difficult to identify using phenotypic methods (Van Belkum et al. 2007). Ribotyping may not be the best method to discriminate against different strains and species but has proven to be a rapid way of screening large bacteria isolates as shown by studies with *P. aeruginosa, L. monocytogenes* and *Salmonella*. In order to discriminate between various species and strains other methods such as serotyping and PFGE should be incorporated.

7.5 Immunological Methods

These techniques of genetic identification are used to study the immune system of an organism and develop reagents that can be used as experimental apparatuses. Examples of surface structures include extracellular organelles, membrane proteins, capsular polysaccharides and lipopolysaccharides (Swain et al. 2014). Serotyping which is also known as serology and refers to the use of antibodies that create a bacterial or viral infection. By the fact that this method uses antibodies in detection

of bacteria, serotyping is considered a molecular method of identifying bacteria in fermented foods. Gram negative bacteria such as *E. coli, Campylobacter* and *Salmonella* is best detected by serotyping. Gram positive bacteria such as *Listeria* can also be identified using serotyping (Aarnisalo et al. 2003). Immunoassays are methods used for the preciseness of antigen-antibody reactions to identify molecules of biological samples. This method can be used for the identification of *B cereus an C perfringens, staphylococcal toxins, cholera* and *botulinum*. This method can be used for both metabolites such as toxins and surface antigens. More recent methods of immunological techniques have been developed to the advantage of processing samples in less effort and time. Food can now be used to identify the present bacteria instead of pure and isolated samples of bacteria. Currently, enzyme linked immunosorbent assay is the best immunological technique to use for whole antigen products or targets. Such bacteria include *Salmonella, Campylobacter, B. Cereus,* and *E. coli.*

7.6 Future Methods in Bacteria Identification

Although these methods are modern, they are based on the genotypic and phenotypic identification of DNA in bacteria, the difference is in the set up or instrumentation of sample analysis (Fig. 7.5). They include biosensors, microarrays (Bachand et al. 2006) and flow cytometry.

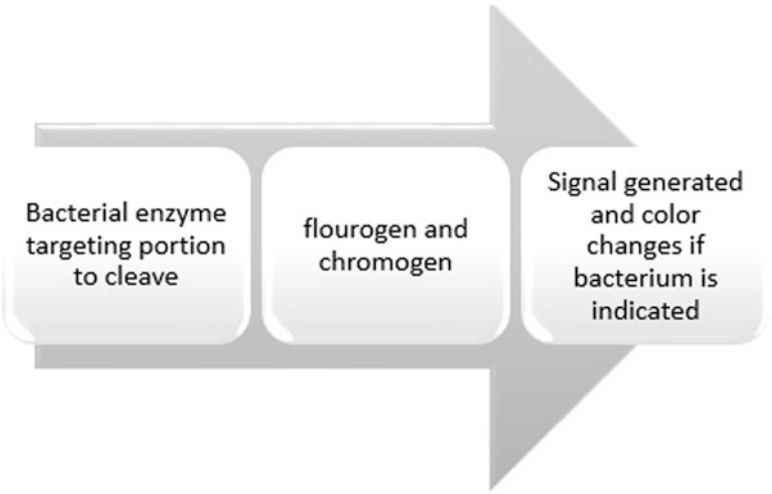


Fig. 7.5 Future of bacteria identification

7.7 Flow Cytometry

Commonly applied in chromosomes and mammalian cells, this method is common in medical diagnosis and cycle analysis. The instrumentation of this method involves using a fluorescent microscope with a focus and cells flowing through it. The main elements include a computer, an electric network, controls, fluid lines and a light source. Samples are illuminated one at a time as they pass through the focused light (Rahman et al. 2006). Protein amounts in bacterial cells were first studied using flow cytometry and have become common ever since. This technique is preferred due to its ability to integrate aspects of both homogenous and heterogeneous samples like cellular structure analysis, cell counting and cell detection. Yeast from bacterial cells will be scattered by the light according to the granularity and size of cells. Algae can easily be identified using the photosynthetic pigments due to its auto fluorescent characteristics. FCM is commonly used in the serological distinction between parasites, viruses, fungi and bacteria (Ferris et al. 2004). One major advantage of flow cytometry is that it is used to differentiate between VNC, dead and viable cells using fluorescent dyes indicating enzyme activity, intracellular pH, membrane potential, membrane integrity and respiration.

7.8 Biosensors

In comparison to gel electrophoresis, culture methods, immunology, and PCR, this is the fastest growing technology in identification of bacteria. The equipment consists of a biological material that is integrated into a transducer that senses chemical or biological change and converts the change into a signal. Drugs, carcinogens, pesticides, pollutants and pathogens from food, water and soil are examples of analyses that can be detected using biosensors based on phage display peptides, immunology and DNA. Biota such as animal tissues, nematodes, algae and plant tissue have been used as biosensor detectors (Rahman et al. 2006). Acids, ammonia and carbohydrates are examples of metabolites that can be produced by viable microbes. A microbe such as *Vibrio fischeri* becomes bioluminescent and can be used to detect viability of fluorescent bacteria (expresses luminescent proteins like luciferase protein and green fluorescent protein). For the detection of pathogens in food, there are various types of biosensors such as antibody and receptor-oriented biosensors, enzyme based and DNA based biosensors.

In fermentation processes, microorganisms produce various metabolites like carbohydrates, enzymes, alcohols and acids. Microbes that help in carrying out the fermentation process include yeasts, molds and lactic acid bacteria (LAB) (Yilmaz and Velioglu 2009). LAB is particularly involved in the fermentation of sourdough fermentation, vegetable fermentation, and dairy products. Pediococci and lactobacilli are starter cultures that are involved in the fermentation of meat. In monitoring microorganisms, it is important to; identify the bacterial flora of foods and starter

cultures, establish the count of bacteria present in foods and lastly, identify the specific biotype and strains in foods. It is also important to detect hazardous microorganisms such as molds, yeasts, viruses and various bacteria. Below are some examples of fermented foods and their ecology. Some of the applications of a biosensor include;

- Prosthetic devices
- Water quality management
- Toxins of defense interest
- Food quality monitoring
- Soil quality monitoring
- Environmental monitoring
- Disease detection
- Drug testing

7.9 Conclusion

At a systems level, in situ visualization, targeted profiling of food, community profiling modern molecular tools have the capacity to provide a high resolution evaluation of fermentation processes. We are able to provide a groundbreaking realization of the selection of targets, analysis tools and techniques for experimental purposes. Researchers still face many challenges in respect to careful selection of these analysis tools, molecular targets and techniques. PCR is a process that can be used to amplify nucleic acid (Singh et al. 2012). Targeted areas of a bacterial gene can be amplified in an arbitrary sequence and can be a sequence of an oligonucleotide. It is easy for an oligonucleotide (a small sequence of nucleotides) to be detected. Various aspects of bacterial pathogens DNA can be tested, such as, multicopy ribosomal RNA (rRNA), cellular metabolites, toxins and virulence factors. Basic structure of DNA contains a deoxyribose sugar, a phosphate and either one of the four nucleotides. In gel electrophoresis, separation of molecules on the agarose gel is dependent on the size of DNA and independent from the sieving process. Separation of molecules also depends on different factors such as the voltage of electric current, concentration of gel, concentration of interpolating dye used, for example, ethidium bromide and buffer strength. Sometimes, the electric current may have to be increased to have a better separation and view of fragments. The analyzed fingerprints can be compared against bacterial fingerprints and their identity be established. Isolation of large DNA molecules can be done using pulse field gel electrophoresis (PFGE) which helps to produce a fingerprint of an isolate of a bacteria sample. Restriction Fragment Length Polymorphism is a technique of gene identification that looks into the variations of homologous DNA and differentiates between species, populations, and individuals. The use of restriction enzyme sites can help to illustrate the differences between DNA. Using RFLP, restriction enzymes digest fragments and using gel electrophoresis, they are separated based on their

size. The fragments are then passed through a membrane in a procedure known as Southern Blot (Van Belkum et al. 2007). The actual length of the fragment is determined through hybridization to a DNA probe which is labelled. Although the emergence of modern and inexpensive methods of DNA fingerprinting has made this method obsolete, RFLP was an important tool for paternity testing, localization of diseases and genetic disorders and genome mapping. Apart from the fact that restriction enzymes are of different types, another benefit of using endonucleases is that the actual DNA sequence of an organism does not have to be known for a plasmid to be investigated. These enzymes split DNA at recognized sites to create a fingerprint (a fragment of DNA). Various fingerprints can be compared against one another to establish their identity. Even though plasmids do not encode housekeeping or survival genes, they encode other cell properties such as their metabolic activities, bacteriocin and antibiotic resistance. In addition, to plasmids being unstable, various strains of bacteria might lack plasmids and therefore the method of DNA fingerprinting becomes unreliable in some cases. These methods have made it easier and increased the effectiveness of microbial food profiling.

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Chapter 8
Enzymes in Food Fermentations

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8.1 Introduction

Microorganisms have been serving human needs from pre-historic times, though their role in fermented foods and beverages was elucidated only during the nineteenth century (Mota de Carvalho et al. 2018; Tamang et al. 2020). Wine, cheese, vinegar, bread, fermented milk, beverages and some oriental foods are prepared employing fermentation techniques, and are well-known for several centuries (Tamang et al. 2020). However, several enzymes are involved in the food fermentation and plays an important role in food industries; such as dairy, baking, juice,

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brewing, meat, beverages, dietary supplements, oils, pharmaceutical, chemical and others (Raveendran et al. 2018). It has well-known that microorganisms (bacteria, yeasts and fungi) are the main source of enzymes, and are able to naturally produce large quantity of the enzymes within the short period (Peng et al. 2005; Singh et al. 2019). The stability of enzymes produced by microorganisms are more stable, compared to plant and animal enzymes (Dhawan and Kaur 2007; Singh et al. 2019). Enzymes are proteins that acts as biological catalysts. First enzyme was discovered in 1833, i.e., diastase by the French chemist Anselme Paven (Punekar 2018). Later, Louis Pasteur explained that (fermentation of sugar to alcohol by yeast) this fermentation was initiated by an energetic force contained within the yeast cells called ferments (Boyer and Krebs 1986; Punekar 2018). The term "enzyme" was firstly given by Wilhelm Kuhne in 1878 (Punekar 2018).

Now a days, the demand and consumption of enzymes is more due to numerous applications and uses in different industrial sectors such as food, paper, textile, detergent and others due to their potential activities (Gurung et al. 2013; Raveendran et al. 2018). Most of the microorganisms are incapable to produce and develop the enzymes under tough environment conditions (Lorenzo et al. 2018; Matthews et al. 2017), however, some of them are capable to produce under harsh conditions after changing or adapting the environmental conditions (Robinson 2014; Waites et al. 2009). Currently, there are many known microorganisms, which are able to initiate or produce enzymes in the harsh conditions like high temperature, different pH, heavy metals, organic solvents and salinity (Hill 2015; Robinson 2015; Bruinsma et al. 1983; Singh et al. 2016a; Tamang et al. 2020). Fermentation is a tool and technology that uses the growth and metabolic kinetics of microorganisms such as bacteria, yeasts and fungi for the conservation and makeover of food products (Sharma et al. 2020; Tshikantwa et al. 2018). Furthermore, this fermentation technology enhances the shelf-life of perishable food products by inhibiting/preventing the growth of spoilage and other pathogenic microorganisms (Sharma et al. 2020). However, fermentation industries and industrial microbiology was developed only after the recognition of the potential of antibiotics production by fungi and actinomycetes, following the discovery of penicillin, as well as large scale production of acetone-butanol and glycerol employing bacteria during the Ist and IInd world wars (El-Mansi et al. 2018; Okafor and Okeke 2020). Fermentation is an anaerobic digestion of carbohydrates and the related substances into products, which are not further digested by the enzymes unless molecular oxygen is available (Allobergenova 2006; Józefiak et al. 2004). Carbohydrates are digested by a series of processes, each of which is catalyzed by its own enzyme (Józefiak et al. 2004). These processes are either oxidation-reduction or those connected with the transfer of phosphates (Raza et al. 2019a). The former reaction supplies the energy, while the latter reaction transfers this energy. Adenosine is the carrier of phosphate ions (Kaim et al. 2013). Adenosine which can be either of the three forms (adenosine monophosphate, adenylic acid, adenosine diphosphate, or adenosine triphosphate) can transfer one, two or three phosphate groups respectively (Wheeler and Simpson-Herren 1973).

All these esterification processes are endothermic. The conversion of adenosine into adenosine monophosphate, diphosphate and triphosphate requires 3, 9 and 11 calories per mole of energy respectively. Anaerobic processes are used to digest sewage of the reaction, the anerobic process is convenient in the treatment of sewage,

because large amount of organic matter are processed during the synthesis of microbial cells (Li et al. 2019b). Some authors explain anaerobic by the absence of the enzyme catalase in anaerobes, which break down the H_2O_2 (McCord et al. 1971; Ślesak et al. 2016). They suggested that organic matter gives H_2O_2 at the first stage of its oxidation with atmospheric oxygen, while H_2O_2 is toxic for all living cells (Ślesak et al. 2016). But all microbes, except anaerobes, can release the enzyme catalase into the surrounding medium, which breaks down the H_2O_2 to H_2O and O_2 (Ślesak et al. 2016). All processes are classified as aerobic and anaerobic depending on whether anaerobic microorganisms are involved (Ślesak et al. 2016). Fermentation has occupied a place of pride in food preservation practices from time immemorial (SARMA and PARASAR 2019). It enhances the nutritional value and enriches flavour and texture of the product. Fermented milk products have been reported to have many therapeutic properties (Shiby and Mishra, 2013). This is accomplished by multifarious biological activities of desirable microorganisms. As a net result, they may add certain vitamins and may enhance the digestion and assimilation of major food constituents namely: carbohydrates, proteins and fats (Fig. 8.1) (Melini et al. 2019).

Primitive man knew the methods to prepare alcohol from cereals, grains and fruits (Melini et al. 2019; Valamoti 2018). What he did not know were the details of the biological changes brought about during fermentation. Nonetheless, without even being aware of the role of microbes, he improved the fermentation processes to obtain alcoholic beverages with pleasant flavours (McCord et al. 1971; Valamoti 2018). The history of many fermented products dates back to ancient times: Chinese and Indian records go back to 3000 B. C., Greek to 1550 B.C. and Roman to 750 B. C. Bread was baked probably as far back as 7000 B. C. (Farnworth 2008; Prajapati and Nair 2003). The Egyptians discovered that if dough was allowed to cure for

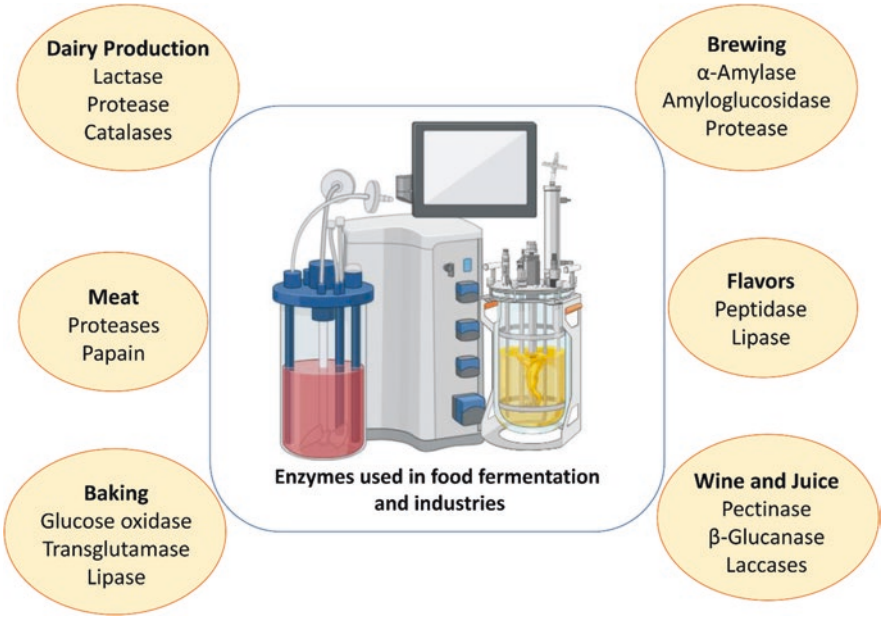


Fig. 8.1 Usage of different enzymes in various food industries

several hours, it expanded when baked, resulting in spongy light loaves (Belderok et al. 2013). Bread was a staple food of the ancient Egyptians and was often given in lieu of wages (Belderok et al. 2013). The documentation of the science of microbiology and fermentation, came only about few hundred years ago with the invention of the microscope (Buchholz and Collins 2013). This instrument literally introduced man to a strange, new world. Industrial fermentation processes were applied initially in the production of food and alcoholic beverages (Tamang et al. 2020). Today these have been extended to many other products.

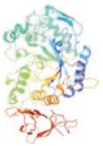
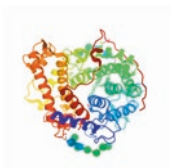
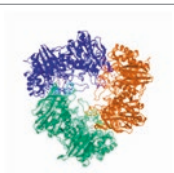
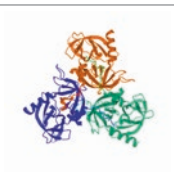
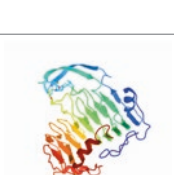
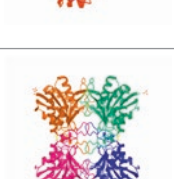
The list of fermentation products is very long and is growing day-by-day. Other principal product categories are enzymes, organic acids, Baker's yeast, ethanol, vitamins, and steroid hormones (Solomons 2002). It is impossible to describe or to even merely list all the fermentation products in each category. Hence, only a few of them are dealt here to get an idea of the impact made by fermentation on the quality of our life. Food is a basic requirement for human beings. The use of fermented food or rather the use of microorganisms for food processing is a long story dating from ancient days (Punekar 2018). Over the ages each civilization has developed a line of fermented food items (Prajapati and Nair 2003). The variation is because of diversity in raw materials, knowledge, perception, taste, and eating habits (Matthews et al. 2017). Food was not thought to be a marketable item in ancient days, and as a result traditional fermented foods and beverages are household fermentations (Kariyawasam et al. 2020). Even today the microbiology and biochemistry of some household fermentations are poorly understood. Nevertheless, some of the traditional products have now become industrial products as a result of modernization and greater demand. A good example of a fermentation product that has successfully bridged the gap from the kitchen to the supermarket is the soya sauce (Gingerelli et al. 2016). The yeast used for dough fermentation is *Saccharomyces cerevisiae (S. cerevisiae)*, popularly known as Baker's yeast (Zhang et al. 2018). Baker's yeast is available either as yeast cake, yeast cream or active dry Baker's yeast (Zhang et al. 2018; Struyf et al. 2017). The concentration of the yeast during leavening is between 1% and 6% depending upon the type of flour and the product desired. Similarly, the fermentation temperature, pH and the time varies with the raw materials and the type of product (Struyf et al. 2017). Higher concentration of yeast is always avoided, as it gives undesirable results. During the leavening process, the yeast breaks down the carbohydrates in the dough and generates carbon dioxide, which more than doubles the size of the loaf giving the spongy structure (Struyf et al. 2017; Battcock 1998). The processing of dough can also be accelerated by the addition of enzymes, such as amylase, which degrades the starch present in the flour to glucose. Glucose is then consumed more easily by the yeast (Goesaert et al. 2006). A mixture of yeast and bacteria (*Lactobacillus*) is also used, specially when we want to develop a sour taste. Baker's yeast is produced fermentatively on a very large scale and sold to the bakers (Souza 2010).

Enzymes are powerful catalysts, they can accelerate the rate of reaction over a million times (Romero et al. 2015). Enzymes have become very handy in many industries because of their unique properties of specificity, ability of perform the reaction under normal conditions of temperature, pressure and pH, and minimum by-product formation (Matthews et al. 2017). In the old days, enzymes were

obtained from plant and animal tissues (Sergeeva and Vreugdenhil 2002), but such sources could not meet the increasing demands (Pandey et al. 1999; Wohlgemuth 2010). Many yeasts, fungi and bacteria are used for production of various enzymes (Ferdeș et al. 2020; Underkofler et al. 1958). In order to improve the microbial strain, genetic engineering is employed to transfer a gene responsible for synthesis of a desired enzyme, from one microorganism to another or from animals to microorganisms (El-Mansi et al. 2018; Ferdeș et al. 2020). The aim of using such a technique is to obtain the desired enzyme in greater quantities and at a lower cost. Each enzyme catalyzes a specific reaction. This property finds many applications, for example, amylases converts starch to glucose (Van Der Maarel et al. 2002). This ability of amylases has enabled their use in production of glucose from starch (Van Der Maarel et al. 2002). Bakeries uses it for the hydrolysis of grain starch to enhance leavening, while the textile industries uses it for the removal of starch during the desizing process (Mitidieri et al. 2006). It is also used in detergents to aid the removal of starchy stains from clothes. It is used in animal feed as a digestive aid (Mitidieri et al. 2006). Similarly, proteases hydrolyse proteins to give simpler smaller fragments, (polypeptides) and even amino acids (Collados et al. 2020). Proteases active under alkaline conditions are used widely in detergents for removal of starchy stains from clothes (Blay and Pei 2019; Collados et al. 2020). In fact detergent enzymes constitute about 40% of the total market value of enzymes.

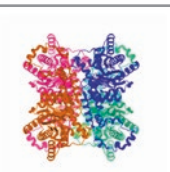
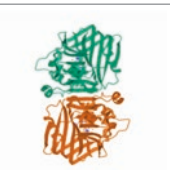
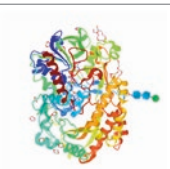
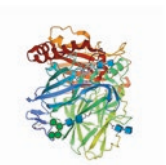
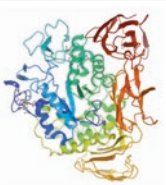
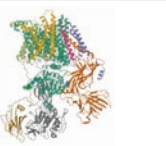
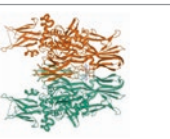
Furthermore, around 60% commercial enzymes are obtained from fungi, 24% from bacteria, 2% *Streptomyces*, 4% from yeast, 4% from plants and 6% from higher animals respectively. Previously, plants and animal derived enzymes were used at large scale, and even today for some specific enzymes they are known as the main source to obtain. Animal's organs and tissues-derived enzymes like lipases, proteases and esterases are abundantly used enzymes in food industries. Lysozyme is the example of animal-derived enzyme, which is obtained from the egg of hen. In the same way, plants are also good source of enzymes like papain, bromelain, which are obtained from papaya and pineapple respectively. Sometimes, it is found to be difficult in distribution due to limited quantity. Moreover, the isolation and purification of the enzymes are also cost-effective. Enzymes used in food industries can be divided into two parts, food additives and processing aids. Food related enzymes are recognized as processing aids consumed during the manufacturing process of foodstuffs, while only a some consumed as additives, like invertase and lysozyme. The importance of enzymes usages have been recognized in starch liquefaction, food, sugar, pharmaceuticals and paper industries. However, in the food fermentation industry, amylolytic enzymes have been used in large scale, and there are several applications, like yielding of maltose syrup, glucose syrups, reduction of viscosity of sugar syrups, to produce clarified fruit juice for longer shelf-life, solubilization of starch in the brewing industry etc. Furthermore, the baking food industry uses the enzyme to delay the staling of bread and other baked products. Other enzyme such as proteases used in the dairy industry and for the manufacturing of cheeses. Chymosin had been ideal in cheese production, due to its great specificity, however microbial proteases are also used. Chymosin is an aspartic acid protease, which causes the coagulation of milk. Hence, the application and importance of enzymes in food fermentation and food industries are described in detail in this chapter and shown in Table 8.1.

Table 8.1 Details and overview of enzymes used in food fermentation and industrial processing

Class	Enzyme	Structure	Role	References
Hydrolases	Amylases		Starch liquefaction and saccharification, increasing shelf-life and improving quality by retaining moisture, elasticity and softness. Other application in bread softness and volume, flour adjustment, ensuring uniform yeast fermentation, juice treatment, low calorie beer	Reddy et al. (2003), Roth et al. (2019)
	Glucoamylase		Saccharification	Aleshin et al. (1996), Coutinho and Reilly (1997)
	Lactase		Lactose hydrolysis, whey hydrolysis	Godoy et al. (2016), Olds and Sibley (2003)
	Lipase		Cheese flavor, in-situ emulsification for dough conditioning, support for lipid digestion in young animals, synthesis of aromatic esters production	Aoki et al. (2007), Kumar et al. (2015)
	Proteases		Protein hydrolysis, milk clotting, low-allergenic infant food formulation, enhance digestibility and utilization, flavor improvement in milk and cheese, meat tenderizer, prevention of chill haze formation	Brix and Stöcker (2013), Sohn et al. (2010)
	Pectinase		Mash treatment, juice clarification	van Pouderoyen et al. (2003), Voragen et al. (2013)
	Peptidase		Hydrolysis of proteins (soy, gluten) for savory flavors, cheese ripening	Rockel et al. (2012), Singleton et al. (1999)

(continued)

Table 8.1 (continued)

Class	Enzyme	Structure	Role	References
Isomerases	Xylose (glucose) isomerase		Glucose isomerization to fructose	(Jenkins et al. 1992; Lee et al. 1990)
Lyases	Acetolactate decarboxylase		Beer maturation	Ji et al. (2018), Marlow et al. (2013)
Oxidoreductases	Glucose oxidase		Dough strengthening	Holland et al. (2012), Petrović et al. (2017)
	Laccases		Clarification of juices, flavor enhancer (beer)	Kallio et al. (2011), Thurston (1994)
Transferases	Cyclodextrins		Cyclodextrin production	Parsiegla et al. (1998), Wenz (1994)
	Glycosyltransferase		Catalyzes the chemical reaction and transformation of acetolactate into the acetone and release carbon dioxide	Keenleyside et al. (2001), Tam et al. (2015)
	Transglutaminase		Modification of viscoelastic properties, dough processing, meat processing	DeJong and Koppelman (2002), Liu et al. (2002)

8.2 Amylase

It is well-known that starch is the source of carbohydrate, containing two molecules; amylopectin and amylose. A single straight chain starch is called as amylose, and a branched chain starch is called as amylopectin (Mitidieri et al. 2006; Reddy et al. 2003). Amylose belongs to a linear polymer containing approximately 6000 glucose elements with α, 1–4 glycosidic bonds. However, amylopectin contains small α, 1–4 linked linear chains of 10–60 glucose elements and also α, 1–6 linked side chains containing with 15–45 glucose elements. Amylase is an enzyme which hydrolyses the starch molecules to produce dextrin and gradually smaller polymers composed of glucose units (Souza 2010; Windish and Mhatre 1965). However, the history of amylase began in 1811, when the first starch degrading enzyme was discovered by Kirchhoff (Reddy et al. 2003). Amylase enzyme has two categories; endoamylase and exoamylase (Reddy et al. 2003). Endoamylase catalyse hydrolysis in a numerous ways in the interior of the starch molecule (Van Der Maarel et al. 2002). However, this function causes the establishment of linear as well as branched oligosaccharides of several chain lengths. While, it is well-known that this enzyme cleaves the α, 1–4 glycosidic bonds occur in the internal portion of the amylose or amylopectin chain (Reddy et al. 2003). α-amylase is an endoamylase enzyme, and is found in variety of microorganisms such as archaea and bacteria (Pandey et al. 2000). Final yields of α-amylase action are oligosaccharides, which are in variable length with α-configuration and limit dextrin.

On the other hand, exoamylase hydrolyse from the non-reducing end, successively giving in small final products. Exoamylase either entirely split α, 1–4 glycosidic bonds like as β-amylase or split both α, 1–4 and α, 1–6 glycosidic bonds such as amyloglucosidase and α-glycosidase (Pandey et al. 2000). It acts on the outward glucose residues of amylose or amylopectin, and hence it yields only glucose (α-glucosidase and glucoamylase), or maltose and β limit dextrin (β-amylase). While, it has been known that these β-amylase and glycoamylase converts the anomeric configuration of the liberated maltose from α to β (Pandey et al. 2000; Reddy et al. 2003). α-amylase acts on the starch and splits in to sugars, this process is termed as saccharification. However, it has been known that approximately around 200,000 glucose elements, thereby being one of the biggest molecules in nature. Cluster model of amylopectin is best-accepted model of the structure (Li et al. 2019a; Wu et al. 2013).

8.2.1 Sources of Amylases

Amylases are mostly found in nature and it is collected from different sources like animals, plants and microorganisms (Kunamneni et al. 2005; Reddy et al. 2003). The industrial sector mostly prefer enzymes collected from bacterial and fungal sources and has been most dominated in various applications (Biazus et al. 2009). Amylases isolated from *Aspergillus* species, which is a fungal source is able to

produce huge amount of different types of extracellular enzymes, and these enzymes are the ones noteworthy for industries (Hutcheon et al. 2005). Furthermore, *Aspergillus oryzae (A. oryzae)* and *Aspergillus niger (A. niger)*; filamentous fungi are known to produce large amount of enzymes that are mostly used in different industrial sectors. *A. niger* has important hydrolytic capacities in the α-amylase production and due to its tolerance of acidity, it allows the avoidance of bacterial contamination (Djekrif-Dakhmouche et al. 2006; Souza 2010). α-amylases from fungal sources are most preferred over other microbial sources, because of their recognition as Generally Recognized As Safe (GRAS) prominence (Gupta et al. 2003). On the other hand, β-amylase mostly derived from *Bacillus* species are also known for various commercial applications (Gopinath et al. 2017; Najafian et al. 2011; Souza 2010). *Bacillus* species based enzymes make up approximately 50% of the total international enzyme market (Schallmey et al. 2004). Amylases, biosynthesized by the bacteria, display unique characteristics such as thermophilic, thermotolerant, alkaline and acidophilic properties (Kandra 2003; Mehta and Satyanarayana 2016). Stability of enzyme plays an important role in commercial applications and for that thermostable enzymes isolated from thermophilic bacterial species is crucial. Microorganisms such as *B. licheniformis*, *B. amyloliquifaciens, B. subtilis* and *B. stearothermophilus*, are well recognized to be good producers of α-amylase (Asgher et al. 2007; Msarah et al. 2020; Souza 2010). β-amylase can be produced by different microorganisms, belonging to *Pseudomonas*, *Bacillus* and *Clostridium* species (bacterial strains) and *Rhizopus* (fungal strain) (Fogarty and Kelly 1990; Oliveira et al. 2010; Reddy et al. 2003).

8.2.2 Fermentative Production of Amylases

Amylases produced by fermentation technique; meaning technique usage for biological transformation of complex substrates/molecules into simple molecules by fungi and bacteria. During fermentation process, they are also able to release some other compounds apart from usual products that are alcohol and carbon dioxide. However, all these supplementary compounds/molecules belongs to secondary metabolites ranging from numerous antibiotics to peptides, enzymes and growth factors (Singh et al. 2017; Subramaniyam and Vimala 2012). Two types of fermentation techniques or methods are used; submerged fermentation (SmF) and solid state fermentation (SSF) for the microbial α-amylase enzyme yield/production (Norouzian et al. 2006). The production of industrial enzymes is mostly done by SmF method using liquid nutrient broth for the growth of microbial strain. Furthermore, this comprises growing of selected microorganisms by prudently in closed like vessels that are having a rich broth medium of nutrients and most concentration of oxygen. However, when the microorganisms break down the nutrients, they start releasing the desired quantity of enzymes into the broth medium. The production of enzyme amylases traditionally occur through submerged culture method, due to ease of handling and better control of environmental conditions like

pH and temperature (Reddy et al. 2003). However, mostly synthetic media have been used for the production of bacterial amylase through SmF method (Gopinath et al. 2017; Hamilton et al. 1999; Souza 2010; Sharma et al. 2020). It is well known that nutrient broth, soluble starch and other components are called synthetic media, and these are expensive substrates. However, the second method is SSF, which is used for good yield/production of different enzymes. In recent years, this method (SSF) has developed as a good biotechnological tool for the better and more production of enzymes (Bhatnagar et al. 2010). SSF has been used for long periods to convert moist agricultural polymeric substrates to fermented food products containing industrial enzymes, where moist agricultural polymer substrates are rice bran, corn meal, soy meal, sweet potato residue, banana meal, rice, cassava, wheat and bran (Lizardi-Jiménez and Hernández-Martínez 2017; Pandey et al. 1999). Furthermore, it has been known that SSF method is commonly well-defined as the growth of microorganisms on moist/wet solid substrates with negligible free water. SSF method is economically cheaper for concentrated and purification procedures when compare with other methods (Nigam and Singh 1995; Pandey et al. 2000). Due to better performance of SSF method, it is the most preferable technique and used in fermentation compared to SmF method. SSF is a simple technique with low waste water output, better product recovery, less capital investment, lower levels of catabolic repression, end product inhibition and high quality production (Kumar and Kanwar 2012; Lonsane et al. 1985).

8.3 Lactase

Lactase enzyme is mainly produced by the microorganisms. The main function of this enzyme is the breakdown of a disaccharide sugar molecule that exists in milk known as lactose (Savaiano 2014; Xiao et al. 2019). However, this disaccharide sugar molecule converts into two monosaccharide sugars; galactose and glucose by adding of a water molecule. Furthermore, the oxygen bridge linking the two sides of the lactose molecule is cleaved and this phenomenon is called as hydrolysis. Yin et al. (2017) reported that the microbial lactase was used as biocatalyst in industry to produce prebiotic galactooligosaccharides (GOS) from lactose (Yin et al. 2017). While, it has been known that the lactase (E.C. 3. 2. 1. 23) (such as β-D-galactohydrolase, galactosyltransferase, β-D-galactoside galactohydrolase, and β-galactosidase), has capability to catalyze both hydrolytic and transfer reactions (Uchil et al. 2017). Furthermore, lactase enzyme is also able to breakdown o-glycosidic bond of other β-D-galactopyranosides (Uchil et al. 2017). The resulting sugars, glucose and galactose are sweeter, more soluble and easily digested (Uchil et al. 2017). Hence, this enzyme has ability to increase sweetness, solubility as well as digestibility of the final product.

8.3.1 Structural Properties of Lactase

Lactase produced mainly by microorganisms and are released from the cytosol as oligomeric proteins. Lactase is composed of four polypeptide chains which is a tetramer and each monomer contains 1023 amino acids which form the distinct regions of structural domains with a compact three dimensional structure (Syukur et al. 2013). The overall structure has horizontal two-fold axis of symmetry which forms long interface, one vertical which activate interface and third is perpendicular. The crystal structure was initially proposed with four asymmetrical units of tetramers (Juers et al. 2012). Later the structure was elucidated with confined resolution as a single unit of tetramer, but asymmetrically. Deletion of amino acid residue from the lactase at the vertical position leads to weakening of active site and further dissociates into dimmers (Juers et al. 2012). The molecular weight of lactase is a 464 kDa as a homotetramer. However, each subunit of lactase enzyme contains of 5 domains while domain 1 is a jelly-roll type barrel, domain 2 and 4 are fibronectin type III-like barrels, domain 3 is a TIM-type barrel and last domain 5 is a β-sandwich. The active site is present in the third domain of lactase and made up of elements from two subunits of the tetramer. The amino-terminal sequences of lactase and the α-peptide involved in α-complementation. The residue in active site region helps in stabilization of a four-helix bundle (Juers et al. 2012; Matthews 2005).

8.3.2 Applications of Lactase

The application of lactase enzyme is mainly used in dairy industry for the yield of low-lactose milk to fulfill the requirement of people who suffers from lactose intolerance and sensitivity to lactose, due to their lack of intestinal lactase (Albayrak and Yang 2002). However, this lactose can also be hydrolyzed by using with acid, but this acid cause‘s color formation and fouling of the ion-exchange resins used in processing. Therefore, this lactase enzyme has much more demand in industries due to the enzymes are capable to hydrolyze lactose without side reactions. However, low-lactose milk is yield in processing plant by providing the lactase to milk and also the other products such as lactose able to reduce processed cheese, ice-creams, cottage cheese were commercially manufactured (Dekker et al. 2019). Furthermore, the other usages of lactase enzyme in dairy industry is to enhance digestibility and sweetness of the final product. Thus, lactase is also able to enhance the quality and consumption of high protein supplements containing milk (Dekker et al. 2019). Moreover, lactose is also applied in bread manufacturing because of its physiological properties like providing appropriate color and texture. Therefore, it has been found that the lactose is not able to ferment by ordinary Baker‘s yeast. Lactase can also be utilized in other ways, since its hydrolysis products can be readily fermented and improve the toasting of bread (Rangel et al. 2016; Singh et al. 2016a). Lactase was also used in the design of amperometric lactose biosensors, which are

used for estimation of lactose in milk and also milk-based products to prevent lactose intolerance (Göktuğ et al. 2005). Pharmaceutical industries use the lactase enzyme for capsulation of chewable lactase tablets, which helps in the digestion of milk and dairy foods without gas, cramps, bloating, or diarrhea (Nath et al. 2014; Nath et al. 2017).

8.4 Proteases

The enzyme proteases are categorized into two main groups on the basis of their mode of action; proteinases and peptidases. Proteinases on one hand, breaks down the intact proteins (Barrett 2000), while peptidases hydrolyze only peptides. This indicates that proteases enzymes belong to the category of extracellular or intracellular (Barrett 2000). The enzyme proteinase refers to vastly multifaceted group of enzymes which have different physico-chemical and catalytic properties. The function of proteases vary from generalized protein digestion to more specific physiological processes such as blood coagulation, activation of pro-enzymes, release of hormones etc. (Brix and Stöcker 2013; Rao et al. 1998). In addition, microbial proteases have profound application in many other fields (Razzaq et al. 2019). Today, majority of the industrially important proteases are produced commercially using microorganisms, often containing plasmids coding for the enzymes, by well-developed fermentation processes (Banerjee and Ray 2017; Rao et al. 1998; Razzaq et al. 2019; Singh et al. 2016b). However animal proteases such as rennet, pancreatin and a few plant proteases such as papain, ficin, bromelain etc. are also considered to be commercially important (Singh et al. 2016b).

8.4.1 Application of Protease

Proteolytic enzymes has widespread industrial applications (Chapman et al. 2018). The most widely used application is in detergent industries (Gupta et al. 2002; Maurer 2004), followed by cheese manufacturing industries (Alemu 2015). Bacterial proteases are used for the desizing of acetate rayon fabrics which are usually sized with 6 gelatin or casein and used for degumming of silk. They are also helpful in hydrolysing gelatin for the recovery of silver and cellulose triacetate from used photographic and X-Ray films (Alemu 2015; Steck et al. 2012). Proteases are employed in the production of predigested protein food for medical patients (Pedroche et al. 2004). Bacterial proteases are also used in the production of fish hydrolysate (Rebeca et al. 1991), meat tenderization (Arshad et al. 2016; Bekhit et al. 2014), removal of haziness from beer (Thakur et al. 2018), recovery of protein from food processing wastes (Kaur and Gill 2019), detergent aid (Niyonzima and More 2015) and in the baking of cereal products.

8.5 Pectinase

Pectinases are produced by microbes (Amin et al. 2019; Chiliveri et al. 2016), plants (Amid et al. 2014) and insects (Evangelista et al. 2015). Among them, microorganisms are considered to be prospective pectinase producing sources, because these are not influenced by climatic and seasonal factors as compared to plants, and can be subjected to genetic and environmental change or manipulations to enhance the enzyme production (Raza et al. 2019b). Among the microorganisms, bacteria and fungi are the common producers of pectinase. A survey of the literature revealed that much of the work on pectinases has been focused on fungi and a very few reports are available on fungal pectinases. Pectinases from bacteria exhibit several advantages over fungal pectinases. Bacterial pectinases are often stable over a wide range of temperature and pH, while fungal pectinases exhibit lower stability at high temperature and high pH values (Garg et al. 2016). Therefore, bacterial pectinases are also useful in various industrial applications that require high temperature and/or high pH (Garg et al. 2016; Rebello et al. 2017). However, it has been found that pectin lyases have been produced mostly from fungal species such as *Fusarium*, *Penicillium*, and *Aspergillus*. While ample studies are existing on bacterial pectin lyases (Demir et al. 2014; Nadaroğlu et al. 2010; Selim et al. 2016), it is worthwhile to characterize, produce and segregate these industrially significant enzymes from bacterial sources. On an industrial scale, pectinases can be yielded by both ways that are SSF and SmF methods (Kumar et al. 2011). SSF method involves the growth and fermentation of microorganisms on moistened solid substrate in the absence or closely absence of free water, whereas SmF involves the microbial growth and fermentation in the presence of sufficient water so as to dissolve the whole medium components in it. Each method has its own advantages and limitations. Whenever, these two methods SmF and SSF have been compared, SSF is considered more suitable owing to higher enzyme yields, minor operation expenses, lower energy requirements, fewer effluent generation and easier plant and equipment projects (Pandey et al. 1999; Pérez-Guerra et al. 2003). SSF process mimics natural environment and offers the best possible use of agro-industrial wastes as substrate for enzyme production. Large amounts of agro-industrial wastes are generated every year which, if not managed, will cause environmental pollution. The agro-industrial remains being eco-friendly and economical (Ravindran et al. 2018). Furthermore, there are many agro-residues such as, sugar cane bagasse, corncob, citrus wastes, wheat bran, sugar beet, as well as wheat straw which have been consumed for pectinolytic enzymes yielding (Ajila et al. 2012; Cho et al. 2020; El-Bakry et al. 2015; El-Shishtawy et al. 2014). There are many sources present for pectin substrate such as orange peel, contain pectin approximately 20–30% pectic substances (Funcia et al. 2020), and this is excellent inducer of pectinase enzyme. Though coconut fiber also contains approximately 3–4% pectic content (Faruk et al. 2012; Schomburg and Salzmann 1991), but it has not been exploited for pectinase and pectin lyase yielding in SSF method. This can be consumed in yielding of enzymes such as pectinase and pectin lyase. However, on a laboratory scale submerged fermentation

system are more applicable due to their ease of operation. Fermentation conditions and growth parameters need to be optimized for obtaining maximum enzyme yield as the enzyme production depends on several nutritional and physico-chemical factors (Prakasham et al. 2007). The optimization can be done by either one variable at a time approach or statistical methods. The latter approach is preferred, since it also takes into consideration the interaction among the factors. In some applications like retting of bast fibers, bioscouring of cotton etc. crude pectinase can be used whereas in fruit juice and oil processing industries, purified pectinase is generally required (Ahmed and Sohail 2020; Hoondal et al. 2002). Therefore, the important and effective protein purification is to choose the important and most suitable method/techniques, optimize their performance to suit the necessities and combine them in a rational way to make best use of production and reduces the number of obligatory steps (Berg et al. 2002; Mesbah and Wiegel 2014). Purification removes contaminants, which are not required in the application of pectinases. It also concentrates the desired protein and transfers it to an environment where it is stable and is present in a form ready for the intended application. Moreover, for knowing the physicochemical properties and characteristics, enzyme needs to be purified (Mesbah and Wiegel 2014). Though pectinases offer wide industrial applications with excellent catalytic properties, but their uses get restricted in soluble form as it causes few limitations like trouble of product yield recovery, low stability under operational conditions and unfeasibility of multiple recycles in an industrial process (Sheldon and van Pelt 2013). However, all these limitations can be overwhelmed by immobilizing the pectinase to some appropriate support, which deals commercial viability due to promising enhancements in catalytic activity, stability, simpler product yielding and enzyme recovery, non-stop operation of enzymatic processes, accessibility in handling, reusability and decreased susceptibility to microbial contamination (Dal Magro et al. 2019; Sheldon and van Pelt 2013; Verma et al. 2018). Immobilization of pectinase on suitable matrix can broaden its exploitation in industries. In view of the wide utility of pectinases in the industrial sector, further research must be focused on the isolation of efficient microbial strains which are capable of synthesizing novel pectinases having desirable properties for their exploitation in industries (Dal Magro et al. 2019). Natural microbial diversity can be utilized for isolation of superior bacterial strains (Das and Kazy 2014). Several pectinolytic bacterial strains have been isolated and characterized by several researchers from various natural sources (El-Shishtawy et al. 2014; Pedrolli et al. 2009; Sakiyama et al. 2001; Sharma et al. 2013). However, as a consequence of the microbial activity in natural environments due to continuous evolution of bacteria at cellular and molecular levels, robust pectinolytic bacterial strains may be produced, which will secrete the enzyme of industrial utility (Satapathy et al. 2020).

8.6 Lipases

The enzyme lipases firstly recognized in bacteria (such as *Bacillus prodigiosus*, *Bacillus pyocyneus*, and *Bacillus fluorescens*) as early as 1901 A.D. (Jaeger and Eggert 2002; Jaeger et al. 1994). It has known that some of microorganism such as *Serratia marcescens*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* are able to produce high quality of lipase (Machado et al. 2017). Lipase belongs to the group of hydrolases and the important function of this enzyme to catalyze hydrolysis of insoluble triacylglycerols to produce mono-acyl-glycerol, di-acyl-glycerol, glycerol and free fatty acids (Jaeger and Eggert 2002). It varies in enzymatic properties and also substrate specificity. In addition to their natural function of hydrolyzing carboxylic ester bonds, lipases can catalyze esterification; inter-esterification, and trans-esterification reactions in non-aqueous media to synthesize a growing range of products of potential industrial interest (Chandra et al. 2020; Schmidt-Dannert 1999; Zhang et al. 2011). The lipases enzyme mostly found in animals, plant and microorganism, while lipases produced from microorganisms are commercially important (Chandra et al. 2020; Jaeger and Eggert 2002), due to availability of wide range of hydrolytic and synthetic activities, the large produces possible by the genetic manipulation (Chandra et al. 2020; Kirana et al. 2016; Neang et al. 2014; Rychlicka et al. 2020). However, it has been well-known that microorganisms have the capacity to produce extracellular lipase, including fungi, bacteria and yeast (Ghosh et al. 1996). The microorganism lipases are normally more stable than plant or animal lipases. Some important lipase- producing bacterial genera include *Bacillus, Pseudomonas* and *Burkholderia.* Bacterial lipases from *Bacillus* possess interesting properties that make them potential candidates for biotechnological applications. They have emerged as important enzymes which have been utilized in different industrial sectors, such as detergent industries, wastewater treatment, pharmaceuticals, cosmetics, food, yield of fine chemicals, paper, textile and others (Athawale et al. 2003; Chandra et al. 2020; Hasan et al. 2006; May 2019; Pandey et al. 1999; Raveendran et al. 2018). Furthermore, the chemo-, regio- and enantio-selective behavior of this enzyme has initiated remarkable interest amid industrialists, scientists and researchers. Lipases can also be useful for biocatalyst in transesterification esterification reactions (Kumar et al. 2016; Rajendran et al. 2009). In textile industry, lipase is used to assist in the removal of size lubricants, in order to be responsible for a fabric with bigger absorbency for better-quality levelness in dyeing (Chandra et al. 2020; Kumar et al. 2016). Furthermore, this enzyme is also used to reduce the frequency of streaks and cracks in the denim abrasion systems. In paper and pulp industry, lipase is used to remove the ‘pitch’ or the hydrophobic components of wood that interfere during the elaboration of paper pulp mainly triglycerides and waxes (Anbu et al. 2017; Chandra et al. 2020).

8.7 Laccases

Laccases are the most common enzymes found in nature. They are commonly found in bacteria, plants, fungi and some insects (Desai and Nityanand 2011). In 1883, laccase was firstly identified from *Rhus vernicifera* species that is Japanese lacquer tree (Giardina and Sannia 2015). Most of the known laccases enzymes have been obtained from fungi, for example white-rot basidiomycetes that are potential lignin degraders (Bassanini et al. 2021). Some other fungi are also notable laccase-producers, such as *Botrytis cinerea* (Gochev and Krastanov 2007), *Agaricus bisporus* (Othman et al. 2018), *Coprinus cinereus Phlebia radiata*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus*, *Trametes versicolor Chaetomium thermophilum*, *Neurospora crassa* (Alves et al. 2004; Kiiskinen et al. 2004; Mousa 2020; Slomczynski et al. 1995). However, these two white-rot fungi *Trametes versicolor* and *Pycnoporus cinnabarinus* are able to degrade lignin (where they generally oxidize the phenolic subunits of lignin) to produce laccases (Mousa 2020). On the other hand, bacterial laccase was firstly obtained in the plant root-related bacterium i.e. *Azospirillum lipoferum* and where it plays an important part in melanin formation (Bugg et al. 2011; Kellner et al. 2008).

8.8 Xylose (Glucose) Isomerase

Glucose (xylose) isomerase (EC 5.3.1.5) has attracted considerable attention largely due to its ability in the conversion of glucose to fructose (Chanitnun and Pinphanichakarn 2012). This enzyme also catalyzes the isomerization of xylose to xylulose and prefers xylose as substrate (Chanitnun and Pinphanichakarn 2012). Xylose isomerase has also been evaluated for its potential application in the conversion of cellulosic biomass to ethanol (Seike et al. 2019). However, due to the extensive application of this enzyme in the industrial production of high fructose syrups, it is generally referred to as glucose isomerase. Prior to enzymatic isomerization, conversion of glucose to fructose was carried out under alkaline conditions (Bhasin and Modi 2012; Dai et al. 2020; Miller et al. 2012; Seike et al. 2019). Based on this approach, the yield of fructose was relatively low (40%) and the syrup contained non-dextrose and non-fructose degradation products as well as coloured impurities resulting in syrups with reduced sweetness (Jin et al. 2017). On the other hand, enzymes being specific, their use in isomerization of glucose not only gives high yields of fructose, but also allows the production of isomerized syrups of varying fructose content. Xylose isomerase activity was initially reported in the extracts of *Lactobacillus pentosus*, *Pseudomonas hydrophila* and *Pasteurella pestis* (Sayyed et al. 2010; Shakoor et al. 2018). However, the presence of an enzyme capable of converting glucose to fructose was first demonstrated by Marshall and Kooi (1957) in the extracts of *P. hydrophijal* (Marshall and Kooi 1957). This discovery generated considerable excitement since glucose could be isomerized to fructose without prior

phosphorylation. Subsequently, it was found that though the glucose isomerase described by Marshall and Kooi (1957) could convert unphosphorylated glucose to fructose, it required arsenate for isomerization (Marshall and Kooi 1957). Similarly, glucose isomerases from *Aerobacter cloacae*, *Aerobacter aerogenes*, *Escherichia freundii* and *Escherichia intermedia* required arsenate for their activity, thus making them unsuitable for commercial exploitation (Bhosale et al. 1996; Dai et al. 2020; Neifar et al. 2019; Shakoor et al. 2018). Hence, attempts were made to look for glucose isomerases which do not require arsenate for their activity. Finally, Yamanaka (1963) demonstrated that several lactic acid bacteria viz. *Lactobacillus pentoaceticus*, *L. brevis*, *L. femeriti*, *L. mannitopoeus*, *L. gayonii*, *L. buchneri* and *Leuconostoc mesenteroides* are capable of producing glucose isomerase (Holz et al. 2017; Kawai et al. 2004; Yamanaka 1963). Amongst them, maximum glucose isomerase activity was observed in *L. brevis*. Glucose isomerase from *L. brevis* seemed to have desirable properties for commercial exploitation but its low pH, optimum and inferior stability at higher temperature prevented its further commercial development (Bhosale et al. 1996; Jia et al. 2017). Since then, several *Bacillus* and *Streptomyces* species were studied extensively for their ability to produce glucose isomerase (Bankar et al. 2009; Bhosale et al. 1996; Dehkordi et al. 2009). Other genera namely, *Nocardia, Hicromonospora, Microbispora, Micromonospora* and *Actinomyces* have also been shown to produce glucose isomerase (Chanitnun and Pinphanichakarn 2012). Apart from aforementioned species, glucose isomerases from *Actinoplanes missouriensis* and *Arthrobacter* are also of commercial importance. However, enzymes from *Bacillus megaterium* and *Paracolobacterium aerogenoides* required NAD and ATP respectively, as cofactors (Wingard 2012). Thus, early discoveries revealed that among the three types of glucose isomerases namely, (1) arsenate requiring (2) cofactors like NAD and ATP requiring and (3) those which do not require arsenate or cofactors, only the third type of enzymes are of commercial importance (Wingard 2012). The other factors which favour their commercial use is their desirable properties like low pH optimum and high optimum temperature (Chanitnun and Pinphanichakarn 2012).

8.8.1 Glucose Isomerase Producing Microorganisms

Firstly this microorganism based glucose isomerase enzyme was reported by Marshall and Kooi in 1957 and the example of microorganism which produced this enzyme is *Pseudomonas hydrophila* (Marshall and Kooi 1957). Further, researcher also investigated and screened many other microbes and check the involvement of enzyme production. The first choice of most of the research is *Streptomycetes* for enzyme production like glucose isomerase. The members of *Actinomycete* group able to produce excellent quality of glucose isomerase enzyme that are *Actinomyces phaeochromogenes*, *Actinoplanes missouriensis*, *Streptomyces olivochromogenes* and *Actinomyces olivocinereus* (Bhosale et al. 1996; Dong 2004). Moreover, some other bacterial strains are also able to produce good quality of glucose isomerase

enzyme such as *Brevibacterium incertum*, *B. pentosoaminoacidicum*, *Lactobacillus brevis*, *L. bifermentans, L. buchneri*, *L. fermenti*, *Bacillus stearothermophilus*, *B. coagulans* and *B. megabacterium* (Bhosale et al. 1996; Sheetal and Modi 2012).

8.9 Cyclodextrin Glycosyl Transferase

Cyclodextrin glycosyl transferase (CGTase) belongs to an extracellular enzyme and is able to convert starch into non-reducing and cyclic malto-oligosaccharides called cyclodextrins (CDs) (Uitdehaag et al. 1999). It is well-known that, this important hydrolytic enzyme able to carry out reversible intramolecular and intermolecular trans-glycosylation and also carry out cyclization, disproportionation and coupling of malto-oligosaccharides (Biwer et al. 2002; Moriwaki et al. 2009). However, CDs have their systematic names of cyclic a-d-(l,4)-linked d-glucose oligosaccharides and they containing of 6–8 glycosyl units. Furthermore, this CD molecules have potential to form inclusion complex with different compounds. This CD molecules are involved in a various range of applications such as food, pharmaceutical, cosmetic and agricultural industries (Buschmann and Schollmeyer 2002; Li et al. 2007; Wang et al. 2013). The enzyme, CGTase is produced by many species such as *Clostridium*, *Thermo-anaerobacter*, *Bacillus*, *Corynebacterium*, *Brevibacterium*, *Klebsiella*, *Micrococcus*, and *Pseudomonas* (Szejtli 1992). The enzyme related to CGTase produces α-, β, and γ-CD from the starch molecule in various ratios. Therefore, the vital role of CGTases enzyme is for synthesis of CDs (Leemhuis et al. 2010; Szerman et al. 2007). The three types of CDs such as cycloamyloses, cyclomaltoses and Schardinger dextrins are best manageable with the cheapest-priced and commonly the greatest useful. Therefore, this enzyme is of prime concern due to the magnitude of its non-polar cavity which is suitable to accommodate several molecules like aromatics and drugs; its low solubility in water which facilitate its separation from the reaction mixture (Jemli et al. 2008).

8.10 Catalase

Catalase is very common enzyme, and it is found in all living organisms exposed to oxygen. This enzyme is able to break down hydrogen peroxide (H_2O_2) into oxygen and water molecules, and they defends cells from oxidative destruction by the ROS (reactive oxygen species) (Aebi 1974; Grigoras 2017). This enzyme belongs to tetramer of four polypeptide chain and each in excess of 500 amino acid long chain. It comprises four iron containing heme groups, that permit the catalase enzyme to start reaction with the H_2O_2 (Grigoras 2017). It is well-known that commercial catalases are yielded from *Aspergillus niger* species by the process of SSF method (Bučková et al. 2005; Vatsyayan and Goswami 2016). The applications of this catalase includes various food sectors and especially in the food-processing industry.

Some example of this catalase in food industries includes working with other enzymes such as glucose oxidase, which is beneficial in food preservation as well as egg processing (Kaushal et al. 2018; Raveendran et al. 2018). However, sulfhydryl oxidase, which under aseptic conditions can eradicate the effect of volatile sulfhydryl groups, they produce from thermal generation and are accountable for the cooked/off-flavor in ultra-pasteurized milk (Clare et al. 2005; Deeth 2017).

8.11 Glucose Oxidase

Glucose oxidase exists in most of the aerobic organisms and is much useful enzyme for its varied applications especially in pharmaceutical and food industries (Bankar et al. 2009). Several number of microbes including Actinomycetes, filamentous fungi and bacteria are used for yielding glucose oxidase. It is produced on huge scale by using *Penicillium amagasakiene* and *Aspergillus niger*. Among eukaryotic organisms, only few species of fungi have the ability to thrive at temperature between 45 and 55°C (Bankar et al. 2009; Wong et al. 2008). Therefore, 1486 mould strains were extracted from various natural sources (screened for extracellular glucose oxidase) and only 119 out of 1486 (*Aspergillus* and *Penicillium*) exhibited this enzyme activity (Wong et al. 2008). Furthermore, the greatest glucose oxidase producer, *Aspergillus niger* have been isolated from decaying tree (Sousa et al. 2017). A large number of filamentous fungi such as *Aspergillus niger* UAF-1, *Aspergillus terrus*, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium* Sp.CBS120262, *Penicillium notatum*, *Penicillium amagasakiense*, *Penicillium chrysogenum* SRT-1, *Penicillum variabiles*, *Penicillum funiculosum*, *Penicillium fellutanum*, *Penicillium adametzii* LF F-2044.1, *Penicillium glaucum*, *F. lini*, white-rot fungus *Pleurotus ostreatus*, *Talaromyces flavus* and other genus *Gliocadium*, *Scopulariopsis*, *Gonatobotyrs* and yeast cell *Aureobasidium pullulans* are glucose-oxidase producers (Karmali and Oliveira 1999; Sousa et al. 2017). Additionally, several bacteria also yield this enzyme; some of these are *Zymomonas mobilis*, *Micrococcus* and *Enterobacter*, *G. oxydans*, *A. methanolicus*, *Pullularia*, *Scopulariopsis*, *Pseudomonas flurescence*, *Pseudomonas putida*, producing glucose oxidase (Bankar et al. 2009; Comotti et al. 2006; Hatzinikolaou and Macris 1995; Petruccioli et al. 1999). The accomplishment of *Aspergillus niger* group for industrial yielding of modern biotechnological products is largely due to the metabolic versatility of this strain. It is well-known that *Aspergillus niger* produces a different types of enzymes, mycotoxins, antibiotics as well as organic acids (Liu et al. 2003). Moreover, the *Aspergillus niger* groups are the most significant in terms of their industrial importance due to more yield and also commercialization of the new products, which are derived by modern bioprocess and molecular biology techniques (Kona et al. 2001; Wong et al. 2008). Glucose oxidase is purified from a range of different fungal sources, mainly from the genus *Aspergillus* and *Penicillium*. Glucose oxidase has been produced by a variability of filamentous fungi and *Aspergillus niger* is the very common fungus consumed for the yielding of glucose

oxidase (Kona et al. 2001). However, the *Penicillium* species has displayed to exhibit more advantageous kinetics for glucose oxidation than that of *Aspergillus niger*. The reported glucose oxidase was found to be produced at temperature between 30 and 40 °C and pH up to 6.0 (Kona et al. 2001). The most important applications for glucose oxidase includes, the enzymatic observation of glucose by using biosensors, for the yielding of gluconic acid and as food preservative (Wong et al. 2008). Furthermore, implantable glucose sensors have found application with diabetes patients. Thus, glucose oxidase in new forms with beneficial properties in biotechnology continues to be of considerable interest, despite the abundant availability of commercial glucose oxidase (Wong et al. 2008).

8.12 Acetolactate Decarboxylase

This enzyme acetolactate decarboxylase that catalyzes chemical reaction and transformation of acetolactate into the acitoine and release carbon dioxide a type of decarboxylation reaction (Goupil-Feuillerat et al. 1997). However, this α-Acetolactate decarboxylase is profitably yielded by the SmF method of *Bacillus subtilis* and naturally/genetically better-improved quality of *Enterobacter aerogenes* and *Bacillus brevis* strain. Therefore, in traditional brewing methods, α-diacetyl is yield from α-acetolactate and this further decreases to acetoin over a 2–4 week maturation period, but α-acetolactate decarboxylase causes direct decarboxylation of α-acetolactate to acetoin and avoiding maturation period (Dulieu et al. 2000; Goupil-Feuillerat et al. 1997; Meng et al. 2020).

8.13 Transglutaminase

The enzyme transglutaminase mostly occurs in eukaryotic and prokaryotic organisms and it has been found in many tissues of plant animals and bacterial origin (Yokoyama et al. 2004). Transglutaminases are a group of thiol enzymes that are capable of post translational modification of proteins as well as peptides and various primary amines by cross linking and covalent conjugation of polyamines, lipid esterification or the deamidation of glutamine residues (Fesus and Piacentini 2002; Yokoyama et al. 2004). In 1957, Clarke et al. firstly introduced this term and they reported trans-amidating activity in animal liver (Beninati et al. 2009). The physiological role of TGs isolated from different sources appear to be miscellaneous. Although, Gs enzyme from plants are supposed to be involved in the formation of cytoskeletal as well as cell wall structures (Cai et al. 2013), on the other hand, the bacterial TG yielded by the sporulating cells of *Bacillus subtilis* is thought to cross-link proteins during coat assembly (Cai et al. 2013). Furthermore, Pisano et al. (1969) verified that trans-amidation is taken about by enzymes, which cross link proteins via an acyl-transfer reaction among the γ-carboxamide group of

peptide-bound glutamine and the α-amino group of peptide bound lysine, resulting in a α-(γ-glutamyl)-lysine isopeptide bond (Folk and Finlayson 1977; Pisano et al. 1969; Savoca et al. 2018). The inter or intra molecular cross linking action of these enzymes lead to the formation of insoluble supramolecular structures and much of the interest of commercial sector in TG is due to its action as biological/molecular glues thus modifying biomolecules (Rachel and Pelletier 2013).

Transglutaminases are capable of catalyzing the acyl-transfer reaction between the γ-carboxyamide group of the peptide-bound glutamine residue and a primary amine, where the glutamine side chain serves as the acyl donor, and the primary amine functions as acceptor (Rachel and Pelletier 2013; Savoca et al. 2018). Although, the isopeptide bonds made among the glutamine and lysine residues in proteins, thus presenting both inter as well as intra molecular covalent cross-links is the most common reaction. The fibrin polymer formation during blood clotting is based on this cross linking reaction (Martins and Choupina 2018; Savoca et al. 2018). However, application of TG in food industry is not feasible, due to the relatively small quantities obtained, the extensive separation and purification steps required, and the costs involved (Kieliszek and Misiewicz 2014).

The major applications of transglutaminase-catalysed reactions can be utilized to change the functional properties of the several food proteins (Kieliszek and Misiewicz 2014; Kuraishi et al. 2000). TG has been used to catalyze the cross-linking of a number of proteins, like gluten, whey proteins, myosin, actomyosin and soya proteins. The changes of food proteins by TG may lead to textured products, help to protect lysine in food proteins from different chemical reactions, encapsulate lipids and/or lipid-soluble materials, form heat and water resistant films, avoid heat treatment for gelation, improve elasticity and water holding capacity, modify solubility and functional properties, and produce food proteins of higher nutritive value through cross linking of different proteins containing complementary limiting essential amino acids (Duarte et al. 2020; Kuraishi et al. 2000). Although, several current patent applications and research reports in present years, clearly reveal the capacity of cross-linked proteins for making novel foodstuffs and improved processing methodologies, techniques that permit the manufacturing of products with high convenience, improved sensory and nutritional properties (Mirzaei 2011). Therefore, TG is highly appropriate for improving the techno-functional properties of proteins and its employment in food industry are well-known like dairy, egg, bread, bakery, meat, fish products as well as in soybean processing (Duarte et al. 2020; Mirzaei 2011; Rachel and Pelletier 2013).

8.14 Conclusion

Commercially useful enzymes are yet another useful gift of microbial fermentation. Enzymes are powerful catalysts, they can accelerate the rate of reaction over a million times. Enzymes have become very handy in many industries, because of their

unique properties of specificity, ability of perform the reaction under normal conditions of temperature, pressure and pH, and minimum by-products formation. Therefore, the fermentation process has the ability to change the characteristics of the variety of the food by the action of the different enzymes produced by different microorganisms such as bacteria, yeast and mould, which can take place in two conditions that are aerobic or anaerobic. Furthermore, this technique can produce different kinds of products such as ethanol, acetic acid, lactate, and other products. Thus, forthcoming of these fermented based foods give the impression to be bright, fueled by the growing interest/demand by the public, all thing apparent as natural and to encourage health as well as long life.

Competitive Interest Statement The authors declare that they have no conflict of interest.

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Chapter 9
Bioactive Components of Fermented Food Products

Suzy Munir Salama and Abdalbasit Adam Mariod

9.1 Introduction

Anciently, fermentation of food was practiced by indigenous people in many countries as a method of preserving food longer time and improves its organoleptic properties (Achi and Ukwuru 2015). In addition, fermentation removes noxious components from food products such as cyanogenic glycosides and phytic acid in some legumes, and supplies fermented food products with beneficial compounds resulting from the metabolic activities of micro-organisms such as antioxidant phenolic compounds, vitamins and minerals (Gänzle 2020; Verardo et al. 2020). Further, the probiotic activity of micro-organisms provides the fermented food product with its specific taste and improves its digestibility (Gänzle 2020) Many studies focused on analyzing fermented foods to explore the bioactive compounds from them trying to expose their contribution in pharmacology. Fermentation conventional techniques are either natural through the endogenous micro-organisms of food or through inoculation of a starter culture into the food need to be fermented (Adebo and Gabriela Medina-Meza 2020). Lactic acid bacteria used in fermentation process of milk and milk products, cereals, legumes, vegetables and meat provide the fermented food with high percentage of cellulase and amylase enzymes that promote digestion of cellulose in the food and treat patients with metabolic disorders (Jayasekara and Ratnayake 2019; Sivamaruthi et al. 2018). Moreover, probiotics showed various health benefits and therapeutic potential against diarrhea, allergies,

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urinary tract infections, gastro-intestinal disorders and hypercholesterolemia as per many studies (Avadhani and Miley 2011; Dylag et al. 2014; Grin et al. 2013; Sivamaruthi et al. 2018).

9.2 Bioactive Antioxidants

Based on Scientific researches, phenolic compounds are the most common antioxidants that exhibit many therapeutic activities such as antidiabetic, antimicrobial, antioxidative and anticancer (James et al. 2020). Plants and plant products such as vegetables, fruits and grains are considered large group of reservoirs for bioactive antioxidants (Carbonell-Capella et al. 2014; Verni et al. 2019). Cereal-based fermented foods play an important role in traditional food worldwide especially in developing countries in making their local bread and other cereal food products. Microbial fermentation of plant-based fermented foods elevates the level of antioxidant compounds *via* the hydrolysis activity of micro-organism involved in fermentation and the breakdown of plant cell walls. Liberation of antioxidant compounds from plant cells play key role in inducing their free radical scavenging and metal chelating activities (Hur et al. 2014). Sorghum is one of the cereals used locally in many African food products such as *kisra* and *Hulu-mur*, *Khamir*, *Ogi* and *Nasha* (Adebo 2020). *Sorghum bicolor* grains are rich in health-promoting factors and phytochemicals such as phenolic acids, phytosterols and anthocyanins (Salih et al. 2020). Several studies reported that fermentation enhances the antioxidant power, phenolic and flavonoid contents of plant-based fermented foods. (Xiao et al. 2015) reported that fermentation of soy whey with lactic acid bacteria significantly increased the total phenolic and isoflavoneaglycone content and soy whey. Additionally, the hydroxyl scavenging radical and ferric reducing power of soy whey remarkably increased compared to unfermented sample. Yeast-fermented coffee beans exhibit higher total polyphenols and total flavonoid contents compared to unfermented coffee beans (Haile and Kang 2019). Based on (Verni et al. 2019), yeast and bacterial fermentation improved the free-radical scavenging activity, increased the vitamins content of wheat germ and bran and enhanced the *in vivo* antioxidant activity of wheat powder in both liver and kidney of healthy rabbits (Pozzo et al. 2015). Further, *Aspergillusoryzae*-fermented soy flour recorded sevenfold increase in its content of phenolic acids compared to the unfermented flour (Verni et al. 2019). Fermentation of rice bran with *Rhizopusoligosporus* and *Monascuspurpureus* fungi significantly increase the phenolic acid content of the fermented food such as ferulic, caffeic, vanillic, sinapic and 4-hydroxybenzoic acid. Accordingly, the antioxidant capacity increases recording high total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing power (FRAP) (Abd Razak et al. 2015). Isoflavones are the most known flavonoid contents of soybean for their health benefits (Yang et al. 2019). Fermentation of tofu by *Lactobacillus casei* and *Lactobacillus acidophilus* increased the ratio between aglycone and glycosylated flavonoid compounds fivefold than

traditional soybean and tenfold higher than soybean flour. Additionally, fermentation elevated the concentration of the major isoflavone compounds, daidzein and genistein in soybean (Riciputi et al. 2016).

9.2.1 Bioactive Peptides

Scientifically, many studies isolated, identified and evaluated the biological activities of many peptides from plant an animal sources. Studies showed that the antioxidant properties of peptides isolated from hydrolysates are higher than purified peptides (Karami and Akbari-Adergani 2019). Biologically active peptides from protein-rich food products play crucial role in promoting health benefits in addition to their nutritive values (Singh et al. 2021). The complex enzymatic system such as proteases and peptidases of bacterial fermentation of food using *Lactobacillus spp* play key role in the release of biotic peptides from food substrates (Moore et al. 2021). Chromatography techniques were able to identify and characterize 25 biotic peptides from whole wheat sourdough for their ex-vivo antioxidant activities in mouse fibroblast cell lines exposed to oxidative stress using hydroxide peroxide 150 μM for a couple of hours (Coda et al. 2012). Sourdough prepared from bacterial bioprocessing of fermented lentils showed inhibition in the *in vitro* release of reactive oxygen species (ROS) from murine macrophage cell line RAW-264.7 and human intestinal cell line Caco-2 cells as monitored for 3 hours after exposing the cells to the fermented lentil samples. The study attributed the significant inhibition of ROS production to the release of antioxidant peptides along with phenolic compounds (Bautista-Expósito et al. 2018). Sourdough obtained from fermentation of quinoa flour using *Lactobacillus plantarum* identified 5 peptides with highly significant antioxidant activity in reducing the intracellular release of ROS from human keratinocytes NCTC 2544 exposed tohydrogen peroxide oxidative stress. In addition, those isolated bioactive peptides reported resistance to hydrolysis by *in vitro* treatment with digestive enzymes (Rizzello et al. 2017). Moreover, *ex-vivo* study confirmed by 13 days *in vivo* study on rats proved that fungal fermentation of quinoa seeds using *Rhizopus oliogosporus* increased the production of intracellular antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Matsuo 2005). *Bacillus subtilis* fermentation of meat protein obtained from some fishe species, *Sardinella aurita*, zebra and ray fish has produced hydrolysates rich in bioactive peptides that have antioxidant capacity and were able to inhibit Angiotensin I-converting enzyme (ACE) and significantly suppress hypertension (Jemil et al. 2016). *Lactobacillus plantarum* fermentation of camel sausages has antioxidant hydrolysates that is rich in bioactive peptides with remarkable free radical scavenging and ACE-inhibitory activities (Mejri et al. 2017). Fermentation of nonfat milk with lactic acid bacteria elevates the concentration of multifunctional peptides with anti-inflammatory, immunomodulatory, anti-hemolytic, anti-mutagenic, anti-microbial and antioxidant activities *in vitro* assays (Aguilar-Toalá et al. 2017). Soybean fermentation produces characteristic peptides that have

various biological activities. The hypocholesterolemic activity of soybean products reported improvement in glucose metabolism *via* enhancement of glucose absorption by experimentally cultured liver cells (Chatterjee et al. 2018). Significant improvement of blood glucose and serum insulin levels were observed in experimentally-induced gestational diabetes mellitus in animals given diet containing equal ratios of animal protein and soy protein (Chatterjee et al. 2018). Based on the liquid chromatography and mass spectrometry (LC-Ms/Ms)technology, isolated bioactive peptides from *Lactobacillus* fermentation of cucumber were identified and quantified from fermentation and proved for their hypotensive activity through their ACE-inhibitory activity (Fideler et al. 2019). Bioactive peptides from fermentation of Cocoa beans have shown significant antiobesogenic activity in experimentally-induced obesity in rats through reduction in serum lipids and body weight (Domínguez-Pérez et al. 2020). Fermentation of germinated brown rice by *Lactobacillus acidophilus* showed considerable enhancement in the well-known neurotransmitter and bioactive non-proteinogenic compound γ-aminobutyric acid (GABA) content of the rice used as dietary supplement for treating nervous system disorders and colon cancer (Grewal 2020). A current study published that the protein hydrolysates of pseudo-cereals such as chia, amaranth and quinoa contain high content of bioactive peptides (Morales et al. 2021).

9.2.2 Bioactive Lipids

Short chain fatty acids such as butyric acid, acetic acid and propionic acid suggested biological activity in controlling glycaemia and cholesterol synthesis. In addition they have anti-inflammatory and anticancer activities. Fermentation of Guava fruit and carrot juice with lactic acid bacteria has increased the byturic acid of guava fruit and carrot juice to about 18 ng/100 mL and 1 mg/mL respectively compared to 1 ng/100 mL and 0.6 mg/mL of the unfermented samples respectively (Annunziata et al. 2020). Further, polar lipids are considered as bioactive lipids too based on the literature. *Lactobacillus acidophius* and *Streptococcus thermophilus* fermentation of ovine milk product has recently reported playing important role in the enhancement of polar lipids production in the produced yoghurt showing antithromboic and antiinflammatory potentials in yoghurt-consuming people. The study attributed result to the bacterial metabolic activity in biosynthesizing polar lipids and improving thee fatty acid profile of milk (Lordan et al. 2019). Studies published that the anticarcenogenic potential of cheese is regarded mainly to its content of sphingo lipids and conjugate linoleic acids (Şanlier et al. 2019). Fungal fermentation of the apricot's kernels *Prunusarmeniaca* L. has shown elevation in the linoleic fatty acid (omega-6) content of the resulting oil and better oil quality (Dulf et al. 2017). Another study found that fermentation of rice with *Saccharomyces bouldardii* and *Monascus purpureus* reported increase in the rice content of bioactive sterols and unsaturated fatty acids which possess hypocholesterolemic potential and protect

against cardiovascular diseases and type-2 diabetes. Non-microbial fermentation of sausages with encapsulated fish oil or flaxseed oil reported high continent of the bioactive fatty acids (*n*-3 fatty acids) (Xiang et al. 2019).

9.2.3 Bioactive Carbohydrates

Long-chain polysaccharides (exo-polysaccharides) are by-products of micro-organisms using such bacteria and fungi when they are exposed to sugar substrates and stress from the surrounding environmental depending on the micro-organism used. Documented evidences published about the antimicrobial, antioxidant, anti-cancer, hypochelesterolemic and hypoglycemic potentials (Verni et al. 2019; Wang et al. 2016). Fermentation of aqueous wheat grain extract using *Preussia aemulans* has increased the exo-polysaccharide content of the fermented sample 36% in comparison to the unfermented sample and accordingly the antioxidant capacity of the fermented extract remarkably increased comparably to that of Ascorbic acid (Li et al. 2014). A recent study exposed that submerged fermentation improved the antioxidant activity of the polysaccharide content of the mushroom *Shiraia bambusicola* (Zhang et al. 2020). Another study showed that fermentation of ginseng polysaccharides using *Saccharomyces cerevsiae* recorded anti-inflammatory activity in lipopolysaccharide-induced liver damage in mice (Ai et al. 2020). The novel acid polysaccharide isolated from fermented broth of the medicinal fungus *Pleurotus citrinopileatus* has shown *in vitro* effective hypoglycemic effect and regulation of insulin resistance in HepG2-IR cell line (Hao et al. 2020). The exo-polysaccharide isolated from fermented liquor of *Rhizopus nigricans* proved activation of the immune activity of peritoneal macrophage *in vitro* (Yu et al. 2020). The major biologically active components isolated from fermented food products are illustrated on (Fig. 9.1).

9.3 Conclusion

In conclusion, fermented food product using different types and species of micro-organisms were found to have higher concentration of biologically active compounds such as antioxidants, peptides, unsaturated fatty acids, polar lipids and exo-polysaccharides. These respected components play important role in the improvement of the pharmacological profile of the fermented food product as proved by the different studies mentioned in the present chapter. However, further studies are required on fermented foods and food by-products to isolate many natural bioactive compounds from them and study their effect on health.

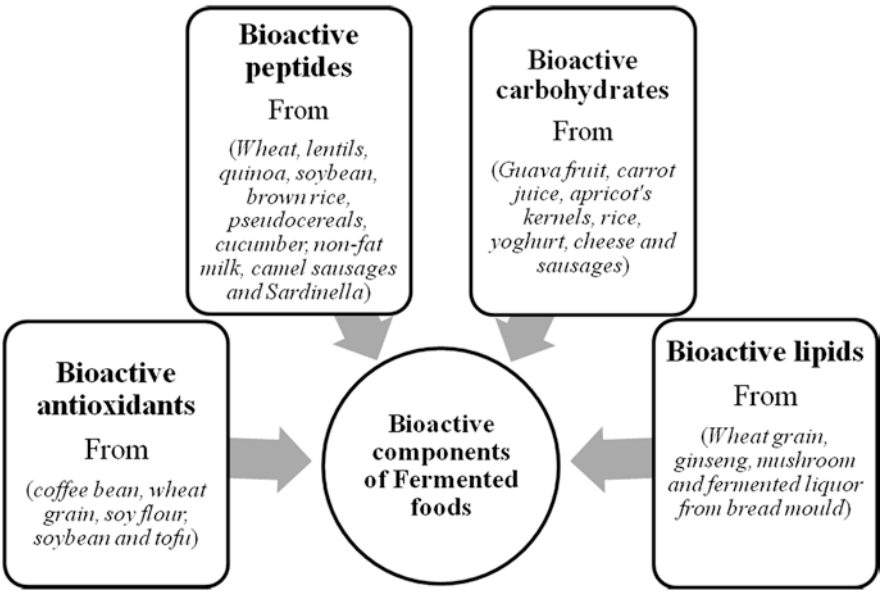

Fig. 9.1 The major bioactive components from fermented food products

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Chapter 10
Transcriptome-Based Characterization of Interaction Between Fermenting Microorganisms during Production of Bakery Products

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10.1 Introduction

Fermentation is one of the oldest food processing method. The term fermentation is basically origin of the Latin verb "fevere", which signify the term "to boil". Following the history, fermentation method is in use since Neolithic period. Archaeologists found the clay tools, which were used in the making of cheese. Throughout the human history, the distinctive quality of enhancement in the sensory properties of raw materials, as well as preservation of the developed products has been recognized as numerous fermented products, which have been part of cookery and cultural heritage of various countries worldwide (Chaves-López et al. 2014; Plé et al. 2015). Fermentation methods includes different subcategories on the bases of its primary metabolite production such as; (1) alcoholic fermentation, through yeasts, with ethanol and CO_2 as the primary products; (2) acetic fermentation, through *Acetobacter* bacteria with acetic acid as the primary product; (3) lactic fermentation, through lactic acid bacteria (LAB) in which lactic acid is the main metabolic product; and (4) ammonia or alkali fermentation of proteinaceous substrates through different *Bacillus* as well as fungal species, and releases ammonia giving strong ammonia like smell to the food (Marco et al. 2017).

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From a biochemical point of view, their common aspect is using microorganism's metabolic pathways to procure energy through organic compounds in the absence of oxidizing agents. Due to these aspects, any raw material having organic compounds could be fermented through the required enzymatic system possessing microorganisms to degrade respective carbon sources. Currently, this fermentation technology has been practised, as it provides solid background for the development of safe products having unique nutritional and functional characteristics. There are around 3500 types of traditionally fermented foods available in the global markets having animal or vegan origin and have become part of our routine life (Blandino et al. 2003). All the fermented products available in the market, amongst them the cereal-based products highly regained its marketability with the increasing health consciousness in consumers. Cereals are vital source of carbohydrates, vitamins, proteins, minerals and fibers. As compared to the animal origin or dairy food products, fermented types of cereals are nutritionally superior to their raw materials (Blandino et al. 2003).

People with lactose intolerance, milk allergies or people who follow low lipid or pure vegan diet, fermented cereal products being plant-based matrices can provide all the nutrition they deficient with. They have been mentioned as novel probiotic delivery vehicles, as well as potential functional foods (Wuyts et al. 2020). Consumption of fermented food has a lot of health benefits (Nyanzi and Jooste 2012) and they also represent 20–40% of the international food supply (Kohajdová 2017). Fermentation enhances the scrumptiousness of the grains by textural and flavour changes that reduces the demand of external additives or flavouring. Raw form of cereal grains consist low amount of organoleptically-active compounds that offers flat flavour and for the consumers it often finds unpleasant. Although, the enzymatic activity of LAB on the components of cereals produces volatile compounds such as alcohols, carboxylic acids, aldehydes, esters, ketones and/or non-volatile compounds like sugars and carboxylic acids, which lands sweet and sour taste with specific aroma of each product (Peyer et al. 2016). There are many foods involving fermentation in their making process like alcohol, cocoa beans, coffee grains, tea leaves, cheese, breads, cakes, biscuits etc. This chapter concentrates on the solid-state processes, such as bakery product and interaction of microorganisms in its production.

10.2 Microorganisms and Fermentation in Bakery Products

There are wide variety of bakery products available in the global market such as breads, buns, croissants, English muffins, cakes, cup-cakes, pan-cakes, pastries, crumpets, rolls, waffles, sweet rolls etc. In terms of nutrition, these bakery products have been very important asset, as they contain good amount of proteins, iron, calcium, vitamins and most importantly high calories. Generally, yeast (*S. cerevisiae)* and different types of LAB are used in baking process in which dough rises, increases in volume and flavour is developed. Fermentation take places when

microorganisms converts sugar like starch into ethyl alcohol and carbon dioxide (CO_2). Released CO_2 entrapped by gluten present in the flour which causes the dough to rise (Peyer et al. 2016).

10.2.1 Baker's Yeast Fermentation

Commercial yeast used in the fermentation of dough was introduced in late nineteenth century. To obtain particularly suitable strains of yeast, they were grown in aseptic systems. Today *S. cerevisiae* is considered as baker's yeast. For the cultivation of this yeast cells, media should be composed of salts, minerals, vitamins and importantly sugars. These components are used to increase their amount with thorough aeration. Some other parameters must also be carefully watched and precisely maintained such as pH, temperature and nutrient feed. Baker's yeast can be produced in wet as well as dry forms. The cream yeast (wet form of yeast) consist 82% moisture with a shelf-life of 3–4 weeks at 2–4.5 °C, while dry yeast can be kept at ambient temperature for longer period of time (Kulp 2003). In the making of dry yeast, tunnel or conveyor belt dryer is used to remove moisture from wet yeast. Before using this dry yeast in dough making process, it is subsequently rehydrated. Until the yeast adjusts to the new environment, its lag phase can be extended. Instant dry yeast can be used to eliminate the rehydration step in which the fluidized bed dryer is suggested to escalate particle porosity, resulting in instant dry yeast. Instant dry yeast can be mixed directly with other ingredients. Additionally some antioxidant agents get added and its packaging may designed in such way that it can reduce the effects of oxygen; this type of yeast is known as protected active dry yeast (Kulp 2003).

10.2.2 Lactic Acid Bacteria Fermentation

To develop varieties of flavours and textures in bakery products, LAB have been used in traditional sourdough. LAB is generally classified into 3 groups, namely obligate homo-fermentative bacteria (*L. amylovorus*, *L. farciminis*, *L. acidophilus*, *L. mindensis*, *L. johnsonii*, *L. crispatus*, and *L. amylolyticus*), obligate hetero-fermentative bacteria (*L. acidifarinae*, *L. fermentum*, *L. reuteri*, *L. brevis*, *L. frumenti*, *L. rossiae* and *L. zymae*) and facultative hetero-fermentive bacteria (*L. casei*, *L. pentosus*, *L. plantarum*, *L. alimentarius* and *L. paralimentarius*). According to the literature, *L. plantarum, L. paracasei, L. sanfranciscensis, L. curvatus, L. pentosus, L. sakei, L. paraplantarum,* and *L. brevis* are the dominant *Lactobacillus* species in sourdoughs made up of wheat flour, whereas *L. reuteri* was recognised as dominant and stable microorganism in sourdough of rye flour. *L. reuteri*'s this characteristic is possibly due to the production of reutericyclin antibiotic (Robert et al. 2009). *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and

Weissella species are less frequently used (Sterr et al. 2009). *L. plantarum, L. sakei* and *Pediococcus pentosaceus* were described as the dominant microorganisms in the sourdough of Amaranth (Sterr et al. 2009). Effectiveness of microorganisms on sourdough chiefly depends on the type of substrate, acidity, fermentation temperature and communication between microorganisms (De Vuyst et al. 2009).

Lactic acid fermentation begins with conversion of glucose to lactic acid via glycolysis using homo-fermentative LAB and results in two molecules of ATP (adenosine triphosphate). Due to the content of fructose-1, 6-diphosphate aldolase, a key enzyme for glycolysis present in facultative hetero-fermentative LAB leads to hexose fermentation. In the process of hetero-fermentation, ethanol with lactic acid, carbon dioxide gas, and acetic acid produces via 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway. Regulation of the ratio of acetic acid to ethanol performed by redox potential of the fermentation system, this pathway also releases one molecule of ATP. However, when fermentation starts at pentose, 2 molecules of ATP synthesised. Content of phosphoketolase is observed in the obligate heterofermentative LAB, which is a key enzyme in 6-PG/PK pathway (Corsetti and Settanni 2007). Phosphorylation and conversion of pentose into ribulose-5-phosphate or xylulose-5-phosphate occurs and then ribulose-5-phosphate goes in the lower half of 6-PG/PK pathway. Further, this pentose phosphate divided into two; one is glyceraldehyde-3-phosphate and the second is acetyl phosphate in order to produce lactic acid and ethanol, accordingly. End product of glyceraldehyde-3-phosphate phosphorylation consist two molecules of ATP. Phosphoketolase reacts with pentose in the facultative hetero-fermentation and therefore, facultative heterofermentative LAB can ferment pentose in the similar way as the obligate heterofermentative LAB. Equal amount of lactic acid and acetic acid produces without formation of CO_2 as a result of pentose fermentation (Corsetti and Settanni, 2007).

10.2.3 Sourdough

Generally, sourdough is prepared with wheat flour or rye flour mixing with water and fermented with LAB with/without addition of yeast. Sourdough can be categorized into 3 types on the bases of their preparation process and LAB's metabolic activity; type 1:in which flour and water mixture incubated below 30 °C with a nonstop back slopping utilizing mother sponge from previous fermentation to keep microorganisms metabolic activity at its high rate. In this, *L. brevis*, *L. paralimentarius*, *L. plantarum*, *L. rossiae* and *L. sanfranciscensis* can be used; type 2: water and flour mixture incubated at more than 30 °C for longer period of time for fermentation, approximately up to 5 days with addition of leavening agent. This can be found in liquid form and widely used for aroma carrier and as acidifier. Microbes those are heat and acid tolerant, for example *L. amylovorus*, *L. fermentum*, *L. pontis*, *L. reuteri* can be used for leavening of the dough. *S. cerevisiae* has also been used; type 3: a dry sourdough that contains LAB and leavening agent. In this, microorganisms from their active state transformed to latent state through drying and reactivate

again after sometime, this type of sourdough needs heat tolerant microorganisms, and can be used as starter in premixes. For the making of bakery products, type 3 sourdough is used. In this, making ratio of dried sourdough to flour has been recommended as 1:9. For the revitalization of selected microorganisms, sourdough used to keep in 40 °C approximately then after it should reduce by 30 °C for normal fermentation of the selected microorganisms (Decock and Cappelle, 2005).

10.3 Genomic Analysis of Baker's Yeast during Production of Bakery Products

Genetic profile of *S. cerevisiae* at the time of dough fermentation was analysed in dough and liquid fermentation medium (Aslankoohi et al. 2013; Pérez-Torrado et al. 2010; Tanaka et al. 2006). In dough and liquid medium as fermentation medium, similar kind of trends were obtained. Expression of genes changes drastically at the onset of fermentation. Genes concerned in glycolysis are up-regulated, while genes concerned in TCA cycle are down-regulated. At the initiation of fermentation, genes concerned in glycerol synthesis are up-regulated which indicates the activation of osmotic response. Therefore, cells of baker's yeast in dough suffer from osmotic stress and induction of glycerol synthesis is required for optimal fermentation (Aslankoohi et al. 2015; Aslankoohi et al. 2013). During the middle phase of fermentation, genes concerned in vitamin and amino acid metabolism are up-regulated. At the end of fermentation, cells suffer from the depletion of nutrients and the pathways associated with stress responses and starvation are activated (Aslankoohi et al. 2013, 2015). Moreover, the transcriptional response of baker's yeast is empirically similar for fermentation in high-sucrose and lean dough (Pérez-Torrado et al. 2010). This might be due to the activity of water, as the low water activity is the major driving force for the transcriptional program triggered by yeast cells in bread dough (Pérez-Torrado et al. 2010).

10.4 Interaction Between Yeast and Lactic Acid Bacteria in Sourdough

Fermentation system that includes yeast and LAB together shows reduced growth rate of yeast in the presence of LAB. Production of ethanol by yeast gets also affected, but the cell count of yeast remains unaffected. Besides, production of mannitol and acetic acid enhances with the help of LAB in the presence of yeast fermentation (Paramithiotis et al. 2006). LAB population stability determines the intensity and rate of acidification. Stability of LAB needs to be checked every few days (De Vuyst et al. 2009). This acidification might be depending on some of the environmental parameters like temperature, time, and content of water. To acquire

particular flavour and maintain high degree acidification in the sourdough, the microorganisms should be chosen from their transition and stationary phases.

In the sourdough fermentation, *S. cerevisiae* is one of the most commonly found yeast because it is used so commonly in bakeries which can effectively become a contaminant. In the presence of lactose and whey protein in sourdough, superior proofing activity of *Kluyveromyces marxianus* compared to *S. cerevisiae* (Caballero et al. 1995). *Kazachstania exigua* and *Candida humilis* are other yeasts reported. *Kazachstania exigua* and *Candida humilis* should be fermented combined with maltose-positive LAB like *L. sanfranciscensis,* because of their maltose-negative and acid-tolerant characterization. In the fermentation process. *L. sanfranciscensis* hydrolyses maltose by the activity of an intracellular maltose phosphorylase deprived of ATP employment, causing in unphosphorylated glucose plus glucose-1-phosphate. The unphosphorylated glucose is then emitted out of the cells and utilized by *Kazachstania exigua* and *Candida humilis*, the maltose negative yeasts, as without the maltose-negative yeasts, the unphosphorylated glucose might excreted and as a result, glucose repression is induced in maltose-positive yeasts (De Vuyst et al. 2009). Hence, communication between yeasts and LAB ominously shakes the stability of the sourdough fermentation.

10.5 Transcriptome Analysis of Microbial Interaction During Production of Bakery Products

Now a days, for the better understanding about the microbial interaction within the mixed cultures, transcriptomic analysis has come out as an essential method (Dennis et al. 2003; Johnson et al. 2006; Maligoy et al. 2008) (Fig. 10.1). Recently, physiological and transcriptome analysis to infer the interactions between *S. cerevisiae* Hb3 and *L. sanfranciscensis* Sx14 isolated from Chinese traditional sourdoughs was carried out (Yang et al. 2020). With the help of RNA sequencing, they determined the transcriptional changes within *S. cerevisiae* during its growth in pure culture and co-culture with *L. sanfranciscensis* during sourdough fermentation. Totally, 1196 genes (625-upregulated, 571-downregulated) were notably found to express differentially between pure and mixed culture of *S. cerevisiae* (Table 10.1).

Genes which are concerned with galactose metabolism such as, GAL2 (encoding galactose permease) and GAL7 (encoding galactose-1-phosphate uridyl transferase) were highly up regulated, which stipulating the rise in the consumption of galactose by *S. cerevisiae* as a consequence of its growth with *L. sanfranciscensis*. The expression level of HOR2 (encoding glycerol-3-phosphatase) which dephosphorylates glycerol-3-phosphate to glycerol (Nevoigt et al. 2002) was higher in mixed culture compared to pure culture, showing that mixed culture increased the synthesis of glycerol. Glycerol plays an immense role in keeping the cytosolic redox balance during the anaerobic growth of yeast (Bakker et al. 2001). It also improves the shelf life and texture of bread (Corsetti et al. 2000). Therefore, raise in its

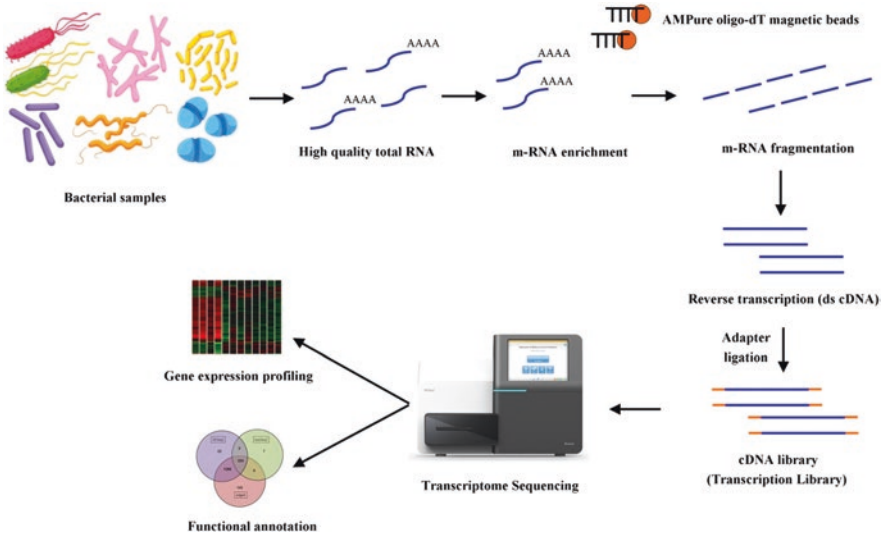

Fig. 10.1 Diagrammatic workflow of the transcriptome analysis of bacterial culture

production may have a positive influence on the quality of steamed bread. The expression level of CAR1 (encodes arginase) was also found significantly high in mixed culture. Arginase intensifies the roasty flavour of steamed bread via catalysing the conversion from arginine to ornithine (De Angelis et al. 2002). Moreover, the expression level of IDH2 (encoding isocitrate dehydrogenase 2) was also found significantly high in mixed culture. It is the key enzyme of TCA cycle, carries out oxidation of isocitrate to α-ketoglutarate and carbon dioxide, while α-ketoglutarate further participates in the glutamate metabolism (Heitmann et al. 2017).

On the other side, expression level of few genes were found notably suppressed in mixed culture compared to pure culture of *S. cerevisiae*. The expression level of BIO4 (encoding dethiobiotin synthase) and BIO2 (encoding biotin synthase) was lower in mixed culture compared to pure culture, showing that mixed culture reduces the synthesis of biotin. The expression level of PDC6 (encoding pyruvate decarboxylases) which catalyses the conversion of pyruvic acid to aldehyde was lower in mixed culture compared to pure culture. This aldehyde can be additionally transformed into ethanol via the activity of alcohol dehydrogenase, stipulating that co-culture had inauspicious activity on the production of ethanol by *S. cerevisiae*. Such finding seemed to furnish a sufficient clarification for the observed reduction in ethanol production in mixed culture, when the volatile compounds were present. On the other side, it can be converted in to acetic acid via the activity of acetaldehyde dehydrogenase produced by genes ALD6 and ALD4, whose expression were also significantly down-regulated in mixed culture, having a negative effect on the production of acetic acid by *S. cerevisiae*. GPX2 (encoding glutathione peroxidase) and GTT1 (encoding glutathione transferase) genes implicated in degradation of glutathione were also significantly reduced in mixed culture. Thus, the content of

Table 10.1 The most changed genes in *S. cerevisiae* during co-culture and monoculture dough fermentation (co-culture vs. monoculture) (Yang et al. 2020)

Up-regulated genes	Down-regulated genes
GAL2 (galactose permease GAL2)	BIO4 (dethiobiotin synthase)
GAL7 (galactose-1-phosphate uridyl transferase)	BIO2 (biotin synthase)
YPS3 (aspartyl protease)	POT1 (acetyl-CoA C-acyltransferase)
PFK27 (6-phosphofructo-2-kinase)	BIO3 (mitogen-activated protein kinase kinase PBS2)
HOR2 (glycerol-3-phosphatase HOR2)	CLB1 (B-type cyclin CLB1)
INO1 (inositol-3-phosphate synthase INO1)	MET3 (sulfate adenylyltransferase)
PDR17 (phosphatidylinositol transporter)	YAT1 (carnitine O-acetyltransferase YAT1)
CAR1 (arginase)	OYE3 (NADPH dehydrogenase)
YPK2 (putative protein kinase YPK2)	PCK1 (phosphoenolpyruvate carboxykinase PCK1)
RGS2 (GTPase-activating protein RGS2)	MET5 (sulfite reductase (NADPH) subunit beta)
PLB3 (lysophospholipase)	ALD6 (aldehyde dehydrogenase (NADP(+)) ALD6)
ARI1 (carbonyl reductase (NADPHdependent ARI1)	PDC6 (indolepyruvate decarboxylase 6)
CTL1 (polynucleotide 5′-phosphatase)	ALD4 (aldehyde dehydrogenase (NADP(+)) ALD4)
UFO1 (SCF ubiquitin ligase complex subunit UFO1)	MET14 (adenylyl-sulfate kinase)
IDH2 (isocitrate dehydrogenase (NAD(+)) IDH2)	GUT1 (glycerol kinase)
TDH1 (glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) TDH1)	GPX2 (glutathione peroxidase GPX2)
PGM2 (phosphoglucomutase PGM2)	SNZ1 (phosphate-sensing transcription factor PHO4)
NDE1 (NADH-ubiquinone reductase (H(+)-translocating) NDE1	GDH3 (glutamate dehydrogenase (NADP(+)) GDH3)
	CMK1 (calmodulin-dependent protein kinase CMK1)
	GTT1 (bifunctional glutathione transferase/peroxidase)

glutathione was increased in the dough fermented by *S. cerevisiae* and *L. sanfranciscensis*. Glutathione carried out modification in the viscoelastic gluten network of the fermented dough via enhancing the rate of thiol-disulfide interchange reactions, as it is a strong reducing agent (Verheyen et al. 2015). Thus, enhanced glutathione content in the mixed culture may have a positive effect on the texture of steamed bread. The products of *S. cerevisiae* by the Ehrlich pathway, including 3-methyl-1-butanol, phenylethyl alcohol and 2-methyl-1-propanol, decreased in co-fermented dough, the expressions of related genes were not significantly down-regulated in mixed culture. The reason for the reduced contents of the Ehrlich pathway products should be further investigated.

Table 10.2 The functional processes in *S. cerevisiae* co-cultured with *L. sanfranciscensis* obtained by GO enrichment (Yang et al. 2020)

Category	GO_Term	Process
biological_process	GO:0044281	Small molecule metabolic process
biological_process	GO:0043436	Oxoacid metabolic process
biological_process	GO:0006082	Organic acid metabolic process
biological_process	GO:0019752	Carboxylic acid metabolic process
biological_process	GO:0055114	Oxidation-reduction process
biological_process	GO:0044283	Small molecule biosynthetic process
biological_process	GO:0006520	Cellular amino acid metabolic process
biological_process	GO:0046394	Carboxylic acid biosynthetic process
biological_process	GO:0016053	Organic acid biosynthetic process
biological_process	GO:1901605	Alpha-amino acid metabolic process
biological_process	GO:0005996	Monosaccharide metabolic process
biological_process	GO:0019318	Hexose metabolic process

The Gene Ontology (GO) is a main bioinformatics inventiveness to unite the representation of gene and gene product assigns across all species. On research carried out GO annotations of the significantly differentially expressed genes for their functional classification (Yang et al. 2020) (Table 10.2). In their analysis, they revealed that differentially expressed genes were mainly enriched in various biological activities such as, organic and carboxylic acid metabolic and biosynthetic processes, alpha-amino acid metabolic process, hexose metabolic process and others. Apart from GO analysis, they also performed KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis to identify changes in major metabolic pathways between single and mixed culture in which pathways of amino acid metabolism (alanine, aspartate and glutamate metabolism, arginine biosynthesis, glycine, serine and threonine metabolism, cysteine and methionine metabolism, arginine and proline metabolism, glutathione metabolism), carbohydrate metabolism (glycolysis, TCA cycle, galactose metabolism, amino sugar and nucleotide sugar metabolism, glyoxylate and dicarboxylate metabolism, pyruvate metabolism) indicating that co-fermentation had a significant effect on *S. cerevisiae* metabolism of carbohydrate and amino acid (Fig. 10.2). Apart from this, the expression of genes related to arginase metabolism and glutamate metabolism increases, whereas genes related to ethanol and acetic acid and glutathione metabolism decreases in mixed culture, when compared the same in pure culture of *S. cerevisiae*. Genes such as, CAR1 and IDH2 were up regulated and PDC6, ALD6, ALD4, GPX2, GTT1 were down-regulated.

In another study, scientists investigated the effect of co-cultivation of *S. cerevisiae* with *L. delbrueckii* subsp. *bulgaricus* in lactose-grown chemostat co-cultures. To study the effect of co-cultivation with *L. delbrueckii* subsp. *bulgaricus*, the comparision of transcriptomes of *S. cerevisiae* in galactose grown monocultures and lactose-grown co-cultures were made. With the help of RNA sequencing, they revealed 17 genes which showed a significantly different transcript level under these two different cultivation condition (Table 10.3) (Mendes et al. 2013). Out of them,

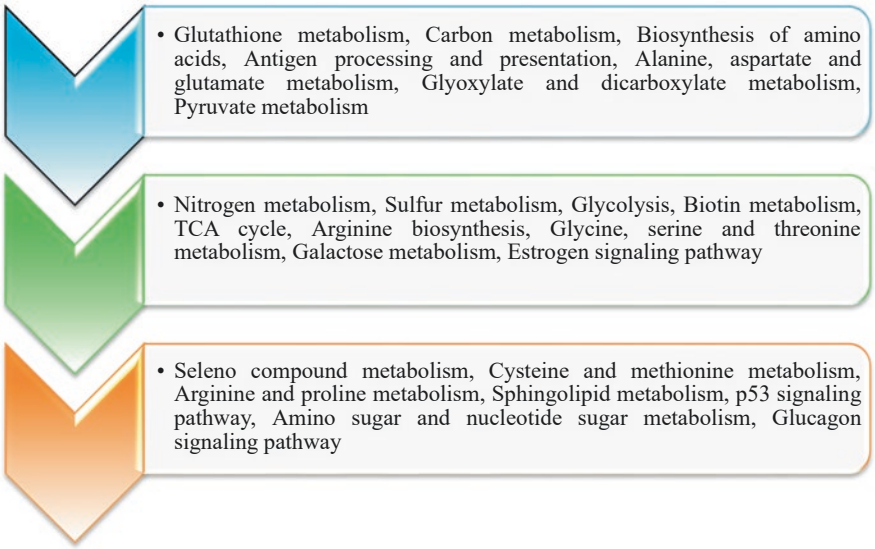

Fig. 10.2 KEGG enrichment of the differentially expressed genes in *S. cerevisiae* (co-culture vs. monoculture) (Yang et al. 2020)

11 genes whose expression were higher in the co-culture were associated with the iron and copper transport and homeostasis. Apart from it, 83 genes of *L. delbrueckii* subsp. *bulgaricus* showed a significant diverse transcript level in co-culture with *S. cerevisiae*. Out of them, 27 genes encode hypothetical proteins whereas, remaining genes were found to involved in biosynthesis of amino acid metabolism and transport.

Another main functional category enriched among differentially expressed genes covered lipid metabolism, especially the biosynthesis of low chain fatty acids. Moreover, when *L. delbrueckii* subsp. *bulgaricus* was grown in the presence of *S. cerevisiae*, the expression of several genes related to the synthesis of extracellular polysaccharide were also down-regulated (Table 10.4). Therefore, different computational techniques have facilitated high throughput data analyses in innumerable ways. Bioinformatics application to data acquired from Next Generation Sequencing (NGS) provided clear predictions of genes, pathways, networks, biological processes and cellular components under single or mixed culture fermentation process during production of bakery products.

10.6 Conclusion

Transcriptome analysis experiments enable researchers to characterize transcriptional activity (coding and non-coding), focus on a subset of relevant target genes and transcripts, or profile thousands of genes at once to create a global picture of cell function.

Table 10.3 Genes significantly differentially expressed in *S. cerevisiae* in response to co-cultivation with *L. delbrueckii* subsp. *Bulgaricus* (Mendes et al. 2013)

Upregulated genes	Downregulated genes
CTR3[e] (High-affinity copper transporter of the plasma membrane, acts as a trimer)	SUL1 (High-affinity sulfate permease)
STL1 (Glycerol importer)	CAR2 (L-Ornithine transaminase, catalyzes the second step of arginine degradation)
FIT1[e] (Cell wall mannoprotein, involved in the retention of siderophore iron in the cell wall)	ATO3 (Plasma membrane protein, possible role in export of ammonia from the cell)
EEB1 (Alcohol acyltransferase involved in ethyl ester biosynthesis)	ADH2 (Glucose-repressible alcohol dehydrogenase II, catalyzes the conversion of ethanol to acetaldehyde, involved in the production of certain carboxylate esters, regulated by Adr1p)
FMP23[e] (Mitochondrial protein of unknown function, contains Rcs1p and Aft2p binding domains)	PHM8 (Lysophosphatidic acid phosphatase involved in lysophosphatidic acid hydrolysis in response to phosphate starvation)
CIN5 (Transcription factor of the YAP-1 family, mediates pleiotropic drug resistance and salt tolerance)	XBP1 (Transcriptional repressor that binds to promoter sequences of the cyclin genes CYS3 and SMF2)
HMX1[e] (Endoplasmic reticulum localized, heme-binding peroxidase involved in the degradation of heme, expression regulated by Aft1p)	
SIT1[e] (Transporters that specifically recognize siderophore iron chelates, transcription is induced during iron deprivation and diauxic shift)	
TIS11[e] (mRNA-binding protein expressed during iron starvation and involved in mRNA degradation)	
CCC2[e] (Plasma membrane Cu2-transporting P-type ATPase, required for export of copper from the cytosol)	
CTR1[e] (High-affinity copper transporter of the plasma membrane, mediates nearly all copper uptake under low-copper conditions)	

Gene expression analysis studies can provide a snapshot of actively expressed genes and transcripts under various conditions. Therefore, this approach can be extended to determine interactions of other microorganisms that occur together in food fermentation either synergistically or as contaminants. Moreover, this approach appears to be exceptionally suitable to learn the mixed-cultivation of antibiotic-producing microbes and appropriate target organisms and the deprivation of complex substrates by defined or natural consortia and appraise metabolic engineering approach for accomplishing steady co-cultivation of industrially significant microbes.

Table 10.4 Genes significantly differentially expressed in *L. delbrueckii* subsp. *bulgaricus* in response to co-cultivation with *S. cerevisiae* for the enriched functional categories amino acid transport and metabolism and lipid metabolism (Mendes et al. 2013)

Amino acid transport and metabolism
LBUL_0215 (ABC-type amino acid transport system permease component)
LBUL_0216 (ABC-type polar amino acid transport system ATPase component)
LBUL_0737 (Transcriptional regulators containing a DNA-binding helix-turn-helix domain and an aminotransferase domain (MocR family) and their eukaryotic orthologs)
LBUL_0915 (Dipeptidyl aminopeptidases/acylaminoacyl peptidases)
LBUL_1181 (Diaminopimelate decarboxylase)
LBUL_1235 (Cysteine synthase)
LBUL_1236 (Cystathionine beta-lyases/cystathionine gamma-synthases)
LBUL_1257 (Aspartate semialdehyde dehydrogenase)
LBUL_1353 (Homoserine trans-succinylase)
LBUL_1619 (Acetylornithine deacetylase/succinyl-diaminopimelate desuccinylase and related deacylases/dipeptidase PepV)
LBUL_1630 (Homoserine kinase)
LBUL_1631 (Threonine synthase)
LBUL_1646 (Amino acid transporters)
LBUL_1668 (Gamma-aminobutyrate permease and related permeases)
Lipid metabolism
LBUL_0784 (Predicted acyltransferases)
LBUL_0806 (3-Hydroxy-3-methylglutaryl coenzyme A synthase)
LBUL_0818d (3-Oxoacyl-(acyl carrier protein)
LBUL_0819d (Acyl carrier protein)
LBUL_0821d (3-Oxoacyl-(acyl carrier protein) reductase/dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases))
LBUL_0822d (3-Oxoacyl-(acyl carrier protein) synthase)
LBUL_0823d (Biotin carboxyl carrier protein)
LBUL_0824d (3-Hydroxymyristoyl/3-hydroxydecanoyl-(acyl carrier protein) dehydratases)
LBUL_0825d (Acetyl/propionyl coenzyme A carboxylase alpha subunit)
LBUL_1607d (3-Hydroxymyristoyl/3-hydroxydecanoyl-(acyl carrier protein) dehydratases)

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Chapter 11
Quality Evaluation of Semi-Indigeous Proceesed Cheese (*Gibna-Beida*) in Sudan

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11.1 Introduction

Sudan assumes the top place among the countries of Middle East regarding the animal wealth and rank the second in Africa. Within the animal resource sector, the largest component is estimated 133.640.00 heads: cattle 39.667.000, sheep, 48.440.000, goat, 42.030.000 and camel 3.503.000 heads (MRF 2003). According to Federal Ministry for Animal Resources Fishery and Range (2012), the livestock population in Sudan was estimated to be about 29.618.000 cows, 39.296.000 sheep, 30.649.000 goats and 40.715.000 camels the total is 140.278.000 head. The indigenous herds found in Sudan belong to the Zibo group in northern Sudan (Sudanimals 2007). Examples are Butana, Kenana and Baggara; multipurpose breeds that are used for milk and meat production as well as draught power (Payne and Hodges 1999). The Butana cow is considered the best milk producer of the Sudanese Zebu breeds (Sudanimals 2006).

11.2 Milk Production in the Sudan

The milk production in the Sudanese indigenous cattle breeds Kenana and Butanna (*B. indiucs*) was found to be lower than that of Holstein Friesian cattle (*B. Tarurs*), even under the same climatic conditions (average lactation milk yield 1405 ± 695 kg

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compared to 4784 ± 81 kg in Holstein Friesian) (Ageeb and Hillers 1991; Ageeb and Hayes 2000). Low productivity (total lactation milk yield: 1597 kg) was also shown in another study concerning the Kenana breed (Wilson 1984). However, the same authors suggested that with improved management, feeding and breeding, the Kenana breed has a high potential as a milk producer under tropical climatic conditions.

The available estimate of milks production in the Sudan vary widely and no accurate statistics are kept. It has been estimated in 1982, 2.9 million tons of milk was produced of which 2.5 million tons (82% were cows milk, the bulk of which is in the hands of the nomadic tribes. The annual production of milk obtained from cows, sheep, goats and camels is estimated at 20249.91 million tons (AOAD 2002).

A great proportion of milk produced in the world is converted into skimmed milk and fermented milk products like cheese and yoghurt that can be kept for longer periods without cooling. Milk is an in dispensable food item and is considered as nature's perfect food for human beings as well as other animals. Mammals secretes milk for the nourishment of their young ones and milk of animals like cattle, buffalo, goat, sheep, camel, yak, etc. are being used as food for human beings (Van Soest 2018).

11.3 Milk Composition

Milk is approximately 87 percent water and 13 percent solids. Milk fat carries the fat soluble vitamins A, D, E, and K (Hurley 2015). The solids-not fat consists of protein, lactose, and minerals. Milk also contains other water soluble vitamins (Mehta 2015). Federal definitions and standards of identity specify the minimum levels of milk fat and solids not- fat for the various milks shipped in interstate commerce (Vaclavik and Christian 2014). Fat gives milk its characteristic smoothness, flavour and colour. Milk fat contains about 66 different fatty acids, emulsified and dispersed in water in small globules. All the essential amino acids are found in milk protein. Eighty percent of milk protein is casein which is found in combination with calcium and phosphorus. The other important milk proteins are lactalbumin and lactoglobulin (whey proteins) (Payne 1990). Lactose is the most stable component of milk.

11.4 Fermented Milk

Fermented milk was warm, raw milk from cow, sheep, goat, camels or horses of the nomads roaming the area, which was turned, into clabber, or curd by bacteria and their end products. Fermentation process, which occurs in fermented milk, results in conversion of lactose to lactic acid. This acid has a preservative effect on milk. The low pH of fermented milk inhibits the growth of undesirable bacteria and

pathogenic organisms. The starter cultures used for fermentation of milk convert a part of lactose to lactic acid, carbon dioxide, acetic acid, acetaldehyde and several other materials (Walstra et al. 1999).

Fermented milk foods with desirable characteristics of flavor, texture, and probiotic profiles can be created by formulating the desired chemical composition of the milk substrate mix, judicious selection of lactic acid bacteria (starter), and fermentation conditions (Chandan 2014). A starter is made up of one or more strains of food-grade microorganisms. Individual microorganisms utilized as a single culture (single or multiple strains) or in combination with other microorganisms, exhibit characteristics impacting the technology of manufacture of fermented milks. Fermented milks have long been used as the main vehicles for probiotic strains. The supplementation of cheeses with probiotic bacteria represents the aggregation of added value to a product that already has benefits inherent in its composition (Balthazar et al. 2017; Gomes et al. 2011).

11.5 Starters

A starter is a culture of one or many types or strains of lactic acid bacteria that is added to milk to ferment it. Sometimes the inoculum also contains no-lactic acid bacteria, whereas in other cases the latter are added separately to the milk. Traditionally, a starter is obtained via growth of lactic acid bacteria in milk at a suitable temperature. The starter is subsequently maintained by propagating and growing it in a fresh portion of milk. Currently, special growth media rather than milk are also utilized to avoid multiplication of bacteriaphages during starter manufacture (Axel 1998).

11.5.1 Properties

The biochemical conversion of milk components by lactic acid bacteria naturally causes changes in the fermented products. These changes depend on the properties of the starter bacteria involved and on the type of product made. The followings are the main aspects:

1. **Production of acid from lactose:**

   The production affects of the preservation of the product, the texture of the product and the flavour of the product.

2. **Formation of other compounds during the fermentation of lactose and citric acid:**

   - Flavour compounds: They involve several metabolites, diacetyl in particular. The desired types of aroma bacteria present in such starters may vary. In

aromatic starters, the ratios between the bacterial strains involved are very critical with respect to the formation of diacetyl from citric acid.
- Carbon dioxide: The production of CO_2 by aroma bacteria is essential for the texture of cheese in which the formation of few "eyes" is desirable like Gouda cheese.
- Bacteria exopolysaccharides: The consistency of stirred yoghurt greatly depends on the strains of bacteria used, and the exopolysaccharides produced by them are held to be responsible.

3. **Proteolysis: Protein degradation affects**:

- The consistency of the product.
- The flavour of the product.
- The mutual growth stimulation of lactic acid bacteria.

4. **Lipolysis:**

The formation of fatty acids is important for flavour of ripened cheese. Hydrolysis of fat during storage is undesirable for products that are consumed shortly after manufacture. Most of the lactic acid bacteria cannot hydrolyze triglycerides, but they can hydrolyse mono- and diglycerides; hence, they can enhance ongoing hydrolysis of fat. Therefore, the conversions by lactic acid bacteria strongly determine shelf life, safety, consistency, and development of flavour and texture of fermented products. The selection of a starter must be based on the properties desired in the product to be made.

11.6 Cheese

Cheese is a product that made from the curd obtained from milk by coagulating the casein with the help of rennet or similar enzymes in the presence of lactic acid microorganism (Guinee and Fox 2017). Fox et al. (2000) defined cheese as the fresh or ripened product obtained after coagulation and whey separation of milk, cream or partly skimmed milk, butter milk or a mixture of these products, it can also be made from the milk of cows, sheep, goats and camels or mixture of two of these (Herrington 2000). Each type of milk imparts the characteristics quality of cheese made from it and the resulting cheese will diver in its proprieties, body texture, and flavor (Andrew 2010). The annual reports of Federal Ministry of Animal Resources (FMAR) 2001 pointed that the total annual production of cheese in the Sudan was (650) thousand metric tons (Sambo 2009).

The cheese industry in Sudan is spread in the rural areas with rich resources in large livestock. Most of these areas are located around cities in small villages that lack electricity and depend on water sources to harvest rainwater and store it in large pits around the village, as well as water wells of groundwater with high salinity. Most of the white cheese factories that depend in production process on primitive tools. This is an industry that is continuously inherited from grandparents to

children and grandchildren. Therefore, white cheese in Sudan is an important traditional industry that competed with the big companies when they introduced technological methods in their production.

11.6.1 Muddafara Cheese

Muddafara cheese was manufactured traditional from both types of milk and from a mixture of the two types. The samples were then ripened in an incubator (40 °C) for 35 minutes before addition of rennet at a rate of 0.07 g/l at the same temperature. After complete coagulation, the curd was cut into small cubes and was salted at 40o until the required acidity for kneed ling was reached (0.46–0.60%). Ripening was assayed by teasing the ability of the curd to be kneed led into a 4-metre rope while any breakage before this length was reached would indicate inadequate ripening. Whey was drained from the ripened curd which was then placed into a wooden plate and cut into slices. The curd was cooked in water at 75%C for 5 minutes and then black cumin (Nigella Sativa) was added to the curd which was then hand-kneed led and pulled to from a long rope. This was washed and left for 48 hours at room temperature in salted whey (10% v/v NaCl) (Suleiman et al. 2005). According to Suleiman et al. (2005) Muddafara cheese content 33% moisture, 1.2% ash, 26% fat, 4.6 total nitrogen, 24% total protein, 42% soluble nitrogen, 41% total solids, 0.88% titrable acidity. pH 5.39 and yield 12.4 kg/100 liter milk.

11.6.2 White Cheese (Gibna-Beida)

White Cheese (Gibna-Bayda) is practically the only kind of cheese on the market available to the public at large in the Sudan and is thus normally referred to simply as jibna-beida. Many Sudanese think that the product is truly indigenous fermented food of the Sudan, but this contention has no basis. It is believed that the beginning of the white cheese industry in Sudan for the first time was by a Greek family, Catherine and Banyuti Maestro, who settled in 1908 in the town of El-Dueim, on the White Nile, 225 km south of Khartoum, and who established in 1920. The first cheese factory dedicated to commercial production (Ali 1987) (Fig. 11.1).

Manufacture of Jibna-Beida

The white cheese industry begins with receiving milk from shepherds in the early morning hours and it is emptied into large plastic barrels with a capacity of 470–500 lbs. After that, coarse salt is added directly to the milk by 18–20 kg and stirred for a period to ensure its dissolution, followed by the addition of the resonance enzyme at a rate of 2–3 strips one barrel, then cover the barrel and Leave until frustration is complete within 6–8 h (Fig. 11.2).

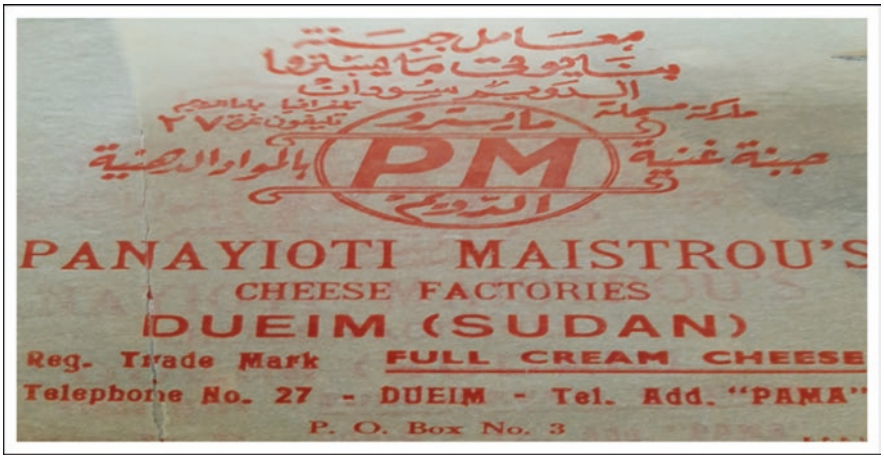

Fig. 11.1 Explains the first preamble to the white cheese product by the Greek Maestro Baniotti in the city of El-Dueim in 1953

Fig. 11.2 Explain Preparing the salt, filtering the milk on it and stirring to dissolve it, then preparing the starting and adding it with stirring again to make the mixture homogeneous

After the frustration period ends, a cleaning process begins on the upper surface of the curd by excluding all the impurities on the surface from the remains of insects and animal dung from the milk during the milking process, as well as extracting the fatty layer on the surface of the curd and storing it and producing margarine (Semin) from it as a by-product of the manufacture of white cheese.

This is followed by cutting the curd with plastic containers, which are then used to transfer the curd to wooden boxes, with plastic sacks (strainers) underneath, to filter the water from the curd. Wooden boxes are used to form white cheese into large rectangular shapes. After completing the filling of the wooden boxes, the curd is moved with your hands to filter part of the water to add another amount of coagulated milk to ensure that the box is filled with the largest amount of coagulated milk.

Fig. 11.3 Explain the purification and extraction of fat in top of coagulate

Fig. 11.4 Explains the process of filling wooden boxes and the process of preliminary filtering of water by stirring with hands

After that, the curdled milk is left inside the boxes after covering it for the next day (overnight) (Figs. 11.3, 11.4 and 11.5).

Finally, the filling process of white cheese is done in plastic containers, and the product takes square shapes of semi-equal sizes, then the salted water resulting from filtering is added so that the whole product is covered. The packages weighing 11 kg will be reduced to about 9 kg after ripening within 7–10 days (Fig. 11.6).

11.7 Chemical Composition of White Cheese

The chemical analysis of collected Gibna-Bayda samples collected from different area showed in Fig. 11.7. The moisture content of cheese samples ranged between 53.27 ± 0.262% and 57.83 ± 0.127% with an average value of 55.83%. There was

Fig. 11.5 Explain cutting the curds and placing them in storage containers, then adding a portion of whey water for ripening and preservation

Fig. 11.6 Explain forms and packaging of white cheese produced

significant variation ($P \geq 0.05$) between collected cheese samples in moisture content. The protein content of cheese ranged with an average value of 14.57%. And statistically, there were no significant differences between the samples collected except for sample D, which was significantly different ($P \leq 0.05$). The fat content of cheese samples ranged with an average value of 20.84%. Statistical analysis showed

significant differences ($P \leq 0.05$) in fat content of collected cheese samples except samples A and B. The difference in the fat content can be attributed to several factors such as the animal's nutrition, individuality of animal, health and age of the animal when the milk was taken. The ash content of cheese samples ranged from 3.77 ± 0.012% to 5.60 ± 0.087% with an average of 4.45%. Statistically no significant difference ($P \geq 0.05$) was found in the ash content of the various cheese samples except samples C and F. Lactose content of cheese samples varied from 1.19 ± 0.000% to 5.77 ± 0.035% with an average of 4.31%. However, significant ($P \geq 0.05$) variations were found in lactose content of the cheese from different cheese samples. The total solid of cheese samples varied from 42.17 ± 0.012% to 46.73 ± 0.064% with an average of 44.17%. Statistical analysis showed significant differences ($P \leq 0.05$) in total solids content of different cheese samples. The variation in total solids content might be due to the lack of standard procedure followed by producers. Solid non fats (SNF) content of cheese samples varied from 20.33 ± 0.144% to 24.60 ± 0.046% with an average 22.01%. Statistical analysis showed significant differences ($P \leq 0.05$) in solid non fat content of different cheese samples. Also Fig. 11.8 showed that pH values of cheese samples varied between 3.9 ± 0.000 and 6.0 ± 0.058 with an average of 5.0. There were Significant differences ($P \leq 0.05$) in pH values of cheese in different samples. The range of titratable acidity of the collected cheese samples varied between 1.21 ± 0.006 percent to 2.06 ± 0.100% with an average of 1.57%.

11.8 Mineral Contents of Collected Chesse Samples

Figure 11.9 presents the mineral content of different cheese samples collected from different area. The analysis of cheese samples for minerals reflected high concentration of most minerals especially macro-elements sodium, potassium, calcium, lead and phosphorus with an average value of 244.8, 63.07, 458.25, 0.52 and 97.35 ppm, respectively, whereas most micro-element manganize, iron and zinc was very low in cheese samples, with an average value of 0.05, 0.50 and 6.56 ppm, respectively.

11.9 Microorganisms Associated with Cheese

Microbiological characteristics of the collected cheese samples are shown in Table 11.1. The total bacterial count (TBC) varied between $4.5 \times 10^5 \pm 1.048$ and $41.0 \times 10^5 \pm 17.786$ cfu/g with an average of 18.76×10^5 cfu/g. Statistical analysis showed that there were significant difference ($P \leq 0.05$) in total bacterial count of the collected cheese samples. The average lactic acid bacterial count (LAB) of cheese samples was 3.49×10^5 cfu/g. The coliform were not detected in cheese samples (A, B, C and D), whereas, it was found in cheese samples F (23 cfu/g). The

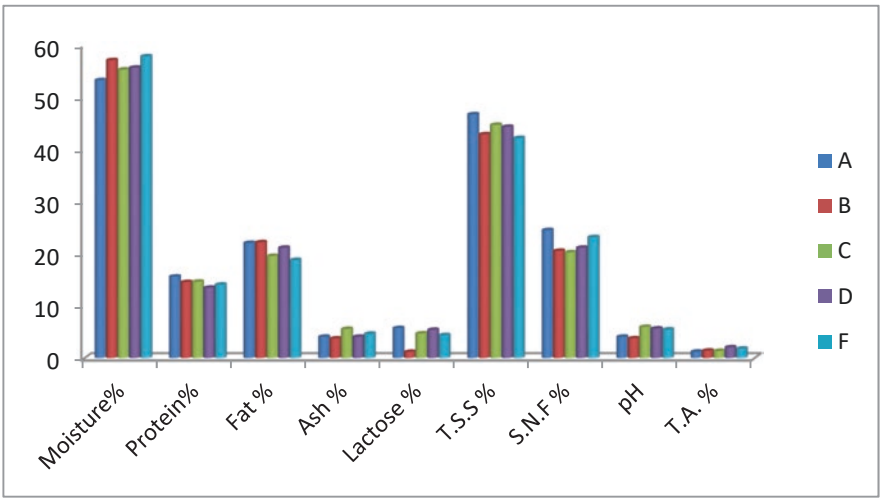

Fig. 11.7 The chemical analysis of collected Jibna-beida samples from different area

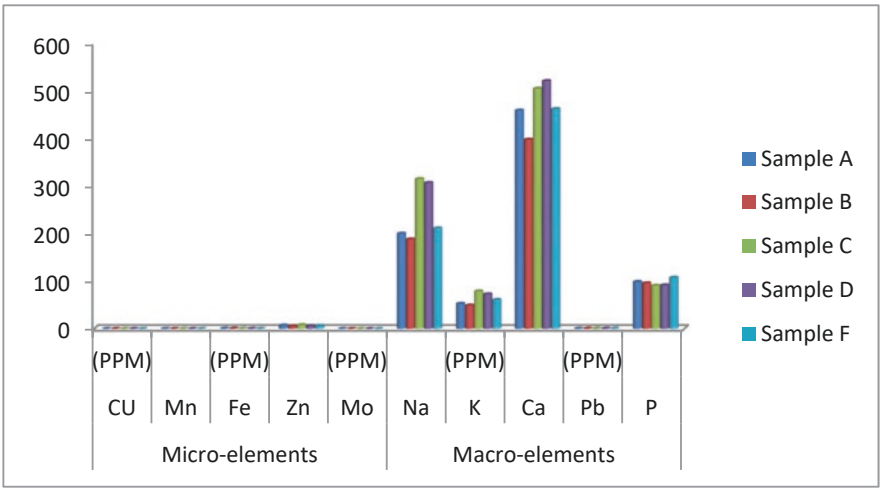

Fig. 11.8 The mineral content of different cheese samples collected from different area

absence of coliform in the different cheese samples could be attributed to the salt added in the processing. The presence of coliform cells in cheese sample F was probably due to milking and production of cheese under poor condition (Ceylan et al. 2003; Warsma et al. 2006). According to the international standards, white cheese should not contain more than 100 cfu/g coliform bacteria (Lau et al. 1991). The result shows the absence of *Salmonella* in cheese samples (A, B, C, and D), while, sample (F) showed positive result. The presence of *Salmonella* will create health risks to the cheese consumers, and when consumed, can cause symptoms

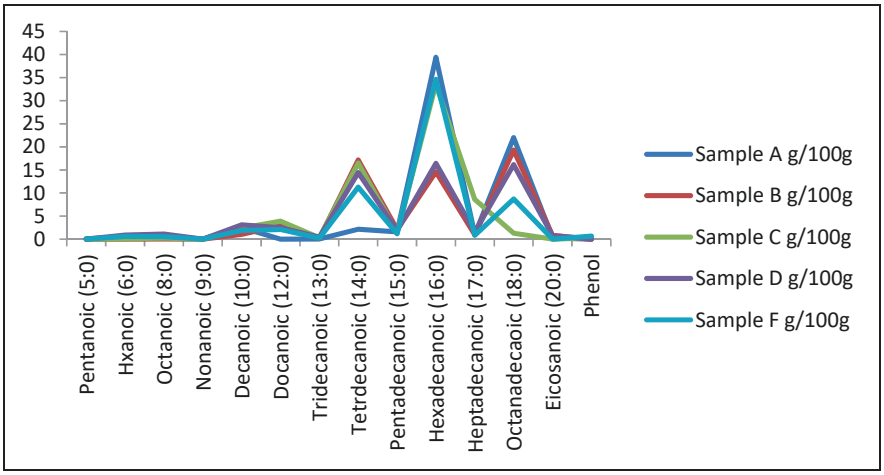

Fig. 11.9 Show Saturated fatty acids composition of jibna-beida samples obtained from different area

such as diarrhea, stomach pains, nausea and vomiting and stomach infections. In very serious case, it can cause death. On the other hand the absence of Salmonella in the other cheese samples examined probably due to high levels of salt and titratable acidity. The high count of *staphylococcus aureus* found in some cheese samples might be attributed to the high initial numbers of *staphylococcus aureus* in milk contamination during processing (Santos and Genigeorgis 1981). The yeasts and moulds cells were detected in all cheese samples with an average count was 4.47×10^4 cfu/g. Statistical analysis showed that there were significant differences at ($P \leq 0.05$) in yeasts and moulds count of different cheese samples. The high count of yeasts and moulds in the cheese samples indicates poor hygienic conditions.

11.10 Packaging

Packaging or packing of cheese is one of the more important steps in the long journey from the producer to the consumer, since most of the cheese plants are far away from the consumption. Packaging of natural cheese must afford general protection of the product from mechanical damage and poor environmental conditions during handling and distribution (Abdalmagid 2019). Also may prevent moisture loss, improve appearance, protect against microorganisms, and prevent oxygen transmission, also may serve as a marketing tool, which provide useful information about the producer name, brand size, variety, net weight, count, shipper and country of origin (Ghemawat et al. 2003). What is noticed in the traditional white cheese industry in Sudan is the use of traditional and recycled packages with the intention of reducing the costs of production by manufacturers and their lack of knowledge of

Table 11.1 The microbiological characteristics of Sudanese cheese Jibna-beida samples

Sample	Total viable count of bacteria (cfu/g)	Coli-form MPN index per gram		Yeast and mould (cfu/g)	*Staphylococcus* (cfu/g)	Detection of Salmonella	Lactic Acid Bacteria (cfu/g)
		Total	*E.Coli*				
A	$41.00 \times 10^{5a} \pm 17.786$	0	0	$5.80 \times 10^{4ab} \pm 1.453$	$5.27 \times 10^{2ab} \pm 2.021$	-ve	$6.67 \times 10^{5b} \pm 0.811$
B	$4.53 \times 10^{5b} \pm 1.048$	0	0	$7.00 \times 10^{4a} \pm 1.562$	$5.30 \times 10^{2ab} \pm 1.877$	-ve	$45.00 \times 10^{5a} \pm 15.044$
C	$6.00 \times 10^{5b} \pm 1.155$	0	0	$4.67 \times 10^{4b} \pm 0.811$	$5.00 \times 10^{2ab} \pm 0.577$	-ve	$41.67 \times 10^{5a} \pm 13.956$
D	$5.28 \times 10^{5b} \pm 1.525$	0	0	$0.43 \times 10^{4c} \pm 0.079$	$6.00 \times 10^{2a} \pm 0.577$	-ve	$5.63 \times 10^{5b} \pm 1.880$
F	$37.33 \times 10^{5a} \pm 5.812$	23	11	$4.47 \times 10^{4b} \pm 0.786$	$3.80 \times 10^{2b} \pm 1.348$	+ve	$4.28 \times 10^{5b} \pm 1.748$
Error mean square	5.53	–	–	2.04	1.52	–	4.96
LSD value	1.70	–	–	0.63	0.47	–	1.52

A, B, C, D and F ≡ Cheese sample collected from different area

the importance of the packaging in preserving the product. However, we find that it is required and preferred by all consumers.

11.11 Fatty Acids Composition of Collected Cheese Samples

The positive flavor of cheese is the result of the balance between the different flavors compounds produced during re-condensation. Two important classes of compounds that contribute to flavor are volatile sulfur compounds and fatty acids (Weimer et al. 1999). Because milk is not heat treated in the production of traditional white cheese, it is likely that the free fatty acids only contribute to the flavor and aroma of the cheese (Zhao 2009).

11.11.1 Saturated Fatty Acids

Figure 11.9 shows the saturated fatty acid composition of bulk cheese samples in total fatty acid content g/100 g. The fatty acid content differed in all samples, and the most abundant fatty acid samples examined were palmitic acid (C16: 0), stearic acid (C18: 0) and Myristic acid (C14: 0), which ranged from 14.56 to 39.41 and 0.04 to 19.31 and from 0.59 to 1.30 g/100 g respectively, palmitic acid was found to contain the highest level of saturated fatty acid in all cheese samples. Palmitic acid is one of the main saturated fatty acids that raise blood cholesterol, while citric acid does not (Grundy 1997). The level of capric acid and lauric acid for the selected cheese samples was lower than that reported by Kinik et al. (2005) for Turkish white cheese, which reported a range of 2.24–13.11 g/100 g and 7.31–12.00 g/100 g, respectively. On the other hand, the concentrations of long-chain saturated fatty acids (C17:0) and arachidic for the cheese samples were higher than that reported by Kinik et al. (2005), who found a range from 0.45 to 0.71 g/100 g and 0.20–0.27 g/100. In fresh husk cheese, turkey cheese, respectively.

11.11.2 Unsaturated Fatty Acid

There were lower proportions of unsaturated fatty acids compared to saturated fatty acids (Fig. 11.10), with the exception of oleic acid (18:1) which had a high proportion. These results were in agreement with those reported by Sulieman (2001) for the traditional Sudanese milk product “Rob”. Oleic acid was found at a higher concentration (21.95–36.89 g/100 g) in all cheese samples. Results were higher than those reported by Sagdic et al. (2004) and Molkentin (2006) who reported values ranging from (20–24 g/100 g) for Turkish and German dairy products. However, the collected cheese samples contained low values for some unsaturated fatty acids

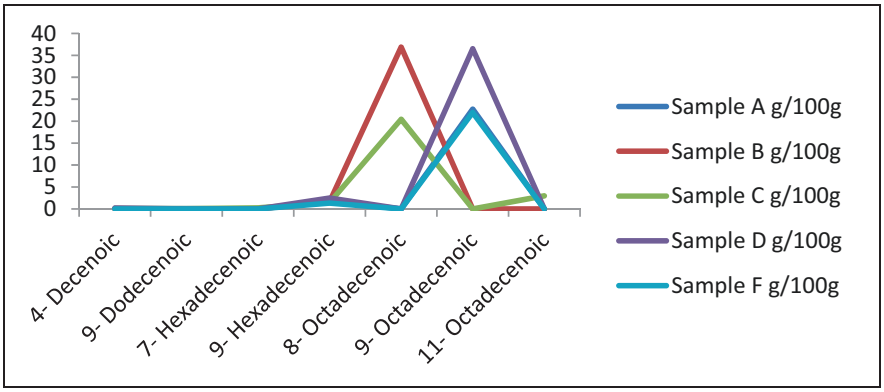

Fig. 11.10 Show Unsaturated fatty acids composition of jibna-beida samples obtained from different area

such as 4-Decenoic acid (10:1), 9-Dodecenoic acid (12:1) and Hexadecenoic (16:1) which ranged from (0–0.10), (0–0.06) and (1.32–2.46) g /100 g, respectively. Organic acids, transporting flavorings to many cheese as a result of the hydrolysis of fatty acids (acetic butter), or natural cows, or biochemistry (Citric, urine) or bacterial growth (lactic, acetic, pyruvic, propionic and formic). These are the main products of the dismantling of carbohydrates from lactic acid bacteria (LAB). The resulting acidity prevents the development of spoilage and pathogenic microorganisms, which improves the healthy quality of cheese (Adde et al. 1982).

11.12 Amino Acids Composition of Collected Cheese Samples

The amount of free amino acids in cheese depends on several factors. These include the quantities of proteins in the raw materials used in production, the activity of proteolytic enzymes in dairy procedures and the microorganisms involved in this process (Yvon and Rijnen 2001). Protein hydrolysis in cheese during ripening plays a vital role in the development of texture as well as flavor and has been the subject of numerous reviews (Fox et al. 1993; Souza et al. 2001; Fox and Ms Sweeney 1998). The determination of free amino acids plays an important role in assessing the nutritional quality of foods (Erbe and Bruckner 2000; Casella and Contursi 2003). In addition, the identification of amino acids also gives an indication of the adulteration and potential transformation that occurs during the processing and storage procedures (Butikofer and Ardo 1999). Figure 11.11 shows the free amino acid content of the pooled cheese samples, expressed as mg of amino acid/100 g sample.

The content of the essential amino acids “threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine” differed in the different cheese samples. The highest percentage of threonine, lysine was found in cheese samples D (40.41

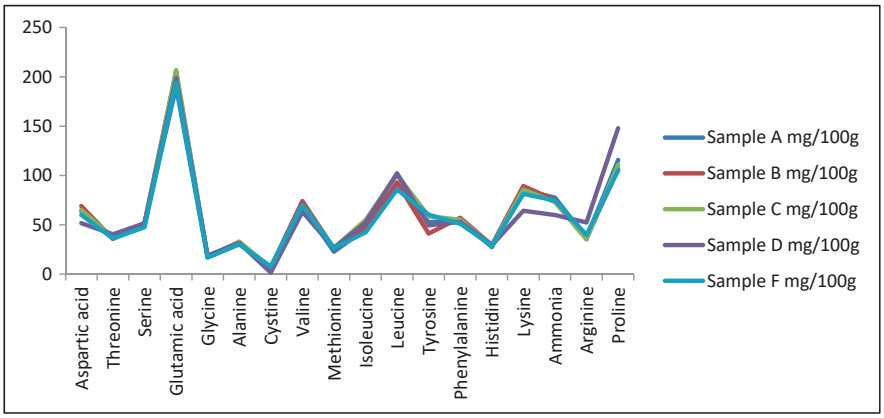

Fig. 11.11 Show Amino acids composition of collected cheese samples

and 102.09) mg/100 g respectively, and the highest percentage of phenylalanine, valine and lysine were found in cheese sample B which were 57.18, 74.07 and 89.38, respectively. The lowest values for threonine (35.78), leucine (85.89), phenylanine (51.13), valine (63.25) and leucine (64.23) were found in the cheese samples (B, F, and D), respectively. The values obtained from essential amino acids in cheese samples were higher than those determined by Ohaj (2009) and Ahmed (2010) in Gouda cheese and white cheese produced in Sudan, where it was found that the content of Thyronine (6.82 and 2.35), Isoleucine (4.79 and 10.48), Phenylalanine (3.73 and 15.84), leucine (8.11and 49.39) and valine (4.62 and 4.76) mg /100 g, respectively.

The content of non essential amino acids "serine, glutamic acid, glysine, alanine, cystine, tyrosine, histidine, NH_4 and arginine" also varied in different cheese samples. The content of non-essential amino acid for collected cheese samples were as follows: serine (51.57), glycine (18.89), arginine (52.29) recorded in sample D, alanine (33.10), glutamic acid (206.77) recorded in sample C, cystine (7.62), tyrosine (60.32) recorded in sample F, histidine (29.52) recorded in sample B. On the other hand, the lowest non-essential amino acids content obtained for collected cheese samples were: glutamic acid, tyrosine, histidine and arginine content were 20.17 mg/100 g, 18.43 mg/100 g, 13.80 mg/100 g and 17.12 mg/100 g, respectively. Also, these values are higher than those reported by Kabelova et al. (2009) who found the content of serine was (9.8 g/kg), glutamic acid (2.3 g/kg), alanine (29.0 g/kg), tyrosine (1.5 g/gk), valine, (7.1 g/kg), henylalnine (9.7 g/kg), and leucine (14.1 g/kg). However, the result have not agreed with those of Sulieman (2001) for Sudanese traditional fermented milk product "Rob" who found that the average content of threonine, valine, methionine, leucine, tyrosine, serine, glutamic acid, histidine and arginine were 1.8 mg/100 g, 1.07 mg/100 g, 0.12 mg/100 g, 2.3 mg/100 g, 4.6 mg/100 g, 2.3 mg/100 g, 3.0 mg/100 g, 0.12 mg/100 g and 0.2 mg/100 g, respectively. Generally the data in Fig. 11.11 show that the collected

cheese samples are rich in most of amino acids. These amino acids may be having a role in development of cheese flavour.

11.13 Conclusions and Recommendations

11.13.1 Conclusions

- From the results obtained, it can be concluded that the Sudanese white cheese collected from traditional small factories from several regions has a high nutritional value, as the chemical analysis indicated that most of the chemical components were closely compatible with the literary values with slight differences.
- Microbiological analysis showed the presence of some pathogenic bacteria such as Staphylococcus aureus, Salmonella and Coliform bacteria in some of the cheese samples collected, in addition to the high number of total bacteria, lactic acid bacteria, and the number of yeasts and fungi. This may be due to the use of low-quality milk in the preparation of cheese, or it may be due to unsanitary conditions during cheese processing or to the failure to use heating of milk as a method of preservation.

11.13.2 Recommendations

The following are recommended:

- The use of milk in rural areas to produce Gibna-Bayda under controlled conditions.
- Sanitation, handling of equipment and utensils, application of good manufacturing practices and the production of milk and cheese in good hygienic conditions can increase the shelf life of Gibna-Bayda and make it safer for human consumption.
- Establishing training centers for the production of Gibna-Bayda at the level of small production areas in Sudan by applying health conditions for product safety.
- Educate cheesemakers on good manufacturing practices under good hygienic conditions.
- Future work should include studying Gibna-Bayda production under controlled conditions, using pasteurization of milk and comparing it with conventional production, as well as studying the activities of enzymes to determine their role in cheese production.

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Chapter 12
Nutritional, Antimicrobial and Bioactive Components of *Gariss*, a Fermented Camel Milk Product

Abdel Moneim Elhadi Sulieman and Abdalla Ali Alayan

12.1 Introduction

Milk is generally considered a staple food, and milk and its derivatives can be obtained from many sources, including: cattle, sheep, camels, goats, etc., as may be obtained from other non-animal sources; such as: soy milk, coconut milk, almonds, flax, and rice. Camel milk has been utilized for numerous centuries and it is very similar to mother's milk, as it has a dark color and has a sweet and sharp taste, and it might be salty at times. Moreover, camel milk can be kept for a while, the first without the need to cool it compared to cow's milk, as it acidifies in a longer period of time. Camel's milk production varies according to its breed, the stage of lactation, and the feeding conditions to which it is exposed, but in general, camels produce about 17–26 liters per day (Megan 2017; Wajiha et al. 2015).

Camel milk has usually a desirable taste, is usually sweet in taste, but its sweet taste changes according to the type of herbs that camels eat (Yagil and Etzion 1980). The acidity of fresh camel milk (pH) ranges between 6.5–6.7 and it is slightly lower than that of fresh cow's milk. Its average density is 1.029 g / cm 3 (Farah 1996. Its viscosity at 20 ° C is 1.72 MPa per second, which is less than cow's milk viscosity at the same temperature and is equivalent to 2.04 MPa per second (Kherouatou and Attia 2003). Camel milk's content of lactose and salts controls the sweetness of milk. When the sugar lactose is 5.8%, the milk is sweet and when it drops to 4.2%, it is salty. As for the salt content of camel milk, it really depends on the amount of drinking water that the camel drank and the stage of milk production, and it ranges

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between 0.6–0.8% and may drop to 0.25% in thirsty camels whose milk is salty due to an increase in the concentration of sodium chloride and a decrease in calcium and magnesium phosphate.

Camel milk is associated with many health benefits and it has high nutritive value, hence its products will have a relatively high nutritive value. Investigations have shown that camel milk possess multiple medicinal functions for the human body, including: lowering blood cholesterol, preventing the generation of cancer cells, reducing the incidence of diabetes and preventing high blood pressure, and it is also recommended for children who suffer from allergies to drinking cow's milk and inhibit the enzyme angiotensin activity.

The percentage of water in camel milk decreases, reaching 84% under normal conditions of the availability of drinking water. While the percentage of water increases to reach 91% in the event of water scarcity and lack of availability for camels. This is one of the advantages of camels in their adaptation to the harsh desert conditions and the need to provide food for their babies constantly. The decrease in the solids percentage is due to the lack of fat formation from 2.4% -1.1% in thirsty camel milk. The wisdom of that is that when the Bedouins in the wild are far from water resources, so camel milk becomes light and sufficient for them to drink water. And when it is about water resources, they need food, so God Almighty makes milk as food.

Camel milk is utilized for treatment of various disorders, including dropsy, jaundice, tuberculosis, asthma, and visceral leishmaniasis, or what is called black fever. In addition, camel milk inhibits the growth of pathogenic microorganisms and many groups of bacteria with Gram positive and negative stains as well. In summary, camel milk has many benefits on human health, which makes it a distinctive functional food (Azzeh 2012). It is also effective in the treatment of And chest diseases such as tuberculosis and asthma as well as internal diseases such as stomach, duodenal and colon ulcers, digestive disorders, sugar and pressure reducer, and heart rate regulator, respiratory rates and sunstroke. An Omani study has shown the superiority of camel milk in treating chronic hepatitis compared to using camel milk to treat ascites, jaundice, spleen problems, tuberculosis, asthma, anemia and hemorrhoids, and special clinics have been established in which camel milk is used for such treatments (https://www.alriyadh.com/296947).

Fresh camel milk maintains its quality longer than other types of milk. Camel milk may remain for eight hours until the pH value reaches 5.7 (Lakosa and Shokin, 1964). This is attributed to the presence in camel milk of appreciable amounts of inhibitors for microbial growth such as lysozyme, lactoferrin, and immunoglobulin than in other types of milk such as cow and buffalo milk El-Agamy, 2000; Konuspayeva, et al. 2008b; Shori 2012) (Fig. 12.1).

Fig. 12.1 Sudanese camels from Tambul area

12.2 Benefits of *Garris*

Camel milk is associated with many health benefits, and hence, *Garris t*he fermented camel's milk product, the most important of which are:

1- The possibility of treating liver disease: Hepatitis C is one of the diseases spread all over the world. This disease requires vaccinations to prevent it, and scientific publications show that camel milk cures viral hepatitis B and C, and its fat content has beneficial effects on Chronic liver disease, in addition to that, camel milk might enhance liver function in general. This is because it comprises high concentrations of vitamin C, or ascorbic acid. According to the observed favorable impacts of fermented camel milk on liver enzymes, its utilization might be regarded as a functional food supplement in related circumstances (Falah et al. 2018).
2- The possibility of preventing diabetes: Camel milk contains appreciable quantity of insulin; which helps prevent or even treat type I and type II diabetes. The reason for this protective ability, indicated that one liter of camel milk contains 52 units of insulin; which is equivalent to 60% of the external insulin recommended for patients with type 1 diabetes (Daniel 2014).
3- The possibility of strengthening the immune system: Regular drinking of camel milk is one of the things that may lead to a stronger immune system. This is

because it contains many protective proteins. The most prominent of which is lactoferrin is considered anti-bacterial, anti-inflammatory, and it has anti-disease activity such as HIV and hepatitis. In addition to this milk containing immunoglobulin, which is transmitted from breastfeeding camels to milk, and has many therapeutic functions as well. On the other hand, camel milk does not contain beta-casein protein, and other substances found in cow's milk, which are associated with causing allergic disorders in children (Zafar 2019).

4- The potential for cancer prevention: As it was recently observed that most of the positive effects of camel milk come in particular from its nanoparticles known as exosomes, and in a study conducted to find out the effect of these substances, it was found that the content of camel milk and its derivatives of exosomes showed anti-cancer impacts. This impact might be due to inducing apoptosis, which saves the body from damaged cells, and limits their development into cancer cells, in addition to inhibiting oxidative stress and inflammation (Abdelnaser et al. 1998).
5- Possessing the therapeutic effects of autism spectrum disorder: It is an acute disorder that occurs in neurodevelopment associated with impaired social behavior. In addition, it is associated with a high incidence of autoimmune diseases and gastrointestinal diseases. The studies have shown that oxidative stress plays a significant role in the incidence of many neurological diseases; among them, autism spectrum disorder. Moreover, it was found in a study that camel milk may have the effect of reducing oxidative stress through enzymes and non-enzymatic antioxidant molecules, which leads to improving autism behavior and thus the treatment of autism spectrum disorder (Laila and Nadra 2019).

12.3 Preparation of Garris

Garris is a product made from camel's milk and consumed by pastoralists, and can live on this product for months as the solitary source of nutrition (Sulieman et al. 2007; Dirar 1993). Camel's milk is regularly depicted as not handily fermented and that its butter can not be effectively separated. *Gariss* is processed in large skin bags or *siin*, that contains an enormous amount of a previously soured product. Without starter, especially when utilizing another si'in fermentation is started by adding to the compartment a couple of seeds of black cumin and one onion bulb (Dirar, 1993) (Fig. 12.2).

Garris differs from other sorts of Sudanese fermented milks in that it has significant amounts of ethanol. The product is in this way an individual of the acid alcoholic fermented milks, which include kefir, koumiss and *bukhsa* of central Asia (Kosikowski, 1982). Showed that 10.5% of the nomadic camel herders utilized *Bukhsa* (wooden Gourd) for getting ready *Gariss*, 42.1% utilized plastic compartments, and 42.1% used *Siin* and only 5.3% of the nomadic camel herders utilized stainless steel containers.

Fig. 12.2 Siin (leather bag) image. (Source: https://www.ammonnews.net/image/183617)

12.4 Nutritive Value of Gariss

Camel milk bears an opaque white color and salty taste at times (Abdel Galil et al. 2016) as its taste varies according to the feed type and drinking water avaiabilityfor it, and butter appears when it is shaken a little (Sisay and Awoke 2015). As for the nutritional composition of camel milk, it consists on average of about 3.1% protein, 4.4% lactose sugar, 0.79% minerals, so that it contains about 11.9% as a total of solids, a ratio that is close to the percentage of total solids in breast milk. It is viewed as plentiful in vitamin C and poor in Carotene (Abdel Galil et al. 2016; Sulieman et al. 2004). The water in camel milk ranges between 84–90%, so that the percentage of water in it is higher the less water is available for drinking, in order to ensure that the infant's needs of water are met in cases of dehydration.

Camel milk has a distinctive nutritional composition; this is because it contains less fat, and is often composed of unsaturated fats, omega-3 fatty acid, cholesterol and lactose. And it contains appreciable quantities of minerals and vitamins. As for its protein content; Camel milk contains a number of protective proteins, which contribute in maintenance of the body, in addition to containing antibacterials, antiviruses, and antifungal compounds (Wajiha et al. 2015).

Nutritive value of Gariss is essentially that of fresh camels' milk as changed by fermentation (Sulieman et al. 2006). The average chemical composition of Gariss

ranged between 1.4–1.35% lactose, 2.15–2.9% fat, 3.4–3.85% protein, 0.75–0.8% ash, 91.7–92.65% moisture, 1.3–1.4% ethanol, 0.13–0.2% volatile fatty acids, 1.0–1.8% total acidity (as lactic acid) and the product has a pH value of 3.25–3.4 (Mirghani (1994).

Camel milk is plentiful in vitamin B group, explicitly vitamin B2, it is higher than that found in goat milk, concerning vitamin B1 it is lower and contains vitamin B12 in an amount of 2.3–3.9 micrograms, additionally extremely plentiful in iron, and it contains vitamin A by 0.037–1.264 mg / Ml, it is also rich in vitamin C by 5.03–9.8 mg and three times more than cow's milk.

With regard to salt, it is more than cow's milk, as nomads need to compensate for the lack of salts as a result of travel, as well as less fat than other types of milk, compared positively with human milk, and the total protein in it is similar to cow's milk 2–5.5%, and the taste and quality of the milk is affected by the forage and the nature pasture.

12.4.1 Amino Acid Content of Garris *Protein*

The free amino acids content in camel's milk were as per the following: tyrosine 1.3, aspartic acid 0.7, proline 0.4, alanine 10.8, glutamic acid 13.1, glutamine 3.8, valine 1.6, isoleucine 0.9, leucine 0.5 and arginine 0.7 ug/100 ml (Mehaia and Al-kahal (1992). Glutamic acid was the most plentiful free amino acid in the milk of camel, cow and man. As a rule, of all species studied, human milk had the highest concentration of total free amino acids, while the cow and camel milk has the least concentration.

Garris protein contains appreciable amounts of the essential amino acids (histidine and isoleucine) and the non-essential amino acids (serine, glycine, cystine and aspartic acid) (Sulieman 2001; Alyan et al. 2009). Table (3) show amino acids content of fermented camel milk (Gariss) from Sudan (Table 12.1).

The *Garris* amino acids may have a role in the development of *Gariss* flavour. The capacity to enhance the release of specific free amino acids would encourage analysis of their possible tangible impacts. The increase in free amino acids content in *Gariss* contrasted with those of milk isn't unexpected since numerous investigators announced increments in free amino acids of dairy products as a result of fermentation such as Aim (1982) revealed that, because of bacterial proteolysis during fermentation, yoghurt has higher levels of free amino acids compared to milk.

12.5 Microbiology of *Garris*

It was accounted for that Gariss contained rod-shaped, non-sporing bacterial cells as single cells, pairs or short chains. In less bountiful numbers yet still various are discovered yeasts, mostly of the elongated cell types, in addition to others of oval or

Table 12.1 Amino acids content of fermented camel milk (*Gariss*) from Sudan

Amino acids	Content
Essential amino acids	
Histidine	0.06–0.04
Threonine	0.20–0.07
Valine	0.14–0.09
Methionine	0.09–0.13
Leucine	0.20–0.05
Isoleucine	0.08–0.05
Tyrosine	0.16–0.17
Phenylalanine	0.28–0.17
Lysine	0.10–0.03
Non-essential amino acids	
Arginine	0.16–0.11
Alanine	0.27–0.33
Serine	0.03–0.08
Glycine	0.06–0.09
Praline	0.14–0.15
Cystine	0.06 0.03
Aspartic acid	0.05 0.80
Glutamic acid	0.34 0.24

Source: Alyan et al. 2009

spherical shapes. Three bacterial types are isolated, one of which grows at 45 °C and was tentatively identified as Lactobacillus helveticus. Among the yeast isolates, one belonged to the genus Candida. Lactococci or Streptococci were found in just constrained numbers in Gariss (Mirghani, 1994).

Abdelgadir et al. (1998) made an uncommon note on the presence in Gariss from the Butana (Eastern Sudan) of the regular isolation of yeast and rod-shaped bacteria that were non-fermentative, strictly aerobic, ca.talase-positive and heavy pellicle-forming.

Sulieman et al. (2006) found that LAB dominated the microflora of Garrissamples, and the significant genera were Lactobacillus (74%), trailed by Lactococcus (12%), Enterococcus (10%) and Leuconostocs (4%). The most dominating Lactobacillus species were identified as *Lactobacillus paracasei* ssp. paracasei (64 strains), *L. fermentum* (seven strains) and just three strains as L. plantarum. Most strains produced the enzymes that are relevant to cultured dairy product processing. The Lactococcus species were identified as *Lactococcu slactis.*

In another study, Sulieman et al. (2007) in their study on the microbiology of *Garris* samples, obtained from two production sites in Sudan, they found that Butana *Garris* (BG) contained relatively high numbers of lactobacilli (S.22 ± 0.2S $10g_{10}$cfu mL $^{-1}$), when compared with that found in Kordufa**n** *Garris* (KG) (7.S5 ± 0.45 $10g_{10}$cfu mL $^{-1}$).On the other hand, KG contained relatively higher

Table 12.2 Strains and results of Lactobacillus genus-specific or species-species PCR assays. Identification results based on PCR-based assays

No.	Strain	Source	Genus (ref.1)	Group (ref.2)	Species
1	SL 1–1	KG	±	IV*3	*L. plantarum*
2	SL 1–2	KG	±	III*2	*L. Plantarum*
3	SL 1–3	KG	±	1lI	*L. paracasei*
4	SL 1–4	KG	±	1lI	*L. Paracasei*
5	SL 1–5	KG	±		*L. Paracasei*
6	SL 1–6	KG	±	IV	ND
7	SL 1–7	KG	±	IV	*L. Plantarum*
8	SL 1–8	KG	±	IV	*L. Plantarum*
9	SL 1–9	KG	?	?	ND
10	SL 1–0	KG	±		*L. Plantarum*
11	SL 1–1	BG	±	1lI	ND
12	SL 1–2	BG	±	1lI	ND
13	SL 1–3	BG	±	1lI	*L. Paracasei*
14	SL 1–4	BG	±	IV	*L. Paracasei*
15	SL 1–5	BG	±	1lI	*L. Paracasei*
16	SL 1–6	BG	±	1lI	*L. Paracasei*
17	SL 1–7	BG	±	1lI	*L. Paracasei*
18	SL 1–8	BG	±	1lI	ND
19	SL 1–9	BG	±	IV	*L. Paracasei*
20	SL 1–0	BG	±	–	*L. Plantarum*

KG: Kordofan *Garris*; BG: Botana *Garris.*
ND: not tested, *2 Group III includes *Lactobacillus]XU'acasei* subsp. *p(U″acasei, L. paracasei subsp. casei, L. paracasei* subsp. *tolerans* and *L. rhmnnosus.* *3 Group IV includes *Lactobacillus plantum, L. reuteri, L. salivarius* and *L.fermentum.*

numbers of yeasts (S.42 ± 0.55 $1og_{10}$cfu mL $^{-1}$» when compared with that found in BG (7.65 ± 0.32 $10g_{10}$cfu mL^{-1}) (Table 12.2).

12.6 Antimicrobial Activity of *Garris*

Camel milk contains numerous growth inhibitors of viral, fungal and bacterial microorganisms with Gram positive and negative stain (El-Agamy et al. 2009), and most of these microbes are considered pathogens to humans. Among the most important inhibitors of the growth of microorganisms in camel milk are the following: lactoferrin, lysosome, aminoglobulin, hydrogen peroxide, lactoperoxidase, and peptidoglycan (PGRP) protein (El-Agamy et al. 1992; Konuspayeva et al. 2008a). Among the most important types of microbes that are inhibited by the active substances in camel milk are: E. coli, L. monocytogenes, S. aureus, S. typhimurium and hepatitis C virus (Redwan and Tabll, 2007).

According to Rameh et al. (2019), the bacterial genera Enterococcus, Lactococcus and Pediococcus had been discovered in raw camel milk. From their study, they concluded that antimicrobial activity of LAB became specially due to the manufacturing of 1 or active metabolites during their growth for example, organic acids, hydrogen peroxide and bacteriocins.

Fermented camel milk is having antimicrobial impacts against various microbes. In an investigation, in Iraq, antimicrobial, in Iraq, antimicrobial activity of fermented camel milk was accounted for (Lafta et al. 2014; Rubin 1978; Min-Chei et al. 2001). LAB isolated from Tunisian camel raw milk showed antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* likewise repressed *Salmonella typhimurium* (Mahmoudi et al. 2016).

In their study, Alyan et al. (2014) found that lactic acid, acetic acid and citric acid inhibited growth of *E. coli* . The found that lactic acid, acetic acid and citric acid were produced as a result of camel milk fermentation into *Garris*. Moreover, the concentration of these organic acids varies among the different samples, while lactic acid was produced in relatively high amounts, acetic acid and citric acid were produced in low amounts.

As fermented camel milk contains different LAB, might create peptides and bacteriocins, which showed inhibitory activity against many pathogenic bacteria including Bacillus, Staphylococcus, Salmonella and Escherichia, reported by various researchers (Rahmeh et al. 2019). The antimicrobial activities of the LAB isolates from fermented camel milk were additionally assessed against a multidrug-resistant Salmonella strain and identified by 16S rRNA gene sequencing as a strain of *Salmonella enterica* subsp. Enterica. This strain exhibited its resistance to various groups of antibiotics whose modes of action involved the inhibition of either cell wall or protein synthesis. Interestingly, most of the tested isolates demonstrated strong antimicrobial activity against this strain (Al-Zenki et al. 2007).

In their previous study, Tagg and McGiven (1971) indicted that LAB strains were tested for their ability to produce bacteriocins against *Listeria monocytogenes* ATCC 7644 by the well-diffusion assay. Among these isolates, CM16 and CM22, which were identified as *Pediococcus pentosaceus* (NCBI accession number MH023512) and *Lactobacillus brevis* (NCBI accession number MH023515), respectively, showed anti-listerial activity estimated at 1600 and 800 AU/mL after neutralization of their cell-free supernatant at pH 6.5.

Mycobacterium tuberculosis was successfully hindered by camel milk (Sharma and Singh 2014). Yateem et al. (2008) gathered raw camel milk (Arabian camel) samples from Kuwait and permitted them for spontaneous fermentation for one week through endogenous bacteria at room temperature. Using 16S rRNA gene sequencing the bacterial species were identified as *Lactococcus lactis*.

12.7 Antioxidant Activity

Camel milk's high antioxidant content can allow it to fight free radicals and protect against oxidative stress, and what's more, it may help reduce cancer symptoms. According to a study published in the Journal of Biomedicine and Biotechnology, researchers found that camel milk can lead to cell death. Carcinogenicity in both HepG2 cells as well as MCF7 cells (Shori and Baba 2014; NRCC 2013–2014; Jrad et al. 2014; Salami et al. 2011; Kansci et al. 2004). An ongoing similar investigation announced that, camel milk fermented with indigenous camel milk probiotic lactic strains (Lb. reuteri-KX881777, Lb. plantarum-KX881772, Lb. plantarum-KX881779) demonstrated greatest antioxidant activity as compared to the bovine milk (Ayyash et al. 2017).

12.8 Hypocholesterolaemic Impact of *Garris*

Camel milk contains a high amount of healthy fatty acids, it helps to reduce the level of bad cholesterol in the body, which reduces the chances of heart attacks, strokes and atherosclerosis, which protects heart health.

Hypocholesterolaemicimpact of *Gariss* containing Bifidobacteriumlactis (BB-12) has been shown to have a hypocholesterolaemic impact through in vivo experiment in rats (Elayan et al. 2008) and in the bringing down of plasma and liver cholesterol levels (Ali et al. 2013; Abdelrahim et al. 2013). However, the hypocholesterolcontrolling system of camel milk is still unclear. Various hypotheses were discussed by researchers (Li and Papadopoulos 1998; Rao et al. 1981; Buonopane et al. 1992).

12.9 Conclusions

Fermented camel milk (*Garris*) is one of the common foods in many areas of camel herders in Sudan, especially residents of Butana and Kordofan regions, and the demand for it increases with time. The components of the bitter camel vary depending on the breed of camels, the type of bush, the amount of water, climate changes and the method of analysis. It is consumed in other areas of Sudan and has many health benefits for humans. *Garris* ferments with strains of lactic acid bacteria and yeasts and has a high nutritional value. It is a functional food that has a role in treating chronic diseases such as treating some types of cancers, jaundice and others.

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Chapter 13
Production and Quality Assessment of Camel Milk Cheese

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13.1 Introduction

Sudan is appraised the second in numbers of camel population on the planet. Camels in Sudan are concentrated in two primary areas; the Eastern where camels are found in the Butana plains and the Red Sea slopes and the Western districts in Darfour and Kordofan (El-Amin and Wilcox 1992; Eisa and Mustafa 2011). These Camels vary in their potential in milk and meat production; and hustling capacities. Majority of camels (>90%) in Sudan belongs to pack camels; these are characterised by heavy belt and classified as dual purpose animals (for milk and meat production). The Rashaidi camel is found in eastern Sudan (Kasala and Gedaref states) and described as dairy camel.

The number of camels in Sudan is assessed to be more than 4.6 million heads (MARF 2010).The actual camel milk production in Sudan is assessed to be 59,000 tons per year. However, the capability of camel milk creation in Sudan is assess to be 1,700,000 tons per year. Commercialization of camel milk is a relatively new trend in the Sudan. The production of milk is the main reason for sustainability of the current rearing management system which is practiced by camel's owners or laborers. Majority of the milk production is purchased with value which is multiple times higher contrasted with cow's milk (Amasai et al.b 2013). The camel female produces 8–14 calves during her life span and could reach 20 under improved management conditions.

Camel milk has a significant part in human sustenance in the hot locales and arid countries. Most the camel milk in the Sudan is smashed new and some of the time harsh (matured) (Gariss) or with tea (Sbanes). Handling and assembling of camel milk in to drain items like spread, ghee, cheddar, frozen yogurt, and so on., not

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found aside from in some restricted exploration. It was presumed that the creation framework and dairy creation of camel in Sudan deplorably got little consideration .and sometimes sour (fermented) (Gariss) or with tea (Sbanes). It was presumed that the production system and dairy creation of camel in Sudan deplorably got little consideration.

13.2 Camel Milk Composition

Camel milk is a complete food, that is, it covers the nutritional needs of the whole organism, but not for humans, but for young suckling camels, and despite this it is considered a food of high nutritional and healthy value for humans, even if it is not a complete food. Scientists and researchers have been interested in studying the components and composition of camel milk, and the results of the studies showed variation and a clear difference in the values of the analysis, due to the different production conditions such as environmental, geographical and seasonal factors, summer and winter, the abundance of water and the type of feed provided to camels in each study, as well as according to the methods of analysis and methods of sampling. . Looking at the entirety of the published studies, as reported by the scientific review study of the scholars Hajj and Kanhal, and published in the International Journal of Dairy Research for the year 2010, the average values of the nutritional analysis of camel coffee were as follows:

Protein (3.1%): The protein content of camel's milk varies according to the difference in the type of breed to which it belongs, and according to the season in which the grazing is carried out. Studies have shown that the camel breed "Majahim" contains higher amounts of protein than that of other breeds. The amount of protein decreases in the summer while it rises in the spring. Perhaps one of the most prominent features of camel milk protein is that it contains large quantities of the protein known as casein, which represents more than half of the protein types in milk (50–80% of the protein intake), while the other type known as Whey protein represents the least part. The rest of it. One of the characteristics of casein protein that gives camel milk an additional advantage is that it is the easiest type to digest and the least sensitizing to the intestine of a nursing infant, when compared to other types of protein such as those found in cow's milk, which makes camel milk closer and more similar to human milk than cow's milk, and safer. Anti-allergy. Looking at the quality of the amino acids that make up the protein of camel milk, studies have shown similarity between the types of amino acids in both camel and cow's milk, with a slight difference being the lower content of the first amino acids glycine and cysteine compared to the second. As for the other component of the protein, the whey protein, it is present in smaller quantities than it is in cow's milk protein.

Fat (3.5%): The fat content in camel milk ranges between 1.2–6.4%, with an average of 3.5%. The difference in the fat content of camel milk is associated with the protein content in it, and the fat content is significantly lower in cases of

dehydration and thirst for camels, and the decrease reaches 74% from the original content. The white color of camel's milk is less yellow compared to cow's milk, which is more yellowish, due to the low amount of carotene compounds in the first compared to the second. Camel milk contains more long-chain fatty acids than cow's milk fat, as well as contains more unsaturated fatty acids, up to 43%, and less saturated acids compared to cow's milk. This makes camel milk a suitable choice for heart patients, high fats and cholesterol. On the other hand, camel milk contains varying amounts of cholesterol, which are higher or lower at times compared to cow's milk, according to different care factors.

Lactose (4.4%): The milk sugar content is the least affected by grazing conditions, the prevailing climate and the nature of the forage, and the most stable, unlike other nutrients. Looking at the nutritional composition tables, we find that the lactose content in camel milk (4.4%) is less than It is similar to cow's milk (5.26%), which makes camel milk safer and more beneficial for patients suffering from lactose intolerance, which is a disease that spreads in a number of countries of the world, especially East Asian countries, and results in intestinal disorders after consuming milk. . This disease occurs as a result of the absence of the enzyme lactase that degrades galactose in the intestine due to genetic defects in patients with it.

Salts (0.79%): Like other components of milk, the amount of mineral salts varies according to strain and environmental conditions. Camel milk is a distinctive source of chloride, due to the high content of this mineral in the plants on which the camel depends for its food. In general, the content of camel milk is high in trace salts such as zinc, iron, copper, manganese and major such as sodium, potassium and chloride, while the content of camel milk is similar. With cow's milk in calcium, phosphorous and magnesium. Looking at the behavior of camels, the researchers found that they tend to eat salt-loving pastoral plants, as a natural way to compensate for the loss of salts from their body as a result of sweating and exposure to heat for long periods of time, which makes the milk taste sweet and slightly salty.

Vitamins: Camel milk, like other types of milk, contains varying amounts of vitamins (C), (E), (D) and (A) and some vitamins (B) complex, but in contrast it is distinguished from other types of milk because it contains excess quantities of vitamin C. This vitamin is known to prevent oxidative stress and contribute to building connective tissues and strengthening immunity against diseases. Therefore, camel milk can contribute greatly to providing desert dwellers with their need of this vitamin, which rural and urban people used to obtain from vegetables and fruits, and that the presence of vitamin C in this relatively large quantity also contributes to prolonging the shelf life of milk and raising its resistance to perceptual corruption resulting from Air oxidation of fats. In addition to this vitamin, camel milk contains more vitamins of pantothenic acid, folic acid, cobalamin (B12) and niacin (B3) compared to that of cow's milk. In contrast, camel milk has lower amounts of vitamins A and riboflavin (B2). According to American dietary recommendations, one cup of camel milk meets 15.5% of an adult's body's need for vitamin Cobalamin (B12), 8.25 per cent for riboflavin (B2), 5.25 percent for vitamin A, and 10.5 percent for Both vitamins C, thiamine (B1), and pyridoxine (B6).

13.3 Camel Milk Products

Camel milk is just appropriate for drinking (Yagil et al. 1984). However different items delivered from Dromedary camel milk incorporate soft cheese (El-Zubeir and Jabreel 2008; Inayat et al. 2003) fermented milk (Elayan et al. 2008; Farah et al. 1990), yoghurt (Hashim et al. 2008), ice cream (Abu-Lehia 1989) and butter (Farah et al. 1989).

Technical development and scientific interest in camel milk have contributed to the development of many food products from camel, and these products have become the focus of consumer interest. Given what modern science reveals about its benefits, and then the benefits of its products, and the level of health and nutritional awareness among consumers. Despite this, camel milk products remain more limited and less widespread, for the simple reason that is the low quantities of production compared to other types of milk, such as cows and sheep. In addition to a number of factors inherent in it that hinder the development of food products such as cheese and others, including:

1. The length of cheese for protein: This makes the time required for cheese 3–5 times the time required for cow's milk cheese. This is mainly due to the aforementioned difference in the content and nature of camel milk proteins mentioned under the protein item represented by the casein protein responsible for cheese.
2. Weak curd: This is due to the relatively low total solids in camel milk (11.9%) compared with its counterparts in cow and sheep milk, especially for casein protein. This property is also due to the small size of the fat grains in camel milk compared to cow's milk.
3. Weakness of the effect of rennet (the enzyme cheese) that causes cheesiness: studies have shown that the thrombus resulting from camel's milk cheese is weak and inconsistent, which negatively affects the organoleptic properties of the products derived from it.
4. Low amount of cheese production: the amount of soft cheese resulting from camel milk cheese is about half less than that of cow's milk, whereas a kilogram of cow's milk produces about a quarter of a kilogram of cheese. Camel milk produces half of this amount (about 120 grams), which makes camel milk an economically unfeasible source for cheese.

Camel milk has been utilized for production of various products such as yoghurt having a thin, flow able and very soft texture (Hashim et al. 2008).

Ice cream was accounted for to be delivered effectively from camel milk utilizing a blend of 12% fat, 11% milk solids not fat (MSNF) and 37% all out solids (Abu-Lehia 1989). The invade of camel milk frozen yogurt was found to fundamentally rely upon the fat and MSNF levels in the blend (Abu-Lehia 1989). For instance the expansion in fat and MSNF content in the blend prompts an expansion in thickness.

These camel milk products were made at laboratory scale, yet some are normally created at a larger scale in the pastoral areas during the pinnacle period of milk production or when milk production is over that required for human and youthful

calf use. These products are as yet not very much grew enough to arrive at a commercial scale, additionally a need to inspect buyer adequacy of these products.

13.4 Camel Milk Cheese

Camels have been used for thousands of years as a means of transporting people and transporting goods across the desert, but seeing them as mere `` ships of the desert "is a diminution of their true value. The basic principle in cheese making is to coagulate the milk to form curds and whey. Modern methods of cheese-making and the coagulation process are assisted by the addition of yeast, which is a bacterial seed that produces lactic acid, and rennet, a substance extracted from calves that contains flocculation enzymes, and these enzymes ensure the acceleration of the rate of separation of liquids from solids.

Apparently, camel milk is technically more hard to process compared to milk from other dairy animals. Only a few rare cheeses are manufactured by acidic separation and heating of milk proteins (Yagil 1982). There have been various endeavors to produce cheese from camel milk, however a large portion of these preliminaries were fruitless and yielded contradictory outcomes. The issues related with production of cheese include:

1. Long coagulation time. Camel milk shows an a few fold longer rennet coagulation time and bovine milk (Farah et al. 1990). This was credited to the differences in the size of casein particles.
2. Weak curd due to the low total solids content of the coagulum, especially casein (El-Zubeir and Jabreel 2008; Mehaia 2006; Ramet 2001) and/or the small size of the camel fat globules (2.99 mm), or might be due to low elasticity and high fragility of the cheese gel texture (Ramet 2001).
3. Rennet action.
4. Lower cheese yield. % because of higher recovery of proteins, fat, and other milk solids (Mehaia 2006).

A few factors have been accounted for to improve camel milk coagulation, including the expansion of calcium chloride (El-Zubeir and Jabreel 2008). Utilizing camel gastric enzyme extracts (Siboukeur et al. 2005) and rennet rather than bovine rennet brought about in improved camel milk coagulation. This could be ascribed to the pepsin content in the rennet preparation utilized.

The addition of yoghurt culture or other lactic acid bacteria with rennet to camel milk was accounted for to encourage camel milk coagulation expanding the lactic acid content and improving curd firmness (Gassem and Abu-Tarboush 2000; Mehaia 2006), while the addition of yoghurt culture or other lactic acid bacteria alone to camel milk did not coagulate the milk.

Other investigations announced that diminishing pH to 5.6 and expanding temperature to up 420C brought about a decrease in camel milk coagulation time (Farah et al. 1990; Siboukeur et al. 2005). The system behind the decrease of camel milk

coagulation time when bringing down the pH could be because of the improvement of the charge balance cycle and compliance changes happening in the optional period of coagulation (Mehaia 2006). However, expanding the temperature builds the pace of accumulation of the micelles and the arrangement of a gel network through the hydrophobic connections. Expanding the convergence ration of rennet up to multiple times was additionally answered to quicken camel milk coagulation (Larsson-Raznikiewicz and Mohamed 1986; Ramet 1989). The need for high rennet concentration to coagulate camel milk could be could be because of the presence of explicit protease inhibitors in camel milk and/or a specific casein micelle structure restricting access of the protease to the k-CN substrate, notwithstanding, these theories should be affirmed (Ramet 2001). Fermented milk products often contain probiotics, which promote gut health.

13.5 Technology of Cheese Making

Cheese manufacture is fundamentally a dehydration process in which the milk protein (casein), fat and colloidal salts of are concentrated 6 to 12 times with removal of great amount on milk water (90%) milk and mostly all of the lactose, whey proteins and soluble milk salts. It is an easy and simple and does not require much effort, all that the manufacturer needs is preparing the ingredients to start preparing the cheese.

Cheese is formed as a result of curdling milk; where it is transformed from a known liquid state to a semi-cohesive protein (casein). The concentration is achieved by coagulating the casein by the action of enzymes blown (enzyme resonance), or by acidity formed by the action of added precursors, or by acidity and heat with the separation of the yellow liquid known as the grit, when the clot is cut, stirred, heat treated and compressed. Basic cheese compounds are transformed from proteins, lipids and carbohydrates into simple, easily digestible compounds as a result of the biochemical reactions that take place during the cheese settlement process.

The cheeses that can be consumed after being kept shall be soft, semi-dry, dry or very dry. They may be covered with a waxy layer or wrap with plasticine. Ripening is done by milk bacteria, added initiator bacteria, and / or fungi (mold) growing inside and / or on the cheese surface.

13.6 Cheese Preparation

Mahgoub (2015) prepared white cheese using raw camel milk, and a mixture of camel milk and cow milk (1:1). The temperature of milk was brought down to 40 °C. Three types of cheese were prepared; the first type was addition of 10% citric acid to pure camel, the second type was addition 10% citric acid to a mixture of camel milk and cow milk (1:1), third type was addition 5% starter culture to a

Fig. 13.1 Image of camel herd from Tambul area (Central Sudan)

mixture of camel and cow milk (1:1) two methods were then used to manufacture soft white cheese from pure camel milk and mixture of camel and caw (1:1) (Fig. 13.1) One method utilized addition of 10% citric acid solution to one portion of milk till pH came down to 5.5. The starter culture was then added at the rate of 5% to second portion. After about an hour rennet was then added to both portions of milk at the rate of 0.15 ml/litre of milk. The milk was allowed to coagulate for five hours. After curd formation the coagulum was cut and whey was drained off. The coagulum so obtained was cut and scalding was done by gradually raising the temperature of the curd to 38 °C within 30 minutes. The curd was moulded and pressed for 2–3 hours at room temperature (25 °C). Cheese was removed from mould, packaged in packaging, sampled and stored at 4 °C for further revaluation.

13.7 Chemical Composition of Cheese

The chemical characteristics of white cheese prepared from camel and cow milk are presented in (Table 13.1). Most of the tested chemical components of both camel milk and cow's milk were comparable.

These samples included: cheese made using 10% citric acid for pure camel milk (PCM), mixture camel milk and cow milk (MCCM) with citric acid, (MCCM1)

Table 13.1 Chemical composition of cheese

Parameter	PCM	MCCM1	MCM2
Moisture (%)	55.00 ± 0.07	61.42 ± 0.59	67.49 ± 0.007
Ash (%)	2.36 ± 0.01	2.52 ± 0.28	2.67 ± 0 .01
Protein (%)	35.55 ± 0.2	31.55 ± 1.05	25.70 ± 1.8
Fat (%)	1.85 ± 0.01	1.97 ± 0.02	1.73 ± 0.01
Lactose (%)	2.49 ± 0.01	2.47 ± 0.02	2.37 ± 0.01
Total solid	45.00 ± 0.07	38.58 ± 0.59	32.51 ± 0.007
Solid non fat(SNF)	43.16 ± 0.07	36.60 ± 0.59	30.77 ± 0.007
pH	5.52 ± 0.02	4.39 ± 0.01	4.39 ± 0.01
Acidity	3.19 ± 0.01	2.99 ± 0.01	2.79 ± 0.02
Calcium mg / 100 g.	325.50 ± 0.70	299.5 ± 070	449.50 ± 0.70
Sodium mg / 100 g.	48.50 ± 0.70	64.50 ± 0.70	64.50 ± 0.70
Potassium mg / 100 g.	16.50 ± 0.70	14.83 ± 0.70	12.50 ± 0.70

PCM: cheese made from pure camel using citric acid (acidification).
MCCM1: mixture camel and cow milk (1:1) using citric acid (acidification).
MCCM2: mixture starter culture: mixture camel and cow milk (1:1) using starter culture.

using 5% starter culture for mixture of camel milk and cow milk (MCCM2). The moisture content was 55.00% for PCM, this result is in line with those found by Haider et al. (2004) which was 55.64–58.8% for pure camel milk cheese, while it was 61.42% and 67.49%, in MCCM1 and MCCM2, respectively. On the other hand, these results were higher than those results reported by Shahein et al. (2014) which were 54.67% for mixture camel (60%) and cow milk (40%) cheese.

The ash values were 2.36% for PCM, these results were higher than those reported by Derar et al. (2014) which were 1.46% for pure camel cheese while they were 2.52% and 2.67%, in MCCM1 and MCCM2, respectively. However these results were lower than that result reported by Shahein et al. (2014).which was 2.80–3.10%.

The protein contents in PCM, MCCM1 and MCCM2 were 35.55%, 31.55% and 25.70%, respectively. These results were higher than those reported by Haider et al. (2004) which was 21.30%, while these results were lower than that reported by Awad elsid (1996) which was 47.98%.

The fat (%) in PCM, MCCM1 and MCCM2 were 1.85%, 1.97% and 1.73% respectively, these results were extremely lower than those reported by Yonas et al. (2014) which were 12.90% and 13.40%. The variation in fat content in this study could be attributed to several factors like breed, individuality of animal, type of feed, health and age of the animal when the milk was taken.

The Lactose (%) in PCM, MCCM1 and MCCM2 was 2.49%, 2.47% and 2.37%, respectively. However, these results are in line with that found by Haider et al. (2004) which was 2.55%.

Total solid (%) were 45.00%, 38.58% and 32.51%, in PCM, MCCM1 and MCCM2, respectively. These results are in line with those found by Haider et al. (2004) and Shahein et al. (2014) and Yonas et al. (2014) which were 44.36%, 34.03% and 39.90%, respectively.

Table 13.2 Microbial load (c.f.u/ml) of cheese

Parameter	PCM	MCCM1	MCCM2
Total bacteria count	15×10^6	9×10^5	11×10^5
Yeasts and Moulds	2×10^3	6×10^2	8×10^2
Coliform bacterial count	2.5×10^5	1×10^6	2.5×10^5
Staphylococcus aureus	Nill	2×10^5	Nill
Lactic acid bacterial	1.5×10^8	3×10^5	4×10^5

PCM: cheese made from pure camel using citric acid (acidification).
MCCM1: mixture camel and cow milk (1:1) using citric acid (acidification).
MCCM2: mixture starter culture: mixture camel and cow milk (1:1) using starter culture.

The pH value was 5.52, 4.39 and 4.39% in PCM, MCCM1 and MCCM2, respectively. These results were in close agreement with those reported by Yonas et al. (2014) which were 4.87 and 5.27 and also Haider et al. (2004) which were 4.90 and 5.80. The variation of pH values of various cheese samples could be attributed to the variation in composition and properties of milk.

The acidity (lactic acid%) was 3.19, 2.99, and 2.79%, in the PCM, MCCM1 and MCCM2, respectively. These findings are in close agreement to those obtained by Zakaria et al. (2012) who found that acidity in cheese (Jibna-beida) produced in Sudan from cow milk ranged between 1.58% and 2.10%. The high acidity of the cheese samples could be due to extended period of storage of cheese.

The mineral contents of PCM, MCCM1 and MCCM2 are also shown in Table 13.2. The major salt constituents were Calcium (Ca) Sodium (Na) and potassium (K) their concentrations were, 325.50, 299.5 and 449.50 mg/100 g, respectively, for Calcium (Ca), 48.50, 64.50 and 64.50 mg/100 g for Sodium (Na) and 16.50, 14.83 and, 12.50 mg / 100 g for potassium. The results indicated relative increase in calcium in MCCM2 as compared with those of PCM and MCCM2. On the other hand; the results indicated relative decrease of potassium in PCM as compared with those of MCCM1 and MCCM2.

13.8 Sensory Evaluation of White Cheese

Sensory characteristics of cheeses are considered one the most important attributes determining the consumers' choice. Before and during ingestion itself, the consumer can perceive several sensory features of cheese, which are generally grouped under appearance, flavour and texture. All such attributes determine the eating quality of cheeses and consequently their acceptability. There is a wide diversity of cheese types worldwide, each one with a unique sensory profile. It reflects the characteristics of the milk feedstock, the cheese making conditions and the physical and chemical changes throughout ripening Jerónimo and Malcata (2013). The results of sensory evaluation of various cheeses are shown in Figs. 13.2, 13.3 and 13.4. The results show that the cheese samples prepared by using starter culture were more

Fig. 13.2 Image of camel milk

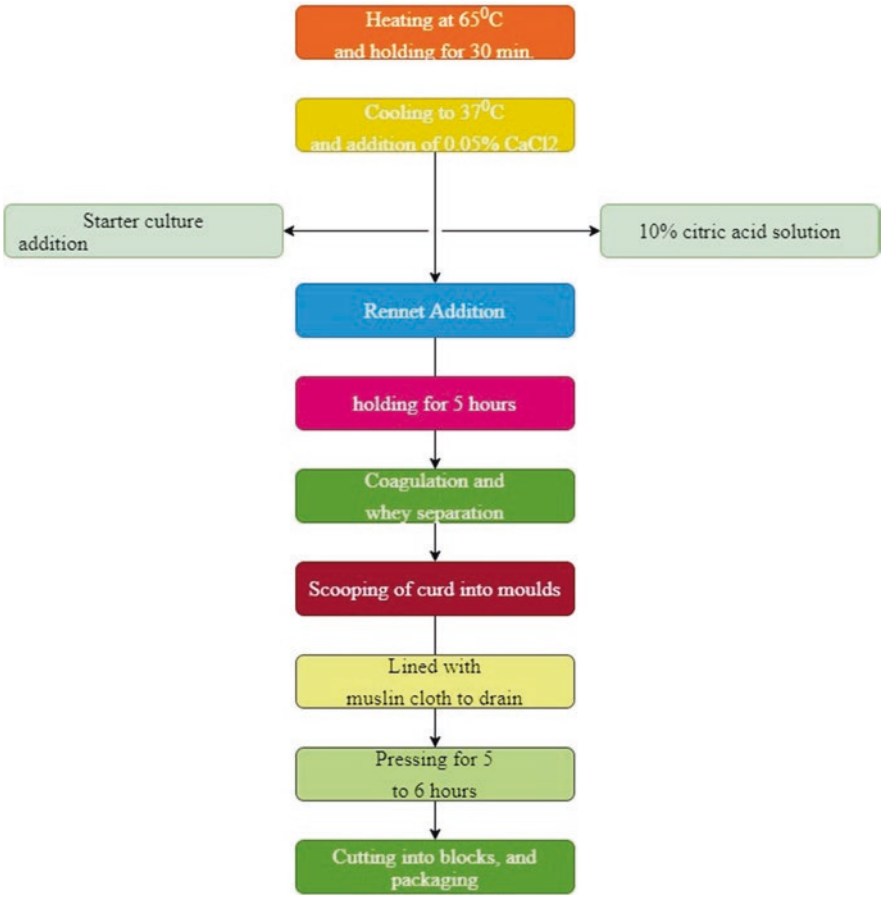

Fig. 13.3 Cheese production flow diagram

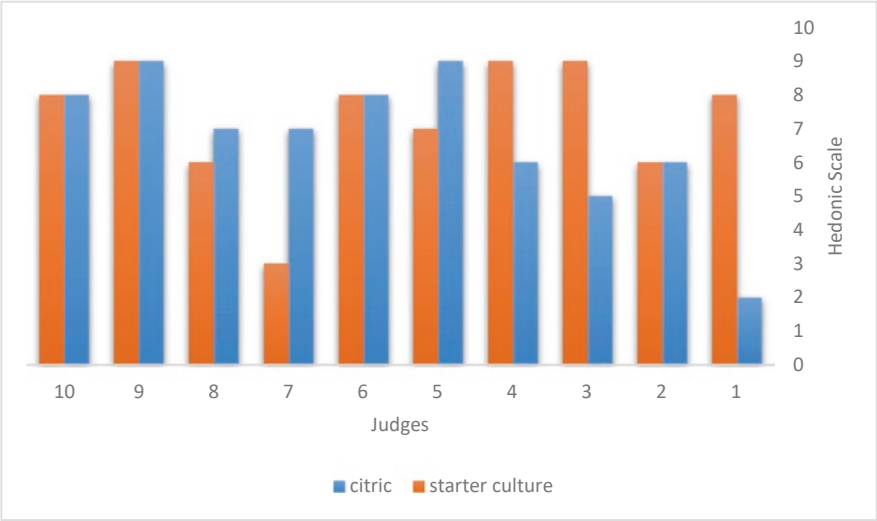

Fig. 13.4 Appearance judgement of cheese appaerance

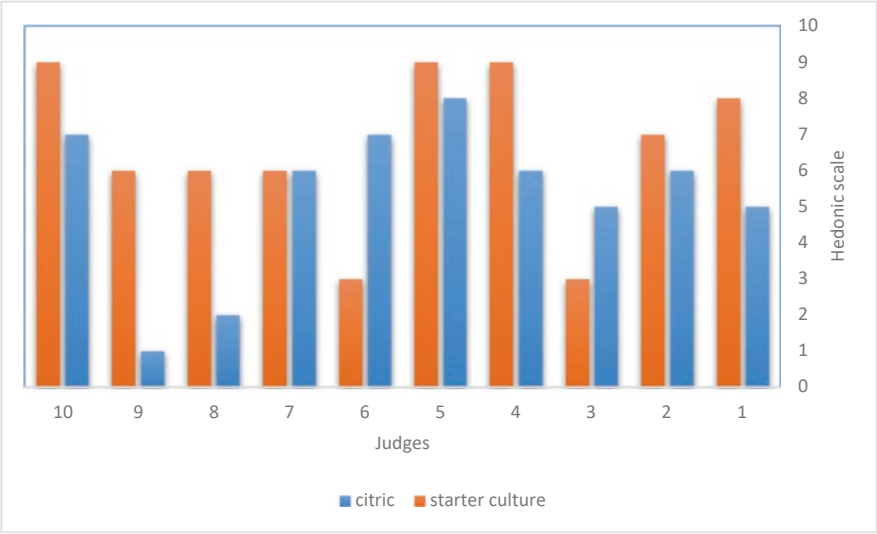

Fig. 13.5 Appearance judgement of cheese taste

preferred on the basis of appearance, flavour and texture as compared to cheeses obtained by direct acidification using citric acid. However, all cheeses were accepted by the panelists (Figs. 13.5, 13.6, 13.7 and 13.8) (Table 13.3).

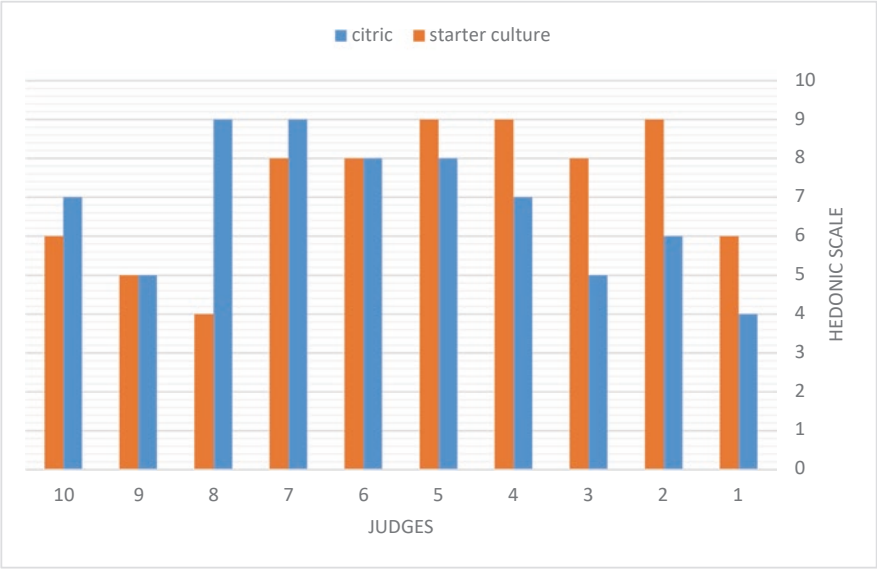

Fig. 13.6 Appearance judgement of cheese Texture

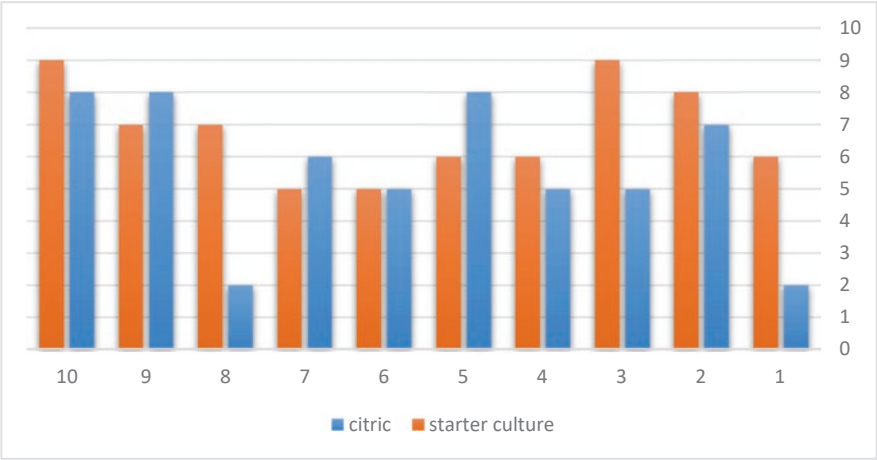

Fig. 13.7 Appearance judgement of cheese smell

13.9 Conclusions

Making cheese from camel milk is a difficult task. The present was a trial to prepare camel milk at the laboratory level. Because of the difficulty of camel milk curdling, it was blended with cows milk (1:1) of was also mixed study confirmed possibility of production of cheese from camel milk with acceptable quality. The manufactured

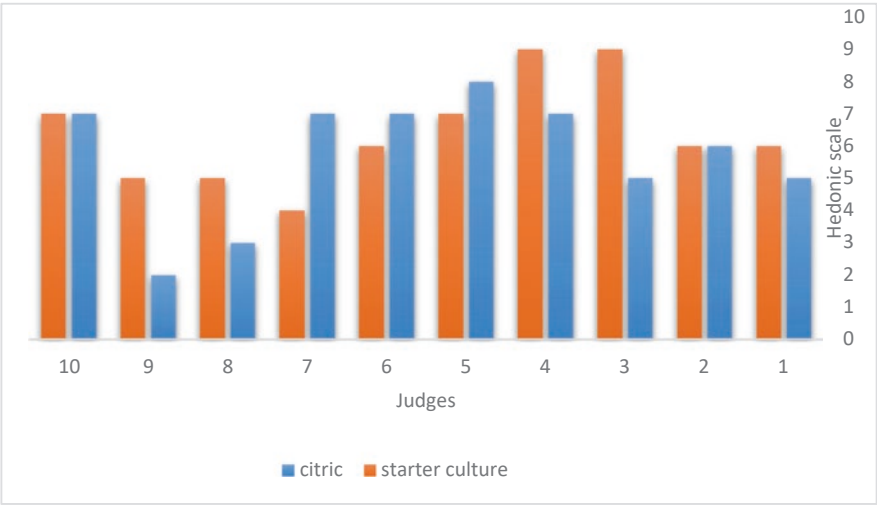

Fig. 13.8 Appearance judgement of cheese overall acceptability

Table 13.3 Mean sensory scores of cheese

Samples	Appearance	Taste	Texture	Flavor	Acceptability
PCM	6.90 ± 0.64	5.60 ± 0.72	5.70 ± 0.67	5.60 ± 0.40	5.80 ± 0.76
MCCM1	6.70 ± 0.67	5.30 ± 0.70	6.80 ± 0.55	5.60 ± 0.72	5.70 ± 0.62
MCCM2	7.30 ± 0.60	5.80 ± 0.85	7.20 ± 0.57	6.80 ± 0.47	6.40 ± 0.52

PCM: cheese made from pure camel using citric acid (acidification).
MCCM1: mixture camel and cow milk (1:1) using citric acid (acidification).
MCCM2: mixture starter culture: mixture camel and cow milk (1:1) using starter culture.

cheese was analyzed chemically, microbiologically and subjected to sensory analysis. From the obtained results, mixing camel milk with cow milk increased and improved the microbiological and chemical quality as well as the organoleptic properties of cheese. Fresh soft white cheese made from camel milk inoculated with starter culture was highly acceptable. The study recommends understanding the mechanism of enzymatic coagulation of camel milk to improve quality and yield of camel milk cheese. In addition, hygienic conditions must be available while making cheese.

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Chapter 14
Selected Fermented Fish Products of the Sudan

Onaheid Ahmed Osman, Walied Abdelrahman Mustafa, and Abdel Moneim Elhadi Sulieman

14.1 Introduction

Water covers about 71.0% of the land area, and water resources are an important source of food. Global fish production (2014) reached about 93.4 tons, of which 81.million5 tons were water and 11.million9 tons were inland watersmillion (FAO 2016). There is an increasing demand for water resources and fish products as a source of dietary protein all over the world (Feldhusen 2000). Fish is a rich source of protein that is easy to digest as well as provides polyunsaturated fatty acids, vitamins and minerals for human nutrition. Although some fish species are used industrially to manufacture fishmeal, the need to conserve and use them for human consumption in order to prevent post-harvest fisheries losses has been recognized (Venugopal and Shahidi 1995). In many African countries, animal protein consumption patterns depend on environmental factors such as animal husbandry practices and traditional beliefs as well as urbanization, income levels and the type of animal protein available in the community (Gomna and Rana 2007; Essuman 1992). Meat and fish are an integral part of the Lvorian diet and are considered a staple protein food, as well as focal point of a family meal. However, the relative contribution of each of these protein sources may vary depending on the household's living activities, income, and fish availability Essuman 1992)).

Fermentation technique practiced in African countries and it is one of the original methods of African cultures. One of the types of fermented foods in Africa is fermented fish, which is one of the oldest and most common products and is often

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used as a spice or main source of animal protein. Fermented, salted, and sun dried fish (fermented fish) are usually known because treatment methods include salting, fermentation and drying. This process is necessary in tropical regions because of its climate and the extreme perishability of fish and autolytic spoilage that occurs quickly after fishing and during processing.

Sudan has a huge potentiality for the development of aquaculture to perform vital role in socio-economic improvement (Khalid et al. 2008). According to (FAO 1999) the conventional salted fish commodities in Sudan entail: Fasseikh; Terkeen (fermented fish), Mandasha (smoked and sun-dried) and Seer (humble fried fish). The salted fish without drying is familiar as Fasseikh in Sudan, Egypt and the Middle East countries (Hasan et al. 1972; Hamed et al. 1973; Campbellplatt 1987). In Sudan Fasseikh is mediated as the most notorious food that is depleted either moist or dried and allotted across the country and its outlay notably exceeds that of fresh Tilapia (FAO 1999). There are two genre of fish proved superior in Fasseikh manufacturing in Sudan, *Hydrocynus Spp* (Tiger fish) recognized locally as "Kass" and *Alestes Spp* (Pebbly fish) certified as Kawwara, the pair regard to the family Characidae (Idris 1981). Fish and fishery products comprehend water, proteins and else nitrogenous compounds, lipids, carbohydrates, minerals and vitamins.

14.2 Nutritional Value

Fishdiet human is a rich source of animal protein, which is an essential nutrient in the. (Steffens 2006; Fawole et al. 2007). also provides polyunsaturated fatty acids It and vitamins such as vitamins A and B, which are found in quantities in the meat of fatty varieties, especially in the livers of cod and halibut. Fish meat, and selenium, in addition to iodine, whichcopper is an especially important source of calcium and phosphorous, as well as iron, is found in high levels in saltwater fish. (Clucas and Ward 1996). Fish and fish products include water, proteins, nitrogenous compounds, fats, carbohydrates, minerals, and vitamins. However, the chemical compatibility of fish varies significantly from species to species depending on antiquity, gender, environment, and season (Human foodClucas and Ward 1996). According to (Rahman et al. 1995; Zenebe et al. 1998 and Fawole et al.; 2007).

Specifications and Standards for the year 2018, the average values of thenutritional analysis for different varieties of F of the analysis, and looking at the entirety of the published studies, as reportedin the scientific study published in the Sudanese Scientific Journal of values The results of the studies showed a clear variation and difference in theasseikh were as follows:

The percentage of moisture content in the different types of fassiekh was found to average 48.31%. This is due to the difference in the amount of added salt and accumulated in the muscles that affect the water activity (Aw).

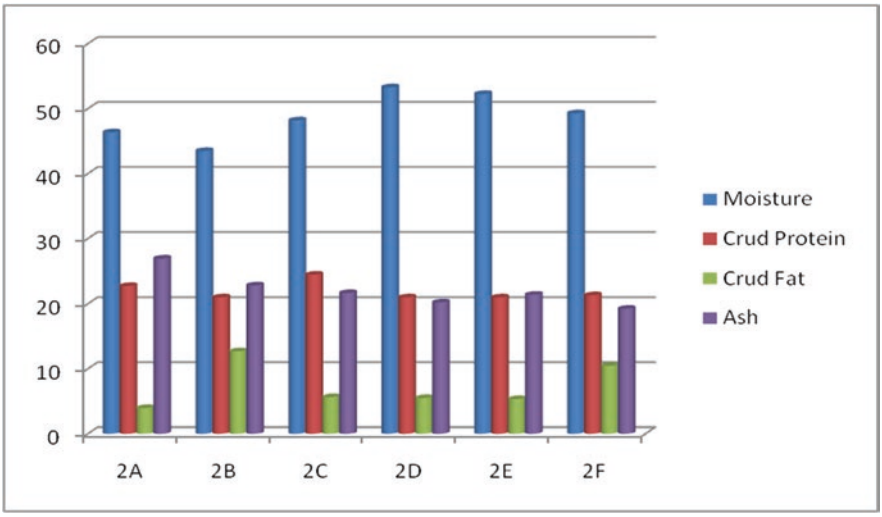

Fig. 14.1 Proximate analysis of fasseikh product

The crude protein of the various fassiekh varieties was found with an average value of 21.93%. Some microorganisms of processed salted fish enhance the nutritional value of produced salted fish (Bedos 1985). In Asia, some microorganisms have been used mainly to enhance the nutritional value of the fish product Zakhia and Kowa 1991).

Ash was an average of 21.55% in the different types of fassiekh, and this increase in salted fish may be due to the salting effect (Fig. 14.1).

The raw fat of the fassiekh varieties showed an average of 7.27%. There was significant ($p \leq 0.05$ in lipid content among the fas, possibly due to the different variety and size of the fish. Different fats from seawater and freshwater fish contain high levels of monounsaturated and polyunsaturated fatty acidsAlestes dentexsikh cultivars tested. The results of the study showed that the fat content was significantly higher in Kara.

It is also characterized by the presence of gamma-linolenic acid GLA) C18:3). The highest concentrations of Linolenic acid C18: 3 distinguish the cultivar (Schilbe intermeddius) with docosahexaenoic acid (DHA C22: 6.) (Suzuki et al. 1998) reported that taking docosahexaenoic acid (22: 6, N-3; DHA) is effective for improving learning ability2008) lower omega-, suggesting that salted fish fat may be a beneficial food source for maintaining human health. According to Simopoulos6 / omega-3 fatty acids are more beneficial in reducing exposure to many diseases of high popularity around the world. Moreover, lipids in dry fish products may play an effective role in improving plasma fat content and treating cardiovascular disease (Nordoff 2001) (Figs. 14.2 and 14.3).

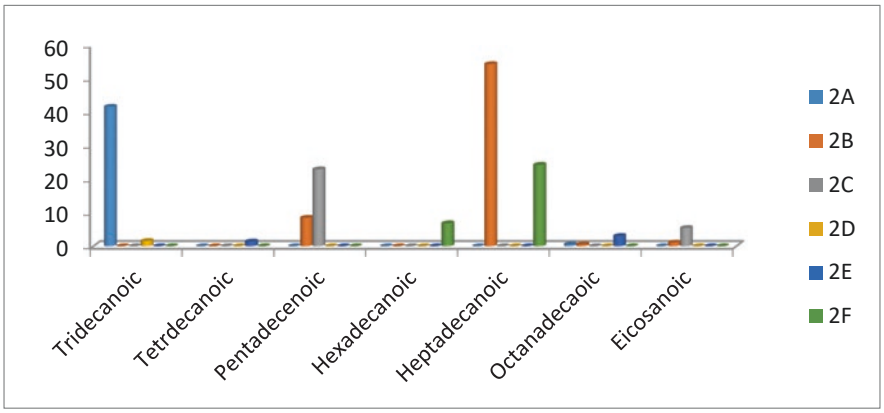

Fig. 14.2 Saturated fatty acids composition of fasseikh samples

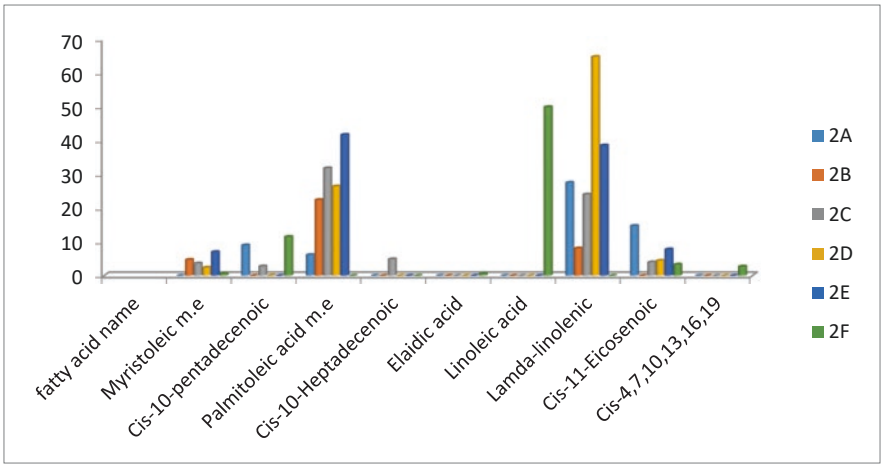

Fig. 14.3 Unsaturated fatty acids composition of fasseikh samples

As for minerals (major and minor), they are found in high proportions. The minerals content of fasseikh (Ca, K, Mg, Na, P, Co, Cu, Fe, Mn, Zn) were significantly (Figs. 14.4 and 14.5).

Note: All these figure show the results of analyzing six samples of fasseikh that were prepared in the area of El-Dueim (White Nile-Sudan) from different types of fish, including: *Hydrocynus forskalii* (Kass), *Alestes dentex* (Kawara), *Labeo niloticus* (Dabis), *Tilapias ssp* (Bulti), *Lates niloticus* (Ijle), *Schilbe intermedius* (Shilba). Osman et al. (2018)

2A ≡ *Hydrocynus forskalii* (Kass)
2B ≡ *Alestes dentex* (Kawara)
2C ≡ *Labeo niloticus* (Dabis)
2 ≡ *Tilapias ssp* (Bulti)

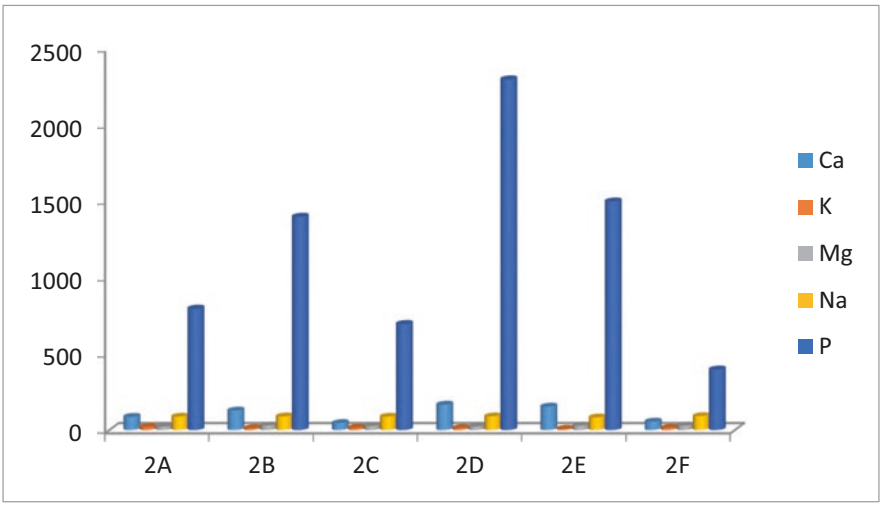

Fig. 14.4 Macro element determination of fasseikh samples

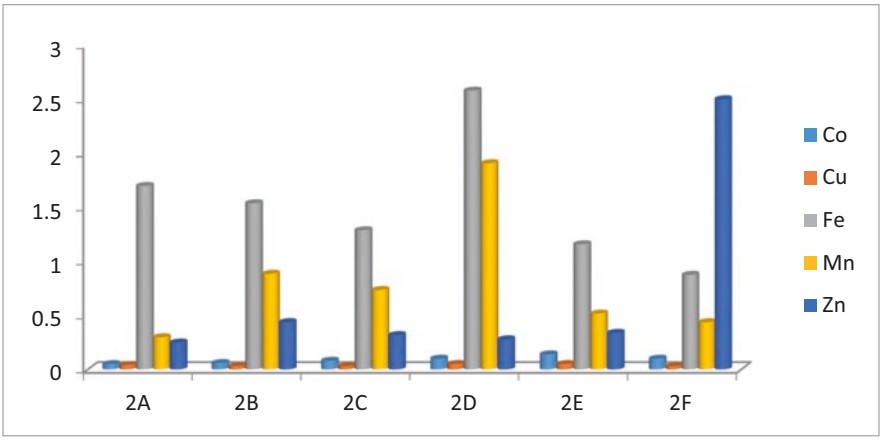

Fig. 14.5 Micro element determination of fasseikh samples

2E ≡ *Lates niloticus* (Ijle)
2F ≡ *Schilbe intermedius* (Shilba)

14.3 Microbiology

The presence of some microbes in a food product can determine its quality and alter its nutritional and sensory properties. Fish and their products can be damaged quickly.

Table 14.1 The Microbiological Characteristic of Fasiekh Samples

Sample	Total viable count of bacteria (aerobic) (cfu/g)	Total viable count of bacteria (anaerobic) (cfu/g)	Coliform MPN per gram		Yeasts and moulds (cfu/g)	*Staphylococcus*	Detection of Salmonella
			Total	*E. coli*			
A	$6.8 \times 10^{4}{}_{b}$	$2.3 \times 10^{4}{}_{a}$	44	6	N.G	$3.6 \times 10^{2}{}_{b}$	-ve
B	$1.4 \times 10^{5}{}_{a}$	$7.0 \times 10^{4}{}_{b}$	0	0	N.G	$1.2 \times 10^{2}{}_{b}$	-ve
C	$6.7 \times 10^{5}{}_{c}$	$2.0 \times 10^{3}{}_{d}$	0	0	N.G	$4.0 \times 10^{2}{}_{c}$	-ve
D	$1.5 \times 10^{6}{}_{c}$	$7.2 \times 10^{4}{}_{c}$	0	0	N.G	$5.6 \times 10^{3}{}_{c}$	+ve
F	$3.7 \times 10^{5}{}_{a}$	$1.6 \times 10^{3}{}_{a}$	0	0	N.G	$3.0 \times 10^{3}{}_{a}$	+ve

Means within the same column bearing the same letter(s) are not significantly different ($p < 0.05$)
A ≡ Fresh fish samples (unsalted)
B ≡ Dry salted Fasiekh samples (fresh)
C ≡ Paste Fasiekh samples
D ≡ Fasiekh samples in salted water (Routo area)
F ≡ Fasiekh samples packed in tin gallon container
N.G ≡ No growth

Microbiological characteristics of the collected fasseikh sample are shown in Table 14.1. The total bacterial (TBC) varied between $8.70 \times 10^{4} \pm 0.075$ and $6.83 \times 10^{6} \pm 0.161$. The highest total bacterial count could be due to improper handling and sanitary conditions during the preparation and moisture content. Similar results were obtained by other workers (Osman et al. 2012; Logesh et al. 2012; Abu-Hassan and Adam Sulieman 2011). Also shows that coliform The absence of coliforms in the different fasseikh samples could be attributed to the salt added during the processing. The *Staphylococcus* count ranged between $4.00 \times 10^{2} \pm 0.100$ cfu/g and $8.00 \times 10^{2} \pm 0.010$ cfu/g, and all collected fasseikh samples showed growth of this microorganism. (Ahmed et al. 2010) studied staphylococcus-micrococcus count in salted kass (*Hydrocynus forskalii*) fish during storage at ambient temperature (37 ± 1°c) they found ˂100 cfu/g. The highest count of *staphylococcus* reflects the poor hygiene of food handlers. According to (Sugumar et al. 2004) unhygienic handling is one of main factors contributing to poor quality of fish in the retails. Many workers reported that *Staphylococcus spp*. It was found in a large number in all over human skin and mucous membrane (Allen et al. 1997; Lamb et al. 1990; Duerden et al. 1992). Varnam and Evans (1991) mentioned that *staphylococcus spp.* can increase up to 5 log 10 cfu/g in food products prepared by hand under bad conditions. Vishwanath et al. (1998) reported that staphylococci grow best in salt and low water activity-containing foods whereas other microorganisms are in lower numbers. The presence of *Staphylococcus aureus* indicates contamination from the skin, mouth or nose of food handlers also contamination of processed food may also occur when contaminated food is processed on surfaces to which food products are exposed. Fasseikh contaminated with the *staphylococcus* toxin makes people sick, with nausea, vomiting and diarrhea usually appearing from two to six hours after eating the staphylococci infected food (O'connell 2002). The spore former bacterial

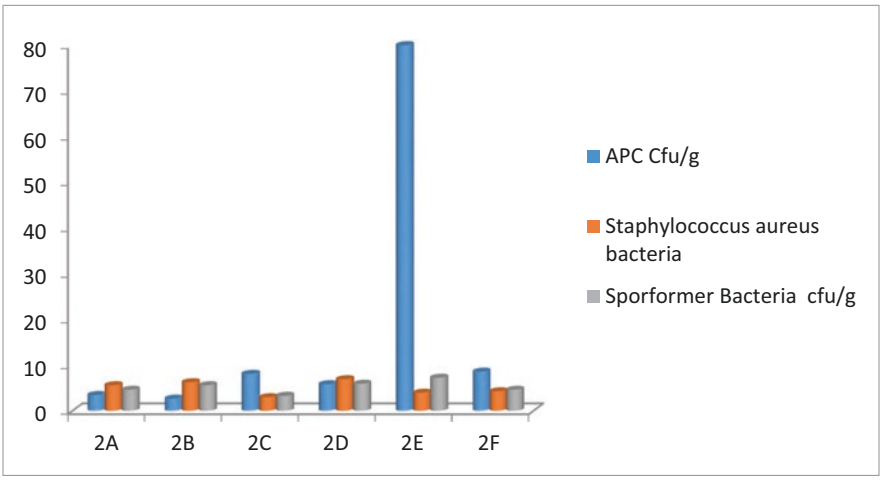

Fig. 14.6 Microbiological analysis of fasseikh samples

count ranged between $3.33 \times 10^2 \pm 0.006$ cfu/g and $5.67 \times 10^2 \pm 0.006$ cfu/g; there was no significant difference between all the samples.

Figure 14.6 shows the microbiological characteristics of the different fasseikh samples, the count of aerobic bacteria in samples 2B, 2C, 2A, 2E, 2F and 2D were $6.00 \times 10^2 \pm 0.026$, $5.67 \times 10^2 \pm 0.006$, $4.67 \times 10^2 \pm 0.015$, $4.00 \times 10^2 \pm 0.01$, $4.00 \times 10^2 \pm 0.01$, and $3.33 \times 10^2 \pm 0.015$ cfu/g, respectively. The high total viable count of aerobic bacterial load of fasseikh samples could be due to improper handling and sanitary conditions during the preparation and moisture content. The total viable count of anaerobic bacteria of fasseikh samples: 2C, 2D, 2F, 2A, 2E and 2B were $6.67 \times 10^2 \pm 0.015$, $6.67 \times 10^2 \pm 0.012$, $6.00 \times 10^2 \pm 0.01$, $5.67 \times 10^2 \pm 0.015$, $4.67 \times 10^2 \pm 0.015$ and $4.00 \times 10^2 \pm 0.01$ cfu/g respectively. Although these fish are from fresh water, they bore considerable numbers of halophilic bacteria. This close range of microbial count among these fish may be due to their living in the same environment. Freshly caught salt water fish, namely, mullet (*Mugialcephalus*) used in fasseikh making in Egypt, were found to contain 3×10^4 to 4×10^5 cuf as total viable count (Hamed et al. 1973). The result also, shows the absence of *Salmonella* in fasseikh samples 2C, 2D and 2F, while sample 2A, 2B, and 2E) showed positive result. The presence of *Salmonella* will create health risks to the fish consumers, and when consumed, can cause symptoms such as diarrhea, stomach pains, nausea and vomiting and stomach infections. In very serious case, it can cause death.

Yeasts and moulds cells were not detected in all fasseikh samples. Yeasts and moulds are responsible for food spoilage and produce mycotoxin (Buchanan and Doyle 1997).

In biochemical tests, both Gram-positive and Gram-negative bacteria were identified Staphylococcus spp., Micrococcus spp., Streptococcus spp., Bacillus

spp. Most Bacillus isolates were identified as (*Bacillus olvei- Bacillus cereus- Bacillus circulans*). And Streptococcus were dominant (*Streptococcus faecium- Streptococcus faecalis- Streptococcus bovis*) and Micrococcus were identified as (*Micrococcus roseus- Micrococcus varians*). Staphylococcus species predominated (*Staphylococcus epidermis- Staphylococcus aureus- Staphylococcus simulans- Staphylococcus xylosus).*

14.4 Sensory Evaluations

The quality of fermented fish is assessed subjectively by visual and/or organoleptic inspection. The main quality parameters are texture, colour, odour and fragility (Essuman 1992).

Texture
Fermented fish intended to be used as food fish is hard dried or semi- dried but firm. (Essuman 1992). The addition of salt affect the texture, and there are no significant differences between the different types of fasseikh.

Colour
The colour of product depends on the species of fish used as well as the processing method. For whole products such as fassiekh, a silvery appearance close to the fresh product is considered high quality. Becomes dark brown after weeks of exposure to the sun (Essuman 1992).

Odour
The odour of fermented fishery product varies from mild to very pungent. Soft, semi- dry products usually have a strong smell but very dry fermented fishery products have a mild odour. (Essuman 1992). on organoleptic quality (appearance, flavor, texture and acceptability) of fish fasseikh. The organoleptic properties of fish fasseikh were found to be affected by the type of fish. In particular, there are slight significant differences between the different fish species (Fig. 14.7).

14.5 Important Types of Nile Fish and Fresh Water Fish

Calf (Lates niloticus),) - Tilapia - Auchenog occidentalisLabeo coubie - Catfish (Clarias) - Hydrocyon - Alests - Hetrotis - Bagrus docma - Mormyrus niloticus SP - Bagrus bagrus - Molasses (Labeo) - Tark (Disichod niloticus) - Schilbe - Gargour (Synodontis) -) - um lip (Marcusenius sypsinoidis). Ministry of Industry and TradeCitharinus citharus - Koya bits (Tetraodon fahaka - Hyperopisus bebe -, Sudan Trade Poin (Fig. 14.8).

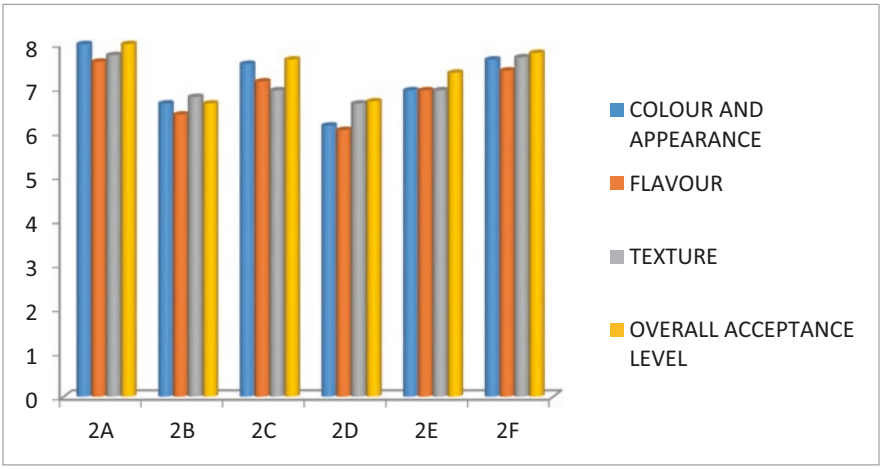

Fig. 14.7 Sensory evaluation of fasseikh meal processing

Fig. 14.8 Pictures explaining the fishing process (White Nile – El-Dueim area)

14.6 Fermentation

The primary goal of fish processing. Fish fermentation techniquestwo weeks in many African countries is to preserve fish and develop a desirable flavor. Usually fermentation, salting or drying is done in order to reduce water activity and inhibit or eliminate proteolytic bacteria and putrefaction. In Africa, the fermentation period is several days to vary from one region to another and there are three common methods in many African countries (Essuman 1992):

1. Fermentation by salting and drying.
2. Fermentation and drying without salting.
3. Fermentation by salting, but without drying.

The fermentation process begins with washing the fish and removing the internal viscera, and this is an essential stage in fish processing, then comes the stage of salting and fermentation, as the added salt is the controller of the ideal fermentation process, and not using it leads to creating an ideal environment for the growth of disease-causing organisms that pose a threat to the health of consumers The product may degrade within a short period.

14.7 Types of Fermented Fish in Sudan

Fermented salted fish without drying is called Fassiekh, It is also known by the same name in the Middle East (Campbell-platt 1987). It is afermentprodumore common in Sudan compared to other fish products. Commonly there are two types of fish used in fasseikh making in Sudan, *Hydrocynus* species (tiger fish) known locally as "Kass" and *Alestes species* (pebbly fish) known as kawara, both belonging to the family Characidae (Idris 1981). These two types of fish are considered the first class in the fassiekh industry*Schilbe intermeddius* and (Shilbaya) *Labeo niloticus*. The second category includes (Dabes). Indicated (Osm an et al. 2018 and Ibrahim, Mustafa; 2019) in their study of the use of other types of fish in the manufacture of fasseikh *Siluriformes*, (Garmoat) *Lates niloticus*, (Aigle) *Tilapia Zilliii*including (Bulti) *spp Mormyrus*and (Kasham-elbanat) *inloticus* respectively. And that these varieties were very acceptable to the participants in the sensory evaluation and they are all Nile fish.

14.7.1 Fassiekh Industry

Fish collected by fishermen The items used in the manufacture of fassiebank places prepared on the river kh are usually sorted and transported to the processing from local materials and are called 'Rakuba', Where the fish are placed on the ground in (plastic sacks) and the inner viscera is still filled with a little salt from the inside and then placed in layers above each other separated by the salt, then covered with a thick layer of sal layers oft, then covered by other palm leaf mats or sometimes jute sacks. In a few hours a solution of salt is formed in the liquid extracted from fish tissues due to the osmotic process, thus the salt is gradually absorbed into the tissues (Yousif; 1986) To maintain the salt concentration, add more salt when the salt layer covering the upper the fish surface ofhas vanished. The salted fish is thus allowed to ferment, on the fifth day, the salted fish are transferred to plastic containers (buckets) with some additional salt. Thus, the product is ready for consumption. This process is affected by seasonality, as it usually takes about 3–4 days in summer and 6–7 days in winter, as indicated by (Dirar; 1989) (Fig. 14.9).

Fig. 14.9 Pictures explaining the process of selecting, cleaning and salting fasseikh items, and the shape of the final product and packages

14.7.2 Terkin

Fermented fish paste) is one of the fermented fish products in Sudan, and the Sudanese fish product that falls into the category of sauces is known as turkin. In fact, it is very difficult to combine the Terkin only with sauces or pastes only, and it can be said at this stage that the Terkin is associated with sauces such as nuoc- mam, Nam-pla, and budu, as well as pastes such as bagoong, pra-hoc, and shiokara. Terkin itself is adongolawi, which is a complete pronunciation of the Donogla (Dongola people) language (Dirar 1993). Terkin usually bring young fish from the kass (*Hyrocynus spp*) and Kwara (*Alestes spp*) both type of fish (Nile carp, Labeo niloticus). The fish are placed in plastic bags and sprayed by a little salt, and then closed tightly. Left for a while until fermentation is complete, then placed in boiling water for a period of time. Minutes until it is cooked with constant stirring until it becomes in the form of a paste, then cooled, then 10% salt is added, then transferred to the burlap, then placed in a clean and sloping place. After the completion of the drainage, the mixture is transferred to a closed plastic barrel and left until it becomes fermented until the desired flavor appears. Usually 3–4 days in the summer and more time in winter.

14.7.3 Kejeik

Also called korki and hout, it is a product obtained by sun drying large fish. Fish Nile, Atbara River and the White Nile)Blue are first spilled asymmetrically along their dorsal axes before drying. Drying fresh fish (and meat) is the most widely used method of meat preservation, not only in Sudan but all over Africa. Kijik is produced in South Sudan by the Nile tribes: the Dinka, Nuer and Shulak. It was also

prepared in the northern part of the country along the Dirar 1993. Babiker and Dirar 1992,1993) gave data for kijik composition, moisture content ranging from 7.1 to 9.0%, protein 55.9–65%, fat 11.3–18.2%, ash 12.6–23.9%.

14.7.4 Mindish

The product comes from southern Sudan, and its production is dominated the Dinka in particular, especially that of the Bahr El Ghazal region. Generally, any type and size can be used, but the young Banat Jawf (Mormyrus spp.) Is usually the best due to its relatively high fat content Dirar 1993)). Fermentation improves the keeping quality of the fish, but in mindeshi's case, the dry state is the main factor for conservation. There are also some types of mindeshi that are made by fermenting a mixture of grain and fish flour Dirar 1993)).

14.8 African Traditional Fermented Fish Products

In most African countries, fish is fermented in a traditional, literal way, and it seems that the method of fermentation is the same in most countries with some small differences, which are three basic methods: fermentation by salting and drying, fermentation with drying without salting and fermentation with salting without drying. And one of the most famous fish fermenters in Africa:

14.8.1 Momone

A product of fermented fish famous in Ghana has a distinctive flavor. The way it is processed is similar to Lanhouin treatment. Usually the fish is used whole or cut into small pieces. The prepared fish is washed well and left overnight or salted immediately after washing and left to ferment for 3–8 days, after which the fish are dried on the ground or nets for a period of 1–3 days, the percentage of salt used is usually between 15–40% of the weight of the fish. It is salted once again ¾ of the weight of the salt used in the first salting. It is usually added when fermentation continues for more than three days. Of the types of fish used in its preparation: mackerel (*Caranx hippos*), Cassava croaker (*Pseudotolithus senegalensis*), Scad mackerel (*Caranx rhoneus*), and barracuda (*Sphyraena spp*). (Anihouvi et al.; 2012).

14.8.2 Lanhouin

It is one of the most common fermented fish in Benin. And the first steps in the preparation scratching and cutting the fish, washing and leaving for a period ranging between 10 and 15 hours, which is the period of ripening, and this step is important because it affects the smell and shape of the tissues of the final product after ripening. Fermentation The amount of salt in the first salting ranges between 20 and 30% of the weight of fresh fish and in the second salting it ranges between 15 and 25% of the weight of the salt used in the first start, then the salted fish are placed in containers or baskets and wrapped in bags of jute or buried in a pit and left until fermentation. This period ranges from 3 to 8 days depending on the environmental conditions. After fermentation, the fermented fish are washed to remove the residual salt on it, and then exposed to the sun for a period of 2–4 days until it is completely dry. There are many types of fish used for processing Lanhouin, but they are mainly used: Atlantic bumper (*Chloroscombrus chrysurus*), Cassava croaker (*Pseudotolithus senegalensis*), Crevalle jack (*Caranx hyppos*), kingfish/ Spanish mackerel (*Scomberomorus tritor*) and lesser African threadfin (*Galeoides decadactylus*). (Anihouvi et al. 2012).

14.8.3 Salanga

A fermented and dried fish product in Chad, with a strong aroma, cohesive texture and a light brown color, made from poor quality fish. The raw fish (*Alestes spp*) is washed and the larger fish are divided and opened dorsally. In the first stage, the prepared fish are dried immediately after washing or left to ferment for a period of 12–24 hours before drying. Usually salt is not used in treating slinga (Anihouvi et al. 2012).

14.9 Conclusion

Coastal African countries are characterized by fermented fish products, which differ in their manufacturing methods from one country to another according to cultures, social status and environmental conditions. Fermentation is practiced as a genuine, inexpensive, economic craft. The fermentation process plays an important role in supporting food security in African countries. Therefore, small traditional industries must be developed to ensure the quality of the final product according to international specifications.

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Chapter 15
Factors Influence the Quality and Safety of Fermented Sausages

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Abbreviations

EC	European Commission
FAO	Food and Agriculture Organization
IFPRI	International Food Policy Research Institute
ISO	International Organization for Standardization
SDS	Sudanese Standard
WHO	World Health Organization

15.1 Introduction

The international request on harmless food free from physical, chemical, and biological hazards (organic food) is on scaling in particular consequently the outbreaks of diseases such as cow-madness, Foot and mouth disease(FMD), Swine flu, Middle East Respiratory Syndrome (MERS) and Dioxin and dioxin-like compounds (DLCs) (WHO 2018). The world populace to be 8 billion people in 2020 (IFPRI 2020). This public overstatement winds up in citified areas, to some extent than rustic, which means that the demanding ramble in merchandise food leftover is in the developing world. Pointless, urbanization, and globalization of the market enumerate another threat in food manufacture and trading (Osman 2017).

Globally, meat expenditure: pork dominated the maximal (15.8 kg/capita/year), pursued by poultry (13.6 kg/capita/year), beef (9.6 kg/capita/year) consequent sheep and goat meat (1.9 kg/capita/year) (FAOSTAT 2014).There is an increase in global meat production expected to be 15% higher in 2027.The demand for cuts, ready to eat, easy to prepare, and distinctive meat products (FAO 2018).

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Fermented sausage, or dry sausage, is a type of sausage created by salting comminuted or ground meat and fatty tissues to remove moisture, while allowing beneficial bacteria to break down sugars into delicious particles. The bacteria comprise Lactobacillus species and Leuconostoc species, which breakdown these sugars to produce lactic acid, which not only affects the flavor of the sausage, but also decreases the pH from 6.0 to 4.5–5.0, which inhibits the growing of bacteria that can spoil the sausage. These effects are multiplied during the drying process, as salt and acidity are concentrated as moisture is extracted (Nikolic et al. 2020).Fermented sausages are meat products of high quality and are really valued among customers. Dry fermented sausages ought to contain under 30% moisture and over 20% meat proteins, while the collagen content in meat proteins ought to be under 15%.The fermentation process additionally has numerous advantages as it improves the practical worth of meat because of biochemical changes. Meat fermentation prompts the splitting of protein into peptides and amino acids (Kononiuk et al., 2020).

The technological process of production creation incorporates arrangement of the stuffing, filling into casings, smoking and drying, trailed by maturing that incorporates physical, chemical and enzymatic processes that empower shelf life and give tangible properties product Gazette of the Republic of Serbia, 2019) (Regulation on the quality of minced meat 2019) (Fig. 15.1).

15.2 Meat Term Contextual

Yardımcı (2019) declared that meat is the eatable part of any slaughtered animal, whether the same is in its crispy state that has been freezing, chilling, salting, canning, or other additives. For the utmost part, meat re-counts to skeletal muscle and combined fat and additional tissues. On the other hand, it may include the meat of mammalian (cattle, lambs) processed for human consumption, also represent other edible tissues such as offal.

15.3 Influence of Meat Product

Meat is critical in economy and development, even though its stack production and dissipation have been decisive to affect human health and the environment (Aberle et al. 2012). The same author reported that mammalian meat entails around 75% water, 19% protein, 2.5% intramuscular fat, 1.2% carbohydrates, and 2.3% other soluble non-protein constituents. The essential amino acids in meat are vital ingredients of proteins. On the other hand, plant food has no Vitamin B12, while animal food is indispensable for children to establish B12 deposits. Animal /food, in particular meat, are rich in iron, which is of utmost importance to prevent anemia, especially in children and pregnant women (Fig. 15.2).

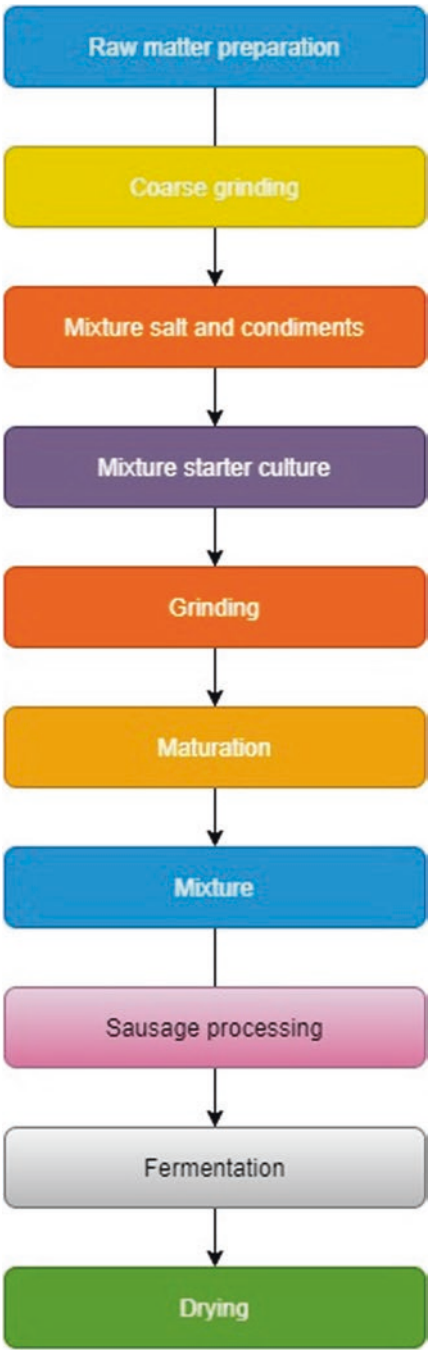

Fig. 15.1 Fermented sausage production flow chart

Fig. 15.2 Fermented sausage pictures. (Source: https://commons.wikimedia.org/)

15.4 Meat Quality

Meat quality is generally distinct by the compositional eminence (lean to fat ratio) and the palatability features such as appearance, smell, firmness, juiciness, tenderness, and flavor. The nutritious quality of meat expected by the customer is very subjective (Legako et al. 2015).

15.5 Meat Safety

The methodology of food safety is that food will not induce detriment to the customer when it is processed or consumed according to its stated use. In addition, it is correlated with the incidences of food safety hazards like: biological, chemical, or physical agent in food, or quality of food, with the likely to cause an awkward effect. (ISO 22000:2005 - E). The WHO implements five significant values for harmless food; avoid polluting food with pathogens dispersal from persons, animals, and pests. Isolate raw and cooked foods to stop contaminating the ready-to-eat foods. To kill pathogens by cooking food for a suitable extent of time and temperature. Store the food at the required temperature. Do use safe water and safe raw ingredients (WHO 2006).

15.6 Fermented Meat

According to Demeyerand Toldrá (2004), the development of fermentation is one of the hoariest procedures used for reservation of meat and meat products. The fermented meats are conserved products resultant of microbes with fats and salt, spices, herbs, and additional components (Zhao et al. 2011).

15.7 Fermented Sausage Classification and Categories

Rust, (1979) detailed that sausage is a cylinder-shaped meat product usually prepared from minced meat, regularly pork, beef, or veal, diversified with salt, spices, additives, and bread crumbs, sheathed by a skin. Generally, a sausage is shaped in an exterior conventionally prepared from the intestine, however sometimes prepared from artificial ingredients. Sausages that retailed raw are cooked in various ways comprising pan-frying, steaming, and roasting (Pearson and Gillette 1999; Acton and Dick 1975, 1976).

15.7.1 Categories of Fermented Sausage

Fermented sausages are a set of minced meat produced by microbial fermentation and have been subjected to drying/aging practice to eliminate 15–25% humidity. They are related to moisture amount dry or semi-dry and summer sausage Stanley and Adam (2012)). The same author mentioned that fermented sausages are produced from lean meat from pork and beef and mixed with fat, spices, salt, sugar, sodium nitrite (sometimes nitrate), and a starter culture. The combination is stuffed into natural or synthetic casings and expose to a fermentation procedure.

15.7.1.1 Dry Sausage

Dry sausage is an old-style dominant South African meat commodity the majority recognized is dry sausages. It contains a pH of 5.0–5.3, lactic acid 0.5–1.0% and an MPR of <2.3:1. The moisture loss is around 25–50%, and the final moisture percent is <35% water activity (aw) range between <0.85 to 0.91. The manufacture of ultramarine of dried or semi-dried sausage regularly includes a fermentation process (Charles et al. 1970). The same writer recounted that the preparation of dried sausage in South Africa, preliminary safeguarding to amount acquired by resources of the remedial constituents (about 0,8f sodium chloride in addition to seasonings such as coriander and cloves).

15.7.1.2 Semidry Sausages

Semidry sausages are stiff than fresh sausage, excluding not hard in material as a dry sausage. The *aw* approximately 0.90 and 0.95 (Demeyer et al. 2000).The American type dry sausages enclose 25–40% moisture, are greatly spiced, are not heated above 26.7∘ C, have a dense texture, and are frequently shelf-stable (Ricke and Keeton 1997).

15.7.2 The Requirement of Fermented Sausage

According to the Sudanese Standard for fermented sausage, a significant proportion of meat should not be less than 55% of the total mixed weight before cooking. The quantity of added fat should not exceed 25% of the total mix weight. The proportion of filler, extender, or binder individually or mixed should not exceed 10% of the total mix weight. The amount of ice or water added should not exceed 10% of the whole mix before cooking. The products should be cooled to the core temperature of (−1)°C to less than (5)°C or should be frozen at (−30)° to (−40)°C to a core temperature of (−18)°C (SDS 2017).

15.8 Factors that Affect the Sensory and Physicochemical Characteristics of Fermented Sausage

The organoleptic features of foodstuff, such as color, and flavour, are critical for consumer acceptance of fermented sausage(Lisa et al., 2016). Meat can also exhibit desirable and undesirable characteristics that can discourage or encourage fermentation.

15.8.1 Nature of Raw Meat

The fermented sausage must be of excellent quality with a negligible bacterial load. The required color of lean and fat is imperative, and the yellow fat is unpleasant while strong red lean desirable. The pH of raw tissue is also vital in the initial stage, and low pH is necessary (Campbell-Platt and Cook 1995**).**

15.8.2 Type of Food Additive

Clients and manufacturers are worried about synthetic food additives in particular foodstuffs to disguise or expand sensory features (Pokorný 1991a). The same author mentioned natural antioxidant, like rosemary, has some detriments like their impact in a sensual quality instant: color, after- taste or off-flavors. Though, the use of additives in fermented sausages can advance sensory appearances. Customers generally believed on fresh (fermented) sausages prepared of sheep and goat meat, with an average of 6 on a scale of 10. No noticeable favorites detected for sheep, goat, or flavor, used to cover some offensive appearances as the palate, odor, and taste (Leite et al. 2015).

15.8.3 Storage Interval and Temperature

According to many authors, Baker et al. (1972[b]); Beckert (2002) Osman et al. 2020 and Pham et al. 2013), the storing temperature is a standpoint for meat and meat foodstuffs production. The chilling storage expressively decreased scores of color in the semi-dry fermented sausages (Ahmad and Amer 2013). Hussein et al. (2017) reported that the flavor scores lessened significantly in all tasters of semi-dry fermented sausage inoculated with *L. casei* (range 6.83–5.63) during storage for 45 days at icy temperature. Osman (2017) suggest that despite food stored according to principle (first in -first out), it will lose the color, texture, flavor, and nutritional quality and become a substandard product.

15.8.4 Type of Culture

Gilliland (1985) submits that the necessities for meat starter culture: non-pathogenic, phage-resistant, free from any microbial or chemical residues that may cause healthiness risk or limit manufacturing, salt-tolerant, and matures fast in a 6% brine, grows well in the presence of 80–100 ppm nitrite, has a growth temperature range from (80 to 109 °F), produces lactic acid from dextrose. A worthy starter culture must also be non-proteolytic. Probiotics are microorganisms mostly related to the strains Bifidobacterium and Lactobacillus ssp., used for human and animal feeding to improve their healthiness (Hempel et al. 2012). The distinctive flavor of fermented sausages mainly creates from the itemization of carbohydrates, lipids, and proteins by microbial and meat enzymes (Ahmad et al. 2012). The starter cultures guarantee food safety and supercilious quality characteristics such as sensorial, nutritive, and technical features. The use of dissimilar starter cultures in the manufacturing of goat meat fermented sausages formed an average value range between

5.5 and 5.9 for comprehensive sensory adequacy, using a 9 points hedonic scale (El-Adab et al. 2014).

15.8.5 Effect of Spices

Flavorings used in fermented sausage for many purposes instant: taste, an antioxidant, improving the progress of lactic bacteria. They can result in a faster rate of lactic acid production and also influence the concluding pH. Particular spices reported having an interesting consequence on fermentation like pepper, mustard, garlic, allspice, nutmeg, ginger, mace, cinnamon (Zaika et al. 1978)**.**

15.8.6 Influence of pH Drop

Dry fermented sausages include two groups with dissimilar acidity, specifically northern-type and southern-type products (Ravyts et al. 2012). The pH in the northern-type drops below 5.0 throughout fermentation and remains more or fewer at that level during ripening. On the other hand, the pH in the southern-type drops only 4 69 moderately during fermentation and increases during the drying phase, resulting in a final pH of 70 between 5.5 and 6.0 (Demeyer et al. 2000). The rate and extent of pH decline influence both sausage texture and gel formation. Saltsolubilizes muscle proteins during comminuting, which later denature due to lower pH (5.3 with 3% salt, (Solignat and Durand 1999). The release of moisture causes coagulation and forms a gel around fat and meat particles. The proteolysis is releasing enzymes from microbial origin. This procedure is of elementary significance since it affects dry fermented sausage taste. The resultant miniature peptides and amino acids, also considered as substrates for microorganisms that renovate them into taste compounds.

15.8.7 Influence of Smoking

Smoking is an old-style usage of Northern-type fermented sausages (Sikorski and Sinkiewicz 2015). It is advantageous to various sorts of semi-dry sausages throughout the dehydrating stage to increases a required smoky taste to delay lipid oxidation and improves color (Price and Scweigret 1987). There is significant progress in the color of the fermented sausage once it is smoked. The interaction among carbonyls and amino groups is comparable to the Maillard reaction and is improved due to the deposit of these compounds on the surface and diffuses inward cause a dark red color (Tóth and Potthast 1984).

15.8.8 Rancidity Development

Approximately 36–40% of the total calories in the food supply originated from fat, half of which is from meat intake. The unsaturated fatty acids are entirely involved in oxidative deviations in the fat of fermented sausages. Hydrogen peroxide and hydrogen sulfide can cause off-color and taste problems (Sheard et al. 1998; Ruiz et al. 2012). Proteolysis and lipolysis usually improve flavor. The same author suggests a particular inherent such as nature of fat in the diet, others extrinsic instant light, temperature, impairment to muscle structures caused by freezing temperatures, and grinding. Judge et al. (1989) mentioned that polyunsaturated fatty acids are significantly predisposed to out-oxidation than monounsaturated or saturated fatty acids due to high moisture. The elimination of fermented sausage fat affects the sensual and technical appearances since fats donate intensely to flavor, texture, and overall acceptance. (Kamal Eldin 2010). Integrating hydrophobic oils can be demanding as meat comprises 75% water and is hydrophilic. Likewise, increasing the content of unsaturated fatty acids increases the potential of lipid oxidation, which shortens the shelf-life. The progress of oxidative rancidity in meat is pretentious by several features (Enser 1974; Morrissey et al. 1998).

15.8.9 Salt Content

WHO (2016) stated that salt provides many functions in fermented sausages, instant: taste, texture, microbiological safety, and overall acceptability. Sodium chloride is an additive that will permit lactic acid bacteria to cultivate and will constrain several undesirable microbes. Fermented sausages comprehend a high salt amount, which subsidizes the bacteriological safety and shelf-life by making water inaccessible for microbes. Also, it influences the industrial quality of the sausages by enabling the proteins to expand the texture and raise the viscosity. Furthermore, potassium ions can afford an improved flavor. No deviations in organoleptic physiognomies of fermented sausages noticed after KCl replacements intended for NaCl were lesser than 40%. An insignificant lessening in the aroma detected when adjudicated the sausage control by 2.7% NaCl (Gelabert et al. 2003; Corral et al. 2013).

15.8.10 Drip Loss and Moisture Defeat

The sensual qualities of fermented sausages depend on the degree of dehydrating of the product. A significant factor after drying sausage is that the amount of moisture loss from the superficial of the product must be equivalent to the rate at which moisture transfers from the sausage's inner (Ambrosaidis et al. 1994). The same author reported that speedy drying is only likely when the pH is low, and the respectively

low solubility of proteins permits moisture downfall. The drying of sausages is occurring at low temperatures, usually less than 20C. Drip loss is a critical influence in meat production, at customers' desire for meat truancy of drip loss favored in many countries. Barringe et al. (1995) and Alizadeh et al. (2007) re-counted that drip loss is a distinct concern in the packed meat for reducing temperature instability. Meat with high drip loss, because of the release of water, might be stiff and declined juicy and, subsequently, none putative by clients.

15.8.11 Effect of Packaging Materials

Modified atmosphere packaging (MAP) is a very substantial and efficient conservation procedure practiced for prolonging the shelf life of food, particularly for fresh or emporium manufactured foods. The MAP extends the shelf-life of meat products by 50–400%, specifically at chill temperatures (Action et al. 2000). Smolander et al. (2004) mentioned that MAP beef steaks were favored by customers in as much as of their increased tenderness and juiciness in comparison with steaks packaged in traditional tray packaging. Jeremiah et al. (1992). The expenditure of meat package in the atmosphere has <0.5% CO merely consequences in an insignificant quantity of carboxyhemoglobin incoming into the bloodstream, and it is unlikely that the CO will cause a toxic hazard to customers (Vainionpää et al. 2004).

15.9 Factor Affecting the Safety of Fermented Sausages

According to Redman, (2007), food-borne contamination and poisoning are grave problems for human healthiness. Osman et al. (2007) stated that The HACCP system ranks hazards into three domains: biological, chemical, and physical hazards. In the past, fermented sausages were considered non-hazardous foods. The consumption of fermented sausages nowadays may show health threats due to the high contents of saturated fats and NaCl, nitrite, and degradation products such as nitrosamines, toxic compounds as a by-product of smoking instant: polycyclic aromatic hydrocarbons in the products (Lucke 1998).

15.9.1 Potential of Physical Hazards

A physical hazard defined as material not frequently originated in a portion of food creates sickness or injury to an individual consuming the product. It caused risk for a limited number of consumers (Osman et al. 2007). Instances of familiar physical dangers: bones and plastics packaging materials (Osman 2017)**.** The same author mentioned that it appears from various points of supply polluted raw materials,

beneath par designed or maintained facilities and equipment and imperfect procedures during processing, and unfitting employee training and manner.

15.9.2 Potential of Chemical Hazards

Chemical hazards may also induce foodborne illnesses, despite the fact generally affecting minority people. Chemical hazards can originate from four general sources: Accidentally added chemicals like Agriculture chemicals (pesticides, herbicides, animal drugs, fertilizers). Environmental contaminants (lead, cadmium, mercury, and arsenic). Naturally-occurring chemical hazards products of plant, animal, or microbial metabolisms such as aflatoxins. Intentionally added chemicals such as preservatives and food additives (Osman 2017).

15.9.2.1 Nitrates and Nitrites

According to the International Agency for Research on Cancer (IARC) nitrates and nitrites are doubtless carcinogenic to humans in some circumstances conditions approving nitrosation where an NO group is covalently bound to carbon, sulfur, oxygen, or nitrogen atoms in a carbon-based molecule. Throughout curing in an acidic atmosphere, disassociated nitrous acid picks up a hydrogen ion and splits off a water molecule. The consequential positively stimulating nitrosonium ion might then react with amino groups to form N-nitrosamines. Some of these N-nitrosamines are carcinogenic (Andree et al. 2010).

15.9.2.2 Hydrocarbons Compounds

A number of hydrocarbons formed in smoke process are hazardous to human health, particularly, the polycyclic aromatic hydrocarbons (PAHs).These hydrophobic compounds include two or further bonded aromatic rings, primarily of hydrogen and carbon atoms. Complexes with four or more rings are less volatile and adsorb on soot and other incineration particles (Singh et al. 2016).

15.9.3 Biological Hazards

Reviews have revealed bacteriological Hazards related to fermented sausages include: Escherichia coli, Salmonella Typhimurium, Staphylococcus aureus, and L. monocytogenes in dry fermented sausages (Pierre 2015).

15.9.3.1 Resistant Bacteria

The increasing resistance to antibiotics in bacteria dowries grave anxiety to human and animal health and presents economic and communal charges. Antibiotic resistance (AR) in food microbes is of worry since they may act as reservoirs for AR genes. This rise may indicate the misapplication of antibiotics in animal farming for years, foremost to the great puddle of AR genes existing in the bacterial populace, dispersal likewise to bacteria in the food sequence (Fraqueza 2015).

15.9.3.2 Biogenic Amines (BAs)

High levels of biogenic amines (BAs) may occur in fermented meat products (EFSA 2011). The contented of BA in the food is a signal of spoilage or hygiene malpractice in the management of the uncooked material (Latorre-Moratalla et al. 2012). According to the same authorthe utmost importance (BAs) existing in fermented sausages of food safety apprehension aretyramine, phenylethylamine, and histamine, with tyramine regularly being the greatest plentiful.

15.9.3.3 Histamine Producers

Histamine inducers are uncommonamongst sausage LAB and histamine, onceat hand in sausage, may be formed by essentiallytoxinenterobacteria (Molenaar et al. 1993).On the other hand, definite strains for instant, *L. buchneri* and *L. parabuchneri* harbor the histidine decarboxylase enzyme and are considered spoilage organisms in cheese (Wuthrich et al. 2017).

15.10 Conclusion

Food quality means that all the requirements set and concerned with the characteristics and recipes of food have been fulfilled (related to taste, smell, appearance, nutritional value and microbial load), while food safety means that all the requirements that are set and concerned with food safety have been met and that the causes of food pollution (physical, chemical or microbiological) has been excluded or controlled. Many factors can influence quality and safety of fermented sausages. However, if these factors are monitored this will enhance consumption of these products and make benefits of their high nutritive value.

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Chapter 16
Technology of Fermented Mango Juice Production

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Abbreviations

%	percentage
° C	Celsius degree
g	Gram
g/l	gram per liter
Kcal	Kilocalorie
me/Kg	milliequivalent per kilogram
me/L	milliequivalent per milliliter
mg	Milligram
mcg	Microgram
IU	International Units

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16.1 Introduction

The fermentation seems to be appeared in Iarq with cheese fabrication in the period 8000–6000 BC. She was largely used as means to preservation and transformation foods. Later, in the period 4000–2000 BC, Egyptians discovered how to use yeasts to make leavened bread and wine (Ross et al. 2020). Today, a large variety of foods obtained through fermentation are consumed in the world and have the good reputation regarding their organoleptic and sensory properties, including cheese, yoghurt, kefir, beer, kombucha, pozol, sourdough, kimchi, olives, sauerkraut, pickles, tempe, gari, fufu or sausages (Tamang et al. 2016).

Fruit consumption has a recognized health benefit which may be associated with the antioxidant and nutritional potential of fruits. It is the mango (*Mangifera Indica L*) case which, with the chemical composition of the pulp, her consumption is highly recommended in cases of skin and retinal diseases, arteriosclerosis, high blood pressure and diabetes (Pamploma 2007). Mango is more nutritious than most fruits in temperate countries (FAO 1993). The recommended daily intake of 5 servings of fruit however seems difficult to achieve. Some of the difficulties commonly mentioned by consumers in achieving the recommended intake of the fruits are their high prices, seasonality, fragility, and low shelf-life. Drinking of fruit juices, is an attractive alternate means of intake of fruits to obtain the desired nutritional and health benefits. Indeed, with fruit consumption, a growing market is developing around fruit juices with new tastes and high nutritional values.

However, after the manufacturing, the fruits juices are confronted with a problem of stability and this until it reaches the table of the consumer, the fruity beverage under goes various types of changes which directly influence the nutritional and organoleptic qualities. In general, the technology of fruit juice preservation is thermal processes that require energy and action of the temperature/time pair (Baron 2002; Aymerich et al. 2008). These thermal methods have drawbacks such as such their energivorous character and the destruction of aromatic compounds due to their volatile nature thereby greatly reducing the nutritional and organoleptic quality of the juices. In addition, these thermal methods require considerable investment in special equipment (Branyik et al. 2010). Thus, the biological method through fermentation with the use of non-*Saccharomyces* yeasts remains an ecologically and economically viable solution. The most wide spread biotechnological approaches rely on the limited or almost zero formation of ethanol by the yeast during fermentation. Limited fermentation is generally carried out in equipment which requires no additional investment. Also, the fermentation brings about the reduction of sugar content and the intensification of flavor (Romano et al. 2003). In addition, the pectinolytic activity (polygalacturonase) of microorganisms involved the fermentation of fruit juice contributes to clarification thereby avoiding a supplementary operation in the production process (Thakur et al. 2010). Use non-*Saccharomyces* yeasts strains appear as the suitable tool to carry out the fruit juices fermentation. In according to

Renouf (2006), "Non-*Saccharomyces*" yeasts include all yeasts with the exception of *Saccharomyces* species and constitute a large and very diverse group. These non-*Saccharomyces* yeasts have high potential impact on biotechnology, namely a significant aromatic contribution and an inhibition of undesirable microorganisms (Bonilla-Sallnas et al. 1995; Renouf 2006). In this review the fermented mango juice production process will be explained.

16.2 Botanical Origin of Mango

The mango tree, *Mangifera indica*, is come from to a region on the Indo-Burmese border. The mango tree can grow up to 30 meters tall and is adapted to a wide tropical climatic range with highly variable annual rainfall and can grow on a wide variety of soils. Optimal temperatures for tree development and fruit growth are ranged between 24 and 30 °C (Fruitrop 2009).

The shape of the fruit is the basis of the paisley pattern. The mango is a fleshy fruit, weighing from 300 g to 2 kg. It is a drupe, its flesh adheres to a large, flat and slippery pit. It can be round, oval or kidney-shaped and has a rind that can be yellow, green or red in colour, which must be removed as it contains irritants and is therefore inedible. The flesh is dark yellow, smooth, fatty and sweet with a false taste of peach and flower. Depending on the variety or when the fruit is overripe, the flesh sometimes becomes stringy. (Fréhaut 2001).

It is also characterized by the presence of lenticels on the entire surface of the skin. The pulp adheres to a large, more or less flattened core and is essentially made up of parenchymal cells (Fig. 16.1). By selection, the mesocarp is nowadays not very fibrous.

Currently, mango is present throughout the intertropical zone and to a lesser extent in the Mediterranean region (Egypt, Spain, Israel, etc.) (Braz 2004). It is found throughout South-East Asia, West Africa, Hawaii, and in all lowlands of Central and South America.

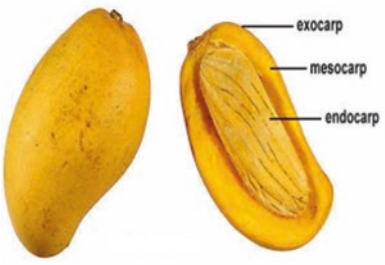

Fig. 16.1 Diagram of mango fruit (Fruitrop 2009; website: http://waynesword.palomar.edu/ecoph9.htm)

16.3 Mango Varieties

More than thousands varieties of mango has been recensed in the world. In terms of the characteristics of the fruit, the differences between these varieties are ranged taste, colour of the flesh and skin, size, format, amount of fiber, resistance to transport and storage, resistance to disease and insects, etc. In many countries, mango is plays an important in the diet of people. Originally, there were two main families of mango, with very different characteristics, which come from two diversification basins, the Indian sub-region and tropical Asia. Currently, several commercial varieties were selected in Florida in the early twentieth century from multiple hybrids using progenitors from these two families. The fruit exported is generally from grafted plants. The main mango varieties are: Tommy Atkins, Kent, Keitt, Osteen, Haden, Valencia Pride, Kumquat, Lime, they are practically present in all regions of the world (Rey et al. 2004).

In Africa, particularly in West and Central Africa, the *Nunkourouni mango* variety characterized through fibers and polyembryos. Her name depended of region where is cultived, example of Sierra Leone to Senegal, she is called German mango, in Adamaoua, Cameroon it N'gaoundéré mango, In Côte d'Ivoire her name is *assabonou.*

16.4 Biochemical Composition and Nutritional Value

The mango is very rich in water more than 80% and polysaccharides but poor in protein and lipids and caloric value is to 56 Kcal for 100 g of fruit. The content of biochemical composition and value nutritional is indicated in Table 16.1. However, these values varying with varieties. In according Lakshminarayana et al., (1970), the starch rate contained in skin and pulp increases continuously between the phases of fruit formation and harvest maturity. On the hand over, Silva et al. (2008) reported that the starch content decreased during ripening under action of some enzyme as β-amylase while the soluble sugars content increases. For example, for Badami variety Shashirekha and Patwardhan (1976) demonstrated an increase in glucose (420–4200 mg/100 g), fructose (560–4300 mg/100 g) and sucrose (16–4400 mg/100 g) during ripening. Moreover, mango fruit is appreciated for its richness in antioxidants (vitamin C, carotenoids and polyphenols) and minerals (calcium and potassium) (Table 16.1).

Table 16.1 Mango pulp composition (USDA, Nutrient Database for Stanadrd Référence 2001)

Constituent	Amount in 100 g fresh pulp
Water	81.7 g
Energy	65 kcal
Protein	0.51 g
Fats	0.27 g
Carbohydrates	17 g
Total dietary fiber	1.8 g
Ash	0.5 g
Minerals	
Calcium	10 mg
Iron	0.13 mg
Magnesium	9 mg
Phosphorus	11 mg
Potassium	136 mg
Sodium	2 mg
Zinc	0.04 mg
Copper	0.11 mg
Manganese	0.027 mg
Selenium	0.6 mcg
Vitamins	
Vitamin C (total ascorbic acid)	27.2 mg
Thiamine	0.056 mg
Riboflavin	0.57 mg
Niacin	0.584 mg
Pantothenic acid	0.16 mg
Vitamin B_6	0.160 mg
Total folate	14 mcg
Vitamin A	3894 IU
Vitamin A	389 mcg RE
Vitamin E	1120 mg ATE
Tocopherols alpha	112 mg
Lipids	
Total saturated fatty acids	0.066 g
Total monounsaturated fatty acids	0.0101 g
Total poly unsaturated fatty acids	0.031 g
Cholesterol	0.00 mg
Amino acids	
Tryptophan	0.008 g
Threonine	0.019 g
Isoleucine	0.018 g
Leucine	0.031 g
Lysine	0.041 g
Methionine	0.005 g

(continued)

Table 16.1 (continued)

Constituent	Amount in 100 g fresh pulp
Phenylalamine	0.017 g
Tyrosine	0.01 g
Valine	0.026 g
Arginine	0.019 g
Histidine	0.012 g
Alanine	0.031 g
Aspartic acid	0.042 g
Glutamic acid	0.06 g
Glycine	0.021 g
Proline	0.018 g
Serine	0.022 g

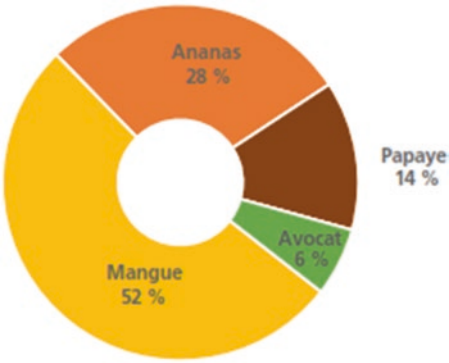

Fig. 16.2 Main Tropical Fruits: production volume by type in 2018

16.5 Mango Production

In according to statistics from the FAO's Intergovernmental Group on Tropical Fruits, world mango production increased by an average of 3.03% per year from 2008 to 2018, from 38 million tons to 52 million tons. In terms of production volumes, the mango has maintained its position as the dominant tropical fruit, due to its popularity in India, where an estimated production to 38% of production worldwide. The world's mango production accounted for more than half of the total world production of the main tropical fruits in 2018 (Fig. 16.2) (FAO 2020). The African continent is the second largest producer of mangoes after Asia. Its production increased by 1.07% from 3.9 million tons in 2008 to 8.2 million tons in 2018. Production in Central American and Caribbean countries has increased slightly, from almost 2.8 million tons in 2008 to 3.3 million tons in 2018. It is concentrated in Mexico, followed by Cuba and Haiti.

16.6 Mango Varieties Used for Juice Production

Several mango varieties exists but all varieties are not used to produce the juice. To be used for juice produce mango must be to satisfy some criteria. Indeed, in Burkina Faso, Traore et al., (2017) reported that the varieties *Amélie, Brooks, Kent, Lippens* and *Springfield* were most transformed. The criteria of these choices were the availability, use easily and growing demand. Also, the pulp must be very homogeneous, of creamy consistency, with a colour ranging from greenish white to orange-yellow. In Congo, an improved variety mango called "Boko" had been used to produce the juice. In fact, the mango "Boko" presented physical and organoleptic characteristics very special particularly taste and aromatic qualities compared to other mangoes (Diakabana et al. 2013). On the hand over, others varieties as *Alphonse, Badami, Lamy N°2, Peter Pasand, Taymour* have been reported in mango juice production in Senegal (Vallet 1965). The Fig. 16.3 illustrated some mango varieties used in mango juice production.

A: Amelie variety; B: Lippens variety; C: Kent variety; D: Brooks variety; E:Springfield variety (Traore et al. 2017).

16.7 Mango Transformation Situation in Developing Countries

World production of mango pulp is estimated at 700000 tons per year, half of which is produced in India. The country consumes around 150,000 tons per year and exports some 200,000 tons. Processing should become a key step in adding value to mango in developing countries, where crop losses regularly exceed one-third of

Fig. 16.3 Some mango varieties used in transformations

production and the subsequent loss of earnings are significant. To date, mango processing remains a marginal activity, using less than 2–5% of the harvest, and includes the manufacture of dried mango, juice, nectar, mango vinegar and jam. Processing takes place in artisanal units (women's groups), semi-industrial units, and very few industrial units. Two main factors that determine this situation are the short harvest seasons, with peaks from May/June to July/August depending on the country and variety, and the lack of possibilities to supply processing units with other fruits outside the mango season. Other factors include the lack of an organized circuit for supplying processing units with quality mangoes at acceptable and non-fluctuating prices; the lack of knowledge of the demand for processed mangoes on national, regional and international markets, as well as the absence of conservation infrastructures and techniques, adapted processing technologies and know-how of operators (ECOWAS 2011).

16.8 Steps of Mango Juice Production Fermented

16.8.1 Mango Juice Production

The mango juice production fermented consists two steps: firstly the mango juice production and the secondly the fermentation. The mango juice is the unfermented filtrate from the previously crushed mango pulp. The mango juice retains the physicochemical, nutritional and organoleptic characteristics (flavors) of the mango variety from which it is extracted. Consumed fresh, the mango juice, rich in carotene, vitamins C, B1, B2 and minerals, is an excellent beverage for people of all ages (FIRCA 2014). The steps of mango juice production are presented in Fig. 16.4. Once produced, the raw juice, which comes from the crushing of the pulp, is in reality a homogenized pulp, because the pieces are so fine in texture that they give up little more than a few fibers. The result is a homogenized cream that is pleasant to taste but impossible to drink. Attempts at partial clarification, by filtration or enzymatic action, have failed. Rapid centrifugation gives only an incomplete separation of 10 to 20% of the supernatant liquid in 15 or 30 minutes; heating to boiling does not precipitate anything and filtration after heating is no faster; similarly, freezing has not destroyed the structure of the suspension (Vallet 1965).

The addition of water gives a nice looking drink, as the decantation of the pulp is very slow. In addition, to obtain a pleasant drink, it is necessary to sweeten and acidify. Indeed, the dry extract of the pulp is around 15%, and its acidity is low (35 me/kg), so that the addition of 3 volumes of water would give a tasteless drink, it is necessary to bring the sugar content between 100 and 150 g/I and the acidity around 100 me/l in the finished drink. The simplest way is to prepare the solution in advance by adding sucrose and citric acid in suitable proportions (Vallet 1965).

On the hand over, pressing alone does not allow for maximum juice extraction, which represents a financial loss for the industry. In addition, some fruits such as

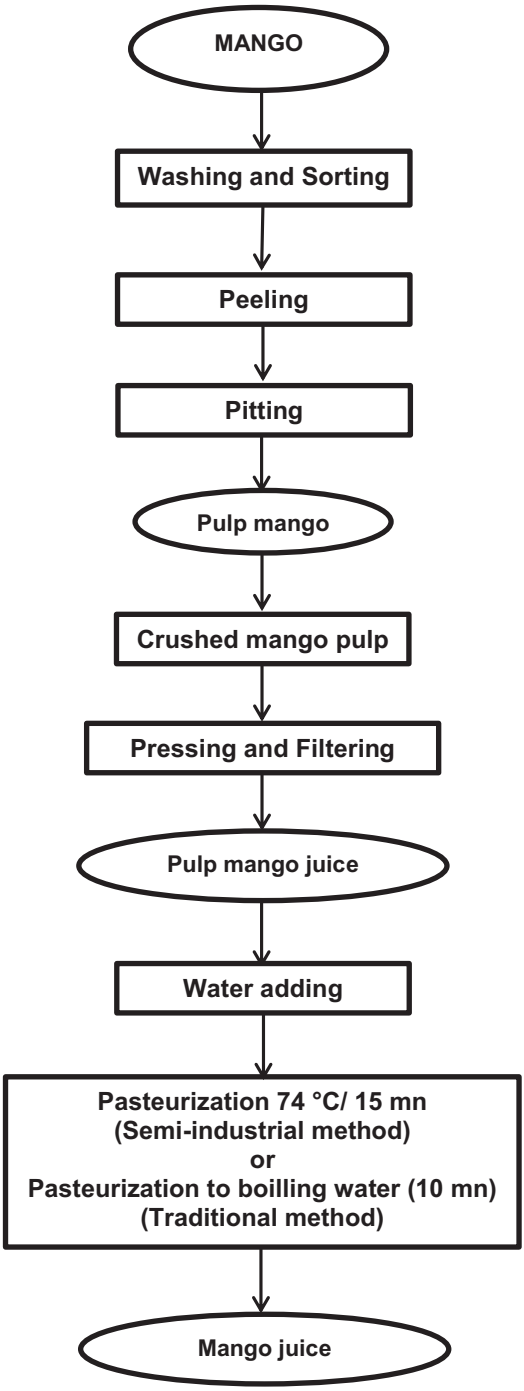

Fig. 16.4 Mango juice production diagram

mango and plum are poorly suited to pressing (Chang et al. 1995; Chauhan et al. 2001; Will and Dietrich 2006). Further research led to the first application of pectolytic enzymes in this technology for apple juice clarification in 1930 (Mehlitz 1930). For juice extraction, it is necessary to act upstream of pressing by physical and/or enzymatic actions in order to increase the yield by facilitating extraction through increased destructuring of the fruit, and/or downstream to remove cloudiness and obtain clarified juices.

16.8.2 Mango Juice Fermentation

The mango juice fermentation can to carry out of two methods, the spontaneous fermentation and the controlled fermentation. La spontaneous fermentation is carried out through the microbial presents in environment and material raw during 5 to 7 days. The problem of this fermentation it the possible present of undesirables microorganisms. Also the high content of sugar in mango juice can give the alcoholic fermentation. For this fermentation, the mango juice is not pasteurized to preamble.

Few studies were focused in mango juice fermentation, however, Coulibaly et al. (2019) were studied the mango juice controlled fermentation by non-*Saccharomyces* yeasts species using. Indeed the non-*Saccharomyces* yeasts are recognized for their contribution to the aromatic quality than for the formation of alcohol during fermentation (Romano et al. 2003). Also, Coulibaly et al. (2019) reported that mango juice fermented with *Hanseniaspora. jacobsenii* had the lowest ethanol content 1.2 ± 0.08% (v/v). In according Arendt et al. (2018), beers produced through non-Saccharomyces strains as *Hanseniaspora valbyensis, Hanseniaspora vineae, Torulaspora delbrueckii, Zygosaccharomyces bailii and Zygosaccharomyces kombuchaensis* species isolated from Kombucha a non-alcoholic medium, have low the ethanol content between 0.34 and 0.5% (v/v). Also, *Pichia kluyveri* strains were used to produce an alcohol-free beer (0.1% v/v alcohol) and a low alcohol beer (0.7% v/v alcohol) (Saerens and Swiegers 2017). However, some studies were focused in alcoholic beverage from mango juice for production of wine and beers (Carle 1924; Vallet 1965; Diakabana et al. 2013).

Also, INRA (Institut National de Recherches Agronomiques) has developed a processing to produce fermented fruit juice which remains applicable to mango. These drinks are obtained by fermenting aqueous extracts of fruits (macerations) or other aromatic plant resources, in the presence of a yeast strain added to control fermentation. This type of drink is traditionally made within the family from macerations in water of fruit remains: pineapple, cytherea, etc. This practice is common in Martinique and makes it possible to make the most of the fruit harvested from domestic gardens and orchards. It produces aromatic, sparkling and refreshing drinks that can be kept for a few days in the refrigerator. The process developed by INRA in 1990 makes it possible to produce in a controlled manner a product of constant quality that can be kept for a long time at room temperature, like the products sold in supermarkets. The fruit pulp is mixed with water. Enzymatic treatments

with pectinases can be performed to better extract the compounds from the pulp. The aqueous extract obtained is clarified by sieving and decanting. Its sugar content is adjusted by adding syrup, which provides sufficient substrate for the alcoholic fermentation yeasts, depending on the desired alcoholic strength.

The sugar extract is inoculated with low-alcohol yeast, such as *Kloeckera apiculata*. Alternatively, the yeasts present on the fruit can be left to do their job. Fermentation will be a little longer and variable. Fermentation is carried out using the closed tank technique. At home, bottles are used and sealed with caps or corks.

After fermentation, the product is pressure-filtered or decanted and then packaged in clean, hermetically sealed bottles.

*16.8.3 Non-*Saccharomyces *Yeasts*

16.8.3.1 Non-*Saccharomyces* Yeasts Strains Used in Fermentation

In according Quoc (2010) Non-*Saccharomyces* yeasts represents a large group of micro-organisms presents in several areas of fermentation, extremely diverse in terms of taxonomy and technological properties. Generally, among non-*Saccharomyces* yeasts genera encountered in fermentation there are: *Hanseniaspora, Candida, Issatchenkia, Pichia, Kluyveromyces, Metschnikowia, Zygosaccharomyces, Zygoascus, Torulaspora, Debaryomyces and Brettanomyces.*

16.8.3.2 The Interest of Non-*Saccharomyces* Species in Fruit Juice Fermentation

Non-*Saccharomyces* species have long been considered harmful for alcoholic fermentation. Recent studies show that some of them have real potential. Non-Saccharomyces can, among other things, release fermentative aromas, contribute to organoleptic complexity, develop enzymatic activities of interest (*Pichia kluyverii* and *Metschnikowia pulcherima*), deacidify (*Hanseniaspora occidentalis*), promote the formation of varietal aromas (*Torulaspora delbrueckii, Metschnikowia pulcherima* and *Kluyveromyces thermotolerans*), produce low volatile acidity (*Torulaspora delbrueckii*) (Lamon 2013). However the characteristics of non-*Saccharomyces* yeasts strains could depend the ecological niche where there have been isolated.

16.8.4 Microbiological Decontamination Processes

In general, because of their low pH, fruit juices present little microbiological risk. A pH below 4.5 is lethal for pathogens such as *Listeria monocytogenes* or *Clostridium botulinum* (Parish and Higgins 1989). However, outbreaks of diarrhea and haemolytic uraemia syndromes in 1991 in Massachusetts (USA) were associated with the

consumption of unpasteurized apple juice containing the pathogenic strain *E. Coli* O157:H7 (Besser et al. 1993). In Libya, 146 unpasteurized juices were found to contain viable microorganisms ranging from 1.7.105 (almond juice) to 5.1.103 (lemon juice) (Ghenghesh et al. 2005). Of these microorganisms found in juices, many are pathogenic to humans, such as the *E. Coli* 0157. At the end of the production chain, fruit juices must be free of microorganisms in quantities that could present a health hazard until the use by date. Controlling micro-organisms is therefore essential to ensure the sanitary and microbiological quality of juices, to ensure their stability and to extend their shelf life. There are several decontamination treatments based on the thermoresistance, baroresistance, etc. of microorganisms. The process classically used for pasteurization is as follows: the full closed bottles are sprayed with increasingly hot water until temperatures of around 90 °C are reached, which heats the product from 82 °C to 85 °C. This "pasteurization" technique, developed by Pasteur, requires lengthy heating and cooling procedures that can cause the fruit juice to cook and degrade its flavors. Pasteurization has negative effects: at 80 °C, non-enzymatic browning can occur, loss of heat-labile nutrients and formation of undesirable products such as 5-hydroxymethylfurfural (5HMF) (Baron 2002). However, many studies show that these new compounds formed during the Maillard reaction have strong antioxidant activity (Kim et al. 2003; Del Caro et al. 2004). The first alternative to conventional pasteurization is rapid heating: the glass containers are filled with juice heated to 95/97 °C. The juice/bottle assembly after closure with a temperature of 82 °C to 85 °C is self-pasteurizing. This combination is then rapidly cooled. This technique, known as "flash pasteurization", reduces the intensity of the heat treatment by half. Good asepsis control allows the thermal scale to be reduced even further (Baron 2002; Aymerich et al. 2008). Flash pasteurization can be followed by aseptic cold filling. The packages are sterilized in an aseptic environment before the filling operation. The fruit juices are sterilized by flash pasteurization (at 95/97 °C) and then cooled to room temperature within seconds before being cold-filled into the aseptic package. However, strict hygiene rules must be followed to avoid post-pasteurization recontamination. Many fruit juice distributors (Andros, Pampryl) have mastered this cold filling process, as their packaging does not withstand the pasteurization temperature.

16.9 Some Definitions

- **Fruit juice**

In according to Codex Stan 247-(2005), fruit juice is a fermentable but unfermented liquid that is obtained from fruit by mechanical processes that retain the essential physical, chemical, organoleptic and nutritional characteristics of the fruit from which it is obtained. A single juice is obtained from a single type of fruit.

- **Cocktail**

A cocktail is a mixture obtained by mixing two or more juices and puree from different types of fruit (Liegeois 2003).

- **Fruits beverages**

The name "fruit beverage"or "fruit juice beverage" or "fruit pulp beverage" is reserved for beverages prepared from drinking water and fruit juice, fruit concentrate or a mixture of both; in a proportion equal to or greater than 10% juice. (International regulations).

16.10 Different Categories of Fruit Juice

The two main categories of fruit juice are pure fruit juice and fruit juice from concentrate.

- **Pure fruit juices**

Identified by the label 100% pure fruit juice: they are distinguished into "pure fresh fruit juice", free pasteurization, and "pure juice" pasteurized after extraction (or pressing). They contain no additives and no added sugar (Liegeois 2003).

- **Fruit juices from concentrate**

These are obtained from concentrated fruit juices from which the water is removed by dehydration at the place of production in order to reduce transport costs. The proportion of water extracted during the concentration process is then restored, this water having appropriate characteristics to guarantee the essential qualities of the juice. The flavor is also restored by means of the aromatic substances recovered during the concentration of the fruit juice, which is fruit juice of the same species (Bourgeois and Leveau 2003).

To these two categories should be added nectars, which are obtained by mixing, in a given ratio, fruit puree and syrup or sugar; as well as: sweetened fruit juice; fruit drink; carbonated juice; sweetened juice (concentrate); fermented juice, milky juice (Benama and Agougou 2003).

16.11 Others Products of Mango Transformations

Excepted the juices, mango is used to produce others several food products as syrup, dried pulp mango, nectar, jam (Litz 1997). Indeed Traoré et al. (2017) reported that use mango to produce dried mango, nectar and jam to Burkina Faso. Also, in Côte d'Ivoire many foods have been produced from mango: jam, syrup, nectar, mango jelly, mango pulp preserves, mango pulp in syrup, mango puree, mango pulp powder (FIRCA 2014).

16.12 Conclusion

Juice and nectar remains the mains products from mango transformation. The mango used for juice production must be answered to some criteria. Thus, the fermentation of mango juice remains a mean to guaranty organoleptic quality and microbiological stability. Also, non-*Saccharomyces* yeasts strains are more suitable for carried out this fermentation.

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Chapter 17
Fermented Fish Products in Sub-Saharan Africa

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Abbreviations

ACE Angiotensin Converting Enzyme
GDP Gross Domestic Product
LAB Lactic Acid Bacteria

17.1 Introduction

Fish is an agricultural commodity that has a great export potential for Africa apart from cotton and coffee (WTO 2014). It also has great potentials that can benefit the locals in Africa if properly harnessed; improved nutrient and food security of Africans, reduced poverty and also generates income for the government (Ayeloja et al. 2020a, 2017). FAO (2006) stated that more than 77% of fish exports come from developing countries including African nations. These nations, therefore, need to improve their local production capacities r to meet the demand of importing countries. The oceans, rivers, floodplains, lakes and fish farms are vital natural resources in Africa from which fishes are sourced. These water bodies provide a wide range of benefits including food and nutrition security, livelihood, exports and biodiversity to many countries. FAO (2014) reported that 9.9 million tonnes of fish were produced in Africa alone in the year 2010 out of which 1.49 million tonnes were collected from aquaculture, 2.7 million tonnes were collected from inland fisheries and the remaining were collected from marine capture fisheries. In the year 2011, about 1.26% of the gross domestic product (GDP) of all African countries (US$24 billion) was obtained from the fisheries subsector (de Graaf and Garibaldi 2014). Despite the great importance of fish, it is however one of the most rapid perishable foods leading to a great loss to fish farmers. According to FAO (2010), close

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to 10% of the world's fish production is lost to fish postharvest loss. The cost of fish postharvest loss goes beyond an economic loss associated with the spoilage of fish. Other losses that are often underestimated or underreported include loss of time and money used to treat patients with fish-borne illnesses after consuming spoilt fish; loss of revenue due to the rejection of exported fish abroad, detention of persons importing substandard fish products to the country, bad publicity for the affected country etc. (Abila 2003). Consumption of unwholesome fish and fishery products is responsible for about 30% of all food-borne illnesses in the world (Iwamoto et al. 2010). To reduce spoilage, different preservation methods have been employed including: drying, smoking, freezing, fermenting, chilling and brining (Ayeloja et al. 2020b). Fermentation as a fish processing method is gaining popularity in many countries of the world due to the low cost of its production, convenience of production and low energy requirement for preservation (Panda et al. 2011). Fermented fish in the human diet was said to have originated thousands of years ago as some researchers have reported the presence of fermented fish in the food consumed by humans during the Yayoi period (300 BC to AD 300) in Japan; in ancient Greece (the fish sauce *aimeteon*) and also during the Roman era (the fish sauce *garum*) (Ishige 1993; Beddows 1997). A variety of fermented fish products now exist all over the world, different communities have varied ways of preparing their fermented fish products, this gives each fermented fish product from different regions and countries its unique sensory properties (Waisundara et al. 2016). Fermented fish products were categorized into three groups by some scientists (Zeng et al. 2013; Giri et al. 2010; Lopetcharat et al. 2001) including fermented fish product where fish retains more of its original structure; the second being fermented fish product where fish was converted to paste-like products and the third being fermented fish product where the fish was completely converted to liquid form. Zang et al. (2019) on their own categorized fermented fish products into two using the processing method for its categorization including: Spontaneous fermentation and fermentation using starter culture.

17.2 Prospects of Fermented Fish Products in Sub-Saharan Africa

Zang et al. (2019) opined that historical and cultural perspectives are very important factors while discussing fermented fish products as fermented fish products have been a staple part of the diets in many countries since the Yayoi period in (300 BCE to 300 CE) due to its ability to extend fish shelf life when there is no access to electricity. Achi (2005) stated that fermentation has the potential of reducing economic loss associated with fish spoilage as fish shelf life is increased through the process of fermentation, digestibility is also improved through the process of fermentation and fish flavours can as well be enhanced through fermentation. Fermented products remain popular in the diet of many Africans till today because electricity is still a

major challenge in many communities in Africa and the fermentation process does not require refrigeration, thereby making it easy to produce and store fermented products including fish. However, globalization and liberalization of the economy is changing people's attitude to the demand for fermented food and fish products as other processed and well=packaged food are being imported to many African countries leading to capital flight out of the continent and reduction in revenue generated by individuals involved in the production of fermented fish products as well as a reduction in government revenue (Ojo 1991). Fermented fish products have the potential of improving the economy and standard of living of people if properly harnessed. Fermented also fish products have the potential of being traded both locally and internationally as there is increasing demand for fermented fish (Zang et al. 2019).This has the potential of increasing foreign exchange earnings for the countries in Sub-Saharan Africa, it can also improve food security, create job opportunities, support agricultural livelihoods and reduce economic loss associated with postharvest loss. Fermentation of fish also influences demand for fish as fish palatability is enhanced through the improvement in fish aroma and flavuor thereby increasing the demand for the fish product (Hasan et al. 2014). In addition, fermented fish have the potential for promoting the health of infants, children and adults if consumed thereby reducing the economic loss associated with poor health condition of the citizens (Hasan et al. 2014). It is therefore suggested that attention should be shifted to the development and production of fermented fish products by all stakeholders in Africa as it has the potential of improving the economy if well harnessed.

17.3 Fermentation

Fermentation is a process that usually occurs during the decomposition of organic matter, it does lead to the release of energy from an organic substrate under the action of microbial enzymes and rejecting products (Mouroufie et al. 2018). Anihouvi (2012) on the other hand see fermented foods as foods that are subjected to the action of microorganisms in other to achieve desirable biochemical changes that will lead to significant modification of the food taste and aroma. Fermented fish is a fish product that has undergone degradative changes through enzymatic or microbiological activity in either the presence or absence of salt (Ohshima 2014). Fermentation is one of the most important methods of food preservation in the world today as it is easier to store and usually taste better than fresh foods. It also leads to reduce the cooking time of food (Ohshima 2014). Camus (2011) divided microorganisms involved in food fermentation into three groups including: bacteria, moulds and yeasts while food fermentation processes were categorized into four groups including: Lactic fermentation, alcoholic fermentation, acetic fermentation and propionic fermentation.

17.4 Process of Fish Fermentation in Africa

The processing steps involved in fish fermentation are similar all over the world (Ohshima 2014). The general steps include the collection of fish, drying or using fresh fish for fermentation. Fermentation could be done with or without gutting or deboning, in salt liquors. Keeping of fish paste at ambient temperature for one to several months depending on fish types and desired product (different bacterial and natural enzymes are involved in the solubilization of fish proteins at this stage). Flow diagram of traditional processing of fermented fish products in Africa as described by Anihouvi et al. (2012) is presented in Fig. 17.1.

17.5 Fermented Fish Products in Africa

Some of the popular fermented fish products in Africa are presented in Table 17.1 below.

17.6 Advantages of Fish Fermentation

17.6.1 Microorganism Activities

Microorganisms naturally present in fermented fish are usually used to improve the acceptability of traditional and commercial fish products by adding flavouring and biogenic amines to fermented fish products. Many of the microorganisms involved in the fermentation of fish belong to the group of lactic acid bacteria (LAB) which includes the genus *Lactobacillus*, with wide metabolic activity and the ability to colonize several habitats (Giraffa et al. 2010). LAB including *Lactobacillus plantarum* and *Lactobacillus pentosus* are involved in the production of *som-fak* (prepared with a mixture of rice, salt, fish, sucrose, and garlic) (Paludan-Müller et al. 2002). *Lactococcus lactis* subsp. *lactis* is one of the starter microorganism used in the fermentation of *Megalobrama amblycephala* in China (Zhao et al. 2014). *L. plantarum* and *Pediococcus pentosaceus* ZY40 GY23 are also good starters in grass carp sausages (Nie et al. 2014). Another species of microorganism commonly found in fermented fish products is the *Staphylococcus* spp. it has been reportedly found as a major microorganism in Malaysian fermented shrimp know as *cincaluk* (Hajar and Hamid 2013). *Enterococcus faecium* and *Enterococcus faecalis*, bacteriocin-producing strains with no amino-acid decarboxylase activity, were isolated from Thai fermented seafood products including fermented shrimp (*kung-jom*), mussel (*hoi-dong*), and fish (*pla-jom*) (Nanasombat et al. 2012).

Fig. 17.1 Flow diagram of traditional processing of fermented fish products in Africa. (**a**). Fermentation with salting and drying, (**b**) Fermentation and drying without salting (**c**) Fermentation with salting without drying. (Source: Anihouvi et al. 2012)

17.6.2 Antimicrobial

Many microorganisms used in the fermentation of fish especially those used starters in the fermentation of fish have great antimicrobial activities, this activity is mainly ascribed to the production of bacteriocins. Zeng et al. (2014) reported that strains of *L. plantarum* which were isolated from a Chinese traditional low-salt fermented whole fish known as *suan-yu* produced bacteriocins against *L. monocytogenes*,

Table 17.1 Common fermented fish product in Africa

SN	Types of fish	Products local name	Country
1	Catfish, croaker, meagre, shark, mullet, skate, rays, triggerfish, horse mackerel, octopus, tuna, sole, Spanish mackerel, seabream, herring	Gyagawere, adjuevan	Ivory coast
2	Nile perch (*Lates niloticus*) or Clarias species (Kanuri Tribe of North Eastern Nigeria)	Bunyi youri	North East Nigeria
3	Catfish, barraccuda, seabream, threadfin, croaker, grouper, bonito, mackerel, herrings, squid, octopus, bumper, snapper, ribbon fish	Momone,	Ghana, Egypt
4	Carp, threadfish	Djegue, jalan	Mali
5	Alestes Nile perch, parch	Aku	South East Nigeria
6	Mackerel, seabream, threadfin, croaker, mullet, catfish, meagre, herrings, skate, rays, shark, Bonito	Guedj, tambadiang, yeet	Gambia, Senegal
7	Tigger fish; Nile perch, Tilapia, parch	Fessiekh, Terkeen/ Mindeshi	Sudan, Egypt
8	Alestes Nile perch, Parch	Salanga	Chad
9	Cassava croaker (*Speudotolithus* sp.) or Spanish mackerel/king fish (*Scomberomorus tritor*)	Lanhouin,	Benin, Togo, Ghana
10	*Heterotis niloticus, Sardinella* sp.	Fermented fish	South-South, Nigeria

Source: Anihouvi et al. (2007), Paul et al. (2016), Nwabueze and Nwabueze (2010), Achinewhu and Oboh (2002) and El Sheikha et al. (2014)

Staphylococcus aureus, and *Escherichia coli*. Crude extract from *Lactobacillus paracasei* LA07 isolated from *budu* was reported to have inhibited the growth of some other microorganisms such as *Bacillus cereus, Lactococcus lactis, S. aureus, Salmonella enterica, L. monocytogenes, and E. coli* (Abbasiliasi et al. 2010). *L. lactis* subsp. *lactis* CWBI B1410 was also used as a nisin-producing starter culture to improve the quality of a traditional fermented-fish product in Senegal known as *guedj*.

17.6.3 Antioxidant Activity

Kleekayai et al. (2014) and Jung et al. (2005) reported that many fermented fish products have a free-radical-scavenging activity which increases the antioxidant potential of the products. During the fermentation process, a lot of small peptides are released leading to their high antioxidative properties (Binsan et al. 2008; Faithong et al. 2010). Kleekayakai et al. (2015) reported the presence of compounds

with masses less than 500 Da in water-soluble extracts of two fermented shrimp pastes (*kapi*), with ABTS radical (2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging capacity which was expressed as EC50 values, of 60.9 and 46.4 μg protein/mL. Prolonged fish fermentation usually makes amino acids and peptides present in fish muscle to interact with reducing sugars to form desirable MRPs with strong antioxidant activity (Peralta et al. 2008). Rajapakse et al. (2005) attributed the high antioxidant property of fermented fish to aromatic amino-acid, histidine residues and the specific positioning of Phe-His in the peptide sequence. Taurine which is another biologically active compound involved in the regulation of oxidative stress was reported to be present in blue-mussel fermented sauce and salt-fermented shrimp paste (Park et al. 2005).

17.6.4 Antihypertensive Activity

Long-term fermented fish products having high concentration of sodium chloride which can be useful as a source of antihypertensive peptides (Ichimura et al. 2003). Noticeable ACE-inhibitory activity has been reported in a number of fish sauces, such as salmon, sardine etc. Naturally occurring ACE inhibitors raise the possibility that hypertension could be modulated through dietary intake, fermented fish products therefore could serve as a natural food that could have antihypertensive activity.

17.6.5 Anticoagulant and Fibrinolytic Activity

Anticoagulant and antiplatelet drugs are used in the management of heart disease and stroke patients to prevent the formation of thrombus by an imbalance in hemostasis. A fermented fish product (salt-fermented anchovy sauce) was reported to have stable fibrin-clotting inhibitor a bioactive substance that may act as an anticoagulant against fibrin clotting by protecting fibrinogen from the thrombin action. This makes the fermented fish product a good food for patients with heart disease (Kim et al. 2004). Singh et al. (2014) and Hwang et al. (2007) also reported a high fibrinolytic activity in fermented small cyprinid fish (*Puntius sophore*), a traditional fermented product from Northeast India (Singh et al. 2014) and in traditional fermented shrimp, anchovy, and yellow corvine Korean *jeotgal* products. Singh et al. (2014) reported that fermented fish are known to have fibrinolytic enzymes which are enzymes responsible for breaking down fibrin and fibrinogen, they are also reported to have anticoagulants and antiplatelet properties thus they can be used for the prevention of thrombus formation (Martınez-Alvarez et al. 2016).

17.6.6 Development of Immune Modulators from Natural Sources

The limitation of most immune-modulatory pharmaceuticals on chronic or preventive use makes the development of immune modulators from natural sources for diet supplementation an area of interest in research. Thongthai and Gildberg (2005) found that small-size peptides from commercial Thai anchovy sauce can stimulate the proliferation of human white blood cells at low concentrations (5 μg/mL).

17.6.7 Enzymatic Activities

Fish naturally have enzymes that are distributed in their body including muscles, tissues, blood, and glands> These enzymes often cause fish deterioration before noticeable spoilage of bacterial origin (Marsh and Flick 2012). Most enzymes are active in dilute solution and do not act in the absence of water and are often destroyed or rendered inactive by concentrated salt solution (Gildberg 2001; Gildberg et al. 2000). Sriket (2014) classified proteolytic enzymes based on the mode of catalysis into four including: (a) aspartic proteases (pepsin and cathepsin D); (b) serine proteases (trypsin and chymotrypsin); (c) cysteine proteases (calpain, m-calpain, and cathepsins B, H, L); and (d) etalloprotease. Proteases play an important role in the production of fermented fish products, particularly during the fermentation process to obtain an acceptable product quality. Proteolytic enzymes are located in the viscera, digestive tract, and fish muscle tissue. Yongsawatdigul et al. (2007) reported major endogenous proteinases in anchovies to include trypsin-like proteinase, pepsin, chymotrypsin, elastase, and aminopeptidase while digestive enzymes of trypsin, chymotrypsin, and pepsin are considered as the three most important enzymes in fish fermentation (de la Parra et al. 2007).

17.6.8 Anti-cancer Activity

Some fermented fish was reported to have peptide fractions with anticarcinogenic activity as it induces apoptosis in a human lymphoma cell line with the potential of preventing cancer (Lee et al. 2004). Fermented fish was reported to be effective in preventing somatic mutations (Zang et al. 2019). Duarte et al. (2006) also reported the fermented fish produced from pacific whiting to have high concentrations of immunomodulatory bioactive substances such as glutamine, glutamic acid, fatty acids etc. which are useful in the commercial dietary supplements.

17.7 Limitations of Fermented Fish Products

17.7.1 Health Hazards Associated with the Consumption of Fermented Fish Products

A lot of poor hygienic practices are involved in the traditional processing of fermented fish products which constitute a health risk to consumers. Some of these practices are related to the processing techniques, the processing environment, the waste disposal of the fish, the unhygienic nature of processing materials and poor packaging. Lack of potable water in many traditional processing units is also another major challenge that make many processors result in the use of water from unhygienic sources to wash the fish. This often leads to contamination of the product including chemical and microbial contamination. The traditional way of drying fermented fish products on the ground or dirty materials also lead to contamination with sand and microorganisms. The fish may be infested by blowflies and other types of insects, in an attempt to get rid of the flies, insecticides are often used thereby creating another health hazard for the consumers.

17.7.2 Presence of Biogenic Amines

Fermentation of fish usually often involves the use of high amounts of salt which could lead to the formation of biogenic amines. Karovicova and Kohajdova (2005) reported that biogenic amines are the potential precursors of carcinogenic nitrosamines, it is known to adversely effects humans if consumed by causing rash, migraine, hypertension, and hypotension.

17.7.3 Source of Heavy Metals

Fermented fish could contain some heavy metals such as cadmium, arsenic, mercury and lead due to the accumulation of these metals in the internal organs of fish (Anihouvi et al. 2012).

17.7.4 Source of Nematodes

Fermented fish products usually contain nematodes and other species of worms as fermented seafood products are not subjected to thermal processing which is the recommended method of killing nematodes (Oh et al. 2014). The presence of

Anisakis L3 larvae in fish and cephalopods has long been recognized as potential human health risk (Oh et al. 2014).

17.8 Conclusion

The fishing industry is important in Africa for the supply of protein and source of revenue for the government and individuals involved in the industry but fish is highly perishable which is a major limitation. Fermentation is one of the methods of preservation that could be used to reduce its spoilage especially in many rural communities where access to electricity for refrigeration is a major challenge. Fermented fish product from Africa is no so popular, infarct no African fermented fishery products are mentioned in the FAO Fisheries Report No. 100 on fermented fish (FAO 1971). This document, therefore, provides brief information on the common fermented fish products from Africa. Scientists from the region should consider more research in the area of fermentation of fish as a method of preservation in other to reduce fish postharvest loss from the region.

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Chapter 18
African Fermented Vegetable Products

Gustav Komla Mahunu and Abdalbasit Adam Mariod

18.1 Introduction

Vegetable products in the diet of people make significant contribution to enrich nutrition and health of the African Populations. Vitamins, minerals, antioxidants and dietary fibres are obtained from vegetable products. The consumption of vegetables can reduce the vulnerability to onset of chronic diseases. World Health Organization (WHO) and the European Food Safety Authority (EFSA) have shown that the intake of daily recommended vegetable has several positive health benefits (Giampieri et al. 2015). They help to prevent chronic diseases from the general gut health, boost immunity, skin health, cholesterol control and lactose intolerance. However, though majority of the vegetables are succulent making their consumption at fresh state palatable, they are prone to accelerated microbial spoilage. The fresh vegetables easily lose moisture and short shelf-life compromising their physiochemical qualities.

Various processing methods (cooking, drying, boiling, microwaving) have been reported for maintaining quality, reducing decay and extending shelf life of vegetables (Animashaun 2015; Guo et al. 2016, 2017; Talens et al. 2017). In addition, several types of preservatives that are used in modern processing of vegetables in order to maintain acceptable level of qualities between time of manufacturing and consumption. Key interest among the vegetable preservation methods is fermentation. Fermentation is an old food preservation technology with long traditional

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history in Africa, where activities of living organisms produce wide-range of metabolites with the capability to suppress the growth and survival of unwanted microflora in raw food materials (Ross et al. 2002). Fermented vegetable food products, globally are valued for their high sensory attributes in addition to what they have long been known for in food preservation and safety. For instance, as a food safety measure fermentation is been used as tool in the reduction of bacterial related food contamination; thus, this type of preservation that can help to reduce the incidence of diarrheal diseases (Zabat et al. 2018). Relatively, fermentation is relatively low-cost and most economical methods of food production and preservation over the years (Russo et al. 2017). In food processing, fermentation has been found to make beneficial contribution in the following ways: (1) Improve nutritive properties, diversity flavor (organoleptic properties) and convert inedible food to edible food (thus, make raw food digestible by possible removal of anti-nutrient factors, where necessary) (Smid and Hugenholtz 2010); (2) protect large quantities of food by lactic acid fermentation, (3) reduce toxicity or detoxify food ingredients and (4) shorten cooking time and reduce energy need.

Compared to the Asian and Mediterranean regions, the fermentation of vegetables in Africa is not well developed scientifically and for that matter no clear standards. The fermented vegetable products are anchored on the local raw vegetable varieties available within the specific to regions/ethnicity. Fermented vegetable products are consumed during the long dry periods when fresh vegetables may be in short supply.

This chapter describes fermented vegetable products in Africa, microorganisms in their fermentation and development of selected fermented vegetable-based products.

18.2 Microorganism for African Vegetable Products Fermentation

The process of fermentation actually involves diverse microorganisms and there is high diversity of the microbiome. Generally, most vegetable fermentation processes are based on the natural occurrence of lactic bacteria on external part of microflora. The presence of lactic acid bacteria (LAB) in several fermented vegetables are purposed to prevent the growth of spoilage bacteria through the production of biopreservatives (Djien 1982). Lactic acid bacteria have a strong anti-bacterial effect as a result of the production of bacteriocins (Agriopoulou et al. 2020). The intake of lactic acid fermented vegetables supports the dietary enhancement of humans, promotion of growth of healthy intestinal flora and the positive health image of probiotics (Yamano et al. 2006). Fermented vegetables contain more vitamin C and the bioavailability of these vitamins also increases with fermentation (REF).

18.2.1 Dry-Salted Fermented Vegetables

Processing of vegetables by dry-salted fermentation is done by intentional addition of dry salt to vegetables. The purpose of the salt is to dehydrate the juice in the vegetables and make it saline. Here, for every vegetables of 100 kg, about 3 kg of salt is added. First the vegetables are cut, then wash in potable water and drained thoroughly. The sliced vegetables are arranged and put in a suitable container in a 2.5 cm layer for fermentation. Then sprinkle salt on the first layer of vegetables. Second layer of vegetables is added and salt is also sprinkled on them. This process is repeated to fill the container up to three quarters. In many instances, stones or heavy objects are placed on the layers to exert weight to compress the vegetables and to also promote the brine formation within 24 h. Shortly after the brine formation, fermentation commences and foams of carbon dioxide start to show. The ambient temperature largely determines fermentation period, which occurs between 7 and 30 days. When the appearance of bubbles completely stops indicates completion of fermentation process, before the pickle is packaged in a variety of blends (i.e., vinegar and spices or oil and spices) (Liu et al. 2011).

18.2.2 Brine-Salted Fermented Vegetables

In the brine-salted fermented vegetables (BSFVs), brine is applied to vegetables that characteristically have lower water content. The salt is dissolved in water to prepare the brine solution (Montet et al. 2014). According to Liu et al. (2011), the best fermentation occurs in brine with approximately 12.5 to 20° Salometer. When brine solution is strong, it induces solutions and carbohydrates out of the vegetables, and eventually decrease the internal salt concentration. It is critical to ensure that the concentration of salt should not drop under 10%; this condition will permit fermentation to occur (Panda et al. 2009).

Intermittently more salt is added to the brine mixture in order to prevent salt concentration from dropping. There is rapid microbial development in the brine as soon as the vegetables were put in the salt water and close the jar. Some of the natural parameters that affect the populations of microbes of the fermenting vegetables are salt concentration and brine temperature, the available fermentable materials and the numbers and kinds of microorganisms that exist at the time fermentation begins. According to Ray and Panda (2007), the speed of the fermentation correlates with the salt concentration in the brine and as well as its temperature. Most vegetables get fermented between 12.5 and 20° Salometer. When the salt concentration is very high (about 40° Salometer), the sequence is slanted to a homo-fermentation development, which is dominated by *Lactobacillus plantarum*. The highest concentrations of salt (60° Salometer), will stop lactic fermentation (Montet et al. 2006). However, they also indicated that any detection of acid (acetic acid) during brine

storage is presumably produced by acid-forming yeasts; which are still active at the highest concentration of salt (60° Salometer) (Sruthi and Rao 2021).

18.2.3 Non-Salted Lactic Acid Fermented Vegetables

Without initial adding of salt or brine LAB can still ferment some vegetables (e.g., some wilted fermented leaves) (Tamang et al. 2005). Detoxifying cassava by fermentation comprise of an acid fermentation, where the cyanogenic glycosides are hydrolyzed to release the toxic cyanide gas (Onabolu et al. 2002). It is important to understand that the fermentation process is hitched on the ability of the LA-producing bacteria to rapidly colonize the food; this will lead to reduction in the pH and the environment become unsuitable for the growth of spoilage microbes. Also, the elimination of oxygen will enable the lactobacilli favour an anaerobic atmosphere. In other words, when oxygen is restricted yeasts do not grow (Montet et al. 2014).

In Table 18.1, various factors have shown to impact on lactic acid fermentation of Africa vegetable products. Definitely, these factors act in combination rather than independently to achieve the desire effect or the contrary.

18.3 Selected African Fermented Vegetable Products

The commonly consumed vegetables in Africa are listed in Table 18.2. These vegetables have very short shelf life and lose their eating quality quickly after harvesting unless they are processed into various products to add value. There are abundant levels of phytate and oxalate in the different vegetables and through fermentations these anti-nutrient substances are degraded (Wakhanu et al. 2014). The presence of these anti-nutrients (such as phytate and oxalate) limit the micronutrients release in the vegetables. The anti-nutrients bind to specific the minerals in the vegetables to reduce the micronutrient bioavailability. The product quality is positively affected by fermentation to retain minerals, β-carotene and α-tocopherol levels (Akello 2014). This section also discusses the fermentation activities in selected vegetables.

*18.3.1 Cowpea (*Vigna Unguiculata*) Leaves*

Cowpea leaves fermentation enhanced with the highest glucose concentration (3%) correlated with the highest concentration of lactic acid (0.6%) and the lowest pH of 4.7 (Kasangi et al. 2010). Also, blanched solar dried cowpea leaves and fresh solar dried cowpea leaves compared with fermented samples recorded the lowest crude protein (13.2%) and moisture content (6.2%) while that of crude fibre, ash and soluble carbohydrates of fermented samples increased. Fermented cowpea leaves also showed a reduction of iron, calcium, magnesium and zinc contents (Wafula et al. 2016).

Table 18.1 Factors that affect lactic acid fermentation of Africa vegetables

Factors	Observations	References
pH	Affects aroma and flavour. Almost neutral pH is benefit from most LAB. Certain bacteria (such as *Lactobacillus* and *Streptococcus*) are acid tolerant and able to survive low pH levels (3.0–4.0). pH	Kobawila et al. (2005), Muyanja et al. (2003) and Ray and Panda (2007)
Oxygen availability	Different species have different O_2 requirements. Most LAB are not sensitive to oxygen. They can grow in the presence and the absence of oxygen. They are known as aero-tolerant anaerobes.	Molenaar et al. (2005)
Temperature	Most LAB require optimum temperature between 20 °C to 30 °C. whiles some are thermophiles; thus, prefer high temperatures (50–55 °C), others tolerate colder temperatures between 15 and 20 °C. However, most LAB work best at temperatures between 18 and 22 °C.	Ray and Panda (2007)
Inoculum concentration	The concentration of HCL is between 20 and 80 g/l during fermentation. LAB can stand high concentrations of salt.	Rao et al. (2004) and Wouters et al. (2013)
Water activity	Mostly, LAB need a fairly high-water activity at 0.9 or higher, in order to survive. Few species can tolerate water activities lower than 0.9. Nevertheless, generally the yeasts and fungi will dominate on foods with a lesser water activity.	Ray and Panda (2007)
Nutrients	Source of nutrients is a requirement for all bacteria to undertake metabolism. Bacteria involve in fermentation require carbohydrates either simple or complex.	Ray and Panda (2007) and Wouters et al. (2013)
Selection of starter culture	The interactive effect between the starter and the natural flora in addition to the sensory properties of the final products are considered in selecting starter cultures for fermenting African vegetables. Also they are selected according to the absence of toxic chemicals production; capacity to produce only (L+) lactic acid; low or zero biogenic amines production; species must have attained their genetic stability; brine acidification must be rapid; ability to duplicate production among the different batch cultures, ability to totally deplete fermentable sugars	McFeeters (2004) and Montet et al. (2014)

*18.3.2 Baobab (*Adansonia digitata*) Leaves*

Baobab is seen as one traditional tree species of Africa's important leafy vegetables and issues related to strategies to manage leaf production have been seriously highlighted. For instance, the unique features of the palatable leaves, and the seasonal dimension of leaf consumption have been reported (Chadare 2010). Processing has effect on the quality of traditionally treated baobab leaves. A comparative study of *in vitro* digestibility and bioavailability of Ca, Fe, and Zn between non-processed

Table 18.2 Types of plant and their respective part fermented

Type of plant	Part fermented		
	Leaves	Fruits	Seeds
Amaranth (*Amaranthus spp*)	x		x
Okra (*Abelmoschus esculentus*)	x	x	
Cocoyam (*Xanthosoma sagittifolium)*	x		
Jute mallow (*Corchorus olitorius*)	x		
Kenaf (*Hibiscus cannabinus*)	x		
Cowpea (*Vigna unguiculata*)	x		
Sweet potato (*Ipomoea batatas*)	x		
Luffa (*Luffa spp*)		x	
Bitter leaf (*Vernonia amygdalina*)	x		
African eggplant (*Solanum melongena*)	x	x	
Water leaf (*Talinum fruticosum*)	x		
African spider plant (*Cleome gynandra*)	x		
West Indian nettle			
Pumpkin (*Cucurbita maxima*)	x	x	
Moringa (*Moringa oleifera*)	x		
Baobab (*Adansonia digitata)*	x		
Cassava *(Manihot esculenta)*	x		
Tomato *(Lycopersicum esculentum L.)*		x	
Onions (*Allium cepa*)	x		
Castor seed (*Ricinus communis*)			x
Cucumber (*Cucumis sativus*)		x	
Roselle (*Hibiscus sabdariffa*)	x	x	x
Turkey berry (*Solanum torvum*)		x	
Black plum (*Vitex doniana*)	x		
Eggplant (*Solanum macrocarpon*)	x		

and processed baobab leaves revealed the availability of total Ca (10–30%) and further stated that lutein and beta-carotene constitute the most vital carotenoids. Although, the *sweet leaves* type is preferred to the *bitter leaves*, the *bitter leaves* contain more nutrients.

The fermented baobab products have been categorized as thus, *Tayohounta* produced from fermented kernels; *Dikouanyouri* produced the fermented seeds, both are used as flavouring agents; *Mutchayan* (sorghum paste with baobab pulp) used as a drink and a main dish; and *Kuka* (processed dry leaves). Fermentation of the kernels and seeds is started mainly by *Bacillus* spp. (8.5 and 9.5 Log cfu/g, respectively) and that of the sorghum paste with baobab pulp mostly fermented by lactic acid bacteria (8.1 Log cfu/g) and yeasts (7.2 Log cfu/g) (Chadare 2010). same studies on the microbes on kernels indicated that the microbiota in-charge of the fermentation were spore forming bacteria, primarily *Bacillus subtilis* and other *Bacillus* spp.

*18.3.3 Cassava (*Manihot esculenta*) Leaves*

Cassava leaves are popular indigenous leafy vegetables in diets among African people. Cassava leaves are high in proteins, minerals and vitamins. It was detected that proteins in the cassava leaves are rich in essential amino acids apart from methionine and phenylalanine (Gómez and Valdivieso 1985; Ravindran and Ravindran 1988). Cassava leaves and roots are also rich in cyanide (Liener 2012). Actually, classification of cassava is based on the cyanhydric acid contents. According to earlier reports by Mlingi et al. (Mlingi et al. 1991), cyanogenic glucosides is the main reason for the toxicity of unfermented cassava leaves. When linamarine is not hydrolyzed, the residuals in the cassava leaves after fermentation process constitute a health risk for the consumers (Gómez and Valdivieso 1985). It was confirmed that the continuous ingestion of cyanide contaminated fresh or minimally processed food poses a high risk to consumer. Intake of non-detoxified cassava products has been found to contribute to certain diseases indirectly caused by the cyanide. These diseases include goitre, dwarfism and the tropical ataxic neuropathy. Especially, in regions where cassava is the main source of energy, these diseases are serious issues of worry (Balagopalan 2002). Breeding programs have been carried out to produce cyanide-free cassava varieties or acceptable cyanide contents. Other traditional methods have been developed in various countries in Africa to remove the cyanhydric acid in the cassava leaves to make is safe for consumption. Traditional method of fermenting cassava leaves, significantly reduced the cyanogenic glucosides content between 70 and 75% (Kobawila et al. 2003). In fermentation of cassava leaves, the cyanogenic glucosides hydrolysis occur in alkaline (pH 8.5) environment (Kobawila et al. 2005).

The occurrence of cassava leaves fermentation in alkaline pH could be attributed to amines produced by *Bacillus* (Louembe et al. 2003). Certain Bacillus strains especially *Bacillus pumilus*, have the ability to use cyanhydric acid for their nourishment (Knowles 1976). Therefore, the Bacillus strains can cause the reduction of the cyanide content in the fermentation medium. Furthermore, the alkaline pH facilitates cyanogenic glucosides content reduction since cyanohydrin acetone production by the hydrolysis of linamarin, cleaves naturally once pH is more than 5.0 or by the action of hydroxynitril lyase to give acetone and cyanhydric acid.

In the Congo (in the Central Africa) a type of vegetable gotten through a semi-solid fermentation process of cassava leaves is called "*ntoba mbodi*" (Kobawila et al. 2005). The type of cassava leaves, conditions of fermentation and the microbes participating in the fermentation have significant effect on the sensory qualities (colour, texture, smell, taste) of the final products.

18.3.4 Cucumbers

Africa also produce pickled cucumbers. Cucumbers an important fruit vegetable and it is processed by lactic acid fermentation; afterwards the product color changes from pale to darker green and more transparent product. Fully mature cucumbers with no physical damaged are cleaned in potable cold water and drained. Then salt (1 kg) is added to the cucumbers. Insertion of the cucumbers in salt brine of 5% is a satisfactory technique. Here, the cucumber has a habit of absorbing salt until there is equilibrium between the salt in the cucumbers and the brine (approximately 3% salt in the brine) (Reina et al. 2005).

Fermentation commences and foams of carbon dioxide appear immediately the brine is formed. Fermentation period is 7 to 16 days depending on the ambient temperature. When the salt concentration is appropriate it places a selective effect on natural flora; this will lead to growth of LAB. As soon as the pH is near 4.7, the brine is inoculated with either *Lb. plantarum* or *P. pentosaceus* or their combination (Steinkraus 2002). Salt is usually added to the LA fermented cucumbers when the fermentation ends. The addition of salt will halt any unwanted bacterial growth during storage (Kapur and Singh 2003). Often, cucumber pickle is kept in clean jars with an overlaid. The quality of final products is maintained when they are stored in a cool place. The risk of food poisoning is also low because of the high acid level (3.1–3.5) of the ultimate product (Tamang et al. 2005).

*18.3.5 Onions (*Allium cepa*)*

Different varieties of onions (*Allium cepa*) such as sweet, white and yellow storage were used for LA fermentation. White and yellow bulb onions are generally processed type due to their high solid content, so they are selected for fermentation. Sweet onions are a spring/summer types with low solids and mild flavour and are often used at the fresh state (Swain et al. 2014). Lactic acid fermentation is used to process different bulb onions to sour onion.

A reported on the fermentation process indicated that first the onions were sliced and salt without or with sugar were added. Temperature condition during fermentation was 18 °C. In the case, where the onions did not have the required LAB for anaerobic fermentation, the sample as inoculated with brine obtained from sauerkraut or slices of cabbage. The final product after fermentation is sour onion with pH of 3.25–3.35 and 1.2–1.5 g LA/100 ml, (all within the range as the sauerkraut). According to the sensory assessment results, the yellow bulb sour onion was the preferred product in terms of color, texture and flavor. Also, the sour onion possessed a tartaric acidic taste, same as sauerkraut, the flavor was onion-like but without the pungency of fresh onions (Roberts and Kidd 2005).

18.3.6 Castor Seed (Ricinus communis)

Ogiri-igbo is a local name in Nigeria for final product of castor oil seeds fermentation, used as a condiment. According to Egwim Evans et al. (Egwim Evans et al. 2013), the microorganisms in the fermentation of castor oil include *Lactobacillus sp. Rhizopus stolonifera, Streptococcus sp., Aspergillus fumigatus, Pediococcus sp. Triscelophorus monosporus, Bacillus sp., Coryneform bacteria*. The raw castor oil seeds are cooked for 2 h until the seed turn to brown colour. After that the seeds are dehulled and rinsed in clean water. The boiled seeds are cooked for another 1 h. Then cooked seeds are cooled and enveloped in adequate banana leaves, and subsequently packed in a hygienic container with cover to ferment at room temperature (Egwim Evans et al. 2013).

18.3.7 Amaranths (Amaranthus *spp.*)

The shelf life for Amaranths after harvest is 3 days at ambient conditions (Peter et al. 2014). Therefore, fermentation among other techniques can be used to process the harvest to reduce postharvest losses and make products availability all-year-round. For more than two decades now, there have been research alternatives to produce bread that is gluten-free to satisfy celiac consumers. Amaranth seeds are among the several alternatives to the commonly gluten-containing grains. Amaranth seeds are considered one of the pseudo-cereals (Alvarez-Jubete et al. 2010; Poutanen et al. 2009) and nutrient-dense whole grains incorporated in gluten-free bread preparations with the potential to increase the dietary value of bread and bread products, in respect of fibre, protein and mineral contents (Moroni et al. 2009). Recently, some researchers (Kiskini et al. 2007; Kiskini et al. 2012) produced iron-fortified amaranth-based bread which met the sensory qualities consumers preferences. The fermentation of amaranth as substrate in bread can also bring a new trend in the development of new functional foods suitable for celiac patients or for people suffering from lactose intolerance.

18.4 Conclusion

African countries require food processing methods that are suitable, conducive for the tropics and relatively inexpensive to rural and urban populations. One of such methods is fermentation; the process has been developed based on indigenous knowledge for a wide variety of food products. Among these food products are vegetables. Fermentation is a component of food processing which contributes to the improvement of food safety, nutritional values, flavour and acceptability. It also helps to reduce anti-nutrients, detoxify poisonous compounds, prolong shelf-life

and enhance the functional properties. Juices in vegetables are appropriate substrates for lactic acid fermentation or as conduit for probiotic bacteria. Various factors have been observed to affect lactic acid fermentation. Besides, lactic acid fermentations occur under three basic types of conditions (dry-salted, brined-salted and non-salted). In particular, addition of salt in vegetable fermentation is an important stage helps the growth of LAB.

Generally, African fermented vegetables are connected to many sociocultural characteristics of different people in the region. Lastly, understanding of the association between raw vegetable materials, beneficial microorganisms, and their environment is important to improve fermentation process and, quality and safety final products.

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Chapter 19
Production and Evaluation of Vinegar Using Nabag as a Raw Material

Abdel Moneim Elhadi Sulieman and Hiba Bokhari Yousif

19.1 Introduction

Zizyphus spina-christi (L.) is a shrub or evergreen tree called Christ's thorn (Al-Wakeel 2008; Anthony and Dweck 2005) with edible sweet fruits. The plant is recognized to be very heat resistant in addition to its drought tolerance (Paroda and Mal 1989). It is a most significant grown local tree species of Arabia with historical, medicinal and religious benefits (Sameera and Mandakini 2015; Youssef et al. 2011; Adzu et al. 2002).

Nabag is a desert fruit, wild in its origin, and it can be grown with ease. It does not need abundant irrigation due to its desert nature, it lives on the banks of rivers and mountainous areas and spreads widely in the Mediterranean basin and the original home of the buckthorn is the regions of southern Europe, the Himalayas, northern China, North Africa, Sudan, Egypt, Iraq, the Emirates and South America (https://suna-sd.net/ar/single?id=434642). Its homeland is the Arabian Peninsula and its fruit is called (Nabag).

The fruits of nabag are sweet in taste and fragrance. They are grown in the winter and collected in the summer. They are planted in fields and gardens, and their fruits are eaten. In addition, its fruits, flowers and leaves are often used to prepare medicinal materials for ailments and diseases, and all these reasons have contributed to surrounding the Sidra tree with an aura of love throughout history.

Nabag fruit has a great position for the inhabitants of the Arabian Peninsula because it is rich in foodstuffs that are linked to stories and narratives, and there are those who believe that those who cut down the Sidr tree preach themselves death because they believe that Sidr is the abode of Paradise.

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Fig. 19.1 Zizyphus spina-christi.jpg

The chemical composition (%) of Nabag various parts as investigated by many researchers indicated that the contents of moisture in fruits, seeds, and leaves were (5.4–16.8%), (4–14.89%), and (7.72%), respectively. The main components include polyphenols, carbohydrates, minerals, oil, fatty acid and vitamins. The big number of these constituents were found in the fruits, followed by seed, stem and finally kernel oil (Ahamed 2016; Duke 1985; Adekunle and Adenike 2012; Ibrahim et al. 2015; Ahmed et al. 2015) (Fig. 19.1).

Nabag fruit taste like a combination of dates and apples and are profoundly valued by the Bedouins and were found to have a high energy value. The fruit can be eaten crude or dried for sometime in the future and has a lovely sub corrosive taste, fairly looking like dried apples (Facciola 1990). The seeds were wealthy in protein and the leaves in calcium, iron and magnesium. The food from this plant is a significant wellspring of energy, protein and minerals.

Nabag fruits can be eaten as a food because of their many health benefits, and they can also be used in preparing desserts, pies and drinks. Nabag, or the oil extracted from it, is also used in the cosmetic industry. The followings are the most important benefits of nabag:

1. It contains many nutrients: The nabag oil extract contains several vitamins, minerals, and antioxidants, which help prevent signs of aging and also contribute to the prevention of heart disease and cancer. The seeds and leaves also contain substances that help reduce blood pressure and protect against cardiovascular disease. Nabag fruits, which are used as a kind of food, contain potassium, calcium, magnesium, iron, phosphorous and many vitamins (B1, B2, B6, C, E) that are beneficial to human health.
2. Promote heart health: Nabag fruits oil helps reduce the risk factors associated with heart disease, it helps to reduce blood clotting, reduce blood pressure and cholesterol.
3. Prevention of diabetes: Nabag fruits oil may help prevent diabetes.

4. Skin care: Nabag fruits oil helps the skin to treat wounds, infections, and sunburn. It is also used in many cosmetic products to moisturize the skin and protect it from drying out.
5. Boost immunity: Nabag oil helps protect the human body from various diseases and microbes, and this benefit is attributed to the presence of antioxidants and other substances known as flavonoids.
6. Prevention of cancer diseases: Flavonoids and antioxidants may contribute to the prevention of various cancers, while their efficacy is not comparable to proven and established cancer treatments.
7. Liver health: Some studies have shown that buckthorn may help reduce the level of enzymes associated with liver inflammation.
8. Aids with digestion: Nabag fruit oil may help prevent and treat stomach ulcers.

This study aimed to elaborate on nabag fruit cultivated in Sudan and the manufacture of vinegar product from its edible part, as well as assessing its quality characteristics.

19.2 Vinegar Industry

The vinegar industry is very old, more than 500 years BC, and it was discovered by coincidence when it was observed that the formation of a sour taste and a pungent odor was observed when wine or beer was exposed to the air, resulting in beer and wine being turned into acetic acid by the acetic acid bacteria. Accordingly, vinegar can be defined as the substance resulting from the double fermentation process of suitable raw materials containing starch or sugar, or both, where in the first stage of alcoholic fermentation is produced by yeast, which often follows genus Saccharomyces. This yeast works to convert sugars into alcohol, while in the second stage of fermentation, the alcohol is oxidized by the vinegar acid bacteria, under aerobic conditions, to acetic acid, which is usually between 5–8% in vinegar.

Vinegar is obtained from apples, sugar cane, rice, barley, coconut, palm, dates, raisins, honey, and kiwi. The vinegar should be clear, transparent, free from solid sediments or suspended or floated membranes or those containing acetic acid bacteria. Vinegar should be old not freshly prepared so that it has the characteristic flavor of good vinegar and that the acid concentration is not less than 6.0%.

Vinegar is widely used in the food industry to give some products a special, desirable taste. It can also be used in the food manufacturing process as one of the components of the ketchup industry, in addition to its uses for many medicinal purposes. It is considered one of the important chemical reagents, and it is an industrial material used in the manufacture of polyethylene terephthalate (polyester), which is used in the manufacture of soft drinks, photographic films, wood glue, fabrics and fibers.

When making it, it is preferable to use utensils that are not subject to rust or corrosion, in order not reacts with salt or acid solutions formed during fermentation

processes. The most suitable utensils used for pickling operations are wooden containers "barrels" which are preferred to be those manufactured from oak or Sidr wood, and it must be cleaned before use and removed any smells in it. These barrels are washed with a caustic soda solution (1/2%), then with soap and water and washed properly with water before use to remove traces of alkali and when mixed with small amounts of vegetables.

There are many types of vinegar, depending on the raw material used in the manufacture:

1. Fruit vinegar: It is the vinegar resulting from the use of fruit juices such as grape juice, orange, strawberry, etc.
2. Vinegar resulting from the use of starchy materials: such as potatoes or starchy vegetables in general, and in this case the starches must be hydrated into fermentable sugars.
3. Malt vinegar: It is the vinegar obtained from the use of a drenched barley, wheat, corn, or others.
4. Vinegar resulting from sugary solutions: such as molasses and honey.
5. Distilled vinegar: It is vinegar resulting from the use of alcohol obtained from alcoholic residues (beer industry) or from yeast manufacture.

Vinegar is corrosive, and its vapor causes eye irritation, dryness and burning nose, sore throat and lungs. It is a weak acid because it is in standard conditions of temperature and pressure, the dissociating acid is in equilibrium with the non-shaped dissolved in the form of aqueous solutions, in contrast to strong acidity, which dissociate completely (FDA 2007).

In general, vinegar can be produced from any material that contains sufficient quantities of fermentable sugars or alcohol, provided that there is nothing to prevent it from being used in food.

19.3 Fruit Vinegar

Fermented juices from many types of fruits can utilized to produce vinegar. It is technically doable to produce them from second quality fruit and even waste fruit (Monspart-Sényi 2006). Nonetheless, the fundamental explanation that fruits are not usually used to produce fruit vinegar is their low sugar content. In spite of the likenesses between the processes and the long tradition and knowledge accessible with respect to the elaboration of wine vinegars, this process isn't completely comparable to the production of fruit vinegars. Aside from the differences in sugar concentration between fruits, there are other factors to be considered as well. These factors incorporate the difficult extraction needed to get the juice of certain fruits, which prompts the utilization of commercial pectinolytic enzymes, and the high convergence of organic acids in some fruits, which can obstruct the development of certain microorganisms. It is imperative to take note of that numerous fruit vinegars are made by refining of an alcoholic solution, and the further addition of fruit juice

or fruit puree is accommodated their aromatization. These sorts of "non-natural" fruit vinegar are normally accessible in some Asian nations, for example, China, where the market has no particular guidelines for this kind of item (Chang et al. 2005). Even in Europe, clear regulation of these products doesn't exist.

As of late, various investigations have been led on these products that mainly concentrated on their sensory attributes and their quality parameters, which has been examined by chemical and sensory methods. Some examples incorporate the studies carried out with rabbiteye blueberry (Min-Sheng and Po-Jung 2010), apple (Liu et al. 2008; Sakanaka and Ishihara 2008), lemon, peach (Liu et al. 2008), persimmon (Sakanaka and Ishihara 2008; Ubeda et al. 2011), plum, and strawberry (Ubeda et al. 2011, 2012) vinegars.

Vinegar has many medicinal uses including:

1. It is either considered a generic or an antibiotic, which was known to be used in treatment scabies, strains, chronic ejaculation, scabies, wound treatment, and some types of poisoning, burning and varicose veins.
2. Used to inhibit the growth of cancer cells and solve some success in treatment of cancer.
3. It helps in treating swelling.
4. Helps to reduce haemorrhage and cosmetics.
5. It is involved in the manufacture of airplanes and stoves

19.4 Vinegar Composition and Specification

The components of vinegar generally depend on the nature of the raw material manufactured from it. Specification for natural vinegar:

1. To have a special flavor with the type of raw material from which it is produced
2. That the acid content of vinegar should not be more than 4% (weight / volume).
3. The ash content should not exceed 0.5% (weight / volume).
4. That the percentage of solid materials does not exceed 2%(weight / volume).
5. That the percentage of alcohol does not exceed 0.5% (weight / volume).
6. That the percentage of phosphoric acid does not exceed 0.5% (weight / volume).

19.5 Microorganisms Associated with the Vinegar Production

The microorganisms associated with the elaboration of vinegars are mostly yeasts and Acetic acid Bacteria (AAB). The former are the responsible for the alcoholic fermentation (AF), and the latter are required for the acetification.

19.5.1 The Yeasts

The yeasts are the most essential microorganisms during AF because they affect fermentation speed, wine flavor and other wine qualities (Pretorius 2000; Fleet 2003; Loureiro and Malfeito-Ferreira 2003; Jolly et al. 2006). The Saccharomyces genus is the most normally utilized genus in beverage industry. The Saccharomyces genus has a few novel attributes that are not found in different genera, for example, their higher ability to ferment sugar. This capacity permits them to colonize sugar-rich media and prevail over different yeasts, which are not as tolerant to alcohol. However, most of the non-Saccharomyces wine-related species have low fermentation activity (Ciani et al. 2010).

19.5.2 Acetic Acid Bacteria (AAB)

AAB are Gram-negative bacillus that is often found in single cell or in pairs. It is compulsively aerobic and thus forms membranes on the surfaces of fermentation tanks. They are not able to form spores, which enables us to eliminate them by pasteurization, and these bacteria are characterized by their tolerance of high acidity, but with a lower percentage of lactic acid bacteria (lactic acid). The optimum temperature for their growth and the production of a good amount of acid ranges between 26–31 °C (Thompson et al. 2001; Nielsen et al. 2007; Yamada and Yukphan 2008).

There are several subdivisions of acetic acid bacteria and one of these is a division Frateur who divided the acetic acid bacteria into four main groups:

1. Oxidans: Ac. Melanogenum
2. Suboxydans: Gluconobacter oxydans
3. Mesooxydans: Ac. aceti, sub sp.xylinum
4. Peroxydans.

The most important characteristics that must be met by vinegar bacteria:

1. To be able to produce vinegar acid in the appropriate quantity and speed without oxidizing the resulting acid.
2. To withstand relatively high concentrations of alcohol.
3. That the material is not sticky to prevent clogging of the openings of the fermentation apparatus.

It is worth mentioning here that some strains of vinegar acid bacteria are distinguished by their ability to oxidize the resulting acetic acid to carbon dioxide and water, that is, they are able to cause complete oxidation.

Among the most important of these types with the ability to cause full oxidation:

A.aceti, *A. xylinum, A.lancens.* Consequently, these aforementioned types are not desirable in the manufacture of vinegar, as the increase in their number in vinegar

in relation to other types leads to oxidation of the resulting vinegar and the formation of pure cellulose films, which appear as fleshy deposits or membranes.

19.6 Materials and Methods

19.6.1 Preparation of Samples

Nabag (*Zizyphus spina –Christi*) fruits were collected from Wad Medani market. To the Department of Food Engineering and Technology, College of Engineering and Technology University of Gezira. The fruits washed and soaked in water for 24 h, then seeds were removed and the pulp was filtered for vinegar production. The pulp was removed from the other fruit samples from the seeds manually, and then the pulp was crushed to powder and kept for further analyses. The pulp had an average sugar content of 9.4 degree Brix (°Bx) and pH = 3.9. The soluble solids were adjusted to 18 °Bx using a sucrose solution. To enhance the sedimentation of the nonfermentable solids, 1 g/L of bentonite was added to the nabag pulp blend (Fig. 19.2).

The following chemicals were utilized in the vinegar production and analyses:

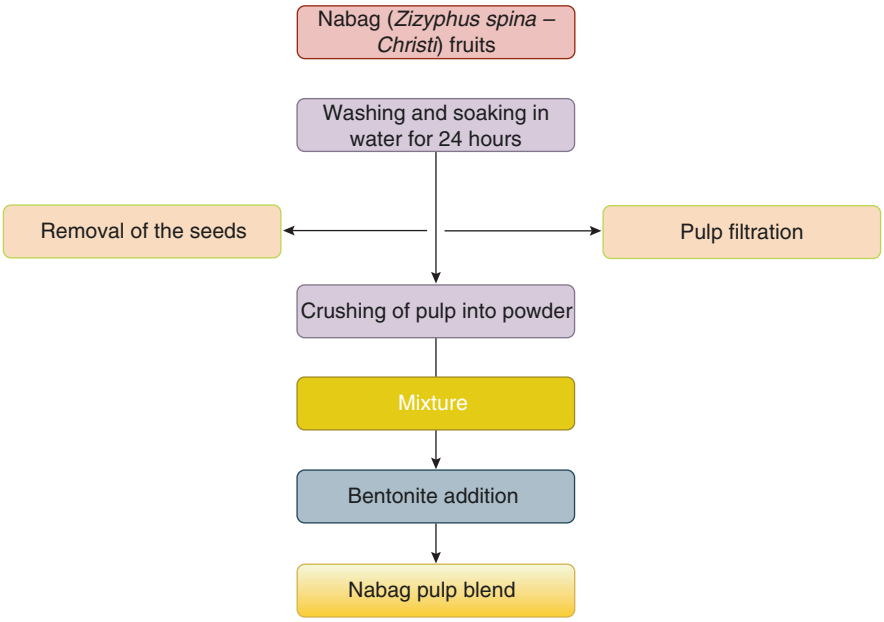

Fig. 19.2 Preparation of Nabag fruit pulp for vinegar production

- Sodium benzoate $C_7H_5O_2NA$ 1.25
- Pheno nphthaline $C_{20}H_{16}O_4$
- Absolute ethanol C_2H_5OH
- Hydrochloric acid HCl 0.02 N
- Boric acid CH_3BO_3
- Ammonium sulphate $(NH_4)_2SO_4$
- Sodium chloride NaCl
- Starch
- Acitic acid CH_3COOH
- Copper sulphate
- Calcium sulphate $CaSO_4$
- Sodium hydroxide NaOH
- Sulphuric acid H_2SO_4 conc.
- Alfa naphthol $C_{10}H_8O$
- Butanol C_3H_7OH
- Hexane C_6H_{14}
- Potassium chloride KCl
- Calcium chloride $CaCl_2$
- Silica gel
- Orthophosphoric acid
- Potassium sulphate

The yeast, (*Saccharomyces cerevisae*) at an initial count of 10^7 cells/mL were utilized for the production of ethanol. The yeast was obtained from the local market, manufactured by Turkish Company, and was utilized according to the manufacturer's instructions. The yeast cells were rehydrated in sterile water at 38 °C for 30 min. and then inoculated into the nabag blend for alcoholic fermentation.

Acetic acid bacteria

19.6.2 Chemical Composition of Nabag Pulp

Nabag fruits were analyzed chemically to determine the contents of moisture, protein and fat according to AOAC (2000) methods. The concentration of free amino nitrogen (FAN) using the formol index method as described by Aerny (1996).

The sugar concentrations (glucose, fructose and sucrose) were measured with enzymatic kits (Boehringer Mannheim, Mannheim, Germany). Titratable acidity was estimated titration with 0.1 N NaOH and phenolphthalein as the indicator.

19.6.3 Fermentation and Vinegar Production

The method described by Paturau (1982) was utilized for the fermentation process and production of vinegar (flow chart 1).

Five kg of nabag fruits were cleaned from extraneous matter, washed and soaked in 10 liter of distilled water for 24 h in stirrer tank, and then seeds were removed from the pulp and filtered. The brix and pH were measured by the refractometer and pH meter, respectively.

Half ml of orthophosophoric acid were added. Then 50 g of yeast were added and well mixed. Then batch fermentation was carried out at room temperature for 72 h in a closed in steel tank. Fermented slurry was kept in the refrigerator at 10–20 °C.

The fermented slurry sample was distilled using a distillation unit, at 78–80 °C. Then produced ethanol was collected weighted and analyzed. After, this, 10 ml of acetic acid was added to the 100 ml of ethanol.

For vinegar production, 10 ml of acetic acid were blended with 100 ml ethanol in a conical flask, and then the flask was closed with a foil paper containing several pores in order to allow oxygen to enter for 72 h (the oxidation process) at a temperature of 37 °C. The vinegar harvest was then weighted and analyzed (Fig. 19.3).

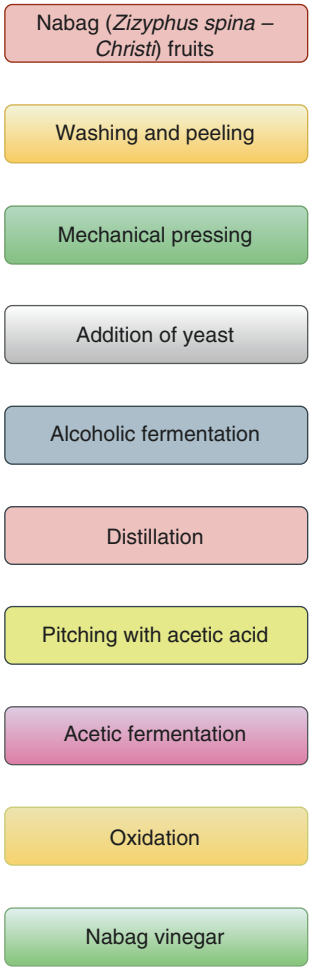

Fig. 19.3 Flow diagram of nabag-vinegar production

19.6.4 Physicochemical Analyses of Ethanol

The pH values of the ethanol was measured using a pH meter (PHS-3C Digital) at ambient temperature according to ICUMSA (1994). The refractive index was estimated using an Abbe bench refractometer (ICUMSA 1998). The ethanol density was measured according to Scann (1971) and was calculated as follows:

$$\text{Density} = \text{weight} / \text{volume}$$

19.6.5 Determination of Ethanol Concentration

Abbe refractometer was used for the determination of the concentration of ethanol. A series of dilutions for absolute alcohol were prepared (10%, 20%, 40%, 60%, 80% and 100%), and the refractive index of the dilutions was recorded, then a curve was plotted. The concentration of the ethanol was determined from the curve (Fig. 19.4):

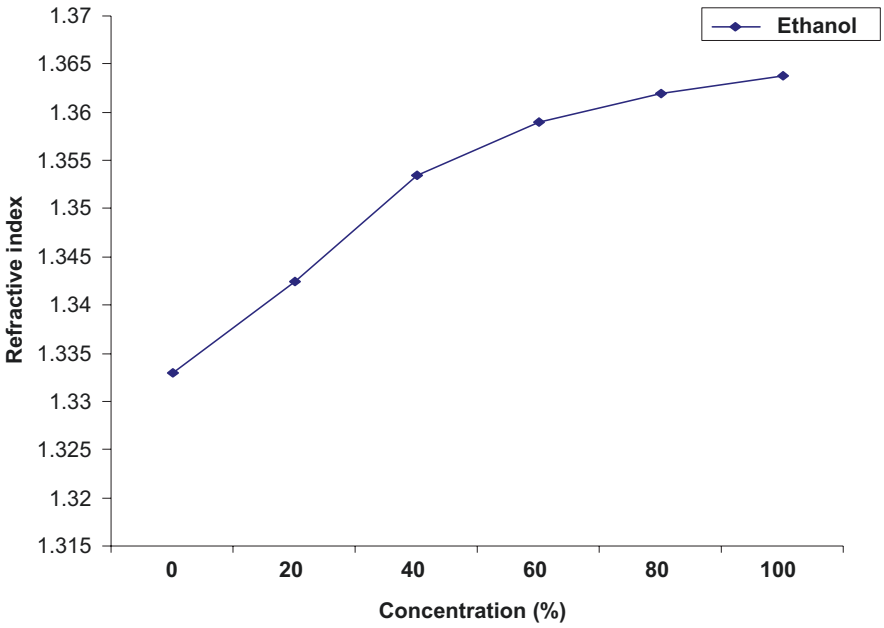

Fig. 19.4 Concentration of ethanol

19.6.6 Physicochemical Analyses of Vinegar

The pH of the vinegar was measured using pH- meter (PHS-3C Digital) at ambient temperature (ICUMSA 1994).

The density of vinegar was measured as described by Scann (1971). The flacon (100 ml) was weighted then filled by ethanol, and weighed again, then the density of ethanol was calculated. The weight per volume of flacon.

$$\text{Density} = \text{weight} / \text{volume}$$

The concentration of acetic acid in vinegar to contrast it with the least required concentration of 4 g acetic acid per 100 ml of vinegar. The analytical technique followed utilizes the neutralization reaction between acetic acid and sodium hydroxide, in this strategy:sodium hydroxide solution of 1.0 molarity was contained in a burette, and the acetic acid solution was contained in an Erlenmeyer flask and phenonapthaline was added. Acetic acid reacts with sodium hydroxide, a base, according to the reaction:

$$\underset{\text{Acetic acid}}{CH_3CO_2H} + \underset{\text{sodium hydroxide}}{NaOH} \rightarrow H_2O + \underset{\text{sodium acetate}}{CH_3CO_2Na} \quad (19.1)$$

This is an example of an acid-base neutralization reaction in which an acid and a base- react to produce water plus a salt.

In the titration strategy, NaOH was added to the acetic acid solution until complete reaction with all of the acid. The point where just enough base has been added to neutralize the acid was called the equivalence point. According to reaction (19.1), one mole of base reacted with one mole of acid. Therefore, at the equivalence point we have the relation.

Moles of base added = moles of acid initially present

Moles of base added = molarity of base x volume of base added and, therefore:

Moles of acid initially present = molarity of base x volume of base added

http://wwwchem.csustan.edu/consumer/vinegar/analysis.htm (1999).

19.7 Results

19.7.1 Chemical Composition of Nabag Pulp

Some of the chemical components of the nabag pulp are indicted in Fig. 19.5. The contents of moisture, protein, total sugars, fructose, sucrose, FAN, Titratable acidity and ascorbic acid were found to be 6.8 ± 0.09%, 7.6%. 7.2%, 112, 50.2, 60, 118 (mg/l), 0.76% and 35.56 (mg/100 g), respectively.

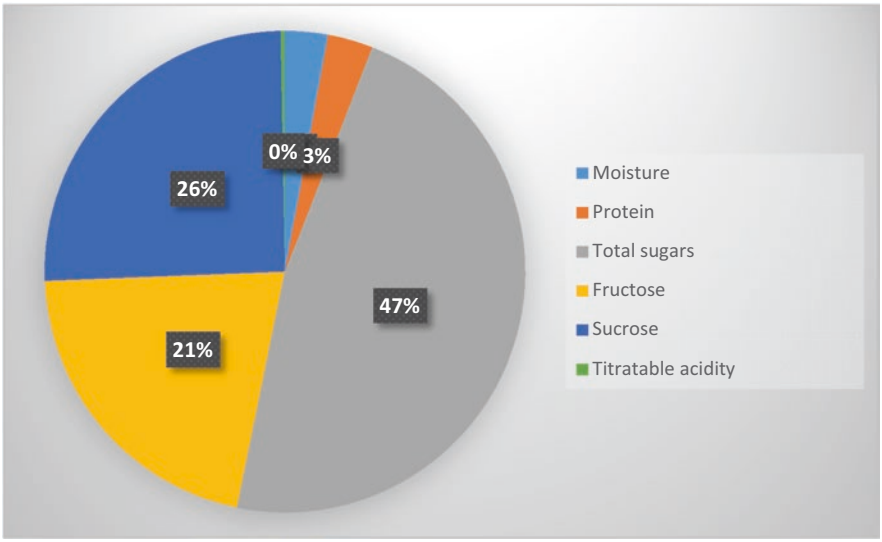

Fig. 19.5 Chemical composition of nabag pulp (per 100 g fruit)

Table 19.1 Physicochemical characteristics of ethanol resulting from nabag fermentation

Character	Value
Concentration	91%
Density	0.83799 at 33 °C g/ml
Refractive index	1.3720
pH	3.7

19.7.2 Physicochemical Properties of Ethanol and Vinegar

Table 19.1 presents some of the physicochemical properties of ethanol produced as nabag pulp fermentation with *Saccharomyces cerevisiae*. The concentration, density and Refractive index of ethanol was 91%, 0.83799 at 33 °C g/ml and 1.3720, respectively.

Table 19.2 shows some of physicochemical characteristics of the vinegar after production. The volume of the produced vinegar was 833 ml/kg of nabag. The vinegar had a density value of, The concentration of acetic acid, pH value and density value of the vinegar was 6.24 g/100 ml, of 2.92 and 0.93344 g/ml at 33 °C, respectively.

Table 19.2 Physicochemical characteristics of vinegar prepared from nabag fruit

Character	Value
Concentration of acetic acid	6.24 g/100 ml
pH	2.92
Density	0.933448 g/ml at 33 °C

19.8 Discussion

In the present study, vinegar was prepared from nabag fruit. All the processes were carried out with fruit pulp. In order to analyse the vinegar process and to prevent side effects caused by wood, we used glass containers glass containers, which were cleaned with boiling water.

Alcoholic fermentation of fruits is affected by the lack of balance between fermentable sugar and accessible nitrogen and the accessibility of various vitamins or minerals (Ribéreau-Gayon et al. 2006). Moreover, the utilization of selected yeast strains is a requirement and makes a critical commitment to the attributes of the final product in beer (Degre 1993). For the alcoholic fermentation,, the greater part of the yeasts accessible for starting cultures have been chosen from brewing in light of the fact that they are acceptable performers, have low nutritional requirements, start fermentations rapidly, give great good fermentation rates, and produce secondary metabolites that are auxiliary metabolites that are valued consumers (Degre 1993). In the present study, the fruit utilized (nabag) has a large amount of available nitrogen, considering the fermentable sugar and comparing with that present in numerous fruits used to prepare vinegar. Other nutrients and vitamins are also available in nabag fruits (Hussein 2019).

The concentration of ethanol, density and Refractive index of ethanol was 91%, 0.83799 at 33 °C g/ml and 1.3720, respectively. The initial pH was 5.6 and decreased sharply to 3.7 after 24 h in the alcoholic fermentation. This value stayed steady all through the alcoholic fermentation.

The concentration of acetic acid in vinegar determined in the present study was within the scope of the standard value which was 4–8% (http:en.wikipedia.org/Acetic acid, 2011), and lower than the value detailed by the US Food and Drug Administration, Code of Federal Regulations (http://www.Fda.gov/org/compliance-ref/cpg fod/cpg525-825-2006) which expressed that vinegar product should contain a minimum of 4% acidity. Typical white distilled vinegar is at least 4% acidity and not more than 7%. Cider and wine vinegars are are normally somewhat more acidic with approximately 5–6% acidity (http://www.versatile vinegar.org/ faqs. Html, 2007).

The density of vinegar was lower than both the standard value (0.96 g/ml) and the house hold vinegar utilized for cooking (1.05 g/ml) (http://en.wikipedia.org/wiki/vinegar, 2009). It was expressed that density, or mass per unit volume for a typical commercial vinegar with 5% acetic acid content, is about1.01 g/ml (http://

www.apple-cider-vinegar-benefitis.com/apple-cider-vinegar-health-benfits.html, 2009).

During vinegar production, the acetification process is still just mostly comprehended. The vast majority of the vinegar is created from alcohol and a blend of supplements in industrial processes in which the seed culture is submerged in in an exceptionally circulated air through tank and kept up constantly all through a batch process, with a daily refilling system. Nonetheless, the high quality vinegars are delivered with the conventional surface culture technique. In this strategy the acetic acid bacteria lie on the fluid air surface and produce a biofilm that utilizes oxygen straightforwardly from the air or from the restricted measures of air that go through the wood pores. Thus, most starter cultures in both cases have restricted accessibility and are inadequately characterized (Mas et al. 2007).

The yield of vinegar is satisfactory as it was in every case well over 60% (Bokhari 2013). The entire cycle was performed at the lab level, with such restricting elements as the strength of the press and the recuperation of fruit mash on a small scale. The final product got in this investigation demonstrated great colour and good sensory characteristics, with compensated pungent smell of the unpredictable acidity.

19.9 Conclusion

Since nabag is utilized as conventional food in the Sudan, however nowdays Sudanese individuals are not keen on nabag fruit such a huge amount with exemption for certain individuals who appreciate eating nabag tissue as nibble food particularly the youngsters. Next to this nabag cost is extremely low in the Sudanese market. Every one of these variables roused the specialist to utilize nabag underway of a significant item, for example, vinegar and consequently improve its economical value. The high nutritional and therapeutic values, beside high sugar content favoured its use in the production of vinegar. The volume of vinegar prepared from nabag fruit pulp was equivalent to 828 ml per kg of nabag. It is highly recommended to process vinegar under controlled conditions and increase the production of vinegar at a large – scale level. Vinegar should not be packed in plastic containers for its acidic properties which may lead to serious problems to the consumers.

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Chapter 20
Selected Fermented Cereal Products of Sudan

Abdel Moneim Elhadi Sulieman, Walied Abdelrahman Mustafa, and Onaheid Ahmed Osman

20.1 Introduction

Africa is one of the lowest producers of cereals worldwide. Cereal crops are used largely as food in African countries, than in the developed world. Main cereals grown in Africa include maize, rice, sorghum and millet. A majority of traditional cereal-based foods consumed in Africa are processed by natural fermentation (FAO 2020a, b). Dietary grains are small, strong, and dry seeds that might be with or without skin or attached to the fruit. It is harvested for human or animal consumption. In addition, agricultural engineers call the plants that produce these seeds "cereal crops". There are two main types of commercial cereal crops, which are grains such as wheat and corn and rye cultivated and legumes such as beans, beans and lentils. After harvesting, dry grains are stronger and more durable than other staple foods, such as starchy fruits such as plantains and breadfruits, and tubers such as sweet potatoes and macaroni. This durability made the grains very suitable for intensive cultivation, as the grains could be harvested automatically, transported by rail or ships and stored for long periods in silos, and the grains were milled to produce flour and squeezed to extract oil. Therefore, we find in the global food commodity markets the availability of canola, corn, rice, soybeans, wheat and other grains, but not for tubers, vegetables or other crops (OECD/FAO 2018).

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Worldwide cereal production is expected to increase to expand by 13% between the base time frame and 2027, fundamentally inferable from more significant returns. Worldwide cereal use is projected to increment by 14% between the base time frame and 2027, predominantly inferable from higher food and feed use in agricultural. Utilization is required is required to increment by 13% contrasted with the base period time frame, and keeps on being generally utilized for human utilization, with food use representing around 66% of all out use all through the projection period (OECD/FAO (2018). As per the discoveries of the Sudan Government drove Annual Crop and Food Supply Assessment Mission, the 2019 aggregate cereal production is estimated at about 5.9 million tonnes, 33% underneath the 2018 bumper harvest and 15% underneath the average of the past 5 years. The decrease underway is because of a blend of diminished plantings and low yields (FAO 2020a, b). The area planted with sorghum and millet declined in 2019 by around 15% contrasted with 2018 as farmers opted to expand plantings of more gainful cash crops, including sesame and groundnuts. Simultaneously, yields were influenced by a combination of unfavorable weather conditions and pest assaults (FAO 2020a, b).

20.2 Fermentation of Cereal Dough

Cereal grains are fermented in different from in Sudan. One of the conventional forms in which sorghum is fermented is the coarse meal or *derish* (Dirar 1993), which is ground material, doesn't shape slurry as does fine flour when blended in with sufficient water. Notwithstanding, the utilization of present day mill flour in urban communities has prompted the slurry form making the reason for sorghum fermentation. Fermentation process for development of a thick batter, and a diluted suspension of maize, followed a similar example (Chaves-López et al. 2020). The Africans who follow these various strategies of cereal fermentation should have strong motivations to choose one form over the other. The choice of the form in which cereal is fermented additionally has bearing on the sort of food to be made later. The release of nutrients, for example, the little molecules, from an intact grain, a coarse meal or fine flour, into the surrounding water is expected to take place at various rates. The amount of soluble so released could lead to the creation of differences in such factors as osmotic pressure, sugar concentration, availability of growth factors, etc. which could in turn influence and select for particular microorganisms. Even if the general course of fermentation is not affected, then perhaps the flavor and the texture of the final dough are.

The ratio of flour to water must also influence the fermentation of cereals as it directly influence the concentration of nutrients and other factors of importance in microbial growth. The ratio varies widely in Africa and even within the boundaries of the same country, for example, the Sudan. The ratios given by various authors for fermentation of *kissra* dough (*ajin*) vary widely in Sudan, Indicating that at least some of the values given are more likely the outcome of guess work rather than actual measurement. Badi et al. (1987) reported one part of sorghum was added to

two parts of water. Abdel Gadir and Mohamed (1983) gave a ratio of 2:3, while Vogel and Graham (1979) gave a ratio of 9:2, flour: water. Various volumetric measurements, however, g a ratio of 1:1 in the case of batter prepared from flour obtained by milling through modern mechanical mills (Dirar 1993).

Cereal fermentations planned for arrangement of porridges in Africa are essentially of souring type. In the Sudan, when one finds that about half of porridges produced using millet are produced using from unfermented batter or flour, sorghum porridges and basically all other sorghum foods and drinks are any remaining sorghum food sources and beverages are for all intents and purposes 100% produced using fermented material. The fermentation of sorghum in the Sudan is acidic. Nonetheless, sour lactic taste insight regarding these foods sources appears to have some refreshing impact. The Sudan and countries lying to the West of it as far as the Atlantic coast are very hot countries with every day temperatures, in numerous occurrences, transcending 40 °C. The inhabitants of these countries have built described by being characterized by being prepared through lactic fermentation (Abah et al. 2020).

20.3 Fermenting Microorganisms

Natural fermentations are started without the addition of starter microorganisms, and their control is restricted to maintenance of external environmental conditions (Nout and Rombouts 1992). According to El Hidai (1978), normal fermentation of sorghum is essentially lactic acid by *Lactobacillus* spp. and yeast, and acetic acid fermentations to lesser extent during the latter stages of fermentation. El Sharif (1993) studied the microorganisms associated with the different stages of fermentation of *Abreh,* a fermented sorghum refreshing drink, and found that the microbial population was mainly composed of lactic acid bacteria, which were the predominate predominant fermenting group throughout fermentation period. Mohammed (1991) isolated and described the microorganisms related with the conventional sorghum fermentation for creation of Sudanese *Kissra*. They found that the microbial population during the 24 h of fermentation consisted of (*Pediococcus pentosacus*, *Lactobacillus confuses*, *Lactobacillus brevis*, *Lactobacillus* spp., *Erwin bananas*, *Klebsiella pneamoniae*, and *Entrobacter cloacae*), yeasts (*Candida intermedia* and *Debaryomyces hansenii*) and molds (*Aspergellus* spp., *Penicillus* sp., *Fusarium* sp. and *Rhizopus* spp). P. pentosacus was the predominant microorganism toward the end of the 24 h fermentation.

In their investigation on the microbial analysis of selected sorghum varieties, Yagoub (2009), they found that before fermentation, the count of bacterial, yeasts and moulds and staphylococci ranged between 5.60×10^6 to 6.25 cfu/g, 2.5 and 6.15 cfu/g, 2.04 × 10 and 9.0510 cfu/g, respectively. The microbiological analysis also revealed that *E. coli* counts exceeded 2.40 cfu/g and *Salmonella* was detected in the tested sorghum varieties. However, after fermentation the total bacterial count increased to the range 2.04 × 10–6.60 × 10cfu/g in 19 h. The moulds and yeasts

count also increased to the range 4.73 × 10–7.30 × 10 cfu/g, Staphylococci count ranged between 5.40 and 6.87 cfu/g, the *E. coli* ranged from 28 to 34 and also *Salmonella* was detected. On the other hand, after 24 h fermentation, the total bacterial count increased to the range of 5.53–6.32c.f.u/g, and the moulds and yeasts increased to the range 3.83–7.93c.f.u/g, while Staphylococci count increased to the range 3.07–7.93c.f.u/g to, and *E. coli* count increased to 5. Moreover, *Salmonella* cells disappeared completely after 24 h fermentation. Hamad et al. (1992) found that the main microorganisms associated with this fermentation were homo- and hetero- fermentative LAB of the genus Lactobacillus. Mohammed et al. (1991) utilizing persistent house hold conventional fermentation strategies, discovered that microbial population was overwhelmed by LAB and the species identified were: *Pediococcu*s, *Pentosacus*, *Lb. confuses*, *Lb. brevis* and *Enterococcus faecium*. The authors suggested that the yeasts *C. intermedia* and *Debaryomyces hansenii* could be significant in the fermentation of sorghum for preparing *Kissra*. Mbugaa (1977) expressed that when sorghum microorganisms were enacted by incubation at 45 °C the dominating microorganism (half of the isolates) were *Lb fermentum*. Yeasts were additionally present yet their role in the fermentation was not satisfactory. El Tinay et al. (1985) discovered Lactobacillus and Streptococcus, while Abdel Gadir and Mohammed (1983) announced the presence of Lactobacillus. All these workers agree on the fact that sorghum fermentation is mainly lactic acid dependent. Agab (1985) investigated Hulu-mur for its microbiological feature, 41 bacterial strains and 5 yeasts were isolated at various stages of fermentation (Elkhalifa et al. 2007) (Table 20.1).

20.4 Effect of Fermentation on Nutrients of Cereal Fermented Foods

Fermenting microorganisms utilized to create new items with improved sensorial and nutritional qualities frequently delivers different metabolites that restrain the development of spoilage and/or pathogenic microbes. These metabolites incorporate organic acids such as lactic acid, propionic acid, acetic acid, etc. that decline the underlying pH value, establishing an acidic environment in the food matrix and therefore extending the shelf-life of the fermented product (Nyanzi and Jooste 2012; Tsafrakidou et al. 2020). Besides, ethanol and hydrogen peroxide, which are strong inhibitory factors for microbial growth, as well as other secondary metabolites that can go about as antimicrobial mixtures, are delivered by some LAB and yeast species. Lactobacillus and Pediococcus strains, having antimicrobial activities, were tested with respect to their productivity to diminish mycotoxin creation from Fusarium just as to limit the development of other mycotoxigenic fungi during malting of wheat grains (used for beverages and bakery products). LAB reduced the Fusarium toxins (deoxynivalenol-vomitoxin-, T-2, HT-2, and zearalenone) by up to 75%, depending on the strain. Antifungal activity was also observed from LAB

Table 20.1 Changes in microbiological characteristics during different fermentation periods of sorghum flour

	Feterita			*Tabat*			*Wad Ajar*		
Fermentation period	0 h	19 h	24 h	0 h	19 h	24 h	0 h	19 h	24 h
Total bacterial count (cfu/g)	$5.60^{ab} \times 10^6$	$2.04^{b} \times 10^8$	$5.83^{ab} \times 10^9$	$6.25^{a} \times 10^5$	$6.60^{a} \times 10^6$	$5.53^{b} \times 10^7$	$5.20^{ab} \times 10^5$	$6.40^{a} \times 10^5$	$6.32^{a} \times 10^5$
Staphylococci count (cfu/g)	$2.04^{b} \times 10^3$	$6.87^{a} \times 10^2$	$2.81^{a} \times 10^2$	$9.05^{a} \times 10^3$	$6.87^{a} \times 10^2$	$4.51^{b} \times 10^2$	$2.27^{b} \times 10^3$	$5.40^{b} \times 10^2$	$3.07^{c} \times 10^2$
E. coli	2.40 < 0	34	15	<2.400	34	11	<2.400	28	15
Yeast and mould count cfu/g))	$2.50^{b} \times 10^4$	$7.30^{a} \times 10^7$	$9.11^{b} \times 10^7$	$6.15^{a} \times 10^5$	$6.86^{b} \times 10^6$	$7.93^{a} \times 10^7$	$4.95^{ab} \times 10^4$	$6.70^{c} \times 10^4$	$6.27^{ab} \times 10^5$

Means having different superscript latter in each row differ significantly (p 0.05 ≥) using DMRT

metabolites (particularly from acetic acid and secondarily from lactic acid) (Juodeikiene et al. 2018).

In the Sudan, it is accepted that fermentation makes the sorghum batter brighter in colour and smoother in texture. The latter change is so dramatic to such an extent that one can tell a stiff porridge made from a fermented batter from one made from unfermented batter, not only by taste or odour, but even by touch. The porridge produced using fermented sorghum dough is exceptionally smooth while that produced using unfermented is rather granular (Dirar 1993). Cant be produced using absolutely unfermented sorghum batter. The matured mixture is more reasonable and can without much of a stretch is extend out into exceptionally far sheets, which can be stripped off the hot plate without any problem. Fermentation definitely affects on the simplicity of baking of very thin *kissra* sheets. Indeed good *kissra* bread cannot be produced using absolutely unfermented sorghum. The fermented batter is more coherent and can easily be spread out into exceptionally thin sheets, which can be stripped off the hot plate easily. The fermented batter has an indisputable, characteristic flavor which goes through a perceptible change on baking the dough. Although, lactic acid gives the desirable sour taste, other minor acidic and impartial substances, such as ethanol, ethanoic acid, butanoic acid and propanoic acid account for much of the organoleptic quality as has been accounted for ting of Southern Africa. It has been accounted for that both germination and regular lactic fermentation improved the nutritional value of cereals. Fermentation was found to cause exceptionally critical improvement ($P \leq 0.05$ in the proximate analysis of three local cultivars of sorghum; namely *Feterita*, *Tabat* and *Wad Ajar* which contained more significant levels of protein, fat, crude fiber and moisture content than the other two varieties. Fermentation was found to cause highly significant improvement ($P \leq 0.05$) in *vitro* protein digestibility and improved nutritional value of sorghum by reducing levels of physic acid and polyphones (Yagoub 2009). The normal lactic acid fermentation of ground grain sorghum showed a huge expansion in available lysine, leucine and methionine, the relative nutritive value, niacin, thiamine and riboflavin were also increased. Additionally Chavan et al. (1988) noticed a critical expansion in water-soluble proteins and free amino acids in sorghum exposed to 24 h of fermentation.

20.5 Important Cereal Crops in Sudan

20.5.1 Sorghum

The agricultural sector in Sudan is considered one of the most important and prominent sectors of food production in Sudan, which works to satisfy the needs of the population, and is also a basic source for many sectors of work and livelihood for a large segment of the Sudanese people. Sorghum is considered one of the main foodstuffs in Sudan, which is included in many of the exported crop combinations that

exploit large areas in the agricultural sector, as the types of sorghum in Sudan are many and varied and their name differs from one place to another, such as (*Wad Ahmed, hybrid sorghum, Gadam Alhamam, Tabat, Feterita, Dabar*). Sorghum is considered one of the most important crops in Sudan, as sorghum is grown in Sudan in the irrigated and rainy sectors, and it is of great importance in securing food in Sudan. It contributes about 75% of the domestic production of cereals. Most of the production is consumed locally as human food and the other part enters into animal feed industries for the excellent quality of grains (FAO 2020a, b). Sorghum cultivation plays a very important role in raising the economy of Sudan. In addition, sorghum in Sudan is a main source of concentrated carbohydrates that are used to provide food for humans and animals in Sudan. Green sorghum is used in Sudan as feed for poultry and animals. In Sudan, sorghum is mainly used as a fuel and as a building material in homes and other real estate. Corn is used in the manufacture of glucose and starch. Sorghum is used in the manufacture of ethanol gas as an alternative to energy. Sorghum flour is used in bread making as wind breaks around vegetables and crops (http://www.tpsudan.gov.sd/index.php/ar/pages/details/179).

20.5.2 Pearl Millet

Pearl millet is one of the most important cereal crops in western Sudan, because it is the only good grain crop for sandy lands. The western states produce 95% of its total area of approximately 5 million acres (2.1 million hectares). Millet is an annual herbaceous plant with small seeds that originated in Asia and Africa. There are several types of millet, where there are at least twelve distinct species, but only six of those types constitute most of the world's production of millet. Millet is one of the plants that produce small, edible grains. Millet grows well in dry sandy soils, and its grain is an important food source in Asia and Africa. Millet grains, leaves and stems are also used as livestock food in some countries. Millet is considered one of the main cereal crops in Sudan, India and some African countries. It is also important in the agricultural cycle and crop rotation system and is considered a summer fodder crop (http://www.tpsudan.gov.sd/index.php/ar/pages/details/179). Due to its rapid growth, resistance to drought and rapid maturation, it is therefore sometimes grown as an emergency crop after the failure of the previous crop. Millet is considered one of the most important cereal crops in western Sudan, as it is the only crop of good yield for sandy lands. The western states produce 95% of the total production in Sudan.

Millet is considered a food crop because it contains carbohydrates, proteins, vitamins, quercetin, and stable oil. It also contains essential and non-essential amino acids and fatty acids. It also contains protein in high amounts and fiber. In addition, millet contains vitamins such as B vitamins: niacin, riboflavin, and thiamin, as well as carbohydrates, and a number of minerals such as calcium, potassium, sodium, zinc, copper, phosphorous, boron, iron and magnesium. Therefore it is considered a good food that contains the elements needed by human body.

20.5.3 Wheat

Wheat is an old crop in Sudan, but for many decades its cultivation remained confined to the far north Sudan for thousands of years. However, its cultivation was confined to the Northern State, and the River Nile State "between latitudes 17 and 22 north". Until the end of the fifties of the twentieth century and in an area not exceeding 30 thousand acres, which was sufficient for the consumption of the inhabitants of the two States in order to provide the environmental conditions for its production and its roots in the consumption habits of the inhabitants of that region, The areas cultivated with wheat in the Northern State were limited in the narrow, irrigated coastal strip Directly from the Nile, where wheat was grown with the aim of self-sufficiency for farmers' families, in the 1960s. The past: The United States of America invaded the third world countries with what is known as the American aid program. This caused a change in the diet of the population, and as a result, the demand of the people of Sudan for wheat increased. The country tended to expand its production, so its cultivation extended to the south until it entered the island project, as it expanded in the east, until the Halfa and Rahad project, the locally produced quantities of wheat were not sufficient to meet the demand. This increase led to the import of additional quantities from abroad to meet the increasing demand for wheat (Ikram 2017). The demand for wheat has increased. This situation has led to the expansion of wheat cultivation south of Khartoum, the "Gezira Project"in areas that are marginal to this crop in terms of temperature (Ikram 2017).

Wheat is an important cereal crop, as more than a third of the world's population depends on it for their food daily because of its high nutritional value. Wheat is included in many meals in one form or another despite that, it is used mainly in making bread because it contains gluten, which makes it elastic (it swells easily when treated with yeast). In addition to making bread, wheat is used as an essential ingredient in making cakes, pies, and pancakes and various pastries. Wheat contains carbohydrates that provide the human body with calories and high energy that helps with activity and vitality, as well as contains an estimated percentage of proteins, which helps in growth. In addition, wheat grains contain essential minerals such as phosphorous and iron.

20.5.4 Maize

Maize has a lower need in agricultural development plans in the Sudan because of low yield potential, restricted nearby and low market price. However, the absences of adjusted lines with high return potential and good resistance to water stress are the significant restricting elements for maize production in the Sudan (Bello et al. 2010). Maize can possess a significant situation in the economy of the economy of the country. Because of the chance of blending maize with wheat for bread- making, the expansion in the interest of maize for poultry feed and for scavenge just as its

incredible potential for export (to give new source of hard currency).This necessities, looking for expanding maize production and productivity in the Sudan (Abdalla et al. 2010). Maize or yellow corn is one of the major cereal crops in the world. Despite the great importance of maize in the world, Sudan considers maize as a secondary crop that is cultivated on a small scale and is considered a staple food only in southern Sudan (http://sudaneconomy.net/sects/agr/mhasil.htm). Most of the world's production of maize is consumed locally. The quantities entering the world market are estimated at about eight million tons, of which Arab countries import about five million tons. Based on the availability of the production components of this crop in Sudan in terms of land, water and a suitable climate, maize is considered one of the promising crops to increase the country's foreign exchange earnings in addition to contributing to achieving food security. (http://sudaneconomy.net/sects/agr/mhasil.htm).

20.6 Fermented Sorghum Products

20.6.1 Kisra

Kisra is the staple Sudanese diet (Figs. 20.1 and 20.2). It is a piece or slice of bread prepared from fermented sorghum flour (Sulieman et al. 2003). The nutritive value of *kisra* is fundamentally a conversation of the nutritive value of sorghum or millet; it was discovered that in Gezira and Managil regions, cereals provided 80% of the protein and together with sugar 84.4% of calories in the diet (Dirar 1993). This bread is the staple of the Sudan; it is prepared from sorghum or millet flour. By far, however, the bulk of kisra is produced using the different kinds of sorghum. The flour is blended with water and a little portion of the previous lot of fermented sorghum batter is added as a starter. The batter is incubated in a warm corner of the

A B C

Fig. 20.1 (**a**) *Mohraka*, a traditional utensil used for milling grains. (**b**) Gargareeba a traditional utensil for making kisra sheets during baking. (**c**) *Raika:* traditional fonds plate for keeping *kisra* sheets

Fig. 20.2 Baked *kisra* sheets

house, then covered and left to ferment over-night. Fresh flour might be added to previous batter, and it is baked on a hot plate to give thin sheets of bread (Dirar 1993, 1996). Fermentation of the batter was found to be mixed, with lactic acid and acetic acid bacteria playing the significant role but yeast have a minor role, (FAO 2006). Average values for the composition of kisra are: 50% moisture, 80% carbohydrates 12% crude protein, 2% crude fiber and 1.5% ash.

Two sorts of kisra can be depicted dependent on the technique for spreading the batter during baking, *kissrat kass* and *kissrat-gergriba*. In the baking of previous, the batter is moved with kass and emptied straightforwardly into focus of the hot plate utilized for baking. The empty kass is then held by the edge in an upstanding position and the hitter spread with the lower part of the gourd by moving the compartment in spin pool movement in logically extending circles until the entire batter has been flattened out into a rippled, roundabout sheet (Dirar 1993). On the other hand, the "Kisra" was found in some traditional Algerian items from durum wheat (Kezih et al. 2014). Today, with developing urbanization, kisra is becoming a commercial home-based industry in Sudan. Globally, in view of the clear expansion in the frequency of celiac illness and bigotry to wheat, interest in without gluten grain items is expanding quickly (Kelly et al. 2008a). Kisra seems to have impressive potential as the basis for improvement of a gluten-free sandwich wrap.

A few different species of lactic acid bacteria can be ensnared in the fermentation of kisra. By utilizing the molecular technique, Lactic acid bacteria and yeast were identified to species level (the most prevailing bacteria was *lactobacillus plantarum* and the most dominant yeast was *Saccharomyces cerevisae* (Kawther et al. 2018).

The sensory evaluation showed that the kisra produced using pure culture of LAB and yeast had high score in all quality attributes. The starch fermenting strains may be significant in the improvement of the starter cultures and for use in the advancement of small-scale commercial production of kisra.

20.6.2 Aceda

Aceda is a stiff porridge made from fermented sorghum or pearl millet flour (Figs. 20.3 and 20.4). Aceda is an Arabic dish of flour mixed with water and is from popular foods and has a special place on social occasions, and it is one of the main meals in Sudan, and there are different types of aceda from one region to another in regions is made of corn flour and other areas or sorghum or pearl millet.

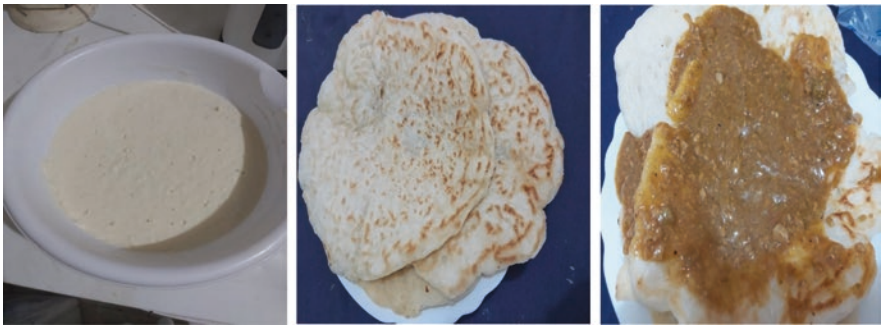
Ingredients include: water, 2 tablespoons salt, 5 tablespoons of keerah oil. The earliest container used for cooking aceda must have been an earthenware pot. Until very recently, in fact, the clay pot called *burma* has been used for the job and even today this container is used by the Hadendowa tribes of the easten Sudan for the purpose.

The Sudanese tajin used to cook aceda is large, hollow iron container. However, aceda may nowadays be cooked in any cooking container, such as saucers and sauce-pans (Elkhalifa et al. 2007).

For preparation of aceda, the sorghum flour is mixed put an appropriate amount of water, salt and oil a day before in a container. After the water boils and over a low heat, we add the dough, fry, gradually, and with constant stirring using manual or wood-scraper to make the texture medium is neither very light nor very thick. Moreover, It will be left until the dough is ripened, with constant stirring so that it does not stick to the bowl. To know the ripening, we wash hands with water and place them on a surface, if the porridge does not stick to the hand, is ripe and if it sticks to the hand it has not ripened yet. After making sure of the ripening, we wipe the molds with oil well to avoid sticking porridge to the molds. Then the porridge is

Fig. 20.3 (**a**) Konsh for mixing aceda; (**b**) Halat Katoosh used for baking aceda; (**c**) Aceda

Fig. 20.4 Aceda covered with molah (cuisine)



A B C

Fig. 20.5 (**a**) Gurrassa wheat dough; (**b**) Baked Gurrassa product; (**c**) Gurrassa covered with *molah*

poured into the molds while it is hot. It is heated and cautiously left to cool completely.

20.6.3 Gurrassa Murra

Gurrassa Murra is restricted toward the Northern State of Sudan, particularly to the region lying between the towns of Dongola and Karima on the grounds that this territory is the date-creating area (Fig. 20.5). In the preparation of Gurrassa Murra, wheat flour is added to water containing a little salt and blended well to make slight slurry. At that point about 10% sour sorghum batter is added as a starter then the batter is placed out in the sun for 4–6 h. Then dates are boiled to make thick slurry. This is then mixed with the sour wheat batter just before baking. The mixture is

baked on a hot plate then the product is scoured with ghee and folded twice to give a triangular shape for storing (Abdel Rahim 2003). The more predominant bacterial genera were Lactobacillus, Bacillus, Staphylococcus and Micrococcus, in descending order. The total viable yeast count was 102–104 cfu/g. The more predominant yeast isolates species were Debaryomyces pseudopolymorphus and Debaryomyces hansenii. The bacterial isolates of the laboratory-adapted samples were Bacillus sp. and Staphylococcus sp. and the yeasts were Debaryomyces hansenii Abdel Rahim 2003). Biochemical changes during fermentation of Gurrassa Murra are characterized by a reduction in pH from 6.4 to 4.2 and there was a notable increment in acidity from 1.3 to 2.9% and a decrease to 2.1% after addition of the dates. Alcohol increased from 0.4 to 2.1% and then reduced to 1.2 after addition of dates.

20.6.4 Abreh

Abreh is typically created from white varieties of sorghum which are cleaned and dehulled then soaked in water overnight then processed to give "*ajin*" (Fig. 20.6). The process additionally includes olives, cooking of one third of *ajin* into thin paste "*madida*", and mixing of *madida* with the remainder of *ajin,* and addition of spices, fermentation and then baking into thin flakes. These flakes are suspended in water, sweetened with sugar and swallowed whole without sieving (El-Sharif 1993). The proximate composition of *Abreh* produced from *Tabat* sorghum cultivar indicated that the dry matter, ash, fiber, protein, oil and carbohydrate contents of Abreh product was 92.56 ± (0.02)%, 1.77 ± (0.08)%, 2.04 ± (0.13)%, 11.66 ± (0.14)%, 3.12 ± (0.25)% and 73.96 ± (0.52)%, respectively (Mariod et al. 2016). During Abreh processing the seeds first decorticated so this decortications had numerous effects on grain composition. The protein content of sorghum flour was 12.24% it

Fig. 20.6 Abreh baking and preparation for consumption

was signed ($P \leq 0.05$) decreased to 11.66% in Abreh batter before the addition of spices, this decrease was related to a fermentation process. The drink abash, which has a fresh so taste, satisfies the role of a nutritious dainty quencher in the sweltering environment of Sudan. The Sudanese effectively accept that a beverage of *Abreh* is a better method of fulfilling thirst than plain water. Moreover, to being nutritious, the acidity developed during the fermentation of sorghum decreases the risks involved in using contaminated watt Moreover, the flakes are lightweight and easily transportable for travelers. Fermentation is significant technique preserving food for times of scarcity, and *Abreh*, like many fermented food products, can be stored for a long time. Fermentation additionally improves the absorbability of a food empowering women to produce high value additions to the diet In the Sudanese context, the traditional knowledge developed by women of fermented foodstuffs has assumed a significant part in adapting to times of starvation.

20.7 Fermented Millet Products

20.7.1 Damirga

Western Sudanese natives process pearl millet in several types of foodstuffs such as fermented or unfermented breads, stiff or thin porridges, alcoholic or non alcoholic beverages and *Damirga*, which is fine sour white flour obtained traditionally from pearl millet grains (Abdalla et al. 1998; Adam et al. 2010). *Damirga* is mainly lactic acid fermentation, as fermentation process lactic acid bacteria count and lactic acid increased so pH decreased from 6.58 to 3.9. Titrable acidity and volatile acids increased as fermentation progressed. *Dagig Damirga* (flour), as this flour is called, is mostly used to prepare *acedat – Damirga* which is a stiff porridge, usually consumed while still warm with some sauce, because if left until cold it becomes rather dense and very compact. The porridge is prepared routinely for the daily meal of many families particularly in Darfur region (Dirar 1993).

For preparation of *Damirga* (Fig. 20.7), millet grains (yellow variety) is cleaned and moistened with water and dehulled by mortar (*funduk*), and the pearled kernels are sun-dried to winnow off the bran. The decorticated kernels are transferred to a clean metal pail and then 2000 ml of tap water is added to give a ratio 1:2; water: grain (w/v), and then allowed to ferment for 72 h. The fermented grains are then washed twice with tap water twice and sun dried. Thereafter the dry grains will be milled in an electric grinder to fine flour. *Damirga* fermentation is mostly lactic acid fermentation caused primarily by lactic acid bacteria activity (Adam et al. 2010). There was an undeniable degree of acetic acid bacteria commitment yet its commitment is lesser significant than that of the lactic acid bacteria. Due to the fermentation interaction, the pH dropped from 6.58 to 3.9. Lactic acid and acetic acid expanded as fermentation progressed. Dehulling has critical impact in in changes of protein digestibility and antinational factors. It increased in vitro protein

Fig. 20.7 Flow diagram of the home process of Damirga flour production

digestibility while protein, polyphenols, phytic acid and tannin diminished. Chemical analysis of fermented Damirga product showed that protein content was expanded while starch content was diminished. In addition, in the fermentation process polyphenols, phytic acid and tannin diminished as fermentation progressed.

The fermenting lactic acid bacteria isolated from *Damirga* fermentation include *Lactobacillus plantarum*, and *L. fermentum*. *Acetobacto*r spp. as well as the yeast *Kluyveromyces* spp., were isolated from *Damirga* product.

20.7.2 Khemiss Tweira

Khemiss Tweira is a food bound State of the Sudan; it is made out of five ingredients: millet grain flour, millet malt flour, cooked sesame seeds (sometimes groundnut), salt and sugar (Dirar 1993, 1996). *Khemiss Tweira* is made by blending millet malt flour and millet grain flour cooked into stiff cool porridge and permitted to ferment overnight then baked into sheets, then sun-dried and 24 crumble and mixed with cooked sesame or groundnuts, salt and sugar, at that point pounded into coarse meal (Mohamed et al. 2016).

Khemiss Tweira generally eaten in Western Sudan as a result of its comfort as a good food for travelers, boarding pupils, soldiers and country laborers. The principle constituents of *Khemiss-Tweira* is thin sweet millet bread locally known as kissra-assala (Honey-sweet Kissra) which is significant in its own privilege right as food for travelers and is ordinarily utilized by farmers and gum Arabic laborers in Western Sudan (Sulieman et al. (2003). Kissra assala may additionally be handled into the more complex *Khemiss-Tweira*. For this reason, sesame seeds are lightly roasted in a hot tajin. Then the dry, crumbled Kissra assala is blended with the roasted sesame in the ratio of 6:1, respectively and a little sugar and a dash of salt are added to taste. The blend is bounded in a mortar to give a coarse meal comprising *Khemiss Tweir*a. The product is a dry meal of a pleasant sweetish and slightly salty flavor. Nutritionally Khemiss-Tweira should provide a balanced meal, containing the necessary carbohydrates, proteins, oil, minerals and vitamins and its name "five birds" probably refers to the five ingredients: millet flour, millet malt flour, sesame, sugar and salt (Mohamed et al. 2016).

The chemical composition and the in vitro protein digestibility (IVPD) of three types of *Khemiss-Tweira* were investigated. The contents of moisture, protein, fats, crude fibre, ash and carbohydrates of three types *Khemiss-Tweira* ranged between 8.00–8.40%, 10.87–23.90%, 7.94–16.93%, 2.64-4-69%, 2.20–3.31% and 51.17 ± 76.35%, respectively. However, the lower the moisture in the various types of *Khemiss-Tweira*, the better will be the quality and the longer shelf life of the product. The food is consumed after the addition of pure water to the level of attaining the consistency desired by the consumer. It is a very useful food and can be utilized as a breakfast cereal or snack in urban areas and as a relief food during famine. *Khemiss-Tweira* is very much liked by children who consume it dry without any added water. It is as well a food for all ages including the toothless elderly and babies (Dirar 1996).

20.8 Conclusion

Traditionally fermented foods and beverages prepared from main cereals are common in numerous parts of Sudan. Some are utilized as beverages and breakfasts or snack foods while some are utilized as staples and weaning foods. A majority of traditional cereal-based foods utilized in Sudan are processed by natural fermentation.

Sudan is a continent in a country, which is why there are many cultures, environments, customs and traditions, and its "popular foods" have different flavors, flavors, methods of preparation, ingredients, and even methods of presentation, tools and form of food. Traditionally, the Sudanese diet is linked to agriculture and agricultural products. In the far reaches of the west, south and center, foods made from "sorghum" such as "porridge, *kissra*", and beverages made from grains "sorghum, millet" and others prevail. The food culture of Sudanese societies has made fermented food palatable to one cultural group and unpalatable for another cultural and geographical group. The great nutritional value of fermented grain products in Sudan was studied and the fermented microbes were identified that gave these products their value and sensory quality, such as flavor, taste, texture and preservation. Research is still ongoing to introduce pure strains of fermented bacteria into other foods.

Although not yet comprehensive, a very abundant literature clearly shows the functional potential of the cereals fermentation under a few points of view. The formation or modification of bioactive compounds during cereal fermentation ought to extend the toolset to develop bake.

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Chapter 21
Production and Quality Assessment of Hulu-mur Fermented Beverage

Abdel Moneim Elhadi Sulieman

21.1 Introduction

Hulu-mur is a traditional drink inherited through generations in Sudan. Hulu-mur is bitter also pronounced (sweet is bitter) or abrih, a popular Sudanese drink, usually drunk in the month of Ramadan according to Dirar (1993). Hulu-mur is the major drink prepared for the special occasion of Siam or sawm Ramadan. In Arabic, the name hulu-mur literally mean sweet-bitter but the true meaning is sweet-sour, for the product is actually sour and not bitter.

The name Hulu means sweet and mur means sour; an acetolactic fermentation gives the required sourness to the drink. Most parts of the Sudan consume this drink during Ramadan (Agab 1985). The *feterita* sorghum cultivar which is utilized in preparation of Hulu-mur when grinding them gives a red color. The Ramadan Iftar table (breakfast) in Sudan is unique with its distinctive varieties. In this vast country alone in its Islamic surroundings, it was discovered in the Blue Nile at the year 1833 AD by chance. Although many traditions are rooted in the legacy of the Sudanese people have disappeared as a result of the successive modern changes and the sweeping monster of globalization, except the Ramadan table is still striving to preserve its traditional public features, with food and drinks. Popularity, inherited through the centuries, such as sweet, the king of this table passed as the Sudanese wish (Al-Bayan 2020).

Hulu-mur name, its preparation goes through unique rituals, and the preparation for it begins months before the Ramadan month begins. In the month of Sha'ban, if

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any one passes through the streets and alleys of Sudanese homes in villages or cities, he will smell Hulu-mur. This pleasant smell on popular dwellings, where women are delighted for their successful completion of this difficult task, as well as a special joy of children, as this smell has a special meaning they foretells the advent of the month of Ramadan, with its delicious foods and drinks of a special nature. Moreover, it means for them many memories and good nights that adults and children gather.

The manufacture of "Hulu-mur" is characterized by something of the complexity, it is a mixture of fermented sorghum flour, spices and some herbs, as it needs quantities a large amount of sugar to reach the desired taste. This chapter there is interchange in usage of the terms traditional and indigenous knowledge. Indigenous knowledge is knowledge that is unique to a given culture or society (Grenier 1998). In this chapter, indigenous knowledge includes local knowledge, skills known, and practiced in food processing, preservation, and consumption which rural people derived from their direct interaction with the local environment. The chapter incorporates a comprehensive review about the product, processing method, microbiology, nutritional value and other aspects.

21.2 Traditional Uses of Sorghum in Food: Needs Rephrasing

Sorghum is utilized in an assortment of foods. The white food sorghum is prepared into flour and different items, including expanded snacks, cookies and ethnic foods, and is picking up Popularity in territories like Japan. In the US, the white sorghum products are utilized to a little degree to fill in for wheat in products for individuals oversensitive to wheat gluten. Different assortments of sorghum are likewise utilized for food in different parts of the world, including parts of Africa, Central and South America China, and India. In Eastern and Southern Africa, for instance customary sorghum assortments of moderate tannin content are generally developed and utilized for staple food and mixed drinks.

In some semiarid zones of the world for example Africa (Nigeria, Sudan, Burkina Faso, Ethiopia), Asia (India, China), and in some different locales of Central and South America, sorghum has discovered to be a significant grain, and human utilization rate is about 40% of the total world production (Awika and Rooney 2004; Schober et al. 2005). Despite the fact that sorghum is the fifth biggest yield delivered worldwide and has been demonstrated to be safe for individuals with celiac sickness (Ciaccia et al. 2007), the literature is generally scant contrasted with that of corn and rice in investigating sorghum for production of foods for Western markets (Liu and Godwin 2012). As of late, the utilization of sorghum in production of gluten-free foods has begun to emerge in some developed countries (Onyango et al. 2020; Taylor et al. 2006).

In some African societies, the tannin sorghum are really favored and the farmer feels full for the majority of the working day. Other pigmented sorghums are likewise favored in some African societies due to the trademark shading they produce in specific nourishments, and furthermore because of customary conviction that they advance the wellbeing of unborn children and are restorative against illnesses of the digestive system.

There is worldwide exertion to improve sorghum cultivating and to discover extra uses of sorghum. Sorghum is currently discovering request essentially as poultry feed, optionally as steers feed and in fermenting applications.

21.3 Malted and Fermented Sorghum

Malting and fermentation were applied to adjust endogenously the sorghum grain components, with the point of easing dirt, dryness and scrap immovability ordinarily connected with incorporation of sorghum flour in composite bread. Malt amylases were viably inactivated by boiling, prior to drying at high temperatures.

A fractionation and reconstitution process uncovered that malting and boiling dextrinized and gelatinized starch, expanded water-soluble pentosans and crude fiber. Dextrinization and gelatinization of starch diminished gelatinization temperature and the rate of starch retro gradation, accordingly diminishing scrap lumpiness and solidness. The expansion in crude fiber and water-soluble pentosans caused by the germinating grain root and shoot growth and the hydrolysis of non-starch polysaccharides, respectively, during malting, expanded water-holding capacity and batter consistency, thus decreasing dryness and the crumb firming rate. Malting and boiling likewise diminished the total protein and the in vitro just a single molecule is released, the other retained by enzyme and it slides along the active site and undergoes another hydrolysis.

21.4 Preparation of Hulu-mur

Hulu-mur dough is prepared at laboratory using two sorghum cultivar (*Feterita*) by a traditional method as shown in Fig. 21.1.

For preparation of Hulu-mur, the following ingredients are generally utilized:

A quarter of sorghum, half of it is a fresh sorghum and the second half is ground, a quarter of a pound of fennel.

1/4 pound cilantro, 1/4 pound ginger, half a pound cinnamon, 1/4 pound honey, A quarter of a pound of lute (sweat), a quarter of a pound of cumin, 2 grams of fenugreek, A pound Aradib (a tamarind), a pound of hibiscus. The spices are ground and mixed (except for tamarind) which is soaked in water until softened and then filtered (Fig. 21.2).

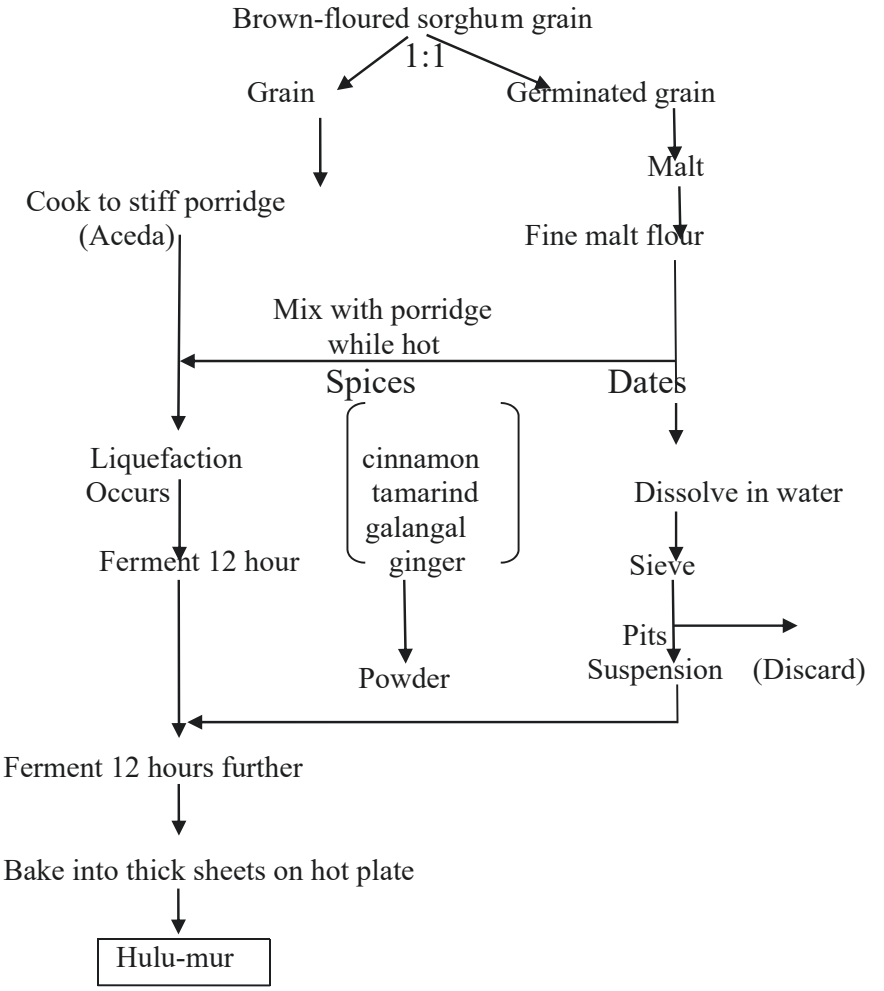

Fig. 21.1 Hulu-mur production process

Preparing the distinctive drink takes several days, as the women start to prepare it a month or two before Ramadan, and the sorghum grains are placed on the canvas and sprinkled with water for several days until they sprout and the planting comes out of them. This process is called locally *Zureea* "implantation". After they sprout and the veins of the plant appear, they are left without being sprayed with water. Then it was turned over and covered for an entire night in the next day, the veins are red. These are left until the mixture thickens and begins to boil. When its texture has become somewhat soft, it will be allowed until it cools, then starter is added to it (the previously fermented Sudanese kisra dough), and left a day or two, and the period depends on the temperature until it becomes bitter. Then the spices and tamarind

A B

Fig. 21.2 (**a**) Germinated Feterita sorghum; (**b**) a Fermented Feterita sorghum dough

"*aradeeb*" solution are added and mixed well until everyone mixes (spices give the dough a distinctive color) (Al-Bayan 2020).

The method of baking Hulu-mur is called *Uwassa* and it is done over. A large flat sheet is placed on a hot fire (usually wood), softens wooden tool, left to level. Then the process of raising it from the sheet begins by folding is into three folds, and and then it is raised from the sheet (flakes).

21.5 Hulu-mur Product

The formula requires the utilization of malt and grain in the proportions of 1:1 or 1:2, separately. Today, the best hulu-mur is supposed to be delivered when equivalent measures of malt and grain are utilized. The malt and the grain are independently processed into fine flour, the flour from ungerminated grain is then cooked straightforwardly into a fairly thick porridge like aceda or the somewhat more slender medida. The hot porridge may is moved from the cooking pot to another pot in which aging and amylolysis will take place.

The malt flour is presently added to the porridge while the latter is as yet hot. The two ingredients are thoroughly blended in with a wooden stirrer. During this process, liquefaction of the porridge occurs very rapidly so that within minutes the mixture is already sweetish and fluid even though water has not been added to it (Dirar 1993). The melted blend is left to ferment in a warm corner of the house or out in the sun in an enormous compartment, leaving head space to accommodate the expected increase in batter volume ensuing from fermentation gases. While fermentation occurs, an assortment of selected spices are ground to powder, in addition to the date slurry are blended into the fermenting batter (when a 1:1 ratio of malt to

Fig. 21.3 An image of Hulu-mur flakes

grain is utilized, additives are mixed in after fermentation proceeded for 12 h, and when a 1:2 ratio is used, the extras are added after 24 h of fermentation).

After through blending, the whole blend is further fermented so that the total fermentation time is 24–36 h. The batter is now ready for baking; it is sour and sweetish, and has red-brown colour and strong bouquet of malt and spices (Figs. 21.3 and 21.4).

21.6 Microbiology of Hulu-mur

Natural sorghum fermentation is mainly lactic acid by *Lactobacillus* spp. and yeast and acetic acid fermentations to lesser degree during the latter stages of fermentation. El Sharif (1993) studied the microorganisms associated with the different stages of fermentation of *Abreh,* a fermented sorghum refreshing drink, and found that the microbial population was mainly composed of lactic acid bacteria, which were the predominate predominant fermenting group throughout fermentation period.

Yagoub et al. (2009) in their study on three sorghum varities namely Feterita, Tabat and Wad-Akar, they found that microbial analysis showed that the total bacterial counts and the counts of yeast and molds increased significantly after

Fig. 21.4 An image of Hulu-mur wastes after extraction of the drink

fermentation time (24 h), while, there was a decrease in staphylococci and coliforms counts. In addition, E. coli counts exceeded 2.400 cfu/g in the raw sorghum flour but the counts were very low in the fermented dough. They indicated also that Salmonella which was detected in the three sorghum varieties disappeared in the fermented dough after 24 h fermentation.

Mohammed (1991) identified micro-organisms related with the traditional sorghum fermentation for production of Sudanese *Kisra* and they. He found that the microbial population during the 24 h of fermentation consisted of (*Pediococcuc pentosacus*, *Lactobacillus confuses*, *Lactobacillus brevis*, *Lactobacillus* sp., *Erwin bananas*, *Klebsiella pneamoniae*, and *Entrobacter cloacae*), yeasts (*Candida intermedia* and *Debaryomyces hansenii*) and molds (*Aspergellus* spp., *Penicillus* sp., *Fusarium* sp. and *Rhizopus* sp.). *P. pentpsacus* was the dominant microorganism at the end of the 24 h fermentation (Fig. 21.5).

Normally, the conceivable pretended by the spices in hulu-mur fermentation captivated the couple researchers who studied the process. The fact that spices are added after fermentation advanced partially through, might be taken as a solid sign

Fig. 21.5 Image of Hulu-mur drink

that these food processors know about the inhibitory and stimulatory impacts of spices on the fermentation process. It is along these lines conceivable that spices are utilized to guide the course of fermentation towards a specific required objective by saddling their particular inhibitory and stimulatory properties to upgrade the development of specific microorganisms and obstruct the development of others.

Marhoum (1987) found that the dominant microorganisms in hulu-mur fermentation were, in descending order of importance, *Lactobacillus, Acetobacter, Leuconostoc*, and the yeasts *Saccharomyces* and *Candida.* Bureng (1987) found that after 16 h of fermentation, *Streptococcus, Leuconostoc* and *Pediococcus* dominated in both spiced and unspiced hulu-mur mix slurries (Dirar 1993) (Table 21.1).

A total of 140 isolates obtained from different stages of hulu-mur prepared by using two sorghum varieties (Feterita and Wad Akar). Among these, seventy percent

Table 21.1 Changes in microbiological characteristics* during different fermentation periods of three varieties of sorghum flour

	Feterita			Tabat			Wad Ajar		
Fermentation period	Hour 0	Hour 19	Hour 24	Hour 0	Hour 19	Hour 24	Hour 0	Hour 19	Hour 24
TBC (c.fu/g)	$5.60^{ab} \times 10^6$	$2.04^{b} \times 10^8$	$5.83^{ab} \times 10^9$	$6.25^{a} \times 10^5$	$6.60^{a} \times 10^6$	$5.53^{b} \times 10^7$	$5.20^{ab} \times 10^5$	$6.40^{a} \times 10^5$	$6.32^{a} \times 10^5$
Staph. count (c.f.u/g)	$2.04^{b} \times 10^3$	$6.87^{a} \times 10^2$	$2.81^{a} \times 10^2$	$9.05^{a} \times 10^3$	$6.87^{a} \times 10^2$	$4.51^{b} \times 10^2$	$2.27^{b} \times 10^3$	$5.40^{b} \times 10^2$	$3.07^{c} \times 10^2$
E. coli	2.40< 0	34	15	<2.400	34	11	<2.400	28	15
Yeast and mould count (C.f.u/g)	$2.50^{b} \times 10^4$	$7.30^{a} \times 10^7$	$9.11^{b} \times 10^7$	$6.15^{a} \times 10^5$	$6.86^{b} \times 10^6$	$7.93^{a} \times 10^7$	$4.95^{ab} \times 10^4$	$6.70^{c} \times 10^4$	$6.27^{ab} \times 10^5$

TBC: Total bacterial count

*Means having different superscript latter in each row differ significantly (p 0.05 ≥) using DMRT

Table 21.2 Identification of LAB isolated from Feterita fermented dough without starter (after 24 h fermentation)

Tests Iso. No	Shape	Motility test	Growth anaerobes	Catalase test	Oxidase test	O/F test	Growth At diff- temp			Genus
							10°	15°	45°	
1	Rod	−ve	+ve	−ve	−ve	F	+ve	+ve	−ve	Lactobacillus
2	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Streptococcus
3	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	−ve	Streptococcus
4	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	−ve	Pediococcus
5	Cocci	−ve	+ve	−ve	−ve	F	+ve	+ve	+ve	Enterococcus
6	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Streptococcus
7	Rod	−ve	+ve	−ve	−ve	F	+ve	+ve	−ve	Lactobacillus
8	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Streptococcus
9	Rod	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Lactobacillus
10	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Streptococcus

(49 isolates) were cocci which occurred either single or in pairs with elongated coccied cell morphology and thirty percent (21 isolates) were rods, occurring either singly or in pairs. On the other side there were thirty three percent (23 isolates) were hetrofermentative and the residual sixty seven percent (47 isolates) were homofermentative. With the growth at different temperature we are found that all isolated genera were growth at 15 °C, while just 34% from these were growth at 10 °C and 86% were growth at 45 °C, this result was confirm with the feature that most of lactic acid bacteria are thermoduric (Table 21.2).

Prepared and analyzed chemical and microbiological characteristics of Hulumur. They found that the yeast counts of Hulu mur prepared from Feterita sorghum (FSH) were 3.3×10^3, 6.1×10^4, 3.6×10^4 and 8.3×10^5 C.F.U/ml, while yeast counts of Hulu- mur prepared from Wad akar sorghum (WSH) were 3.5 × 103, 4.3 × 105, 6.3 × 105 and 1.6 × 106 C.F.U./ml at time intervals of 3, 9, 12 and 24 h, respectively. Moreover, the results of morphological and biochemical identification tests of yeast isolates from different stages of Hulu-mur production indicated that, only two genera of yeast were detected in the various stages of Hulu-mur fermentation. These were Saccharomyces and Candida (Table 21.3).

21.7 Nutritional Value of Hulu-mur Product

Indigenous foods are a significant commitment to adjusting the eating routine. Utilizing of indigenous information in food processing and preservation e.g., fermenting and malting may add to increment of the nutritional potential. Indigenous processed food items add to advance supplement substance and variety and consequently improving health of provincial populations (Ibnouf 2012).

Table 21.3 Identification of LAB isolated from Feterita fermented dough with starter (after 24 h fermentation)

Tests Iso. No	Shape	Motility test	Growth anaerobes	Catalase test	Oxidase test	O/F test	Growth At diff- temp			Genus
							10°	15°	45°	
1	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Pediococcus
2	Rod	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Lactobacillus
3	Rod	−ve	+ve	−ve	−ve	F	−ve	+ve	−ve	Lactobacillus
4	Cocci	−ve	+ve	−ve	−ve	F	+ve	+ve	+ve	Enterococcus
5	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Streptococcus
6	Rod	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Lactobacillus
7	Cocci	−ve	+ve	−ve	−ve	F	+ve	+ve	+ve	Entrococcus
8	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Pediococcus
9	Cocci	−ve	+ve	−ve	−ve	F	−ve	+ve	+ve	Streptococcus
10	Rod	−ve	+ve	−ve	−ve	F	+ve	+ve	−ve	Lactobacillus

As known, nutritional value is a relative term that does not have a specific definition, and it refers to the sum of the components of a food item and the amount of benefit from it to humans, and here we must differentiate between foods with high nutritional value and foods with high energy (or many calories). Among the measures of nutritional value is a comparison of the protein, vitamins and minerals contained in the foodstuff with the calories it contains. So if the first increases and decreases the second, the food item is of high nutritional value and if the calories increase and the proteins, vitamins and minerals are decreased, the food item becomes of low nutritional value (Table 21.4).

The nutritional quality of whole and decorticated sorghum grains and their products was investigated (Eggum et al. 2009). The quality tests concluded that sorghum is low in lysine and thus has a low biological value. On the other hand, the true digestibility of protein, as well as the digestible energy, is very high Values higher than 90%. True digestibility of the protein decreased when cooking These progressions were dispensed with if the pH was acclimated to 3·9 prior to cooking. These impacts of cooking were considerably more articulated for the assortment Feterita (high polyphenol). The Feterita variety was found to have a high level of sugars and other chemical constituents and it thus produced good malt (Sara et al. 2016).

Hulu-mur is meant to be consumed as a drink by adults who have been fasting for more than 14 h. In addition, the product is solely prepared to nourish this particular group of people. Hulu-mur is a drink popular for the Sudanese in the different regions of Sudan with their various tribes and squares, as it constitutes one the most drinks utilized in Sudan. When using, it is wet in the water for a period since that afternoon, and the juice is filtered and sugar is added to it and placed in the refrigerator. It is consumed after breakfast time at Maghrib azan.

Hulu-mur has a sweet-sour taste and a pleasant flavor, as a consequence from the combination of roasted sorghum malt, spices and lactic acid. An individual may

Table 21.4 Some of morphological and biochemical characteristics of yeast isolates from different stages of hulu-mur production

Tests Sample No.	Colony shape	Cell			Sporulation	Growh at		Genus
		Shape	Vegetative growth	Pseudo hypha		37°C	42°C	Saccharomyces
AI	Smooth-White to creamy	Spherical	Budding	−ve	−ve	+ve	+ve	Saccharomyces
A2	"	Spherical	Budding	−ve	−ve	+ve	+ve	Saccharomyces
A3	"	Spherical	Budding	−ve	−ve	+ve	+ve	Saccharomyces
A4	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Saccharomyces
A5	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Saccharomyces
B1	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Saccharomyces
B2	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Candida
B3	White-butyrous	Longer	Budding	−ve	+ve	+ve	−ve	Candida
B4	"	Longer	Budding	−ve	+ve	+ve	−ve	Candida
B5	"	Longer	Budding	−ve	+ve	+ve	−ve	Saccharomyces
C1	Smooth-White to creamy	Spherical	Budding	−ve	−ve	+ve	+ve	Saccharomyces
C2	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Saccharomyces
C3	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Saccharomyces
C4	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Saccaromyces
D1	"	Ovoid	Budding	−ve	−ve	+ve	+ve	Candida
D2	White-butyrous	Elongated	Budding	−ve	+ve	+ve	−ve	Saccharomyces
D3	Smooth-White to creamy	Ovoid	Budding	−ve	−ve	+ve	+ve	Saccharomyces
D4	"	Spherical	Budding	−ve	−ve	+ve	+ve	Saccharomyces

consume about a litre of the extract in the evening Ramadan breakfast. The extract contains an average of 24.3% total soluble solids (Bureng et al. 1987).

Hulu-mur, which removes the thirst in addition to its nutritional value, as it contains a group of vitamins, starches, sugars and minerals important to the body. Sudanese expatriates in the various areas (abroad) are keen that the Hulu-mur is present at their breakfast table, so their relatives send it to them in parcels.

The extracted Hulu-mur residues are considered absolutely useless as human food and are thrown away. This residue which is called *Mushuk*, constitutes the major wasted constituents of sorghum foods in the Sudan. *Mushuk,* however, is considered the most fattening form of sorghum for animals (Dirar 1993).

The major components of Hulu-mur of the air-dry flake are, accordingly: sugars (31%), protein (14.3%),lactic acid (3.8%), ash (3.5%) and starch (41%) as reported by Marhoum (1987) as indicated in Table 21.5. The major component that presumably goes into solution when flakes are soaked in water. It is therefore reasonable to assume that Hulu-mur has been designed to provide a readily absorbable sugar,

Table 21.5 Proximate composition of Hulu-mur

Parameter	% of dry matter
Moisture	6.10
pH	4.50
Acidity (as lactic acid)	3.75
Volatile acids (as acetic acid)	0.16
Ethanol	0.75
Crude protein	14.26
Fibre	1.78
Fat	2.50
Carbohydrates	71.94
Sugar	31.00
Ash	3.45

Source: Marhoum (1987)

needed to bridge the leeway in blood sugar caused by fasting. Of course the drink provides other nutrients beside sugars and acts as a refreshing and cooling agent, as it is often served with ice (Dirar 1993). Hulu-mur contains high levels of carbohydrate, protein, minerals (K, P, Fe, Mn) and essential amino acids (Valine, isoleucine, leucine, tyrosine, and phenylalanine. It is deficient in lysine, threonine and sulfur amino acids (Mariod et al. 2016).

The basic ingredients of Hulu-mur, are sorghum grains and sorghum malt, both of which are strictly of the variety *Feterita*, which is widely considered the most nutritious of all sorghum types in Sudan. However, while un-germinated sorghum is decisive factor in the nutritional value of cereal foods containing no malt, such as kisra, germinated sorghum seems more important in the case of foods containing malt such as Hulu-mur.

Malting and fermentation had been implemented to alter endogenously the sorghum grain additives with the purpose of assuaging grittiness, dryness and crumb firmness normally associated with inclusion of sorghum flour in composite bread. amylases had been efficaciously inactivated via manner of boiling, earlier than drying at excessive temperatures. Germination of sorghum decreased phytate, tannin, and salt via manner of 40%, 16.12% and 49.1%, respectively, while fermentation of sorghum flour decreased higher than via way of means of 77%, 96.7% and 67.85%, respectively. There was no significant change in hydrogen cyanide in malted sorghum flour compared to whole sorghum flour, but fermentation of sorghum flour reduced hydrogen cyanide by 52.3% (Pravin et al. 2018). An important characteristic of germinated sorghum is its content of poisonous hydrocyanic acid (HCN) and therefore the consumption of sorghum sprout or products made from them may be hazardous to health (Panasiuk and Bills 1984). However, Hulu-mur contains very small amounts of total cyanide, for example, a 4-day *Feterita* malt contained 67.6 mg of cyanide per 100 g of dry weight. Obviously, the procedure followed in the preparation of Hulu-mur has a great influence on the cyanide content of the final

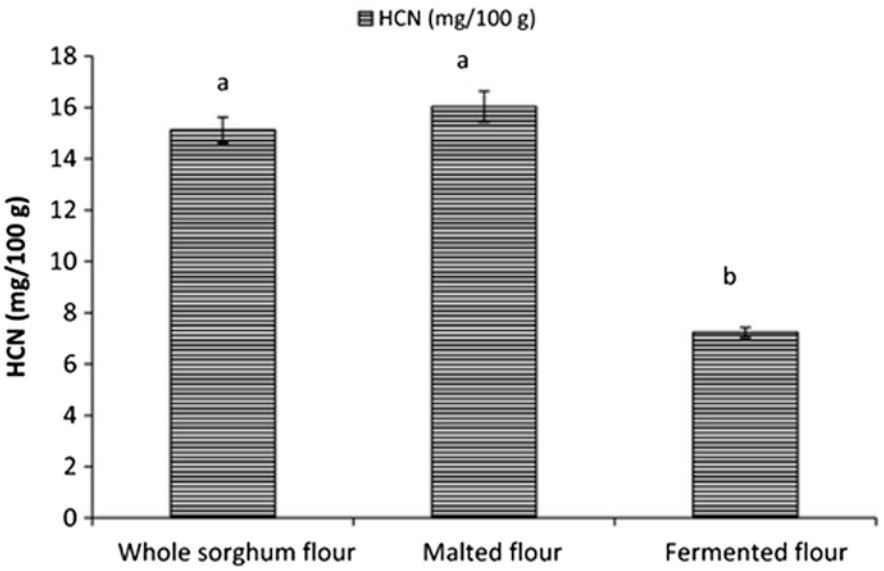

Fig. 21.6 Effect of processing on hydrogen cyanide of sorghum flour. (Source: Pravin et al. (2018))

product. One of the effective steps in the reducing cyanide content is the fermentation step. Ahmed (1988) found that when sorghum malt, initially congaing 16.5 mg per 100 g, was fermented for 2–5 days, total cyanide dropped to 10.3–5.3 mg per 100 g (Fig. 21.6).

21.8 Conclusion

Hulu mur is a food item set up from fermented Sorghum bicolor flour, typically assortment *Fetarita*, which contain the red pigment which gives the trademark colour of the final product. A batter is made, fermented and afterward baked, pasta-like sheets. These are soaked in water and the brownishred supernatant turns into the popular drink Hulu mur. Hulu-mur product has a high nutritional value. The major components of Hulu-mur of the air-dry flake are, accordingly: sugars (31%), protein (14.3%), lactic acid (3.8%), ash (3.5%) and starch (41%). It also contains high levels of carbohydrate, protein, minerals (K, P, Fe, Mn) and essential amino acids (Valine, isoleucine, leucine, tyrosine, and phenylalanine. It is deficient in lysine, threonine and sulfur amino acids. Fermentation of Hulu-mur results in its desirable sensory characteristics and pleasant flavor which are attributed by the fermenting microorganisms which include lactic acid bacteria, acetic acid bacteria and the yeasts (Saccharomyces and Candida). It is strongly prescribed to produce Hulu-mur by the conventional strategy without adding starter culture, and left the batter to ferment by the natural microflora and the malt enzymes, all together not to lose the

natural flavor. The process of Hulu-mur can be performed under control conditions free from contamination and with good quality, also the product can be in a sealed and packaged form to preserve and consume it in other months, in addition to export this product.

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Chapter 22
Nutrient Composition and Bioactive Components of Non-Alcoholic Sorghum Malt Beverage

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Abbreviations

g	gram
GAE	Gallic acid equivalent
mg	milligram
QE	Quercetin equivalent
µg/mL	microgram per milliliter

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22.1 Introduction

Sorghum, *Sorghum bicolor (L) Moench* is an annual herbaceous cereal plant of the grass family (*Poaceae*) and belongs to the *Andropogoneae* tribe. It is a plant of African origin, probably from Ethiopia, from where it spread throughout Africa (Asiedu 1991). The sorghum plays a crucial role in food security in developing countries. It is involved in cooking of many foods such as breads, porridges, pastes and pancakes. On other hand, where sorghum is produced, it is also abundantly used to prepare traditional beers commonly named sorghum beers or opaque beers but known as pito or burukutu in Nigeria, chibuki, in Zimbabwe, dolo in Mali and Burkina Faso, bili bili in Chad and tchapalo in Côte d'Ivoire (Moura et al. 2006; N'guessan et al. 2010). The preparation of sorghum beer involves two fermentations; a first spontaneous lactic and a second alcoholic. The lactic acid bacteria involved in lactic acid fermentation produced the wort sweet and acidify it for the yeasts which performed alcoholic fermentation. If the beer remains the final and major product of sorghum transformation in beverage, another beverage from acid lactic fermentation is produced. This beverage called sweet wort is characterized by alcohol free and taste sugared. This non-alcoholic beverage is appreciated from women and children. These beverages (sweet wort and beer) are consumed at various festivals and African ceremonies (e.g., marriage, birth, baptism, the handing over of a dowry, etc.) and constitute a source of economic return for women beer producers. If several studies have been performed on the traditional sorghum beer process improvement (Sefa-Dedeh et al. 1999; Orji et al. 2003; Glover et al. 2005; Maoura et al. 2005; Sawadogo-Lingani et al. 2008; Glover et al. 2009; N'guessan et al. 2010; Adewara and Ogunbanwo 2013); nutritional values (Aka et al. 2008) and bioactive compounds (Coulibaly et al. 2020) few studies were focused in sweet wort. In this chapter, the nutrient composition and bioactive components of sweet wort will be examined. This will focus entirely on the sweet wort or non-alcoholic sorghum malt beverage produced Africa. ..

22.2 Sorghum Grain Biochemical Composition

The mains components of sorghum grain are the starch, proteins, non-starch polysaccharides and fat (Table 22.1) (Dicko et al. 2006). The content of these compounds are depended by the genetic characteristics and growing conditions of the grain (Hill et al. 2012). The sorghum grain flour has an average energetic value of 356 kcal/100 g (BSTID-NRC 1996). Sorghum macromolecular composition is identical at others cereals such as the maize and wheat (BSTID-NRC 1996). On other hand, sorghum grain contains resistant starch which anti-nutritional compound. In fact this resistant starch is difficultly digestible, precisely for infants (FAO 1995). In others applications, this resistance performs to fight human obesity and to feed diabetic people. In according, Awika and Rooney (2004), foods cooked from

Table 22.1 Proximate composition of sorghum grain[a] (Dicko et al. 2006)

Macro-components (g/ 100 g f. m.)		Essential amino-acids		Vitamins (mg/100 g d. m.)		Minerals (mg/ 100 g d. m.)	
Carbohydrates	65 - 80	Leu	832 -	Vit.-A	21 RE**	Ca	21
Starch	60 - 75	Ile	215 -	Thiamin	0.35	Cl	57
Amylose	12 - 22	Met/Cys*	190 -	Riboflavin	0.14	Cu	1.8
Amylopectin	45 - 55	Lys	126 -	Niacin	2.8	I	0.029
Non starch	2 - 7	Phe/Tyr*	567 -	Pyridoxine	0.5	Fe	5.7
Low M_w carbohydrates	2 - 4	Thr	189 -	Biotin	0.007	Mg	140
Proteins	7 - 15	Trp	63 - 187	Pantothenat	1.0	P	368
-Kafirins	4 - 8	Val	313 -	Vitamin C	<0.001	K	220
ß-Kafirins	0.2 - 0.5	Arg*	500 -			Na	19
-Kafirins	0.7 - 1.6	His*	200 -			Zn	2.5
Other proteins	2 - 5						
Fat	1.5 - 6						
Ash	1 - 4						
Moisture	8 - 12						

[a]Sources: Verbruggen et al. (1993, 1996); FAO (1995), Hamaker et al. (1995), BSTID-NRC (1996), Glew et al. (1997), Duodu et al. (2003), Dicko et al. (2006). *Not strictly essential amino-acids,**RE = retinol equivalent; f.m. = fresh matter, d. m. = dry matter; NSP = non starch poly-saccharides

sorghums varieties which contains high tannin have a longer passage in the stom-ach. In sorghum grain, the non-starch polysaccharides are mainly located in the pericarp and endosperm cell walls, with content ranging from 2 to 7% according on variety (Knudsen and Munck 1985; Verbruggen et al. 1993). Verbruggen et al. (1993) and Hatfield et al. (1999) reported that the non-starch polysaccharides pres-ents in sorghum grain are essentially constituted of arabinoxylans and other ß-glucans which represent 55% and 40% of the total non-starch polysaccharides. The works of Verbruggen et al. (1993, 1998) revealed that arabinoxylans from sor-ghum to be glucuronoarabinoxylans and contained ferulic acid and p-coumaric acid. Such as one of the major non-starch polysaccharides present in sorghum cell walls, arabinoxylans play an important role in the processing of sorghum for baking and brewing (Verbruggen et al. 1998). The proportions of 40% and 55% of ß-glucans comprise cellulose (1,4-ß-D-glucans), curdlan-type glucans (1,3-ß-D-glucans), and lichenantype glucans (1,3; 1,4-ß-D-glucans) (Knudsen and Munck 1985; Verbruggen 1996; Verbruggen et al. 1993, 1998). These ß -glucans form viscous and sticky solu-tions because of their water-unextractable character, thus in brewing, together with arabinoxylans, they are responsibles with processing problems like poor wort and beer filtration rates and the occurrence of haze (Aisien and Muts 1987; Durfour et al. 1992).

Sorghum also contains non-carbohydrate cell-wall polymers such as lignins with proportions constituting up to 20% of the total cell wall materials (Hatfied et al. 1999). Sorghum grain contains between 7 and 15% of protein (FAO 1995; Beta

et al. 1995). In according the works of Jambunatan et al. (1975) on using the solubility-based classification sorghum proteins have been divided into albumins, globulins, kafirins (aqueous alcohol-soluble prolamins), cross-linked kafirins and glutelins. The kafirins comprise about 50–70% of the proteins (Hamaker et al. 1995; Oria et al. 1995; Duodu et al. 2003). aKafirins (23 and 25 kDa) make up about 80% of the total kafirins and are considered the principal storage proteins of sorghum, whereas b-kafirins (16,18, and 20 kDa), and g-kafirin (28 kDa) comprise about 5% and 15% of total kafirins, respectively (Dicko et al. 2006).

Axtell et al. (1981); Taylor and Taylor, (2002) reported that the protein digestibility of sorghum may decrease upon cooking but prefermentation may increase the digestibility. The disgestibility depends of interactions between the compounds. Thus, the low digestibility observed is due to protein-protein, protein-carbohydrate, protein-(poly) phenol and carbohydrate-(poly) phenol interactions (Knudsen et al. 1988; Axtell et al. 1981; Hamaker et al. 1995; Oria et al. 1995; Duodu et al. 2003).

In sorghum grain, the fat is mainly present in the germ which rich in polyunsaturated fatty acids (Glew et al. 1997). Knudsen et al. (1988); Adeyeye and Ajewole, (1992); FAO, (1995) claimed that the fatty acid composition of sorghum fat (linoleic acid 49%, oleic 31%, palmitic 14%, linolenic 2.7%, stearic 2.1%, etc.) is similar in content to that of corn fat. Fatty acid composition of sorghum fat is more unsaturated than corn fat. Sorghum represents a good source of vitamins, notably the B vitamins (thiamin, riboflavin, pyridoxine, etc.), and the liposoluble vitamins A, D, E and K (Dicko et al. 2006). Sorghum is reported to be a good source of more than 20 minerals (BSTID-NRC 1996). Sorghum is also rich in phosphorus, potassium, iron and zinc (Glew et al. 1997; Anglani 1998). Zinc (an important metal for pregnant women) deficiency is more common in corn and wheat than in sorghum (Hopkins et al. 1998).

22.3 Bioactive Components of Sorghum Grain

Sorghum is an excellent source of bioactive compounds that can promote benefits to human health. Several studies showed that the sorghum grain contains an important content of bioactive components particularly the phenolic compounds which have benefits effects on human health (Fall et al. 2016; Dykes and Rooney 2006, 2014; Ba et al. 2009; Alfieri et al. 2018; Devi et al. 2011; Cardoso et al. 2015; Awika et al. 2003). Many authors reported that the phenolic compounds isolated from sorghum grain promote beneficial changes in parameters related to non-communicable diseases such as obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension (Awika et al. 2009; Farrar et al. 2008; Kamath et al. 2007; Kim and Park 2012; Moraes et al. 2012; Muriu et al. 2002; Shih et al. 2007; Woo et al. 2012; Yang et al. 2009). The mains phenolic compounds presents in sorghum grain are phenolic acids, flavonoids and condensed tannins (Dykes and Rooney 2006).

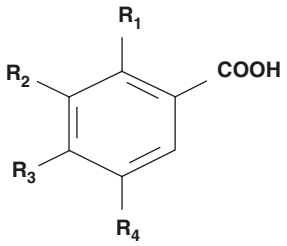
22.4 Phenolic Acids

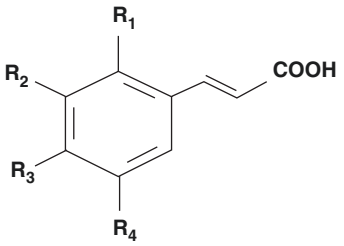
In sorghum grain, the phenolic acids are located in in the pericarp, testa, aleurone layer, and endosperm (Hahn et al. 1984; McDonough et al. 1986). Phenolic acids are regrouped in two classes: hydroxybenzoic and hydroxycinnamic acids. The first class consist of Hydroxybenzoic acids are directly derived from benzoic acid and include gallic, p-hydro- xybenzoic, vanillic, syringic, and protocatechuic acids, among others. The second class includes the hydroxycinnamic acids have a C6–C3 structure and include coumaric, caffeic, ferulic, and sinapic acids (Fig. 22.1). The Table 22.2 reported the phenolic acids in sorghum.

22.5 Flavonoids

In according studies of Awika et al. (2005) and Dykes et al. (2009), most flavonoids of the sorghum are located in the outer layers of the grain. The same authors reported that differences in the color and thickness of the pericarp and presence of the testa influence the concentration and profile of flavonoids. In turn, Taleon et al. (2012) claimed that the physical characteristics of the sorghum are determined by genetic and environmental factors.

In sorghum grain, the flavonoids can subdivide in three groups: anthocyanins, flavones, and flavanones. The most common anthocyanins in sorghum are the 3deoxyanthocyanidins (Gous 1989; Sweeny and Lacobucci 1983), which comprise luteolinidin (orange) and apigeninidin (yellow). At structural view point, the lack of a hydroxyl group at position C-3 confers to sorghum more stable than other anthocyanins (Awika 2008; Awika and Rooney 2004; Dykes et al. 2009; Shih et al. 2007).





Hydroxybenzoic acids

	R_1	R_2	R_3	R_4
Gallic acid	H	OH	H	OH
Gentisic acid	OH	H	H	OH
Salicylic acid	OH	H	H	H
p-hydroxybenzoic acid	H	H	H	OH
Syringic acid	H	OCH_3	OH	OCH_3
Protocatequinic acid	H	OH	OH	H

Hydroxycinnamic acids

	R_1	R_2	R_3	R_4
Caffeic acid	H	OH	OH	H
Feluric acid	H	OCH_3	OH	H
o-coumaric acid	OH	H	H	H
p-coumaric acid	H	H	H	H
Sinapic acid	H	OCH_3	OCH_3	OCH_3
Vanillic acid	H	OCH_3	OCH_3	H

Fig. 22.1 Structure of the major phenolic acids present in sorghum. (Carodoso et al. 2015)

Table 22.2 Phenolic acids detected in sorghum grains (Dykes and Rooney 2006)

Phenolic acids	References
Hydroxybenzoic acids: Gallic	Hahn et al. (1983) and Subba Rao and Muralikrishna (2002)
Protocatechuic	Hahn et al. (1983), McDonough et al. (1986) and Subba Rao and Muralikrishna (2002)
p-Hydroxybenzoic	Hahn et al. (1983) and McDonough et al. (1986)
Gentisic	McDonough et al. (1986) and Waniska et al. (1989)
Salicylic	Waniska et al. (1989)
Vanillic	Hahn et al. (1983) and McDonough et al. (1986),
	Subba Rao and Muralikrishna (2002)
Syringic	Waniska et al. (1989) and McDonough et al. (1986)
Hydroxycinnamic acids	Hahn et al. (1983), McDonough et al. (1986) and Subba Rao and Muralikrishna (2002)
Ferulic	Hahn et al. (1983), McDonough et al. (1986) and Subba Rao and Muralikrishna (2002)
Caffeic	Hahn et al. (1983), McDonough et al. (1986) and Subba Rao and Muralikrishna (2002)
p-Coumaric	Hahn et al. (1983) and McDonough et al. (1986)
Cinnamic Sinapic	Waniska et al. (1989) and McDonough et al.

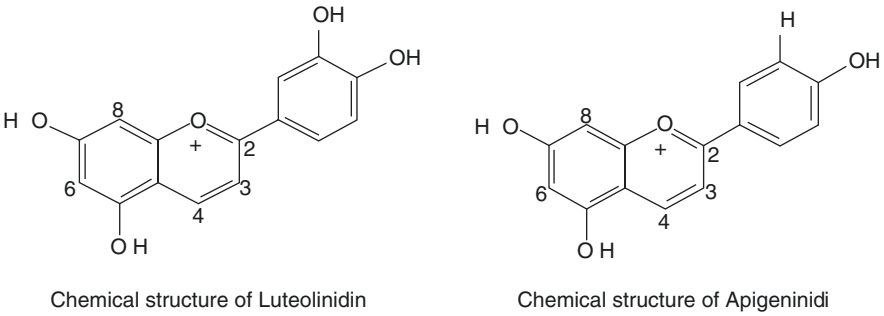


Fig. 22.2 Chemical structure of 3-deoxyanthocyanidins, luteolinidin and apigeninidin

They are considered such as phytoalexins because they are produced as a response to mold invasion or other stresses in sorghum (Lo et al. 1999; Seitz 2004; Waniska and Rooney 2000). Several authors revealed that sorghums with a black pericarp have the highest levels of 3-deoxyanthocyanins which are more concentrated in the bran (Awika et al. 2004a, b, 2005; Dykes et al. 2005; Gous 1989).

The Fig. 22.2 presents non-methoxylated forms (luteolinidin and apigeninidin) of the main 3-deoxyanthocyanidins of the sorghum (Awika et al. 2004). The Table 22.3 listed the anthocyanins in sorghum grain.

Table 22.3 Flavonoids detected in sorghum grains (Dykes and Rooney 2006)

Flavonoids	References
Anthocyanins:	
Apigeninidin	Nip and Burns (1971) and Gous (1989)
Apigeninidin 5-glucoside	Nip and Burns (1969, 1971) and Wu and Prior (2005)
Luteolinidin	Nip and Burns (1971) and Gous (1989)
5-Methoxyluteolinidin	Seitz (2004) and Wu and Prior (2005)
5-Methoxyluteolinidin 7-glucoside	Wu and Prior (2005)
7-Methoxyapigeninidin	Pale et al. (1997), Seitz (2004) and Wu and Prior (2005)
7-Methoxyapigeninidin 5-glucoside	Wu and Prior (2005)
Luteolinidin 5-glucoside	Nip and Burns (1971) and Wu and Prior (2005)
`5-Methoxyapigeninidin	Seitz (2004)
7-Methoxyluteolinidin	Seitz (2004)
Flavan-4-ols Luteoforol	Bate-Smith (1969)
Apiforol	Watterson and Butler (1983)
Flavones	Gujer et al. (1986) and Seitz (2004)
Apigenin	Seitz (2004)
Luteolin	Kambal and Bate-Smith (1976)
Flavanones Eriodictyol	Gujer et al. (1986)
Eriodictyol 5-glucoside	Gujer et al. (1986)
Naringenin *Flavonols*	Nip and Burns (1969)
Kaempferol 3rutinoside-7glucuronide	
Dihydroflavonols Taxifolin	Gujer et al. (1986)
Taxifolin 7-glucoside	Gujer et al. (1986)

22.6 Condensed Tannins

The tannins are the phenolic compounds presents in plants. Contrary others cereals, the tannins are presents in sorghum varieties which have pigmented testa (Awika 2003; Dykes and Rooney 2006; Wu et al. 2012). The presence and content of condensed tannins in sorghum are regulated by the genes *B1_B2*, *S*, and *Tannin1*, among others (Hahn et al. 1984; Hahn and Rooney 1986; Wu et al. 2012). Waniska et al. (1989) reported that the condensed tannins confer some resistance to molds and deterioration of the grain. Tannin contents vary among genotypes. The tannins are classified in three types: there are type I characterized by no significant levels, type II, which tannins that are extractable only in acidified methanol and type III which correspond to tannins that are extractable in methanol and acidified methanol (Hahn and Rooney 1986; Price et al. 1978). In according Awika and Rooney (2004) and Wu et al. (2012), almost all of the tannins in sorghum are condensed and

constituted by oligomers or polymers of catechins (flavan- 3-ols and/or flavan-3,4-diols). Earp et al. (1981) reported that in type II and III sorghums have tannin levels of 0.02–0.19 mg/100 mg and 0.4–3.5 mg/100 mg catechin equivalents, respectively. The tannins have both advantage and disadvantage. Reagrding disadvantage, the tannins have both advantage and disadvantage. Regarding disadvantages, in according Al-Mamary et al. (2001); Barros et al. (2012); Taylor et al. (2007) the tannins reduced the availability of minerals, proteins, and starch of the sorghum. On other hand, this reduction correlates not only with the content of tannins in the grain, but also with degree of polymerization (Kaufman et al. 2013; Mkandawire et al. 2013). However, despite the anti-nutritional effect, the radical scavenging power of tannins are 15–30 times more effective than simple phenolics (Hagerman et al. 1998).

22.7 Benefits Effect of Sorghum

Several authors reported sorghum potentialities in human health (Awika et al. 2003; Dykes and Rooney 2006; Ba et al. 2009; Boua et al. 2010; Devi et al. 2011; Cardoso et al. 2015; Alfieri et al. 2019). Their experiences have been performed *in vitro* and on animal studies.

Among sorghum proprieties more studied there are antioxidant activities (Dykes and Rooney 2006; Ba et al. 2009; Boua et al. 2010; Alfieri et al. 2019). In all these studies, the results showed that the phenolic compounds are responsible of antioxidant activities. In their study, Boua et al. (2010) showed that 10 sorghum varieties in West Africa possessed antioxidant activities determined by FRAP method (ferric reducing-antioxidant power) and DPPH (2,2-diphenyl-1-picryl-hydrazyl). Also, Devi et al. (2011) reported that 3-deoxyanthocyanins from red sorghum (*Sorghum bicolor*) are responsible of free radical-scavenging by DPPH method. On the hand other, "Dykes et al. (2005) reported a strong correlation between antioxidant activity and flavan-4-ol levels ($r = 0.88$) among non-tannin sorghums with a red pericarp. Sorghums containing condensed tannins have consistently shown the highest antioxidant activity *in vitro*". In addition to its antioxidant activities, Awika and Rooney (2004) and Cardoso et al. (2015) have been reported health benefits of sorghum. The results of *in vitro* and animal studies have shown that phenolics or fat soluble compounds isolated from sorghum beneficially balance or stabilize the intestinal microbiota and parameters related to noncommunicable diseases such as oxidative stress, inflammation, obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension (Cardoso et al. 2015).

22.8 Use of Sorghum Grain in Non-alcoholic Beverages Production

The sorghum grain is used to produce the beers mainly. However, non-alcoholic beverage can be produce from sorghum malt. In some countries of West Africa such as Côte d'Ivoire, Burkina-Faso, Mali…this non-alcoholic sorghum malt beverage (sweet wort) is called "for women" or "not strong". This beverage is characterized by her alcohol free and sugared taste. She is produced by acid lactic fermentation (spontaneous fermentation) performed by acid lactic bacteria just before alcoholic fermentation step. If many studies have been focused in traditional sorghum beer which is alcoholized, few studies have been aimed at non-alcoholic sorghum malt beverage (sweet wort). More the sorghum malt is added such as ingredient in non-alcoholic beverage production process. Indeed, Gadaga et al. (1999) reported that the best *mahewu* (non-alcoholic beverage produced in Zimbabwe) was obtained when 30 g of a mixture of finger millet (1 / 3) and sorghum (2 / 3) malts were added to 500 ml of heated porridge. The diagram of the sweet wort production is presented in Fig. 22.3.

22.9 Nutrient Composition of Sweet Wort

Few studies were focused in non-alcoholic sorghum malt beverage. The data existing were focused in sorghum, sorghum malt and sorghum beer. Also the non-alcoholic sorghum malt beverage (sweet wort) data's were different of the beer. Traditional sorghum-based beers in Africa are considered both food and beverage and are therefore sometimes referred to as 'eat-beverage'; in several countries they are a source of energy providing important nutrients to contribute to people's diets (De Lemps 2001; Van der Aa Kühle et al. 2001; Achi 2005; Maoura et al. 2006). For example, they contain a high proportion of starch and sugars and protein (Table 22.4).

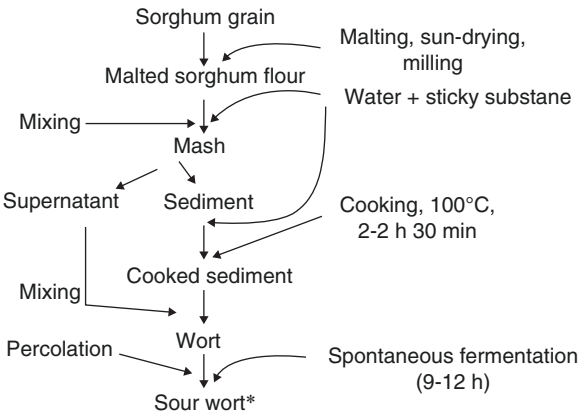


Fig. 22.3 Diagram of sweet wort production (Aka et al. 2008)

Table 22.4 Composition of sorghum malt and sorghum beer (Chevassus-Agnes et al. 1976)

Compounds	Malt	Sorghum beer
Calories (joule)	380	394
Proteins (g)	9.8	8.7
Lysin (g/100 g de protéines)	3.7	7.2
Fat (g)	2.2	0.3
Total carbohydrates (g)	86.2	86.1
Ashes (g)	1.75	4.06
Calcium (mg)	9.3	20.7
Total Phosphore (mg)	327	630
Ca2+/P	0.026	0.021
Phyitc Phosphore (mg)	85	112
Potassium (mg)	361	1101
Sodium (mg)	14.7	26.9
Thiamin (µg)	426	3441
Riboflavin (µg)	231	760
Niacine (mg)	5.3	8

They are also sources of B-group vitamins (thiamin, riboflavin and niacin) and minerals such as iron, manganese, magnesium, phosphorus, calcium, potassium and copper (FAO 1995; Michodjèhoun et al. 2005). According to Taur et al. (1984), traditional sorghum beer contains vitamin C and Aka et al. (2008) reported that the content of vitamin C of sweet wort was 1.1 mg/100 mL. This vitamin is synthesized during germination in the seed and during fermentation there is a further increase in the vitamin C content. Also in Cameroon, Chevassus-Agnes et al. (1976) showed that 'amgba' beers made from sorghum proved to be nutritionally superior to sorghum flour because they provide more riboflavin, thiamine and lysine. Derman et al. (1980), meanwhile, reported that iron uptake from sorghum beer was more than 12 times higher than that from sorghum flour.

22.10 Bioactive Compounds of Sweet Wort

Such as nutrient compounds, few researches aimed the bioactive compounds of sweet wort. Recently, Coulibaly et al. (2020) carried out a study focused in bioactive compounds and antioxidant activities of sweet wort and sorghum beer. Thus, Coulibaly et al. (2020) showed the phenolic compounds content of sweet wort was most higher than sorghum beer. Indeed, the total phenol, total flavonoids, total anthocyanins contents of sweet wort were 1254.69 µg/mL GAE; 106.99 µg/mL QE; 664.12 µg/mL respectively. Also, the antiradical activity of sweet wort was most higher than sorghum beer. Probably, antioxidant activity of the sorghum wort was related to phenolic compounds. There are no studies sighted in literature on traditional sorghum beer and sweet wort antioxidant activities. However a study on red

sorghum grain antioxidant activity, the variety used for traditional sorghum beer preparation showed that the antioxidant activity was related to total phenols content (Boua et al. 2010). It is probable the therapeutic qualities of sweet wort and sorghum beer would be due to the cereal used, which is sorghum. Indeed, Abdoul-Latif et al. (2012) showed that the sorghum was distinguished from other cereals used in breweries by its high content of phenolic compounds whose antioxidants properties are known. Furthermore, significant correlation between total phenols and antioxidant capacity had been demonstrated by many authors (Beretta et al. 2005; Ferreira et al. 2009; Bertoncelj et al. 2007).

22.11 Conclusion

Sorghum is a cereal which plays an important role in diet people. He is used to produce many foods and beverages. Among, the beverages produced from sorghum there are sorghum beer and non-alcoholic sorghum malt beverage (sweet wort). For health and religion view point, the demand of non-alcoholic sorghum malt beverage (sweet wort) a growing demand. The contents important of phenolic compounds correlated to antioxidant activities of sorghum grain which used to produce the sweet wort conferred to this beverage the therapeutic properties.

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Chapter 23
Preparation of Soy Milk *Zabady* and Assessment of Its Quality

Abdel Moneim Elhadi Sulieman, Areej Mohamed Ali, and Elamin Abdullah Elkhalifa

23.1 Introduction

Soybeans (Glycine max) having a place with the family leguminosae constitute one of the most established developed crops of the the jungles and sub-tropical areas, and one of the world's most significant sources of protein and oil. Soybean is a common legume in East Asia, as it grows in tropical, subtropical and hot regions. Soybeans consist of 36% protein, 30% carbohydrates and good content of minerals, vitamins, dietary fiber and 20% oil (Adelakun et al. 2013). It is characterized by three-leaf composite leaves with complete edges, broad or narrow in shape or elongated lanceolate with a sharp end and stalk that differ by cultivar; They may be weak, thin, strong, standing, semi-standing, root and main wedge that is not tall and small white or purple-purple flowers that are odorless and self-pollinating (https://mqalaat.com).

Soybean is especially one of a kind for various explanation and thus classify as a an important and economical horticultural commodity. In the principal occurrence, it has agronomic qualities with its capacity to adjust to a wide scope of soil and environment; and its nitrogen fixing capacity. This makes it to be a good rotational crop for use with high nitrogen – devouring crops like corn and rice. Furthermore, soybean unique chemical composition on an average dry matter basis is about 40% of protein and 20% of oil. This composition makes it to rank most noteworthy regarding protein content among all food crops and second as far as oil content after nut (48%) among all food vegetables. Moreover, soybean is a nutritious food crop (Adelakun et al. 2013).

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Soybean flour has enormous possibilities of being utilized to improve food sources to give sufficient supplements to people not gathering day by day needs. In light of the accessible data on the supplements profile of soybean including the amino profiles, human utilization of soybean flour can be advanced due to its beneficial outcome on nourishing upgrade on various sustained food products.

Recent studies have indicated the role of soy in reducing the risk of cardiovascular disease and prostate cancer, colon and breast, and it is also a factor in preventing osteoporosis associated with menopause in women and reducing the severity of hot flashes associated with this stage (https://www.organicfacts.net/health-benefits/cereal/soybeans.html).

Soymilk is a drink extracted from soybeans, prepared by soaking soybeans in water, and then grinding them. It is characterized by its white color and creamy texture, which makes it similar to cow's milk. Soy milk contains a greater amount of protein compared to cow's milk, it can be used as a substitute for dairy products and meat, moreover, it is free of lactose, easy to digest, and it does not contain cholesterol, and is characterized by containing polyunsaturated fatty acids.

Soy milk is a stable emulsifier of oil, water and protein, as the aroma of soy milk depends on its content of proteins, soluble solids and oil, its original form is a natural by-product of making tofu (also known as bean curd, which is a food prepared by coagulating soy milk and then pressing The resulting curd into solid white lumps of varying smoothness) where it can be silky, smooth, firm, or very hard, outside of these broad categories (https://e3arabi.com/?p=499212).

The soymilk can be drunk as such but can be improved by adding some salt (also, cow's milk contains many salts). With soymilk, it can easily make fruit smoothie. Fruit smoothies are very health because the contain soymilk and many fresh fruits (Del valley et al. 1984).

23.2 Nutritional Value and Benefits of Soymilk

Plain, unfortified soymilk is an excellent source of high-quality protein, B-vitamins and iron. Some brands of soymilk are fortified with vitamins and minerals and are good sources of calcium, vitamin D and vitamin B-12. Soymilk is free of the milk sugar lactose and is a good choice for people who are lactose intolerant. In addition, it is a good alternative for those who are allergic to cow's milk. Children can enjoy home-made or commercially prepared soymilk after the age of 1 year. Infants under 1 year of age should be fed breast milk, commercially prepared infant formula or commercial soymilk infant formula (Nelson et al. 1987).

Soymilk has a greater variety of complex carbohydrates than whole cow's milk. Soymilk and okara (the soy fiber remaining after making soymilk) are good sources of isoflavones. Okara is a good source of dietary fiber (www-soyabe/nutritional value-of-soymilk php, June (2007).

The fat content in soymilk is highly unsaturated and includes concentrations of Omega-3 fatty acids. Omega-3 fatty acids, found in soy, flax, and fish, are being

Fig. 23.1 Soy bean picture: (**a**): Plant; (**b**): Green pods; (**c**): beans

studied for their ability to lower the risk of heart disease and even cancer. In addition, Omega-3 may be essential to brain development in infants (Lidner 1988).

The appropriately prepared soymilk and its subordinates offer numerous neutraceutical and medical advantages. The sort of preparing and following handling conditions like high or low temperatures influence the properties of soymilk (Giri and Mangaraj 2012) (Fig. 23.1).

23.3 Benefits of Soy Milk

There are numerous benefits of soy milk, it contains many essential nutrients, these benefits include the followings:

1- A rich source of protein: Soy milk can be adopted in the diet as a good source of protein made from a group of amino acids that support various body functions. These proteins carry out many functions. They can build new proteins from amino acids to build new proteins; They create antibodies necessary to enhance the function of the immune system, structural proteins that bind body tissues together, in addition to enzymes that help cells to produce energy. It should be noted that soybeans, unlike most plant proteins, contain all the essential amino acids (Kelly 2020).
2- A source of antioxidants: Soy milk contains a type of antioxidant called isoflavones, and it has been observed that this compound possesses properties that can help reduce inflammation within the body (Jessica 2019).
3- A good source of minerals and vitamins: The nutritional content of soy milk varies according to the brand, but generally; it can be said that most of the famous brands on the market are fortified with vitamins and minerals, for example you can find many soy milk products that are fortified with calcium, or some other

nutrient. Such as: vitamin D, vitamin A, vitamin B2, and vitamin B12, in addition to folate, iron, phosphorous, potassium, and magnesium (Dolson 2020)

4- Soymilk contains only vegetables proteins which have the advantage that they cause less loss of calcium through the kidneys.
5- Soymilk contains no lactose: About 65% of the world population can not tolerate lactose. Soymilk contains the prebiotic sugars stachyose and raffinose, these prebiotics sugar boost immunity and help to decrease toxic substances in the body.
6- Less people are allergic to soymilk**:** Only 0.5% of the children are allergic soymilk, whereas 2.5% is allergic to cow's milk.
7- Soymilk reduces cholesterol: The saturated fats in cow's milk are unhealthy and increases the levels of cholesterol, soy protein can decreases cholesterol levels. The FDA (Food and Drug Administration of US) confirms that soy protein, as part of a diet low in saturated fat and cholesterol may significantly reduce the risk of coronary heart disease. The FDA recommends incorporating 25grams of soy protein in daily meals.
8- Soymilk does not cause insulin dependent: Some studies have shown an association between drinking cow's milk in early life and the development of insulin dependent diabetes. The association does not exist with soy milk.
9- Soymilk is rich in isoflavones: The presence of isoflavones is the most important and unique benefit of soymilk. Each cup of soymilk contains about 20 mg isoflavones (mainly genistein and daidzein). Cow's milk does not contain isoflavones (www- Soya be/ benefits-soy- milk php, March 2007).

23.4 Preparation of Soymilk

Soymilk is one of the most versatile products from soybeans. Whole soybeans must be carefully prepared to inactivate the anti-nutritional factors and the enzyme that cause off-flavors. Inadequate preparation results in a product with an objectionable beany flavor and diminished nutritional value. Lo et al. (1968) found that as the soaking time for soybeans increased, larger quantities of water-soluble solids leached out in the soak water and were lost during the manufacturing process. Kapoor et al. (1977) suggested that sodium bicarbonate pre-treatment improves the taste and flavor marginally but reduced the score under appearance and consistency.

The flavor profile of conventionally prepared soymilk is very complex and is largely caused by the action of lipoxygenase and possibly other enzyme on the lipids during soaking and wet grinding of beans prior to soaking (Wolf 1975). There are different methods of preparation of soymilk including the followings:

1- **Traditional method:**

Traditionally, soymilk was used to be prepared from soybeans as follows: the beans are soaked in water overnight, water is drained, beans homogenized with hot water. Then the wet mash is cooked for 10–15 min at 100 °C, and filtered through

Fig. 23.2 Soy milk pictures. (Source: https://commons.wikimedia.org/)

coarse cloth to recover the product. The water: beans ratio extracts about 50% of the protein and oil (Cicrle and Smith 1975). Whole soybeans are thoroughly washed and soaked in distilled water for 6–8 hr. at 5 °C, until absorbed water is 1 ml/ gm of dry soybeans. The soybean -water mixture is blended in a warning blender for 3 min filtered twice through cheese-cloth, and the residue is discarded. The soymilk is autoclaved for 15 min at 120 °C (Fig. 23.2).

2- **Modern method:**

In these method Soybeans (150 gm) are soaked overnight (16 hr) in 500 ml distilled water at 20 °C. The soaked beans are drained, rinsed and blended with 375 ml distilled water in commercial warning blender for 2 min at high speed, followed by the addition of 200 ml boiling water and blending of high speed for other 2 min. The resultant slurry is strained through a small centrifugal juice extractor lined with filter cloth. The final volume of soymilk is adjusted to 100 ml with distilled water (Lim et al. 1990). Commercial full-fat soy flour was mixed with water (1: 20, w/w) and the resultant suspension was heated to 95 °C for 10 min with continuous stirring. The hot suspension was mixed vigorously (warring blender jar, high speed) for 3 min. The resultant soymilk was cooled and stored at 5 °C until used (Del valle et al. 1984) (Fig. 23.3).

23.5 Fermentation of Soymilk

The fermentation of legume flours is done by the food industry for the improvement of the nutritional, functional or sensory properties of legume products. Along these lines, the lactic acid fermentation of faba bean flours has been exhibited as a likely pretreatment for development of the nutritional quality of gluten-free faba breads, without influencing their sensory properties (Sozer et al. 2019). Schlegel et al.

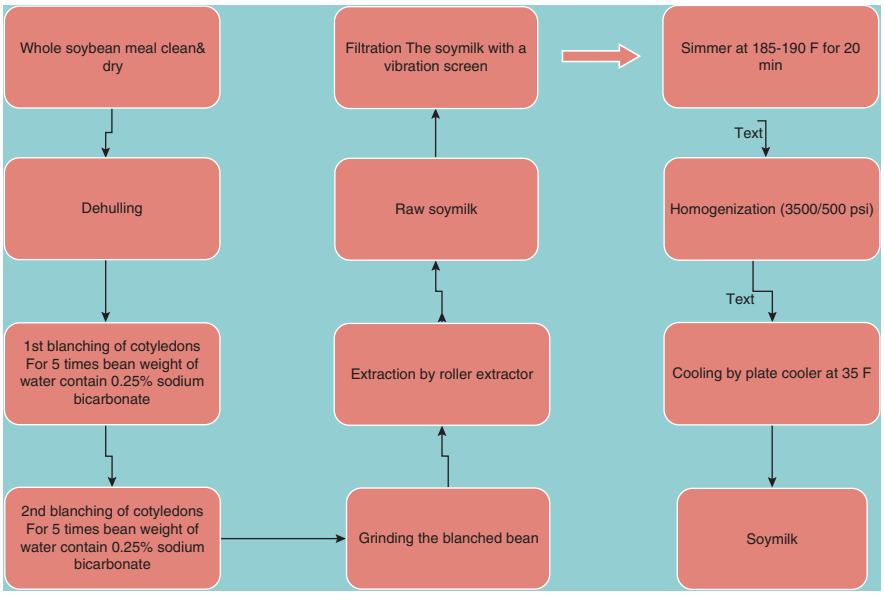

Fig. 23.3 Preparation of soymilk chart

(2019). detailed the fermentation of lupin protein isolated with up to eight distinct microorganisms, and the consequences for the tangible and techno-functional (protein solubility, frothing and emulsifying capacity) properties, and the protein's resistance to the fermentative process, were both assessed. The fermentation of a pea protein solution with a starter culture of lactic bacteria and various yeasts fundamentally diminished the presence of green-off compounds; such outcomes are of interest for as far as expanding the adequacy of plant matrices to consumers (EI Youssef et al. 2020). Kim et al. (2019) announced the utilization of superfine defatted soybean flour got by fly processing for the preparation of fiber-enriched tofu, without influencing the physical and tangible properties of the product.

Soy milk has an undesirable beany flavor, fermentation is the main procedure which can inactivate components which are responsible for these undesirable beany flavors in soymilk (Mitsuru et al. 2017). Numerous bacterial species are utilized for lactic acid fermentation of soymilk, such as *Bifidobacterium* (Hsieh et al. 2007; Kikuchi-Hayakawa et al. 2000; Lai et al. 2013), *Lactobacillus* (Appukutty et al. 2015; Cheng et al. 2013; Kitawaki et al. 2009; Kobayashi et al. 2012; Liu et al. 2016; Wang et al. 2006), and *Enterobacterium* (Martinez-Villaluenga et al. 2008) (Table 23.1).

Mital and Steinkraus (1979) indicated that addition of cow's milk flavor would be expected to further improve the flavor acceptability of the soymilk. Fermented soymilk with unacceptable flavor and yoghurt-like texture can be produced. Flavor acceptability can be further improved by increasing the acidity of the fermented soymilk. Mitsuru et al. (2017) indicated flavor similarity of fermented soymilk and

Table 23.1 Microorganisms used for fermented soymilk

Bacteria	Functions of Fermented Soymilk	References
Bifidobacterium	Hypolipidemic effect	Kikuchi-Hayakawa et al. (2000)
Lactobacillus	Antioxidation	Wang et al. (2006)
Streptococcus and *Bfidobacterium*	Antimutagenicity	Hsieh et al. (2007)
Lactobacillus	Improvement of lipid metabolism	Kitawaki et al. (2009)
Enterobacterium	Antihypertension, antioxidant	Martinez-Villaluenga et al. (2008)
Lactobacillus	Hypocholesterolemic effect	Kobayashi et al. (2012)
Bifidobacterium	Anticancer	Lai et al. (2013)
Lactobacillus	Antiartherioscreosis	Cheng et al. (2013)
Lactobacillus	Immunomodulatory effect	Appukutty et al. (2015)

Source: Mitsuru et al. (2017)

cow milk yogurt which is accessible in various of styles and flavors. O'Toole (2016) reported that acid production in soymilk was not always directly related to the growth rates of the bacteria, but also to those bacteria which are able to utilize the sugars in soymilk e.g. *S. thermophilus*. The poor growth and acid production in soymilk by *L. bulgaricus* was explained on the basis of in ability of the organism to ferment sucrose and other soybean carbohydrates (Mital and Steinkraus 1979). Carmen et al. (2006) found that soymilk is a satisfactory medium for growth of lactic acid bacteria. However, lactic cultures produced less acid in soymilk than in cow's milk.

Mital and Steinkraus (1979) prepared yoghurt from soymilk supplemented with 15% sucrose and fermented with *L bulgaricus* or cow milk yoghurt cultures to obtain a desired product. The fermentation process has suppressed the beany flavor of soybeans. Acid production in fermented soymilk could be enhanced by enrichment of soymilk with sucrose, glucose or lactose and further increased by using selected lactic cultures (Mital and Steinkeraus 1979). Mital et al. (1974) observed that all lactic cultures exhibited higher growth and acid production in soymilk prepared from dehulled defatted soybeans.

Angles'and Marth (1971) suggested that heat treatment of soymilk at 60 °C for 15 min during fermentation enhanced acid production, whereas heating of soymilk to 80 °C for 5–60 min greatly reduced acid production as a result of marked increase in the concentration of inhibiting substances such as sulfhydryte and toxic volatile sulfides.

Fermented soymilk has many therapeutic properties. Ghanem et al. (2020) examined the antioxidant, anticancer, and antiviral properties of fermented extracts of defatted soybean meal. They distinguished 26 compounds with 11,14-octadecadienoic acid and methyl ester (63.63%) and 31 compounds with butylated hydroxytoluene (66.83%) and δ-myrcene (11.43%) as primary

constituents. They likewise tracked down that the antioxidant activities of DSM extract were 3.362 ± 0.05 and 2.11 ± 0.02 mmol TE/mL, FDSM treated with *A. awamori FB-133* were 4.763 ± 0.05 and 3.795 ± 0.03 mmol TE/mL and FDSM treated with *A. fumigatus F-993* were 4.331 ± 0.04 and 3.971 ± 0.02 mmol TE/m.

23.5.1 Effects of Fermentation in the Nutritional and Functional Properties Soybeans

Soybean products have been known good food varieties due to being a magnificent source of excellent protein just as giving different various medical advantages. The protein content of soybean is 32% to 42%, contingent upon the assortment and development, of which around 80% is composed of 2 storage globulins, 7S globulin (β-conglycinin) and 11S globulin (glycinin), having different functional and physicochemical properties (Garcia et al. 1997; Kwon et al. 2003).

Soybean products are considered as a good substitute for animal protein, and their nutritional value with the exception of sulfur amino acids, for example, methionine and cysteine is practically identical to that of animal protein since soy proteins contain the majority of the fundamental amino acids for human nutrition. Notwithstanding excellent protein, soybeans contain significant degrees of unsaturated fats, dietary fiber, isoflavones and minerals, which have various medical advantages (Kim et al. 2021). Specifically, the relationship, the relationship of high-quality protein and phytochemicals, particularly isoflavones, is unique among plant-based proteins because isoflavones are not broadly appropriated in plants other than legumes (Velasquez and Bhathena 2007).

23.6 Disadvantages of Soy Milk

The consumption of soy protein and its products is often considered safe, and it is possible to consume nutritional supplements that contain soy extracts for up to 6 months, however, soy can cause some mild side effects in the stomach and intestines. Such as: constipation, bloating, and nausea. As for pregnant or breast-feeding women, soy is often considered safe if it is consumed in the normal quantities found in food. It is likely unsafe to use soy milk in large quantities during pregnancy. It is also advised to be careful and adhere to the normal quantities found in food during the period of breastfeeding; this is due to the lack of sufficient and reliable information to prove the safety of using soy in large quantities during this period ("SOY", www.webmd.com).

Soybean consumption by children is often considered safe if it is in the normal quantities found in food or infant formula. If health problems occur in the later stages of a child's life, however, soy milk should not be used as a substitute for

Fig. 23.4 Soy milk pictures. (Sources: https://commons.wikimedia.org/)

infant formula; since this can cause a deficiency of nutrients in the child. In addition, it should be noted that using soy as a substitute for cow's milk is unsafe for children who suffer from an allergy to cow's milk. So it is advised not to give soy to children in quantities that exceed the normal quantities; whereas, there is no reliable information about whether or not soy is safe for children in high doses (Soy www.rxlist.com, 17-9-2019) (Fig. 23.4).

23.7 Soy Milk Yoghurt

Soy milk yoghurt is made by fermentation of soymilk with friendly bacteria, mainly *lactobacillus bulgaricus* and *Sterptococcus thermophilus*. The process is similar to the production of yoghurt from cow milk. The sugars are fermented by the bacteria to lactic acid which causes the characteristic curd to form. The acid also restricts the growth of food poisoning bacteria.

Some countries only allow using the name "yoghurt"for products which have not been pasteurized to kill the bacteria after fermentation. This "live" yoghurt is believed to be nutritionally superior. The live bacteria are supposed to improve our immune system and the enzymes help to digest food. It is difficult to find soy yoghurt in supermarkets but it might be found in health food shop. In some countries soy yoghurt is labeled as "cultured soymilk because the term" yoghurt" is reserved for milk products.

A yoghurt - like soybean product developed with lactose content similar to control (commercial plain yoghurt) in acidity, with a sour taste and did not differ in intensity and aroma (www-_oznet.Ksu.edu soyyoghurt.htm. March, 2007).

Marisa and Graciela 2012) demonstrated that some strains of lactic acid bacteria and associated microrganisms (as Bifidobacterias) have the fundamental enzymes neccesaries for produce fermented soymilk or pre-treated soymilk with an improvements in the overall characteristics (low flatulence, good digestibility, enrichment in isoflavones aglicones), besides potential probiotical influences of each strain.

The enzymes as α-glactosidase contribute in a creation of soymilks with low content in α-galactoligosaccharides product or of intestinal disturb in humans. The proteases, peptidases contribute in soy products with an improvement in digestibility, and have some bioactive peptides with healthy contibution. The action of β-glucosidases contribute in the bioconversion of isoflavones in aglicones that can be absorbed by the human organisms, with beneficial aspects in bone mineralization, alleviate menopausial effect, hipoccholesterolemic impact, antioxidant effect (in the anti-eaging).

The effect of fermentation on the level of oligosaccharides present in soymilk has been determined for a number of lactic acid bacteria. *L. bulgaricus* did not grow readily in soymilk without supplementation (by 0.1% yeast extract, 1% glucose of both of them). However by altering the levels of supplementation and the method of starter preparation, a greater reduction in the oligosaccharides were observed (Nsofor and Chukwu 1996) showed that taste improvement in soymilk yoghurt was enhanced by the addition of 4% sucrose before starter inoculation.

Soy milk yoghurt contains live bacteria; these friendly bacteria foster a healthy colon and can even lower the risk of colon cancer. Lactobacteria promote the growth of the healthy bacteria in the colon and reduces the conversion of bile into carcinogenic bile acids. The friendly bacteria in yoghurt seem to inactivate harmful substances before they can become carcinogenic. Bacterial enzymes created by the yoghurt culturing process partially digest the proteins making them easier to absorb (Yu-Jie et al. 2015) (Table 23.2).

Table 23.2 Nutritional values of soymilk-yoghurt (per 100 g)

Water	89.0 g
Cal energy	59 k
Energy	245 k j
Water	89.0 g
Fat (total lipid)	2.7 g
Protein	4.7 g
Fatty acids, mono-unsaturated	0.6 g
Fatty acids, poly-unsaturated	1.6 g
Fatty acids, saturated	0.5 g
Fiber	0.2 g
Sodium, Na cholesterol	0.0 g
Cholesterol	0.0 g
Carbohydrates	3.2 g

Source: USDA Nutrient Database for Standard Reference 2008

23.8 Manufacture of Zabady Fortified with Soymilk

Zabady (fermented milk) was manufactured by cow's milk supplemented with soymilk. Raw cow's milk was collected early in the morning (7a.m) from Nesheshiba farm" The University of Gezira farm -Wad Madani Sudan). Milk sample was collected immediately after milking in steel sterilized containers, the amount of the sample was about 10 Ib and then transported under aseptic conditions to the Dairy Technology laboratory of the Department of food Science and Technology, University of Gezira. Milk was heated to 85–90 ° C for 30 min, then cooled to 45 °C and divided into three parts (1) whole cow's milk (control), (2) 5% soymilk plus 95% cow's milk, (3)15% soymilk plus 75% cow's milk. Zabady was prepared as described by Dirar (1993).

23.8.1 Preparation of Samples

For preparation of soymilk at laboratory, the following steps were followed**:**

1. **Ingredient:**

The ingredient needed include about 25 g whole soybeans to make 1 liter of soymilk (Fi. 24.4).

2. **Soaking and dehulling the soybeans:**

The soybeans were cleaned and soaked in water for 10–16 hr. . The process could remove the hulls by kneading the soybeans and flushing the loose hulls with water. Removing the hulls makes the extraction process more efficient. An alternative is to crack the soybeans before soaking. The hull became easily loose and could be washed away. When cracked soybeans are used, then less soaking time (6–8) hours is needed.

3. **Heating the soybean:**

Heating of the soybeans is necessary to destroy enzymes which are responsible for the development of beany flavor. This heating was achieved by microwaving the wet soaking soybeans during 2 minutes.

4. **Grinding the soybeans:**

The soybeans were ground and soaked and 1-liter water in blender. Then the mixture was sieved through a cheese cloth to recover the soymilk. The insoluble material which remained on the sieve is called okra, and can be used as an ingredient for bread or as cattle feed.

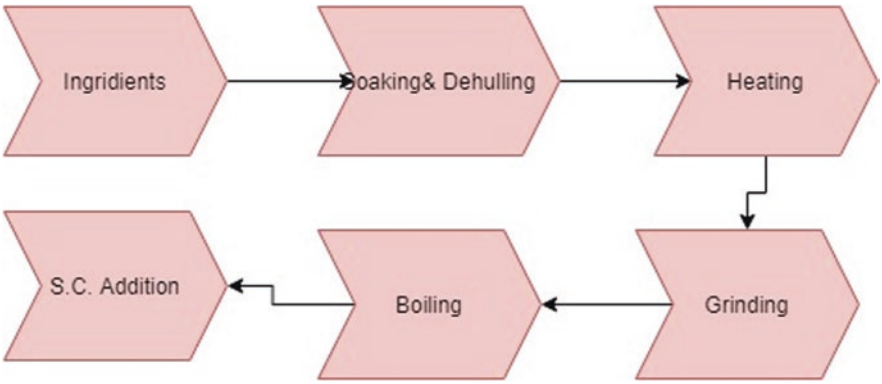

Fig. 23.5 Flow chart of soymilk preparation at laboratory

5. **Boiling the soybeans:**

The soymilk was heated until boiling point and continued for about 5 to 10 minutes. After cooling, the soymilk was ready and could be kept in the refrigerator for another 3 days.

6. **Addition of starter cultures**

Starter culture (3%) of 1:1 combination of *S. Thermophilus* and *L. bulgaricus*) was added. The mixture was poured into a clean dry plastic cup (100 gm). The cups were covered and then placed into an incubator at 42–4 °C until milk was completely coagulated (about 3–4 h). The cups were removed from the incubator and placed in a refrigerator at 4 °C (Fig. 23.5).

23.8.2 Proximate Chemical Analysis of of Zabady Samples

The contents of moisture, ash, protein, fat, total soluble solids, acidity and the pH value were determined in all manufactured zabady samples (control zabady and soymilk zabady) using the AOAC (2005) methods.

23.8.3 Microbiological Analysis Zabady Fortified with Soymilk

The microbiological analyses were carried out according to IOS (2013) on samples consisting of soymilk, control zabady, zabady fortified with 5% soymilk and zabady fortified with 15% soymilk. Appropriate dilutions of the respective sample in 0.1 gm aliquots were spread on pre-poured plates of plate count agar for the presumptive enumeration of total viable count (TVC), Baird-Parker agar for Staphylococci,

Salmonella-Shigella agar for Salmonella spp. and potato dextrose agar (PDA) for yeasts and moulds. Inoculated plates, except for PDA, were incubated for 24–48 h at 37oC. PDA plates were incubated for 72 h at 25oC. Characteristic colonies appearing on the respective selective agar media were counted, multiplied by the dilution factor and expressed as colony forming units per ml (c.f.u/ml). Sensory evaluation of yoghurt samples.

23.8.4 Sensory Evaluation of Zabay

Zabay samples were subjected to sensory evaluation using 10 untrained panelists at the second day of zabady manufacture. The panelists were asked to assess each sample for appearance, texture, color, flavors, and overall acceptability using 9-point hedonic scale with 9 as the high score and 1 as the lowest. A particular testing area was used for the judgements so that distractions could be decreased and conditions could be controlled. The testing area was quiet, comfortable environment with uniform level of lightening and good ventilation. Each panelist was provided with water for rinsing. All these conditions were equalized for all tests. The samples were given codes before being tested.

23.8.5 Sensory Evaluation

All scores of sensory evaluation were analyzed by the analysis of variance (ANOVA) according to SAS (1982) to determine whether there were significant differences between means for each variable. The Least Significant Difference (LSD) test was used to separate means.

23.8.6 Results and Discussion

23.8.6.1 Chemical Composition of Soymilk- Zabady

Chemical analysis of soy zabady (Fig. 23.4) indicated that it has been greatly affected by the incorporation of soymilk. Zabady fortified with 5% and 15% soymilk had high contents of fat when compared with the control sample. These values were similar to those reported by Abu Donia (1980) who stated that fat content increased gradually with increasing levels of soymilk. Protein content was slightly higher in zabady fortified with 15% soymilk compared with that of control and zabady fortified with 5% soymilk samples. Total solid contents increased gradually from 13.23% in control zabady to 14.18% and 14.56% in zabady fortified with 5% and 15% soymilk, respectively. Our findings contradicted with those reported by

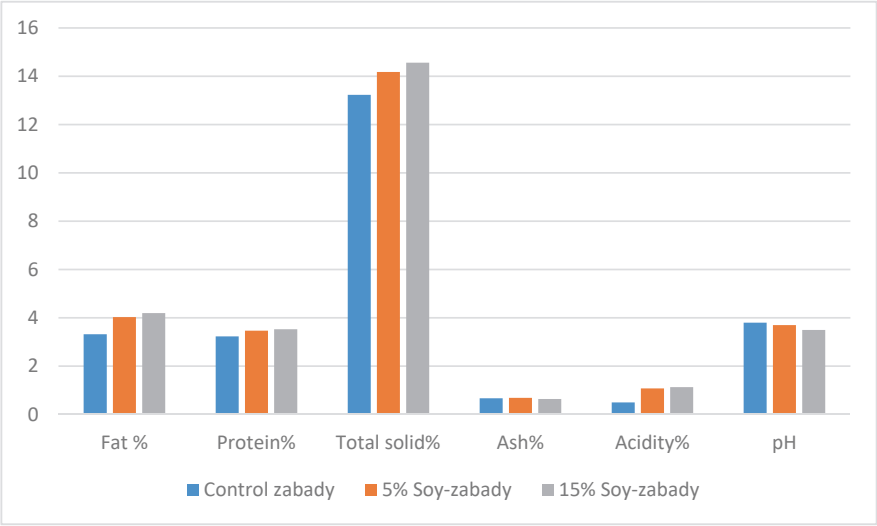

Fig. 23.6 The chemical composition of control zabady and soymilk- zabady
5% Soy-zabady = zabady supplemented with 5% soymilk.
15% Soy-zabady = zabady supplemented with 15% soymilk

Lee et al. (2018) who found that milk based yoghurt contained higher total solid (14.54%) than the soymilk based yoghurt (11.49%). Ash content was similar in three samples. The titratible acidity increased in control zabady from 0.5% to 1.08% in zabady fortified with 5% soymilk and increased to 1.13% in zabady supplemented with 15% soymilk. Whereas this is in full agreement with Abu Donia *et al* (1980), who found that titratible acidity in variably rises with increasing levels of soymilk. However the result in this study are not in agreement with the finding of Gehrke and Weister (1984) who reported that the lactic cultures produced less acid in soymilk than in cow's milk, because of the type of organisms used. The pH values slightly decreased from 3.8% in control yoghurt to 3.7% and 3.5% in zabady fortified with 5% and 15% soymilk, respectively (Fig. 23.6).

23.8.6.2 Sensory Evaluation of Soymilk- Zabady

Table 23.3 presents the effect of supplementation zabady with two levels of soymilk in texture, appearance, flavor, and overall acceptability. The panelists gave the highest scores of texture to the yoghurt supplemented with 15% soymilk. However, there were no significant differences ($p < 0.05$) in texture of zabady fortified with 15% when compared with the control zabady and zabady fortified with 5% soymilk.

The control zabady scored high value in flavor than the fortified samples due to the unacceptability of the beany flavor of the soybeans which were used to supplement the yoghurt samples. The zabady containing 15% soymilk scored high value

Table 23.3 Sensory evaluation of zabady samples

Sample	Color	Flavor	Texture	Overall acceptability
Control zabady	6.5b	7.0a	6.8a	7.2a
5% soy-zabady	5.3b	3.5c	5.2c	4.3c
15% soy-zabady	7.3a	5.8b	7.0a	5.7b

Table 23.4 Microbial load (c.f.u/ml) of soymilk, control zabady and zabady fortified with two levels of soymilk

Samples	TVC (c.f.u/ml)	Salmonella (c.f.u/ml)	Staphylococci counts (c.f.u/ml)	Yeast and moulds counts (c.f.u/ml)
Soymilk	5.3×10^2	NIL	5.0×10^5	2.0×10^2
Control zabady	6.4×10^3	NIL	13×10^5	5.4×10^2
Zabady fortified with 5% soymilk	4.4×10^3	NIL	4.2×10^5	4.7×10^2
Zabady fortified with 15% soymilk	3.8×10^3	NIL	3.5×10^5	4.5×10^2

TVC Total Viable Count

in color than the other treated samples, there was no significant difference ($p< 0.05$) between zabady fortified with 5% soymilk and control yoghurt in color and appearance. It has been stated that yoghurt -type product made from Soya protein dispersion were less sour viscous than milk based yoghurt (Mil et al. 1993). Soy yoghurt generally possessed acceptable color and physical properties (Lee et al. 2018).

Although, all the tested samples were acceptable, yet control zabady received higher scores than did the other samples in the overall acceptability. It has been stated that the undesirable flavors in soy are are brought about by the creation of hexanal and other unstable aldehydes compounds by hydroperoxidation of cis-cis 1,4- pentadiene-containing fatty acids, fundamentally linoleic and linolenic acids, by lipoxygenase in soybeans. The reaction occurs when soybeans are damaged, crushed, ground, or rehydrated during processing and! or storage (Wolf 1975; Wilson 1996; Zhu et aI. 1996.

23.8.6.3 Microbial Analysis of Soymilk-Zabady

The microbiological characteristics of soymilk, control zabady and zabady fortified with 5% and 15% soymilk are presented in Table 23.4. The total viable count of bacteria (TVC) of soymilk was (5.3×10^2 c.f.u /ml) while the control zabady, zabady fortified with 5% and 15% soymilk contained 6.4×10^3c.f.u/ml, 4.4×10^3c.f.u/ml and 3.8×10^3c.f.u/ml, respectively. Increases in the microbial load of various zabady samples could be because of improper sanitary conditions during the manufacturing process. On the other hand, the TVC were higher in the control zabady when compared with that of the soy-zabay samples. The staphylococci count was higher in soymilk (5×10^5 c.f.u/ml) when compared with that reported by Mustafa (2005) who found that staphylococci count of fresh goat's milk was 2.4×10^2 c.f.u/ml.

control yoghurt contained 13.0×10^2 c.f.u/ml staphylococci. The staphylococci of zabady fortified with 5% soymilk were 4.2×10^5 c.f.u/ml and zabady supplemented with 15% soymilk was 3.5×10^3 c.f.u/ml, this reduction could be attributed to the increased acid concentration as a result of fermentation. The presence of staphylococci in such higher levels cloud be due to improper handling or bad hygienic practices pre-or during zabady preparation.

The yeast and mould count of soymilk (2.7×10^2 c.f.u./ml) lower than a value of 4.0×10^3 c.f.u/ml in cow's milk that reported by Mustafa (2005). Yeast and mould grow well in acidic food with low water activity, however, the yeast and mould count in control zabady (5.4×10^2 c.f.u/ml) was lower when compared with that reported by Safa (2005) who found a value 7.0×10^3 c.f.u/ml in control goat's milk zabady and in agreement to the value that reported by Mustafa (2005) who found a value of (5.0×10^3 c.f.u/ml) in control cow's milk yoghurt. The yeast and mould was (4.7×10^2 c.f.u/ml) and (4.5×10^2 c.f.u/ml) in zabady fortified with 5% and 15% soymilk, respectively. Both values were lower than that of the control cow's and goat's milk zabady which were 6.5×10^3 c.f.u/ml and 5.0×10^3 c.f.u/ml, respectively as reported by Safa (2005). Yeast and mould are example of fungi, and moulds are responsible for the food spoilage and produce mycotoxin (Buchanan and Doyle 1997). Yeast and mould are considered as spoilage organisms resulting in flavor and textural deterioration including softening, discoloration and slime formation. This result was not acceptable since the confidence limits of staphylococci of fluid product was greater than 1.00 c.f.u/ml as reported by Rufus (1980)**.** The results showed absence of salmonella in all tested zabady samples.

It has been demonstrated by Momoh et al. (2011) that soy milk can be preserved at for up to 13 days at a refrigerator during which no sensible increase of mesophilic aerobes above 3x103 cfu/mL was notices and a total restraint of yeasts and molds accomplished when preserved between 700–800 parts per million (ppm) of sodium benzoate, pasteurization and refrigeration while a mix of 400 ppm of sodium benzoate and 175 ppm of sodium metabisulphite can accomplish a preservation of the milk for around 11 days during which no sensible increase of mesophilic aerobes above 3x103 cfu/mL was noticed and an all out restraint of yeasts and molds were accomplished when protected with between 700–800 sections for each million (ppm) of sodium benzoate, sanitization and refrigeration while a mix of 400 ppm of sodium benzoate and 175 ppm of sodium metabisulphite can accomplish a safeguarding of the milk for around 11 days.

23.9 Conclusions

The current study was initiated to review the importance of utilization soy milk in fornication of fermented milk zabady with two levels of soymilk (5%and 15%). Soybean which was collected from Kenana "Central Sudan" was subjected to chemical analyses. These analyses indicated that most of the chemical components were in close agreement to literature values with slight variations.

The gap between production and utilization of fluid milk is constantly increasing in Gazira State, central Sudan. In addition, the Sudanese individuals become acquaint with the significance of zabady as one of the widely consumed fermented milk products. However, since the price of raw milk increases daily, it is not possible to produce fermented milks like zabady solely from raw milk or skim milk powder. This investigation focuses on the production of zabady from milk fortified with 5% and 15% soymilk. The fortification of zabady with soymilk improved its nutritive value; however, the fortification with 15% soymilk resulted in unacceptable zabady due to its beany flavor.

Based on the results the study it is recommend to fortify of soymilk with whole milk in the manufacture of zabady at a concentration level of 5%. However, soymilk could efficiently be substituted or supplemented in higher levels if the beany flavor is removed by fermentation. Thus, further studies will be of great economic value in formulating acceptable soy foods milk with the addition of flavoring agents for human consumption in the Sudan.

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Chapter 24
Nutrient Composition and Bioactive Components of *Kejeik*

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24.1 Introduction

Fish is an important food source for humans because it provides essential nutrients such as easily digestible proteins that contain all essential amino acids necessary for humans. It also contains fats that are characterized by their high content of essential fatty acids (unsaturated), vitamins, as well as mineral elements such as phosphorous. Fish, despite their importance, They can be a source of many diseases. This is because of the pathogens they may carry as a result of the way they are caught, marketed, and dealt with improperly. The pollution of the environment in which it lives might cause disorders to human beside many factors that affect fish quality. Therefor fish is a rapidly perishable food item more than red meat due to the nature of its chemical composition, the great activity of its own enzymes, and being an ideal environment for the growth of most types of microbes (Sheem and Mohamed 2017).

Sudan enjoys great sources of fish wealth represented in the Nile and its tributaries Its lakes have a total length of 6400 km and an area of two million hectares. Represent reservoir lakes built on the Nile, with a total area of about one million hectares. In addition, there are territorial waters in the Red Sea coastal within the limits of 720 km and cliff continental 9800 km and exclusive economic zones 96,100 km. There are also sewers The non-Nile water is administrative and charitable, and its water is estimated at about 8.2 billion meters Cubic and

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groundwater estimated at about 9.4 billion and represent the resources mentioned above mainly from inland waters from the culture of finfish and other aquatic organisms.

The annual production of fisheries in Sudan is estimated at about 65 thousand tons per year, and the fish stock, according to the estimates of the International Food and Agriculture Organization (FAO), is about 110 thousand tons of fish.

The importance and role of fish wealth in the economic reality: the fish sector is gaining ground of its importance in being: -

- source of animal protein.
- A source of income that provides job opportunities for large sectors of society in the country
- Areas of forward and backward activities and investment in the areas of fishing and storage, deportation, marketing, manufacturing and exporting.
- Contribute to increasing the national income.
- Provides alternatives to the consumption of animal protein, which provides a greater opportunity for export red meat and live animals.
- Fish can play an important role in the biological control of a problem of aquatic weeds in the irrigation canals of major agricultural projects.

About the flow circumstance and the degree of misuse of fishery assets, Awad (2018) demonstrated that the production of consumed fish comes from the lakes of the seven reservoirs spread along the White and Blue Niles and the Nile River, and that the Jabal Awliya reservoir is exploited of 8 thousand tons, with 53% of the total stock of 15 thousand tons. As for the Rossires reservoir, the exploited percentage is (1500), 36.6% of the total stock of 4100 tons, and the exploited Sennar reservoir is 1100 tons with 100% of the stock. Khashm al-Qirba reservoir has exploited quantities of 400 tons, 50% of the total stock of 800 tons. While the percentage of the exploited quantities from the reservoirs of Upper Atbara and Setit was not specified. The total production reached (two thousand tons) and is constantly increasing. As for Lake Nubia, the percentage of exploitation (two thousand tons) is 39.2% of the total stock of 5100 tons. As for the Merowe reservoir, the exploited quantities amounted to 1500 tons, or 25% of the total stock of six thousand tons (Awad 2018) (Fig. 24.1).

24.2 Fish Consumption and Consumer Preference

In general, human consumption of aquatic organisms and fish has increased In particular, due to its high nutritional protein content (multiple unsaturated fatty acids and vitamins (Bener et al. 2009; Colangelo et al. 2009). And these living organisms, whether hunted, are among the areas of their natural spread, or cultured, protein sources. These organisms contributed more than 18% of total consumed animal protein (FAO 2009). In general, most of the nutrients of seafood can be preserved by cooking it moderately or eat it raw. However, to eat like these fish, may lead to

Fig. 24.1 *Kejeik* at Wad Madani Market

health risks from either the resulting pollution from the environment in which the fish live, or secondary pollution as a result of hunting, processing and handling (Feldhusen 2000).

The consumer inclination and fish utilization design on the planet gained a new incredible significance. Ye 1999 contemplated the recorded utilization and future interest for fish and fishery items and made exploratory estimations for the years 2015/2030. Westlund (2005) laid out the guaging for the fish utilization and request investigation in future possibilities for fish and fishery items, in order to cover approaches and strategies utilized for anticipating utilization and examining interest for fish. He expressed that, disregarding a broad writing search, next to no work was found on quantitative expectations of fish utilization or interest later on.

The Sudan is a wide county with individuals of various and utilizing an enormous assortment of food (Boutros 1972). Medani (1972) demonstrated that, fish represent the only source of animal protein for many Nilotic tribes in southern Sudan and that the characteristic features of fish consumption patterns in the Sudan are of relatively high level of intake in southern Sudan and of generally low level in northern and western Sudan. Abu Gideiri (2001) expressed that, in the Sudan significance of fish in the eating routine appears to follow a notably local significance of fish in the eating routine appears to follow a notably local pattern.

As of now, seven fish products mostly produced in Sudan include: Feseikh, dried fish *Kejeik*, indigenous Turkein "salinity" chilled fish, fish, frozen fish, canned fish, smoked fish, fish waste. Dried fish from minimal effort customary industries and residents practiced on the Blue Nile and White Niles (Dirar 1993). One of these home Products, a dried black or brown Kejeik made from garmut (mud-fish, Clarias angullaris, Clazera); nawk (thick-skinned fish, Heterotis niloticus,) humar-el- hout (black spotted cat fish, H. niloticus bidarsalis). As indicated by the classification of the Nile fish on the basis of market meat grade given by Amirthalingham and Khalifa (1965), *Kejeik*, likewise called korki, and hout is a product obtained by sun drying big fish. The fish are first unevenly split along their dorsal tomahawks prior to

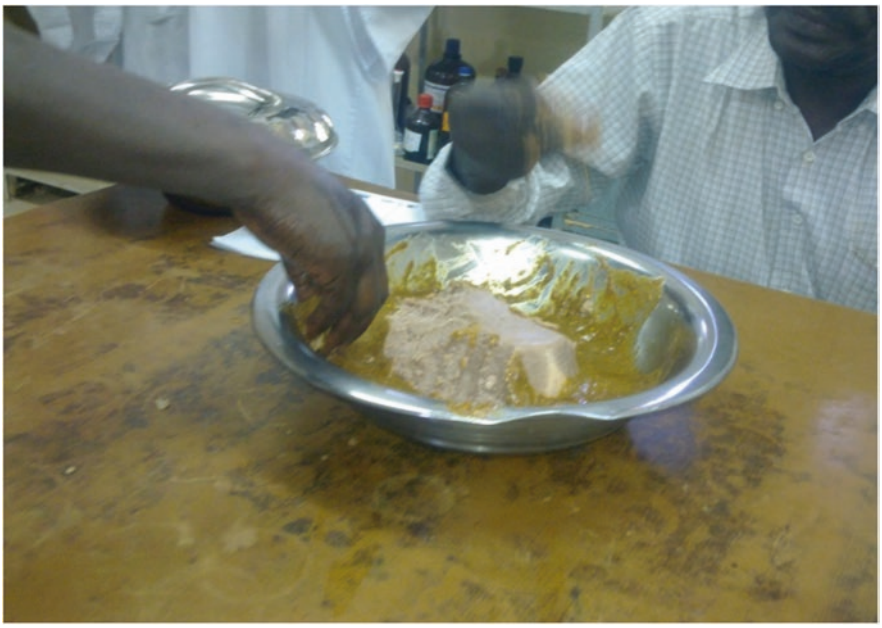


Fig. 24.2 *Kejeik* meal with *aceda* (Stiff fermented sorghum porridge)

drying. The drying of fresh fish (and meat) is the most commonly utilized conservation strategies of food not only in the Sudan as well as all through Afr. Kejeik is delivered in the southern Sudan by the Nilotic tribes the Dinka, Nure and Shulluk. Numerous subgroups of these big tribes live in the immense damp zone of the sudd of the Nile. Here the river fans out immersing a great many hectares, hence supporting an enormous development of aquatic weeds it is here where water are shallow, that individuals from these tribes catch fish by spears. Although the greater part of the product comes from the Southern Sudan. Kejeik is also prepared in some part of northern Sudan along the White Nile, the Blue Nile and Atbara River (Dirar 1993) (Fig. 24.2).

In the Sudan there are various tribes that vary in the sort of utilized food. The tribal impact on fish utilzation pattern in the Sudan was early noticed by numerous investigators (Stubbs 1949). Sandon (1951) expressed that within the Nilotic tribes, fish are esteemed more by amount than by quality. Any sorts of fish being eaten, in any case contrasts in taste do exist between various clans, and presumed that the Nilotic individuals of the Sudan are an incredible illustration of the need to view sustenance as a social as opposed to individual or individual issue.

An impressive number of business fish seem, by all accounts, to be in extraordinary interest among purchasers, and some others which are in restricted interest despite the fact that their substance is of genuinely high nutritive worth (Rekhina 1974). Finne et al. (1980) expressed that the size, shape, and presence of huge numbers of the accessible species make them unacceptable for customary fish items.

Very little work was done on the determination of consumers' preference request of fish as food in the Sudan. El Obeid and Sid Ahmed (1988) examined the interest of fish as food in Khartoum and Port Sudan territories. Karar (1997) investigated the taste, propensities and demeanor of the Sudanese consumers towards fish consumption, and noticed stamped provincial and ancestral impacts on the customers' inclinations and food propensities. These findings were affirmed by El Fazary (2005).

24.3 Fish Industry in Sudan

It is known that fish are quickly spoiled, particularly in hot countries like Sudan, so the quality of fish must be taken care of, from the moment it is caught until it reaches the consumer, in order to achieve several goals, the most important of which are:

1. The delivery of a healthy commodity to the consumer.
2. Achieving a better financial profit for the fisherman as a result of being able to sell fresh fish and maintain its quality at a higher price than the fish that are liable to spoil quickly.
3. Provide quantities of fresh fish in the market for a long period and reduce the waste resulting from spoilage of fish not traded properly.

One of the most effective ways to increase fish production, is the establishment of a fishing industry on a modern scientific basis. In addition, the establishment of an advanced fish processing industry where cooling and freezing occupies, followed by the preservation in cans, smoking, salting, drying, cooling and drying, and the importance of this is summarized in preserving the initial characteristics of the fish in its initial fresh state without changing (https://ipecs.sudanforums.net/t22-topic).

The fish industry has been practicing in Sudan for a long time, but the quantities manufactured are relatively few and most of it is consumed locally. Sudan's export from this industry is weak and does not live up to the possibilities.

The fish industry is currently limited to seven products:

1. Fesikh: The fish suitable for fesikh are available in the fresh water and the Red Sea, so in the water there are goblet and kawara fish, but in the Red Sea there are large quantities of Arabi fish.
2. Dried fish: Dried fish is considered a low-cost traditional industry that is practiced by citizens on the blue and white beaches of the Nile. The main dried product is black cakes

   Which is made from catfish and um Koro, and white gjek, which is made from tilapia, calf and molasses.
3. Torkin "Al-Malouh": fish that are fermented with salt and made from the calyx, kawara, molasses and other species. Malouha, or Al-Turken, was closely linked to northern Sudan. Torkin is a meal that consists of fish, and it is one of the favorite meals of the people of the North, as they eat it greedily in the winter and

autumn seasons because it is warm. It is said that the *Turkin* is a very old meal associated with the pharaohs and shared between Sudan and Egypt, such as fesikh, and some sources indicate that the *Turkins* were the main food during the era of the ancient Nubian kingdoms. *Torkin* is prepared by placing large or medium Nile sardines or seer fish in a large container and sealing it. Coarse salt, chili, vegetable oil and lemon juice are placed on it. It is tightly closed and left for a month or more, and some prefer to bury it under the ground until it decomposes and becomes a "*Turkin*".

4. Chilled fish: fresh fish cooled by ice and can be kept for a period of 2 weeks. This industry requires the establishment of ice factories near the production areas. The industry is affected by the seasons (winter, autumn summer) and the amount of consumer ice and means Anakl. Private ice plants are spread out in the fish sector in each of the Suakin in eastern Sudan, the city of Al-Shajarah in Khartoum and the old Halfa governorate, and isolated carts, refrigerators and modern cooling rooms are used in preserving and transporting these fish.
5. Cooling and freezing fish: an industry based on preserving frozen fish for a period of up to 6 months at a temperature of 18 degrees below zero, which helps to export it to remote areas. There are many method currently used, including primitive and somewhat advanced methods. The primitive methods include the weed and the wet cuttings, and are used to keep fish inside boats immediately after the fishing process. After, reaching the nearest landing area and in the final fishing areas and camps, small rooms of mud are built that are taken as a preservation party with the use of snow as a material preservative, which is the prevailing method used in cooling, with the use of sawdust as an insulating material. Among the most advanced means are preservatives made of wood internally insulated with raspberry material (https://www.gafrd.org/posts/158213).
6. Canned fish: There are some fish species that are suitable for canning. In the Red Sea, for example, there are tuna, sardines, and mackerel, which are seasonal, and there are some fish species in the freshwater suitable for canning, such as carrara, cup and others.
7. Smoked fish: In this industry, a fish grilled by smoking method, meaning smokers are poured on the fish to ripen slowly, and it is produced by traditional methods, and the smoking of fish in modern methods began on the White Nile and Mount Awlia.
8. Fish waste. Fish waste is used in the manufacture of feed, fertilizers, fertilizers and in the manufacture of soap, and the quantities of waste increase when cleaning fish and cutting it into strips, and can also benefit from the meat of shells and zumbags in the manufacture of fish (The General Administration of Fish).
9. Fish farming: it includes:

    1- Fish farming: It is the raising of fish in a limited water environment under human control and with the purpose of increasing its production.
    2- Cultivation of shrimp
    3- Cultivation of pearl oysters

24.4 Production of *Kejeik*

Seven fish merchandise basically produced in Sudan consist of: *Feseikh,* dried fish *Kejeik,* Turkan "salinity" chilled fish, frozen fish.

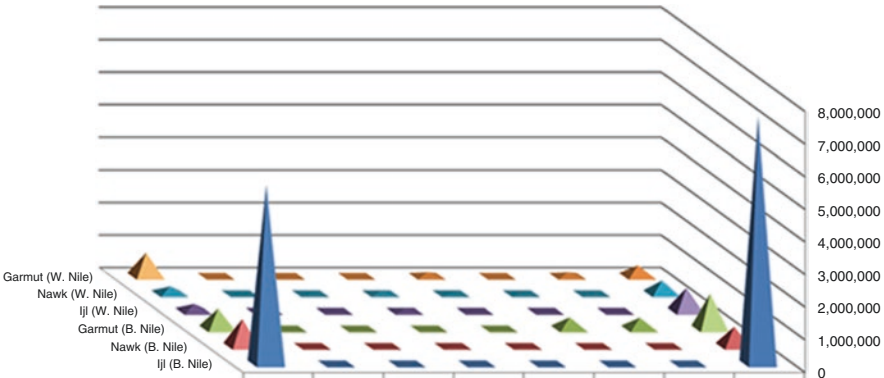
Kejeik is reviewed in the nearby Sudanese market into first grade dark *Kejeik* that is produced using Garmout (Clarias anguillaris and Clarias Lazera), Nauk (Heterotis niloticus), Humar EI Hout (Auchinoglanis biscutatus and Auchinoglanis occidentalis) and Surta (Heterobranchus bidorsalis). The second grade *Kejeik* is produced using Kharish (Distichodus niloticus), Bayad (Bagrus bayad), Dabs (Labeo niloticus and Labeo horie) and Bulti (Tilapia spp.). White *Kejeik* is produced using fish type that gives excellent market meat. The fish included are igil (Nile roost, lates niloticus), bayad or kabavros (Forskal's catfish, Bagrus Bayad), dbas (Nile carp. Labeo niloticus, L.horie), Khreish (unpleasant cast fish, Distichodus Nile carb, Labeo niloticus, L.horie), (nilticus, D. rostratus, D. bervipinnis, D engycephalus) and bulti (roost, Tilapia nilotica. T.zillii, T galilaeaus). (Mirza and Omer 1984). Of these fish, only khreish is used for *Kejeik* to any important extent (Yousif 1988). It should be remembered that this division of fish between the two grades of Kejeik is not always strictly followed. For instance, *Kejeik* prepared from Tilapia is sometimes considered of top grade.

Home Products are dried black or brown *Kejeik* kinds consisting of gamut, Clarias angullaris, Clazera) Nawk) humar-el- hout consistent with the type of the Nile fish on the he idea of marketplace meat grade stated through Amirthalingham and Khalifa (1965).

24.5 Microbiology of *Kejeik*

Fish is soft and easily damaged; therefore, rough handling and bruising results in contamination of fish gentle and without difficulty damaged; consequently, tough managing and bruising outcomes in infection of fish flesh. So, fish will become undeserving for human intake rapidly after capture (1 day), except it's far processed or preserved. Even after fish has been processed, especially if through traditional means, the fish continues to be situation to severa spoilage forms. Microbial flora associated freshly harvested fish is a characteristic a of surroundings wherein the fish are captured and now no longer of the fish species; hence, the indigenous microbial populations of fish can range significantly (Shewan 1962).

Fish, because of their soft tissues and aquatic surroundings are extraordinarily subjected to microbial infection specially through microorganism, on the grounds that numerous of them are potential spoilers, are present within side the floor slime, at the gills and withinside the intestines of stay fish. Bacterial increase and invasion at the fish are averted through the body's natural protection system in the course of lifestyles however after loss of life the protection device breaks down and the microorganism multiply and invade the flesh. Bacterial spoilage is characterised through



	Halophilic bacteria	Acidophlic bacteria	Total Molds	Total Yeast	Bacillus cereus	Coliform	Enterbacteriaceae	Total bacterial count (spc)
Ijl (B. Nile)	5,500,001	0	9,100	21,000	5,000	0	10,901	7,600,000
Nawk (B. Nile)	850,001	0	12,001	36,000	23,000	0	9,300	610,001
Garmut (B. Nile)	620,001	0	12,001	0	10,000	360,000	350,000	1,080,000
Ijl (W. Nile)	230,001	0	16,001	90,000	13,001	6,700	1,700	720,002
Nawk (W. Nile)	230,001	0	16,001	90,000	40,000	32,001	21,000	410,000
Garmut (W. Nile)	730,001	0	12,001	0	130,001	0	130,001	340,000

Fig. 24.3 Microbiological characteristics of *Kejeik* samples fro Blue Nile and White Nile

softening of the muscular tissues and the manufacturing of slime and offensive odours (Gram and Huss 1996).

Kejeik fish product is commonly secure from microbiological view factor due to the fact the processing consists of dehydration in addition to salting; however, the microbial infection may want to arise as a result of pass infection post-processing. Data offered in Fig. 24.1 display the microbiological traits of *Kejeik* samples accumulated from Blue Nile (Zahra 2013) (Figs. 24.3 and 24.4).

The high levels of aerobic bacterial count of Kejeik could be may be attributed to wrong managing sanitary circumstance in the course of the guidance and moisture content. The Enterbacteriaceae isolated from *Kejeik* were identified at species level as Enterobacter cloacae,, and their counts in Ijl *Kejeik*, Nawk Kejeik and Garmut *Kejeik* have been 1.09×10^4, 0.93×10^5 and 3.5×10^5 cfu/gm, respectively. The count of Nawk *Kejeik* (0.93×10^5 cfu/gm.) was higher than those of Ijl *Kejeik* and Garmut *Kejeik* (Sulieman et al. 2014). The coliform bacteria were not detected in most of the *Kejeik,* while the isolated Bacillus from Kejeik samples were identified at species level as B. cereus, their counts ranged between 5×10^5, and 1.0×10^4cfu/gm (Sulieman et al. 2014). Halophilic bacteria found in *Kejeik* samples could be identified as *Kocuria rosea, Streptococcus-dysgalactiae* ssp-*dysgalactiae* and *Enterococcus faecal*is.

Aspergillu niger, Alternaria, and *Penicillium*. sp. fungi, have been efficiently isolated from *Kejeik* samples (Zahra 2013).

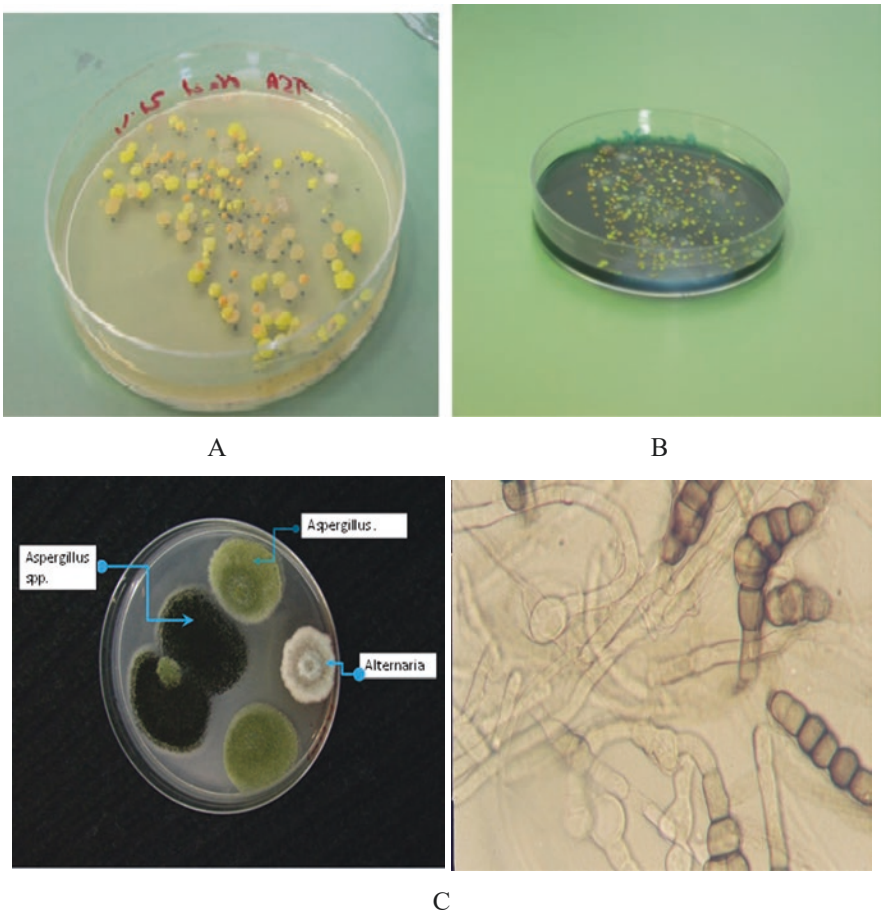


Fig. 24.4 Microbial groups isolated from Kejeik product: (**a**) Halophlic bacteria; (**b**) Bacillus cereus; (**c**) Fungi (Alternaria, Alternaria

The microbiological analyses of *Kejeik* revealed absence of acidophlic bacteria, Escheriaceae coli (E.coli), *Escheriaceae coli* 0157:H7, *Staphyloccoccus aureus*, Salmonella, *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *Vibrio cholera*. The absences of dangerous and pathogenic bacteria suggest protection of the *Kejeik* merchandise and inspire its advice for intake. The impact fish kind withinside the microbial load of 3 kinds of fish is proven.

The effect of fish type in the microbial load of three types of fish is shown in Table (24.1). It is found that, the fish kind and vicinity may have an effect on its microbial load (Mogessie et al. 1995; Zahra 2013).

Table 24.1 The Microbiological Characteristic of *Kejeik* samples (Blue Nile)

Microbial Characteristic	*Kejeik* samples		
(cfu/gm)	Ijl	Nawk	Garmut
Total bacterial count (spc)	$7.6 \times 10^6 \pm 0.77$	$6.1 \times 10^5 \pm 0.48$	$1.08 \times 10^6 \pm 0.06$
Enterbacteriacea	$1.09 \times 10^4 \pm 0.7$	$0.93 \times 10^5 \pm 0.14$	$3.5 \times 10^5 \pm 0.23$
Coliform	Nil	Nil	$3.6 \times 10^5 \pm 0.4$
B. cereus	$5 \times 10^5 \pm 0.001$	$2.3 \times 10^4 \pm 0.23$	$1.0 \times 10^4 \pm 0.01$
Total yeast	$0.21 \times 10^5 \pm 0.21$	$3.6 \times 10^4 \pm 0.33$	Nil
Total molds	$0.91 \times 10^4 \pm 0.001$	$1.2 \times 10^4 \pm 0.52$	$1.2 \times 10^4 \pm 0.52$
Acidophlic bacteria	Nil	Nil	Nil
Halophlic bacteria	$5.5 \times 10^6 \pm 0.6$	$8.5 \times 10^5 \pm 1.1$	$6.2 \times 10^5 \pm 0.59$
Escheriaceae. Coli (E.coli)	Nil	Nil	Nil
Escheriaceae. Coli 0157:H7	−ve	−ve	−ve
Staphyloccoccus aureus	Nil	Nil	Nil
Salmonella	−ve	−ve	−ve
Listeria Monocytogenes	Nil	Nil	Nil
Vibrio Parahaemolyticus	Nil	Nil	Nil
Vibrio Cholorea	Nil	Nil	Nil

Source: Sulieman et al. (2014)

24.6 Chemical Composition *Kejeik*

The processor, the nutritionist, the cook and the customer all have an have a direct hobby withinside the fish. The processor has to realize the concept of the crude fabric earlier than he can follow successfully the techniques of chilling, freezing, smoking or canning. The nutritionist wishes to recognize what dedication fish could make to the ingesting habitual and to fitness health and the cook ought to realize for example whether or not a fish is frequently lean or greasy to; set it up for the table. The consumer is intrigued now no longer simply in whether a selected fish tastes great, which entails assessment, but moreover in whether or not it's far nutritious.

While the patron is intrigued. Fish is one of the maximum treasured reasserts of excessive grade protein handy to guy on this hungry world, and a statistic of its composition is simple if the fullest use is to be manufactured from it (Adeyeye 2014).

While the consumer is intrigued essentially withinside the eatable piece of the fish, this is the flesh or muscle, the fish meal producer is involved with the composition of the complete fish, and the processor of fish oils recognize what's withinside the liver. Estimation of fish gadgets ingredients of is occasionally essential to fulfill details or to comply with guidelines. For instance, the fish content material of fish desserts or the oil content material of fish meal might also additionally want to be regarded which will meet sure business or valid necessities. Fish one of the maximum treasured reassets of high-grade protein accessible to man in this hungry world, and a information of its composition is basic if the fullest use is to be manufactured from it (Adeyeye 2014) (Fig. 24.5).

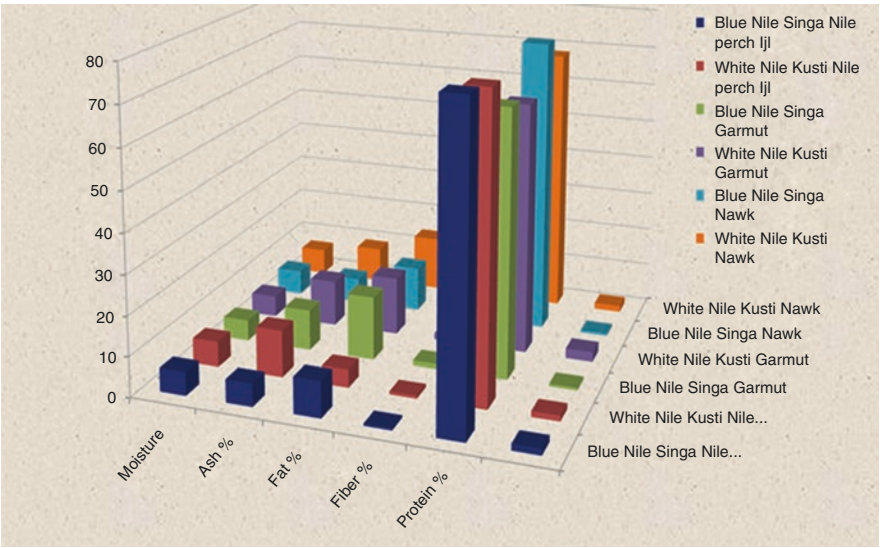


Fig. 24.5 Chemical composition of *Kejeik* samples

The outcomes of chemical analyses recommend that *Kejeik*, despite of deficiencies in sure crucial amino acids, do now no longer lose their dietary value. This truth is even greater essential if this fish product is to be regarded as an factor in balanced diets.

Kejeik samples contained considerable quantities of macro-minerals and the calcium turned into the best in all samples. Moreover, *Kejeik* samples contained most of the micro-mineral. All *Kejeik* samples free from toxic metals such as mercury, arsenic and cadmium (Fig. 24.6).

24.7 Conclusions

Kejeik (a traditionally dried fish product) prepared in rural areas in many parts of Blue Nile and White Nile basins (of Sudan), and consumed by many people in these areas and other parts of Sudan was intensively investigated . The study aimed at the determination of chemical composition of Kejeik samples in addition, to the identification of the product safety through determination of the microbiological characteristics of the product.

The results of the microbiological analyses indicated the absence of acidophlic bacteria, Escheriaceae coli (E.coli), Escheriaceae.Coli 0157:H7, Staphyloccoccus aureus, Salmonella, Listeria Monocytogenes, Vibrio parahaemolyticus and Vibrio cholorea. The absence of harmful and pathogenic bacteria indicate safety of the Kejeik products and encourage its recommendation for consumption. The variations in microbial counts of Kejeik samples from different markets and seasons could

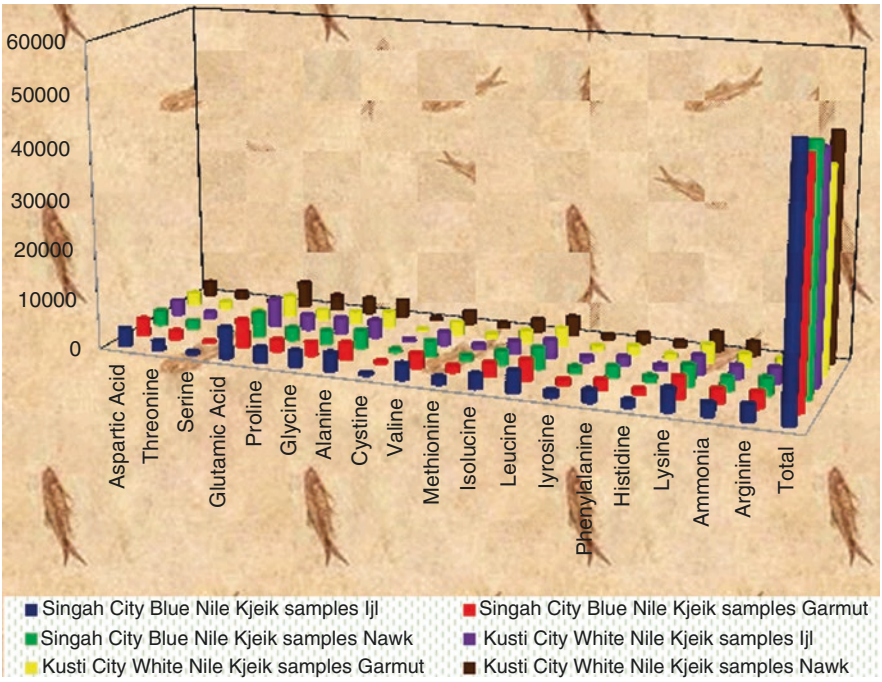


Fig. 24.6 Amino acid composition of Kejeik samples collected from Singah City (Blue Nile) and Kusti city (White Nile)

attributed to a lack of proper procedures adopted by the Kejeik processor and/or improper hygienic in some of these areas. But generally the results indicated that Kejeik is a safe for consumption.

The chemical analyses indicated that there was non-significant difference between the Blue Nile and White Nile *Kejeik* samples in most of the chemical components of *Kejeik* samples which contained significantly higher contents of protein, fat, fiber ash, moisture and carbohydrate. The analysis for amino acid composition showed an increase in glutamic acid, lysine, leucine, alanine, arginine, valine and isoleucine levels, whereas levels of methionine, tyrosine, histidine and serine relatively decreased in *Kejeik* product. The fatty acid content was significantly affected by the different areas. The, results suggest that *Kejeik,* in spite of minor deficiencies in certain essential amino acids, do not lose their nutritional value. This fact is even more important if *Kejeik* are to be considered as an ingredient in balanced diets.

The effect of the production areas and fish genus in the fatty acid composition of fish genus indicated that the fatty acid content of three fish type was affected significantly by the different areas**.** *Kejeik* contained various quantities of minerals, and all *Kejeik* samples free from toxic metals such as mercury, arsenic and cadmium.

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Chapter 25
Nutrient Composition and Bioactive Components of Non-alcoholic Sorghum Malt Beverage

Ahmed G. M. Elgorashi and Abdel Moneim Elhadi Sulieman

25.1 Introduction

Sudan is the third country in Africa and the seventh in the world's largest sorghum producer (Oluwakemi and Omodele 2015). The annual sorghum production ranged between 2.2 and 4.2 million tons and is grown in 10–14,000,000 acres. More than 75% of this production from rainfed sector. This makes sorghum, quantitatively the first most important cereal grain.

Foods prepared from sorghum can be grouped in two categories, traditional products and non-traditional industrial products. A detailed classification of traditional foods from sorghum has been developed (Vogel and Graham 1979; Rooney et al. 1986). They can be classified broadly into breads, porridges, steamed products, boiled products, beverages and snack foods (Rooney et al. 1986; Rooney and McDonough 1987).

Mashing is the process of crushing food, usually after cooking it, so that it forms a soft mass. Some studies on mashing show that starch hydrolysing enzymes, α and β amylase, which developed during malting of sorghum appeared to be in low activities, when assayed using new standard methods (McCleary and Sheehan 1987; McCleary and Codd 1989). However, an adapted mashing procedure developed for extracting sorghum malt which gelatinized sorghum starch and protected the enzymes of sorghum malt, must be used to extract sorghum malt if equivalent starch extract to that achieved for barley malt is to be realized.

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Mashing of sorghum malt at 65 °C rather than the decantation method resulted in the production of high levels of peptides (Agu and Palmer 1996, 1997). Brewing with sorghum malt would consequently require the development of a suitable mashing regime which would be quite different from that of barley malt (Dufour and Melotte 1992).

Traditional African sorghum beers are very rich in calories, B-complex vitamins and essential amino acids such as lysine. The beers are consumed at African ceremonies (*e.g.*, marriage, birth, baptism, the handing over of a dowry, etc.) and constitute a source of economic return for the beer producers. From microbiological aspect, a very varied yeast and lactic bacteria acid flora has been found in African sorghum beers, although *Saccharomyces cerevisiae* and heterofermentative *Lactobacillus* usually predominate.

25.1.1 Sorghum as Traditional Foods

Food sources from sorghum can be gathered in two classes, customary items and non-conventional modern items. Natural or prepared grain can be cooked entire or decorticated and if vital ground to flour by any of the conventional or modern method. A definite characterization of conventional food sources from sorghum has been created (Vogel and Graham 1979; Rooney et al. 1986). They can be ordered comprehensively into breads, porridges, steamed items and snack foods (Rooney et al. 1986; Rooney and McDonough 1987).

Grain fermentation is one of the most established biotechnological processes, going back to old Egypt, where both brew and bread were created by the assistance of yeasts and lactic acid bacteria. Spontaneous fermentation probably been utilized in the very early days, simply initiating the normally happening organisms in processed grains. In the later past, utilization of sourdough has just been more precise, and microbial cultures have been created and kept up by saving part of the ferment for additional utilization.

The main thought processes in utilization of fermentation in baking were raising, flavor arrangement, and enhanced stability. Steadily, with advancement industrial baking, the pattern of utilizing white wheat flour and cook's yeast turned into the significant practice globally. The craft of sourdough and fermentation is again progressively perceived, and now improvement of explicit cultures and control of fermentation process has become the practice. Their utilization in baking, and impact on bread texture and flavour has been of late surveyed. Simultaneously, the worry and information on the wholesome impacts of grain fermentation have expanded.

25.1.2 Non-alcoholic Malt Beverage

The creation and utilization of non-alcoholic beverages have expected disturbing measurements on the planet and decision of the beverage today. Beverages are the food that are recognized by its important qualities from different foods, first they are fluids that are burned-through in the fluid state and furthermore, they are either devoured for refreshing properties or for their animating impact.

Malt drinks are grouped dependent on alcohol content as alcoholic (over 1.2%), low alcohol (0.5–1.2%) and with no alcohol (less than 0.5%). Non-alcoholic malt beverages are created as non-fermentative or fermentative. In the fermentative kind, alcohol is taken out from the beverage utilizing physical technique (by warming, dialysis and reverse osmosis) or organic techniques (by choosing a low alcohol delivering yeast strain and additionally shut down fermentation, by warming of the wort).

Non-alcoholic malt beverage is essentially an unfermented wort and is a sort of soft drink normal in the Caribbean and Latin America. It is likewise called youthful beer or children's beer and prominently known by its trademark "malta". Customarily, since this was treated as a staple, no subordinates were added. Generally, it was utilized as nourishment for kids and the debilitated and has since become the standard drink. It is a carbonated malt beverage, which means it is blended from grain barley, hops and water much like beer; corn and caramel color likewise be addded. In any case, malta is non-alcoholic, and is devoured similarly as pop or cola in its unique carbonated structure, and somewhat, frosted tea in non-carbonated structure. As such, malta is really a brew that has not been fermented. It is comparable in color to stout however is exceptionally sweet, by and large portrayed as possessing a flavor like molasses. In contrast to lager, ice is regularly added to malta when burned-through. A well known way Latin Americans now and then beverage malta is by blending it in with dense or vanished milk.

The nature of a drink is regularly characterized by a mix of components. These elements are significant for consumer's acknowledgment of an item. One of these components recognizing an item from contenders is the flavor. The primary quality issue of malt-based refreshments is the difference in its compound organization during capacity. Other significant elements are froth dependability for non-fermented brews, caloric worth, colloidal steadiness and wellbeing helpful mixes like nutrients or dietary fiber which might be available in such items.

Lately various novel, creative drinks have been dispatched. Because of developing buyer familiarity with the negative effect of hunger in western nations, novel beverages dependent on regular crude materials have pulled in a developing interest. Particularly malted grains and regular organic product juices are reasonable for the creation of such drinks as they are by and large considered as sure and solid food fixings.

The objective of the present study was to produce a non-alcoholic malt beverage using *Feterita* sorghum malt and evaluation of the product quality.

25.2 Materials and Methods

25.2.1 Preparation of Samples

Seeds of local sorghum (*Sorghum bicolor* (L) Moench) cultivar known as *Feterita* were procured from a retail outlet at Wad-Medani market, Sudan. The grains were carefully cleaned and freed from broken seeds and extraneous matter.

25.2.2 Wort Production

Wort was produced from the sorghum seeds, non-aerated (NA) germinated for 5 day at 30 °C by two different mashing procedures according to Igyor et al. (2001) procedure as described below:

25.2.3 Decantation Mashing at 80 °C (Wort A and B)

Decantation mashing of wart A was accomplished at 80 °C. This mashing procedure is a slight modification of the infusion process. Briefly, 50 g of the grist were mashed in 360 ml distilled water at 45 °C for 30 min. Thereafter, 150 ml of the clear "enzymatic supernatant" were removed while the remaining mash was heated at 80 °C and held at the same temperature for 30 min and cooled below 50 °C at which the clear "enzymatic supernatant" was re-added. The mash was mixed and temperature raised to 65 °C as above. After 1 h at 65 °C, the mash then warmed to 75 °C for 10 min and cooled, and the volume was completed to 515 ml with distilled water were blended well and filtered utilizing filter paper.

The procedure of decantation mashing of wart B 100 °C was similar to that reported for wart A, except that the enzymatic wort was removed after mashing at 45 °C and the residue was boiled at 100 °C instead of being heated at 80 °C. The total volume raise up to515 ml with distilled water stirred well and filtered using filter paper (Fig. 25.1).

25.2.4 Physicochemical Properties of Sorghum Wart

Sorghum wort samples were analyzed to determine several physicochemical properties using standard methods, these were:

Wart α -amino nitrogen was determined by Ninhydrin method. Sample was adequately diluted (1 ml sample in 100 ml distilled water) as recommended. Diluted sample or glycine standard solution, two milliliters, were mixed with one milliliter

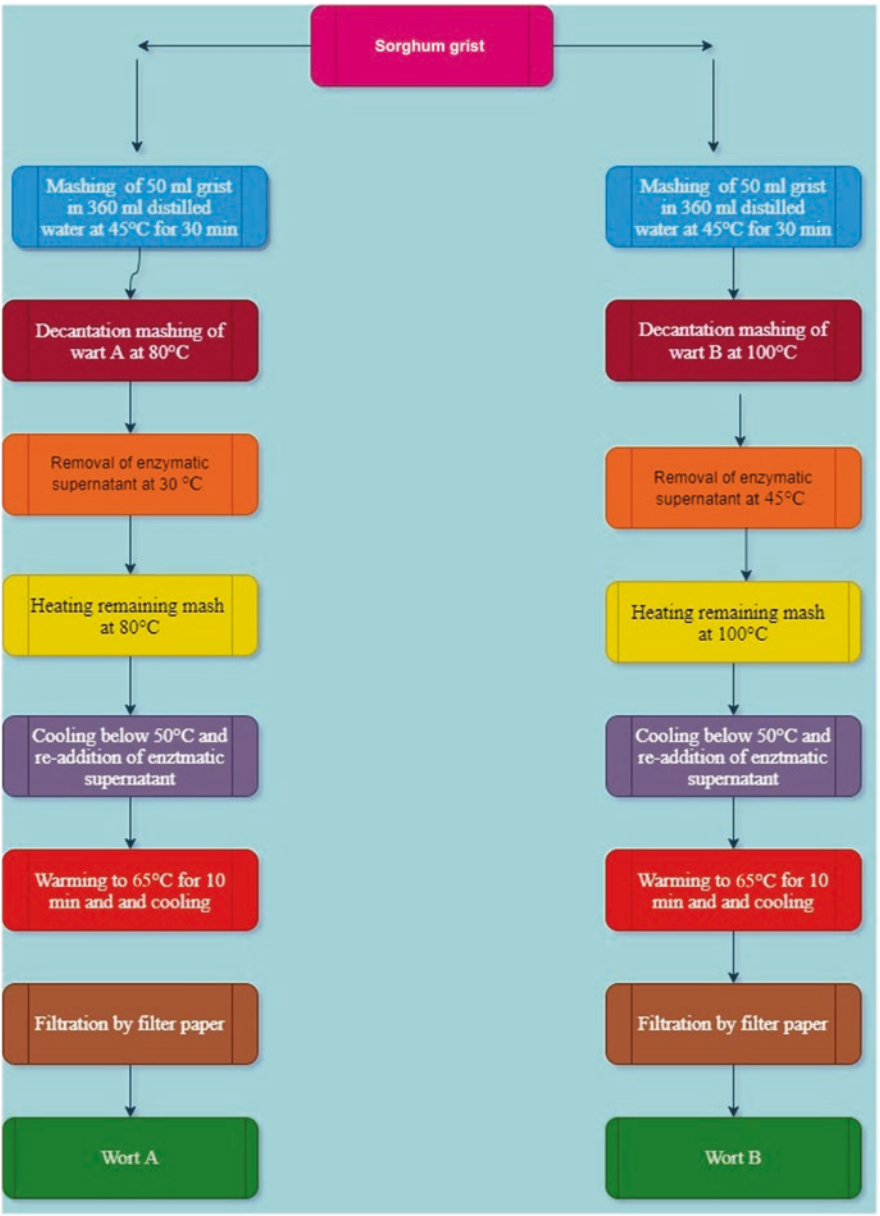

Fig. 25.1 Wart production flow chart

of ninhydrin colour reagent and heated for 16 min in a boiling water bath (Grant W 14, Grant Instrument (Cambridge) Ltd., Barrington, Cambridge). After cooling for 20 min at 20 °C, 5 ml of diluting reagent were added. The absorbance was measured at 570 nm in a spectrophotometer (Spectrumlab 22 pc No. 22pc 08370). Blank solution contained all the reagents except the test solution that was substituted with distilled water. The result was obtained using following formula:

$$\frac{\text{Absorbance of standard}}{\text{Concentration of standard}} = \frac{\text{Absorbance of sample}}{\text{Concentration of sample}}$$

Total soluble nitrogen was determined by the Kjeldhal method. 1.0–1.5 g of malt was digested on a heater block (Tecator Digester System 1007 Digester). The distillation was effected in a Kjeldhal distillation unit (Tecator Kjeltec System 1002 Distillation Unit), and titration of the resulting distillate was carried out using a Metrohm Herisan Multiburette E485 System (Agu and Palmer 1996). Percentage of total soluble nitrogen was calculated using following formula:

$$\frac{\text{Titre} \times 0.0014 \times 6.25 \times \text{dilution factor} \times 100}{\text{Weight of sample}}$$

25.2.5 Sugar Determination

The reducing sugars values were determined using Lane and Eynon constant volume method (Ferdinand 1979).

pH of wort was determined according to AOAC (1980) using a pH meter model (PHSJ-4A) standardized with buffer solution of pH 7. Approximately 25 mL of wort were placed into a 50 ml beaker. The probe was inserted into the liquid and gently stirred until a stable pH was displayed.

Determination of wort colour was carried out using spectrophotometer (Spectrumlab 22 pc No. 22pc 08370) which was set at 430 nm with the visible light on, and zeroed using distilled water as blank. The samples were placed into a 10 mm silica cell, wiped clean and the intensity values recorded. The wort colour were compared to Lovibond scale colour chart (Jean 1957) see page 78.

Original gravity was determined by using an electrical balance. 100 cm^3 of wort were weighed in density bottle and the same volume of distilled water was weighed at 21 °C. Original gravity was expressed mathematically as:

$$\rho_{\text{wort}} / \rho_{\text{water}}$$

Where ρ_{wort} is the density of the wort and ρ_{water} is the density of water (Fellows 2005).Wort viscosity was determined using glass capillary viscometer (U-Viscometer). The driving force in the gravity operated glass capillary viscometer

was the hydrostatic head. Viscosity of wort was expressed in centipoises (cP) and calculated as described by Steffe (1996) as below:

$$\mu_1 = \left(\rho_1 / \rho_2\right) \times \left(t_1 / t_2\right) \times \mu_2$$

where $\mu \equiv$ viscosity

$\rho \equiv$ density
$t \equiv$ flow time through viscometer
subscripts 1 and 2 refer to wort and water respectively

25.3 Results and Discussion

The objectives of mashing are to extract into solution, fermentable sugars, amino acids, vitamins, etc., from malt. Malt normally provides most of the potential fermentable materials and sufficient enzymes to generate a well balanced fermentation medium (François et al. 2012).

Protein hydrolysis measured as α -amino nitrogen achieved suggests that effective enzymatic hydrolysis of the endosperm proteins occurred at the germination temperature. α- Amino nitrogen of wort A and wort B were 114 mg/L and 125 mg/L, respectively (Table 25.1). The results were higher than that achieved by Abdulraheem et al. (2013) who found that α -amino nitrogen of red sorghum malt grain which were sourced at a local market Abuja, Nigeria was 32.3 mg/l. However, α- amino nitrogen of wort A and wort B were within the range of results obtained by Agu and Palmer (1996), who found that α- Amino nitrogen of sorghum wort germinated at 30 °C for 1 day, and mashed at 65 °C were 91 mg/L and 216 mg/L and for wort germinated at 30 °C for 5 days and mashed at 65 °C. Also α- amino nitrogen of wort A and wort B were within the range of the results achieved by Ijasan et al. (2011) who found that less α- amino nitrogen was 96 mg/L when sorghum malt germinated at 30 °C for 5 days using the infusion method and high α- amino nitrogen was 196 mg/L when sorghum malt germinated at 28 °C for 4 days using decantation methods. Odibo et al. (2002) also found that α- Amino nitrogen were 144 and 138 mg/L, respectively for two sorghum varieties studied with a view to producing wort and evaporated wort. Igyor et al. (2001) found in their studies the effect of malting temperature and mashing methods on sorghum wort composition and beer flavour free amino nitrogen ranged between 91 and 177 (mg/L). EtokAkpan (2004) in his study on the changes in sorghum malt during storage reported that α -amino nitrogen levels dropped from 238 to 194 mg/L during the 6 month storage period.

Protein solubilisation measured as total soluble nitrogen also as well as protein hydrolysis measured as α -amino nitrogen achieved suggests that effective enzymic hydrolysis of the endosperm proteins occurred at the germination temperature.

Total soluble nitrogen of wort A and wort B were 0.43% and 0.53%, respectively. Agu and Palmer (1997) found that the total soluble nitrogen of different sorghum

Table 25.1 Important released cultivars of sorghum in Sudan

S. no	Cultivar	Year of release	Area
1	Dwarf white milo	1957	Irrigated and rainfed
2	Wad Akar	1967	Rainfed
3	Tub7(Um Benein7)	1971	Rainfed
4	Tub11(Um Benein11)	1971	Rainfed
5	Tub22(Um Benein22)	1971	Rainfed
6	Dabar/1/1/1/1	1978	Irrigated and rainfed
7	Gadam Alhamam	1978	Rainfed
8	Hageen Dura-1	1983	Irrigated
9	Magawim Buda-1	1991	Rainfed
10	Magawim Buda-2	1991	Rainfed
11	Ingaz	1992	Irrigated and rainfed
12	Sheikan	1992	Irrigated and rainfed
13	Wad Ahmed	1992	Irrigated and rainfed
14	Pioneer877	1992	Irrigated and rainfed
15	Kambal	1994	Irrigated and rainfed
16	Hageen Rabih	1996	Irrigated and rainfed
17	Tabat	1996	Irrigated and rainfed
18	PAN 606	1998	Irrigated and rainfed
19	Aroseelrimal	2000	Rainfed
20	Yarwasha	2003	Rainfed
21	Bashayir	2008	Low rainfall area
22	Butana	2008	Low rainfall area
23	Arfa Gadamak 8	2009	Low rainfall area

varieties (white, yellow and red varieties) germinated for 5 days at 30 °C were 0.43%, 0.57%, 0.45% and 0.71%. Also the total soluble nitrogen of wort A and wort B were more than that obtained by Igyor et al. (2001) who found in their studies on effect of malting temperature and mashing methods on sorghum wort composition and beer flavour, that the total soluble nitrogen was ranged between 0.32% and 0.43%. Odibo et al. (2002) studied two sorghum varieties with a view to producing wort and evaporated wort, found that the total soluble nitrogen were 0.66% and 0.56%. Also the results obtained were less than that achieved by Abdulraheem et al. (2013) who found that total soluble nitrogen of red Sorghum malt grain was 2.12% (Fig. 25.2).

Reducing sugar of wort A and wort B were 39.42 mg/ml and 41.67 mg/ml, respectively. Igyor et al. (2001) examined the impact malting temperature (20 °C and 25 °C) and mashing technique (infusion mash at 65 °C, decantation/mash boiled at 80 °C and decantation/mash boiled at 100 °C) the reducing sugars of sorghum worts went between 186 and 422 µg/l. Owuama and Adeyemo (2009) reported the effect of exogenous enzymes sources, sweet potato (*Ipomea batatas)* and yellow yam *(Discorea cayenesis)* concluded that an increase in the amounts of reducing sugar compared with the untreated malt on the sugar content of wort of a four

Fig. 25.2 Germinated sorghum grains

sorghum varieties. Reducing sugar of untreated malt were 20, 20, 21 and 21 g/l for wort and *Ipomea* were 23, 31, 36 and 43 g/l and for wort and *Discorea* were 128, 97, 119 and 120 g/l. Avicor et al. (2015) recently reported that reducing sugar of sorghum wort at zero time of fermentation was 86 when the quality characteristics of wort pitched with single and mixed Culture yeast strains during 24, 48 and 72 h alcoholic fermentation. In early study Owuama and Asheno (1994) reported that three temperature regimes, 55 °C, 55/65 °C and 65 °C, were used to kiln green malts of three varieties of sorghum, produced from grains steeped for different periods at 30 °C. Maximum reducing sugar values for KSV variety (5.63 mg/ml), FFBL variety (5.89 mg/ml) and CHAKARA variety (5.57 mg/ml) were obtained from malts of grains steeped for 20 h and kilned at 55/65 °C.

The pH which is the level of either acidity or alkalinity of any substance are very important as the body intake of either acid or alkaline are monitor and regulated, to avoid any excess take a disorder. The pH of wort A and wort B were 6.59 and 6.68, respectively. The decantation mashing at 80 °C (wort A) generally gave lower pH than the decantation mashing at 100 °C (wort B). However, there was no clear difference of mashing procedures on pH of wort. The results were near to those reported by Odibo et al. (2002) Who found that the pH of wort produced from two Nigerian sorghum varieties (SK 5912 and Fara fara) were 6.2 and 6.3, respectively. Malomo et al. (2012) found that the effect of commercial enzymes on pH of wort developed from replacement of malted barley (100%) with sorghum as adjunct 50%, 60%, 70%, and 80% were in range of 5.6 and 6.0 with no significant changes at all levels of replacements. Avicor et al. (2015) reported that both the fermentation time and inoculum type affected pH of the fermenting wort. The reduction in pH value obtained by Nkiko et al. (2006) who found that pH of malted and un-malted sorghum wort were 5.62 and 5.73, respectively compared with malted barley and malted barley/sorghum adjunct which were 5.06 and 4.70, respectively. However, fermentation time decrease pH value of wort (Raji et al. 2014) who found that pH

of Pre-fermentative wort of two local Nigerian varieties, red and white were 5.6 and 5.7, respectively.

The average wort colours for sorghum malt mashing at 80 °C and mashing at 100 °C were 9 EBC and 11 EBC and 4–6 on the Lovibond scale, respectively. Odibo et al. (2002) reported that the colour of wort produced from the higher nitrogen sorghum is darker than that obtained from the lower nitrogen sorghum. It is not conclusive at present whether a direct relationship exists between grain nitrogen per free amino nitrogen products and colour development during mashing. There is however, evidence to show that when the soluble nitrogen and/or sugars present in the extract are high, the colour of the extract might be high. Etokakpan (2004) reported that freshly kilned sorghum malt displayed high wort turbidity (4.9 EBC) which dropped to 0.95 EBC and 1 EBC after 2 and 6 months of storage, respectively.

Nandwa et al. (2013) in their study on Malted sorghum as a possible alternative to barley in beer industry found that the average wort colour for sorghum malt was 15.62 ± 0.02 EBC and 6–8 on the Lovibond scale. The value was slightly higher compared to that of barley malt at 13–14 EBC (average value). Wort colour contributes directly to the clarity of final beer. Clarity affects certain final beer characteristics such as beer texture, turbidity and final beer colour.

Information on the density of foods is significant in separation processes and contrasts in density can have important effects on the activity of size decrease and blending equipment. Density of liquids is a measure of mass per volume at a specific temperature can be expressed as specific gravity which is found by dividing the density of a liquid by the density of an equal volume of pure water at the same temperature. Specific gravity is widely used instead of density in brewing and other alcoholic fermentation where the term Original gravity is used to indicate the specific gravity of the liquor before fermentation (Fellows 2005).

Original gravity of wort A and wort B were 1.026 and 1.025, respectively. These outcomes not exactly that accomplished by Aniche and Anih (1994) who announced that specific gravity of wort samples from two malted sorghum assortments (SK5912 and KSW3) and barley were equivalent (1.04). Likewise the outcomes were not exactly that of Odibo et al. (2002) who announced that original gravity of mash of two sorghum cultivars were 1.042 and 1.045, respectively. Avicor et al. (2015) recently reported that original gravity of sorghum wort at zero time of fermentation was 1.0416. Nkiko et al. (2006) found that original gravity of wort made with unmalted sorghum, malted sorghum, malted barley and sorghum/barley malt adjunct were 1.004394, 1.04406, 1.04415 and 1.04412, respectively.

Viscosity plays an important role in theory of filtration and it is taken into account when designing the filters and setting the working pressures. Lowe et al. (2005) reported that a high viscosity makes beer filtration more difficult and may lead to starch hazes in the final beer. Severa et al. (2009) also wrote that viscosity is monitored in several different stages of beer production. Beer has an almost ideally viscous behaviour and is therefore a Newtonian liquid (Steffe 1996). This makes it possible to determine the malt, beer, and filtered wort viscosity using relatively simple measuring principles.

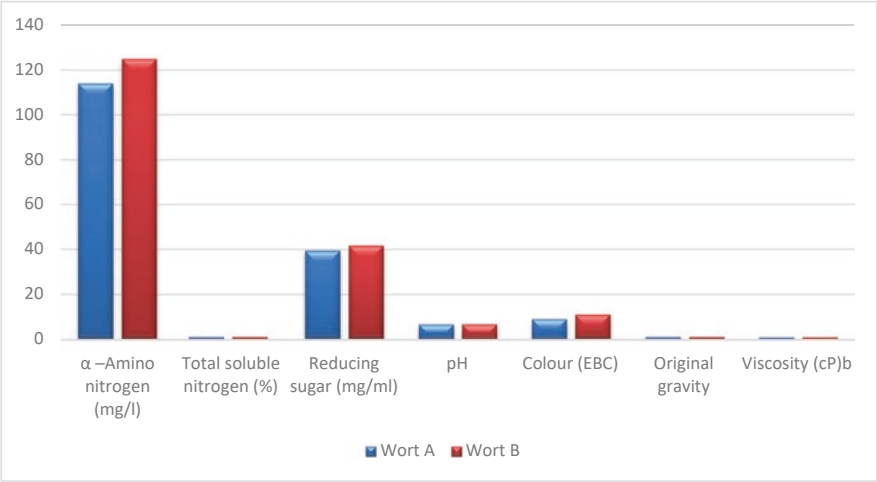

Fig. 25.3 Physicochemical properties of sorghum wart A and B

The viscosity of wort A and wort B were 0.846 cP and 0.864 cP, respectively. The results were less than that of Severa et al. (2009) who reported that dynamic viscosity of wort gradually increased from 1.75 to 2.1 mPas during lager beer base processing which separated in 11 different stages, first stage at 5 min after mashing 52 °C and last stage at the end of wort boiling. Malomo et al. (2012) found that the effect of enzymes on the quality of beer/wort developed from proportions of sorghum adjuncts on viscosity ranged from 1.33 to 1.46. Igyor et al. (2001) in early study on the effect of temperature of malting and mashing methods on sorghum wort composition and beer flavour the less viscosity was 1.30 cP and high viscosity was 1.54 cP. Therefore, they observed from the results that the different mashing procedures did not change the viscosity of either sorghum malt wort produced from either the infusion or decantation mashing at 80 °C. In contrast, a significant increase in wort viscosity of sorghum malt was observed when the decantation mashing was done at 100 °C for unknown reasons. Although the wort β-glucan was not investigated, it is likely that other materials that caused an increase in wort viscosity were extracted at 100 °C. This required further investigations (Fig. 25.3).

25.4 Conclusion

The study confirmed that optimal wort properties were achieved at 30 °C rather than at 25 °C. Decantation mashing at 100 °C produced much better results in terms of malt and wort properties than at 80 °C because boiling the mash at 100 °C adequately gelatinized sorghum malt starch, since sorghum starch has a gelatinization temperature of 80 °C. Further research is needed to- Introduce exogenous enzymes for mashing sorghum malt to yield more sugars in wort.

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Chapter 26
Production and Quality Assessment of Kissra, a Sudanese Fermented Sorghum Product

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26.1 Introduction

Sorghum bicolor (L.) Moench is a significant grain crop, especially on the planet's semi-parched jungles. It is a significant food crop in sub-Saharan Africa and South Asia and is the staple nourishment for the most food-uncertain individuals on the planet (Bibi et al. 2010). It is the world's fifth most significant oat, after wheat, rice, maize and grain (FAO 2010). In excess of 105 nations in Africa, Asia, Oceania and the Americas develop sorghum on 40 million hectares (Kumar et al. 2011), and 60% of this land is in Africa, where it keeps on playing a significant food security role (Assefa et al. 2020).

Sorghum bicolor is considered one of the important food and fodder crops for its use in feeding the livestock sector as grains with concentrates. It is also used as green fodder and in the manufacture of silage, as it is considered as a raw material for extracting starch, cellulose and making alcohol, in addition to the fact that some species have a high percentage of sugar (wcsp.science.kew.org 2020).

Sorghum bicolor exists in dry weather, and is greatly affected by frost, especially during flowering, so it is grown as a summer crop after the end of the frost period. For the success of germination and plant growth, temperatures should not drop below 8–10 ° C, as the appropriate temperature for its success is 30–32 ° C. It also needs rain rates of at least 250 mm / year and may be cultivated under irrigation. It is well cultivated in all types of soil and has the ability to withstand salinity and alkalinity, but fertile muddy lands are preferred at rates of 350 mm / year rain (data.nbn.org.uk. 2020).

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Fig. 26.1 Sorghum bicolour (L.) Monech plant. (Source: https://commons.wikimedia.org/)

The sorghum crop is considered one of the most important crops in Sudan and ranks first in terms of importance as food for the vast majority of Sudanese in terms of cultivated area and total productivity. Corn is grown in Sudan in the irrigated and rainy sectors, and corn is especially important for farms in irrigated projects in terms of securing The food for him, the labor he uses, and his animals. Due to the fluctuation of rains under rainfed conditions, irrigated corn has an important role in securing food for the country in general. Sudan grows about 24% of the corn area in Africa and produces 17% of the production. In addition, it contributes 70–85 to the volume of grain production in Sudan (Fig. 26.1).

26.2 Sorghum Production in Sudan

The sorghum crop is considered one of the most important crops in Sudan and ranks first in terms of importance as food for the vast majority of Sudanese in terms of cultivated area and total productivity. Sorghum is grown in Sudan in the irrigated and rainy sectors, and it is particularly important for farmer in irrigated projects in terms of securing the food for him, the labor he uses, and his animals. Due to the fluctuation of rains under rainfed conditions, irrigated sorghum has an important role in securing food for the country in general. Sudan grows about 24% of the corn area in Africa and produces 17% of the production. In addition, it contributes 70–85 to the volume of grain production in Sudan (http://www.tpsudan.gov.sd/index.php/ar/home/show_export/49).

Fig. 26.2 Sorghum bicolor grains. (Source: https://commons.wikimedia.org/)

Despite the importance of the corn crop and the existence of the basic ingredients for its production in irrigated projects, especially irrigation water, and the suitability of the climatic conditions for production, as Sudan is considered one of the habitats of corn. However, its productivity remained low and not commensurate with what was achieved in many countries that consider corn an alien crop, and the reason for the low productivity is the lack of or incomplete use of the technical packages recommended by the Agricultural Research Authority (http://www.ttea.gov.sd/sorghum.html) (Fig. 26.2).

The cultivation of sorghum is good in all types of light and heavy soils, as well as tolerates salinity and alkalinity in relatively high degrees, and tolerates intense heat and intense thirst, as it continues dormant without any vital activity until we drop rain, so it continues to grow. Therefore, it is found that it is grown in all production areas in the country. In addition, its production is concentrated in all production areas in the country. It is production in the rain sector, both automatic and traditional, at about 78%, and the irrigated sector, about 22% of the total production, in an area that exceeds 40% of the total cultivated areas in Sudan. The country produced, on average, for the period from (97–2000) about 2.5 million tons of corn annually.

The most important uses of sorghum are thus summarized:

1- Mixing white corn flour with wheat flour to make bread.

2- It is used in poultry feeding and mixed with other feeds to feed dairy cattle. This is because of the closeness of the nutritional composition of this crop with the nutritional composition of yellow corn.
3- Its green plants are used as fodder for livestock, provided that the plants are not less than 55 days old, given the toxicity of the leaves, and they are small because they contain toxic durin glucoside, provided that the leaves are dried for twelve hours before use.
4- The plant residues are used after harvesting the crop to feed work animals and livestock.
5- Sorghum is one of the most important agricultural products used in the production of glucose and starch sugar.

26.2.1 Chemical Composition and Nutritional Value of Sorghum

Sorghum plays an important role in the economy of Sudan, and it represents the main food for 65% of the population of the Sudan, particularly in rural areas in central and eastern Sudan, as sorghum is an important source of carbohydrates needed for human and animal food. It is used as fodder, fuel and as a building material in housing. Moreover, sorghum is used in the manufacture of starch and glucose (http://www.ttea.gov.sd/sorghum.html.)

Sorghum is considered as food with low nutritional value (Raihanatu et al. 2011). Further, sorghum contains anti-nutritional factors like tannin, cyanogenicglucoside, phytic acid, trypsin inhibitor, and oxalate (Etuk et al. 2012; Mohammed et al. 2011). Various researches have revealed that the processing condition decreased antinutritional factors and increased the bioavailability of other nutrient in cereals and legumes (Adegunwa et al. 2012; Mubarak 2005; Osman 2007; Yasmin et al. 2008; Ogbonna et al. 2012).

The chemical composition and nutritional value of whole sorghum are similar to rice, corn, and wheat. The energy value of 100 g of sorghum grains varies between 296.1 and 356.0 kcal (Martino et al. 2012; U.S. Department of Agriculture 2012). The main components of sorghum are the polysaccharides (starch and non-starch), followed by proteins and lipids (Martino et al. 2012; U.S. Department of Agriculture 2012).

The content and composition of starch, the main polysaccharide of sorghum, are influenced by the genetic characteristics and growing conditions of the grain (Hill et al. 2012). In some varieties, starch ranges between 32.1 and 72.5 g/100 g and is composed mainly of amylopectin (81.0–96.5%) and amylase (3.5–19.0%) (Shegro et al. 2012; Udachan et al. 2012).

Sorghum has the lowest starch digestibility among cerealsbecause of the solid relationship between the starch granules and proteins and tannins (Barros et al. 2012; Rooney and Pflugfelder 1986). Generally the majority of the starch granules

are gradually edible (30.0–66.2%) and the rest of quickly absorbable (15.3–26.6%) or safe (16.7–43.2%) (Sang et al. 2008; Mkandawire et al. 2013). The non-starch polysaccharides of sorghum (6.0 to 15.0 g/100 g) include insoluble fibers (75.0–90.0%), mainly arabinoxylans, and soluble fibers (10.0–25.0%) (Taylor and Emmambux 2010; Martino et al. 2012; U.S. Department of Agriculture 2012).

Sorghum proteins are classified as prolamins and not prolamins. Prolamins correspond on average to 79% (77–82%) of the total proteins (7 to 15 g/100 g) and the remainder is albumins, globulins, and glutelins (Belton et al. 2006; Martino et al. 2012; U.S. Department of Agriculture 2012; Afify et al. 2012b). The kafirins are the major prolamins of the sorghum and comprise three major classes: a-kafirins (66–84%), b kafirins (8–13%) and g-kafirins (9–21%) (Belton et al. 2006; Mokrane et al. 2010). Sorghum kafirins are stored in the endoplasmic reticulum in spherical protein bodies. The b and g-kafirins are located in the peripheral protein bodies region while a and d-kafirins are encapsulated in the inner region (Wu et al. 2013). This conformation determines the digestibility of sorghum proteins. Overall, the digestibility of sorghum proteins, especially after cooked, is lower than cereals like wheat and maize (Duodu et al. 2003; Mokrane et al. 2010; Afify et al. 2012b; Moraes et al. 2012b). Despite the reduction in protein digestibility of sorghum after cooking in wet heat, processing such as fermentation and germination may increase the digestibility up to 2 times (Correia et al. 2008; Wedad et al. 2008; ELKhier and Abd- ALRaheem 2011; Pranoto et al.; Afify et al. 2012b).

Sorghum has a reduced lipid content (1.24 to 3.07 g/100 g), which is mainly composed of unsaturated fatty acids (83–88%) (Afify et al. 2012a; Martino et al. 2012; U.S. Department of Agriculture 2012). In most of the varieties of sorghum the polyunsaturated fatty acids (PUFA) are higher than monounsaturated fatty acids (MUFA) (Mehmood et al. 2008; Hadbaoui et al. 2010; Afify et al. 2012a). The major fatty acids of sorghum are linoleic (45.6–51.1%), oleic (32.2–42.0%), palmitic(12.4–16.0%), and linolenic acids (1.4–2.8%) (Mehmood et al. 2008; Hadbaoui et al. 2010; Afify et al. 2012a).

Sorghum is a source of minerals (phosphorus, potassium, and zinc) whose content varies according to the place of cultivation (Martino et al. 2012; Shegro et al. 2012; Silva et al. 2012; U.S. Department of Agriculture 2012).

Information on the content of vitamins in sorghum is scarce. However, it is worth noting that it is a source of some B-complex vitamins (thiamine, riboflavin, and pyridoxine) and fatsoluble vitamins (D, E, and K) (Ochanda et al. 2010; Martino et al. 2012; U.S. Department of Agriculture 2012; Cardoso et al. 2014).

26.2.2 Bioactive Compounds of Sorghum

The phenolic compounds are the main bioactive compounds of sorghum and are present in all varieties of this cereal (Dykes and Rooney 2006). Almost all classes of phenolics are found in sorghum (Awika and Rooney 2004; Dykes et al. 2005);

however, the classes of phenolic acids, tannins, and flavonoids are major. The profile and content of phenolic compounds in sorghum are more diverse and higher than those observed in wheat, barley, rice, maize, rye, and oats (Ragaee et al. 2006). Sorghum varieties resistant to biotic and abiotic stresses were found to have on average higher contents of proanthocyanidins, 3-deoxyanthocyanidins, and flavan-4-ols than susceptible varieties (Dicko et al. 2005).

The content of phenolic acids in some sorghum varieties ranged between 135.5 and 479.40 mg/g (Afify et al. 2012c; Chiremba et al. 2012).

Tannins, secondary metabolites found in many plant species, are phenolic compounds that often act as a defense mechanism against pathogens and predators (Kaufman et al. 2013). Overall, these compounds are absent in other major cereals, such as rice, wheat, and maize, but are present in sorghum varieties that have pigmented testa (Awika 2003; Dykes and Rooney 2006; Wu et al. 2012). The presence and content of condensedtannins in sorghum are controlled by the genes S and Tannin1, among others (Hahn and Rooney 1986; Wu et al. 2012).

26.2.3 Traditional Sorghum Use for Food

Sorghum is used in a variety of foods. The white food sorghums are processed into flour and other products, including expanded snacks, cookies and ethnic foods, and are gaining popularity in areas like Japan (United States Grains Council 2001; Rooney 2001).

Traditionally, Africa has employed sorghum in both the malted and unmalted form in wide varieties of porridge and beverages, often using lactic and alcoholic fermentation to enhance their appeal. In the Sudan, sorghum is the staple food of the vast majority of the population and is produced mainly in the central clay plains of the Sudan under rain, with limited amount being produced in the irrigated schemes of Gezira, Rahad and New Half.

26.2.3.1 Sudanese Fermented Sorghum Based Foods

In general, two sorts of fermentation are practices: by addition of starter to the dough or by the natural flora activity of microflora. Sorghum fermentation is principally a lactic acid one (Abdel Gadir and Mohamed 1983; El Mahdi 1985; Mohammed et al. 1991). The raw material and its initial treatment will encourage the growth of an indigenous microbiota (Tamang and Fleet 2009).

It is well established that fermentation enhances the nutritional quality of foods and contributes to food safety particularly under conditions where refrigeration or other foods processing facilities are not available (Motarjemi 2002) such as in arid and semi-arid rural areas in Sudan. Fermentation also develops a new flavor and appearance in 21 food products, and is also utilized as a technique of preservation (Onwurafor et al. 2014).

26.3 Fermented Foods

Fermentation is one of the oldest biotechnology approaches of food processing and preservation that extensively applied in both developed and developing countries. Over thousands of years, the demands of producing and consuming fermented foods has extremely increased, so these products constituted a significant portion of the daily food globally (Elyas et al. 2015).

Fermentation enhances the nutritional quality of foods through the biosynthesis of vitamins, essential amino acids and proteins, improving protein and fiber digestibility, enhancing micronutrient bioavailability, and degrading anti-nutritional factors (Giraffa 2004). It also contributes to food safety and sustainability particularly under conditions where refrigeration or other foods processing facilities are not available such as in arid and semi-arid rural areas in Sudan (Elyas et al. 2015).

Food fermentation covers a wide scope of microbial and enzymatic processing of food and fixings to accomplish desirablecharacteristics such as prolonged shelf-life, improved safety, attractive flavour, nutritional enrichment, and health promotion (Giraffa 2004; Holzapfel 2002). Throughout the fermentation processes, microorganisms played a key role in the production of specific metabolites such as acids, alcohols, enzymes, antibiotics, carbohydrates, which contribute to the safety and nutritional quality of fermented foods. One of these metabolites, is lactic acid bacteria (LAB), play a significant role in the majority of food fermentations and preservation, and a extensive variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable, and bakery products (Elyas et al. 2015; Giraffa 2004; Saeed et al. 2014). This is because of the fact that lactic acid bacteria has numerous essential technological properties, for example, acid production in various media and at different temperatures, proteinase and peptidase activities, autolysis, production of volatile compounds, resistance to bacteriophages and production of inhibitory compounds (Piraino et al. 2008). These properties are significant for the utilization of LAB as starters or assistants to keep up and improve the nourishing, tangible, and security characteristics of end results and their evaluation in the screening of proper starter culture from natural environments has been and still on the rise in recent years. However, these fermented foods are still mainly prepared at the household level under poor sanitary conditions and advertised through casual courses (Elyas et al. 2015; Saeed et al. 2014).In like manner, various polluting microorganism and additionally native microflora engaged with this fermentation processes could be anticipated. In addition, there is a lack of data on the technological properties of microorganisms involved and their metabolic impact on flavour, hygienic safety and shelf life of these products.

26.3.1 Fermented Cereal Foods

The well-documented fermented cereal foods of the world are sourdough of Europe, America, and Australia, *selroti* of India and Nepal (Yonzan and Tamang 2009), *idli* of India and Sri Lanka (Sridevi et al. 2010), *dosa* of India and Sri Lanka (Soni et al. 1986), *mawè* and *gowé* of Benin (Vieira-Dalodé et al. 2007), *ben-saalga* of Burkino Faso and Ghana (Humblot and Guyot 2009), *kissra* of Sudan (Hamad et al. 1997), *kenkey* of Ghana (Oguntoyinbo et al. 2011), *togwa* of Tanzania (Mugula et al. 2003), *ting* of Botswana (Sekwati-Monang and Gänzle 2011), *ogi* and *kunu-zaki* of Nigeria (Oguntoyinbo et al. 2011), and *tarhana* of Turkey, Cyprus and Greece (Sengun et al. 2009). Cereal fermentation is portrayed by a complex microbial environment, chiefly addressed by the types of LAB and yeasts (Corsetti and Settanni 2007), whose fermentation confers to the resulting bread its characteristic features such as palatability and high sensory quality (Blandino et al. 2003). The species of *Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus*, and *Weissella* are commonly associated with cereal fermentation (Guyot 2010). A native strain of *Sacch. cerevisiae* is the principal yeast of most bread fermentations (Hammes et al. 2005). Other non-*Saccharomyces* yeasts are involved in numerous cereal fermentations which include *Candida, Debaryomyces, Hansenula, Pichia, Trichosporon, Yarrowia* (Foschino et al. 2004, Vernocchi et al. 2006). Yeasts produce carbon dioxide and ethanol. Interactions between yeasts and lactobacilli are important for the metabolic activity of the sourdough. The changing conditions during fermentationcontribute to the activation of enzymes present, and adjustment ofpH selectively enhances performance of certain enzymes, such asamylases, proteases, hemicellulases and phytases. The enzymeinducedchanges, together with microbial metabolites, bring aboutthe technological and nutritional effects of fermented cereal foods.Sourdough fermentation can influence the nutritional quality bydecreasing or increasing levels of compounds, and enhancing orretarding the bioavailability of nutrients.

26.4 Kissra

Kissra can be defined in a number of ways (Abdualrahman and Ali 2012):

1- Kissra is the staple Sudanese diet. It is a morsel or piece of bread prepared from fermented sorghum flour (Sulieman et al. 2003).
2- Kisra is a naturally lactic acid bacteria- and yeast-fermented sorghum pancake-like flatbread(AwadElkareem and Taylor 2011).
3- Kisra is an indigenous staple food of the majority of Sudanese people. It is pancake-like bread made from sorghum or millet flour.

The kissra is called ‘al-Rahafa’ and the way it was prepared has not changed for centuries, but its presence on the table in Sudan has declined due to the availability

Fig. 26.3 Pictures of kissra bread

of bread in various forms, after it was scarce. There are many types of corn used in the making of the Sudanese kissra, including (fretrita - yellow hybrid, dr Aker, al-Fahl, and al-Zrizira) (Fig. 26.3).

Today, with growing urbanization, kisra is becoming a commercial home-based industry in Sudan. Internationally, because of the apparent increase in the incidence of celiac disease and intolerance to wheat, interest in gluten-free cereal products is increasing rapidly (Kelly et al. 2008).

Kissra seems to have significant potential as the basis for development of a gluten-free sandwich wrap. As of late, it has been shown that Lactobacillus and Saccharomyces cultures can be used to reduce the fermentation time from 19 to 4 hr. (Ali and Mustafa 2009), which would be useful for commercial production.

26.4.1 Kissra Preparation

Kissra fermentation is a conventional process, whereby sorghum or millet flour is blendid with water in a proportion of about 1:2 (w/v), generally a starter is added by a back-slopping utilizing mother batter from a past fermentation as a starter at a degree of about 10%. Fermentation is finished in around 12–19 hours by which time the pH drops from around six to less than four. Because of the tedious process of kissra perparation, the greater part of the populace deserted kissra utilization and moved to bread (Ali and Mustafa 2009).

The process begins with fermenting the flour at night in a closed container, and in the morning the rest of the flour is added to it, kneaded and reduced until it becomes coherent and ready to make the kissra. The process of making kissra is called "*Awassa*," and the woman or girl who is preparing it is called "*Awassa*". *Awassa*" process starts by pouring of a small amount of dough on the saj (hot plate),

which is placed on a slow fire of charcoal on a canon (wood stove). In the past, the "douka" made of pottery in a flat form was used. The *Awassa* saj is often wiped with oil or *taouk* (animal marrow), which is the material of the spinal cord. This is because it helps to pull out the fracture quickly so that it does not stick to the saj and burn.

After that, the kissra is placed on the plate one by one, and the plate is an airtight, flat round container that can be stored in it for several days.

Nowadays, with developing urbanization, kissra is turning into a business locally situated industry in Sudan. Globally, due to the clear expansion in the frequency of celiac infection and prejudice to wheat, interest in gluten-free cereal items is expanding quickly (Kelly et al. 2008) (Fig. 26.4).

26.4.2 Nutritive Value of Kissra

Abubaker et al., (2019) investigated the chemical compositionof Kissra. They found that contents of moisture, ash, protein, fats, fibres and carbohydrates of kissra ranged between 5.17–5.02%, 1.32–1.29%, 10.01–9.95%, 1.66–1.89%and 79.09–79.26%, respectively. They Furthermore, they found that kisra fermentation improved levels of the protein, fiber, fat, ash, and minerals contents. Moreover, maximum amino acids contents were found in Kisra prepared from Tabat sorghum flour compared with that prepared with Wad-Ahmed sorghum flour.Kissra fermentation also resulted in increasing ascorbic acid content, in vitro protein (IVPD) and in vitro starch digestibilities (IVSD) of Kissra from both sorghum cultivars, with a concomitant decrease in phytate and tannin contents (Figs. 26.5 and 26.6).

Kawthar et al. (2018) analyzed six samples of kissra from Khartoum Bahry markets. Three were made from pure mixed cluture of lactic acid bacteria (LAB) and yeast (*Saccharomyces cerevisiae*). The proximate composition of kissra results showedsignificant ($P < 0.05$) difference in moisture content of most of the various kissra samples.The control showed the highest level of moisture content (8.76%) which was higherthan the values reported for the recommended dietary allowance (RDA) value(<5%) for older infants and young children (FAO/WHO 1991). The lowermoisture content is an indication of the better quality of the products with thelonger shelf life. On the other hand, Mohammed et al. (2017) reported a value of 3.13% for moisture content of kissra.

According to Kawthar et al. (2018), ash content ranged between (1.710–1.333%) which than the values reported by Mohammed et al. (2017) which was (2.86%), butagreed with the recommended dietary allowance (RDA) value (< 3%) for older-infants and young children (FAO/WHO 1991).

Higher protein contents of kissra was reported by both Kawthar et al. (2018) and Mohammed et al. 2017) who reported an average of 10.90–13.37%.

Elkhalifa et al. (2005) announced that customary Sudanese technique for fermentation prompts an expansion in the protein solubility of sorghum flour in the acidic reach (pH 2–4). Additionally they detailed that fermented sorghum flour had

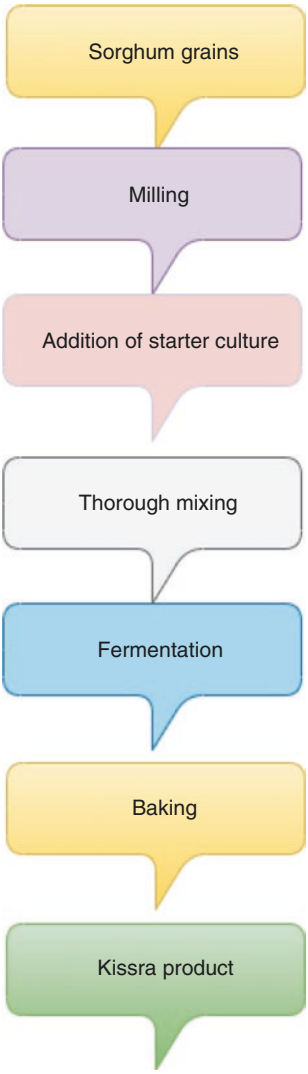

Fig. 26.4 Kissra preparation flow chart

a least gelation concentration of 6% after 16 h of maturation, while it was 18% for unfermented sorghum.

Fermentation likewise expanded oil binding capacity, emulsifying capacity and emulsifying strength, while it diminished the water-binding capacity. Sorghum flour, fermented or unfermented showed no frothing capacity (Elkhalifa et al. 2005). According to study by Kawthar et al. (2018) All types of kissra showed significant difference in fibre content, the kissra which have the highest levels of (1.267 and 1.287%), and the lowest level (0.617%). The fibre content of all kissra was less than the result obtained by Mohammed et al. (2017) which was (2.41%) but agreed with the recommended dietary allowance (RDA) value (< 5%) for older infants and

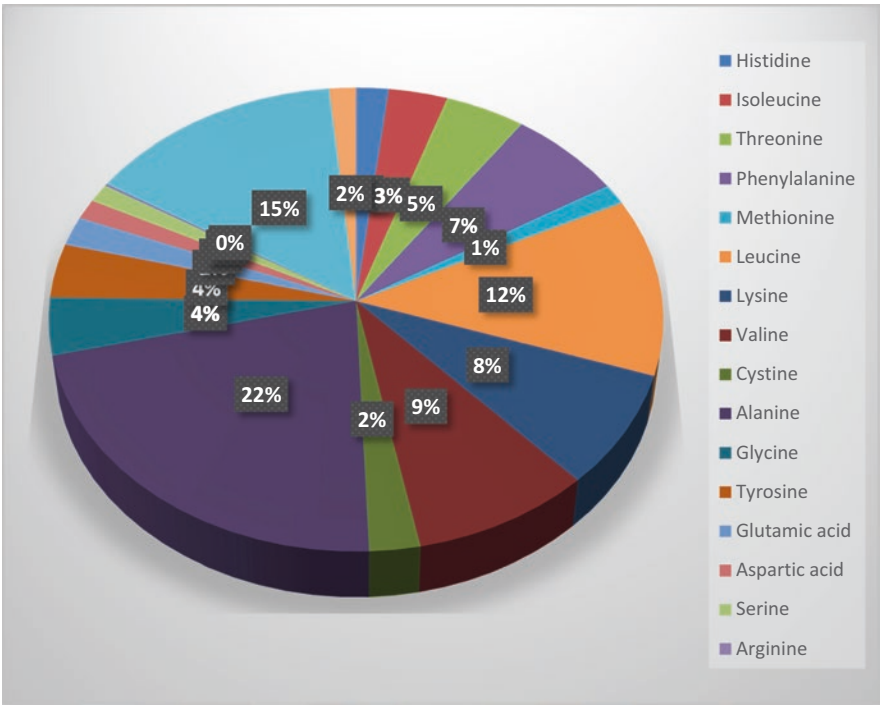

Fig. 26.5 Amino acid contents (mg/100 g) of kissra bread

young children (FAO/WHO 1991). The reduction in fiber content of kissra would enable the children to utilized sufficient amounts, giving an opportunity to meet their daily energy and other vital nutrient requirements.

Fat and carbohydrates content the kissra ranged 1.133% - 5.52% and 73. 63–78.34%, respectively (Kawther et al. 2018; Mohammed et al. 2017). However, these values were higher than that reported (FAO/WHO 1991).

Contents of thiamin (vitamin B1) and riboflavin (vitamin B2) in kissra prepared from two sorghum cultivars, *dabar* and *feterita* were investigated by Salah et al., (1998). They found that contents of both vitamins improved as a result of fermentation process. The riboflavin contents of the two sorghum cultivars were almost the same (1.08 mg/g for *dabar* and 1.07 mg/g for *fetarita*). The difference in the thiamine content of the two cultivars (3.92 mg/g for dabar and 3.47 mg/g for fetarita) was insignificant ($p < 0.01$). On the other hand, germination of fetarita grains for 6 days caused significant effects on the levels of thiamine and riboflavin which increased by 70.6% and 42%, respectively (Fig. 26.7).

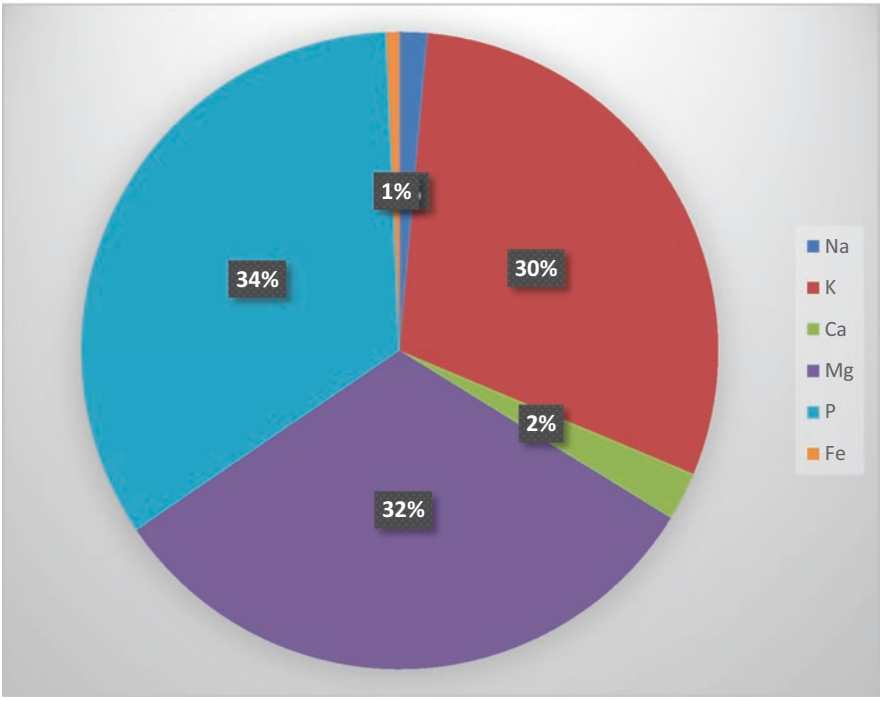

Fig. 26.6 Mineralscontents (mg/100 g) of kissra bread

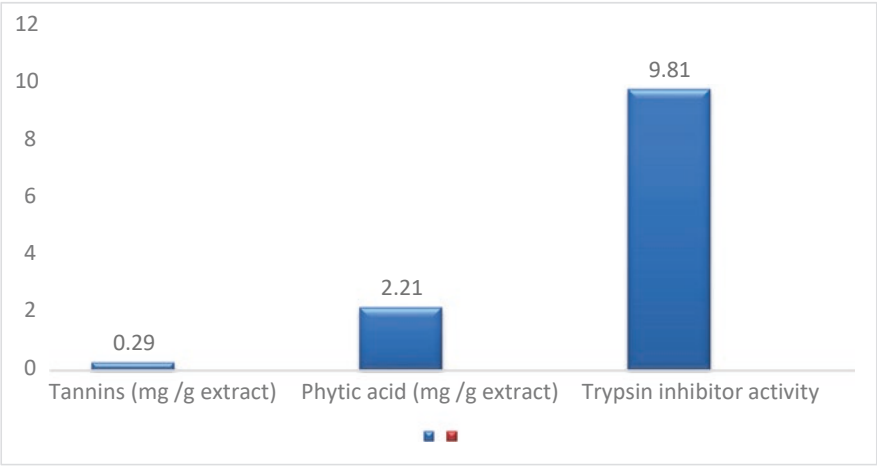

Fig. 26.7 Thiamin and riboflavin of germinated *dabar* and *feterita* sorghum grains

26.4.3 Fermentation Effects on Antinutritional Components

Sorghum is considered as food with low nutritional value (Raihanatu et al. 2011). Poor digestibility of sorghum and limited product diversification compared to other cereals limit the use of sorghum (Mella 2011). Further, sorghum contains antinutritional factors like tannin, cyanogenicglucoside, phytic acid, trypsin inhibitor, and oxalate (Etuk et al. 2012; Mohammed et al. 2011). Due to these and other reasons, sorghum is categorized as of low nutritional value and a food for the poor. Low protein digestibility and mineral absorption are also associated with the presence of antinutritional factors (Mohammed et al. 2011). Different investigates have uncovered that the handling condition reduced antinutritional factors and expanded the bioavailability of other supplement in cereals and vegetables (Adegunwa et al. 2012; Mubarak 2005; Osman 2007; Yasmin et al. 2008; Ogbonna et al. 2012).

According to the study conducted by Pravin et al. 2017 to observe the effect of malting and fermentation on antinutritional component and functional characteristics of sorghum flour, they found that, the lower yield of sorghum flour was obtained compared to whole and malted sorghum flour. Germination of sorghum decreased phytate, tannin, and oxalate by 40%, 16.12% and 49.1%, respectively, whereas fermentation of sorghum flour reduced these compounds by 77%, 96.7% and 67.85%, respectively. Furthermore, in their study on nutritional value, protein quality and antioxidant activity of Sudanese sorghum-based kissra bread fortified with bambara groundnut, Mohamed et al. (2019) indicated that tannins, phytic acid and trypsin inhibitor activity were significantly reduced ($P < 0.05$) (Fig. 26.8).

26.4.4 Microbiology of Kissra Bread

According to many investigations on sorghum flour lactic acid bacteria (LAB) and yeasts dominated fermentation. Mohammed et al., (1991) demonstrated that bacterial burden expanded with fermentation time. Besides the microbial populace during the 24 h of fermentation comprised of bacteria (Pediococcus pentosaceus, Lactobacillus confusus, Lactobacillus brevis, Lactobacillus sp., Erwinia ananas, Klebsiella pneumoniae, and Enterobacter cloacae), yeasts (Candida intermedia and Debaryomyces hansenii), and molds (Aspergillus sp., Penicillium sp., Fusarium sp., and Rhizopus sp.). P. pentosaceus was the dominant microorganism toward the end of the 24-h fermentation.

. Lactic acid bacteria were the dominant microflora and their number increased during fermentation of three sorghum varieties, in the fermentation of the three varieties and at each interval the number of **lactic acid** bacteria was greater than the number of yeasts and molds and they dominated till the end of fermentation. This fact implied that Kisra fermentation is mainly a lactic acid fermentation (Abdel Rahman et al. 2010).

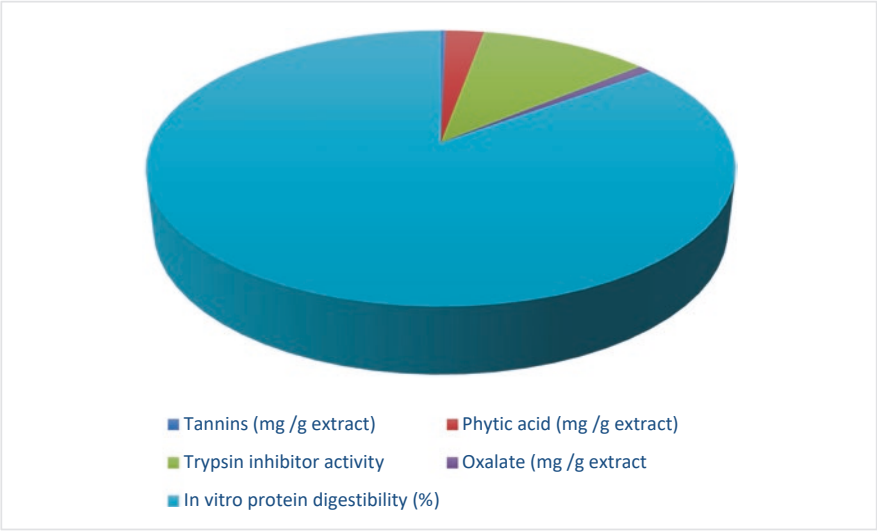

Fig. 26.8 Anti-nutrients content and in vitro protein digestibility of kissra bread

Yagoub et al., (2009) indicated significant drop in pH during fermentation of sorghum dough. Microbial examination of sorghum varieties before fermentation showed that the total bacterial counts and the counts of yeast and molds expanded altogether after fermentation time (24 hours), while, there was a reduction in staphylococcal counts. The microbiological examination additionally uncovered that E. coli counts exceeded 2.400 cfu/g in the raw sorghum flour but the counts were very low in the fermented dough. Salmonella was identified in the three sorghum varieties vanished in the fermented dough after 24 h fermentation.

In their study on the investigation on the microbiological qualities, germinated sorghum (malt) and fermented dough at different timeframes, Sulieman et al., (2015) indicated that three samples out of ten were devoid of any lactic acid bacteria (LAB)development, while the other seven samples indicated development of bacteria, thus, a total of 140 isolates of these bacteria were examined to identify their bacterial genera. Their outcomes demonstrated that a portion of the isolates were cocci while others were rods in shape, not many of the isolates were hetrofermentative, and most of them were homofermentative, all isolates were grow at 15 °C and some growth at 10 °C and 45 °C. And the LAB isolated bacteria recognized in this examination, have a place with five genera; *Streptocpccus, Lactobacillus, Leuconostoc, Enrococcus* and *pediococcus*with various rates .

The concurrence among LAB and yeasts affirmed the synergistic connection between the organisms in a fermenting food matrix (Wood 2004). Yeast is significant for or acceptable batter and raising, while LAB produce acids and other metabolites which hinder the development of spoilage organisms. Lactic acid bacteria (LAB) strains are able to improve the time span of usability of few food items (Lopez et al. 2001; Di Cagno et al. 2004) (Table 26.1).

Table 26.1 Enumeration of counts of total bacteria, LAB, Staphylococci and yeasts and moulds of sorghum dough at different fermentation periods

Fermentation period	hour0	19 hours	24 hours
Total bacterial count(c.fu/g)	5.60^{ab} x10^{6}	2.04^{b} x 10^{8}	5.83^{ab} x 10^{9}
Lactic acid bacterial count (c.fu/g)	2.12^{b} x10^{5}	4.04^{a} x 10^{8}	4.95^{a} x 10^{8}
Staphylococci count (c.f.u/g)	2.81^{a} x 10^{2}	6.87^{a} x 10^{2}	2.04^{b}x 10^{3}
E. coli	2.40<	34	15
Yeast and mould count c.f.u./g)	2.50^{b}x 10^{4}	7.30^{a} x10^{7}	9.11^{b}x10^{7}

26.5 Conclusion

The traditional Sudanese method of fermentation of sorghum significantly improved the functional properties of sorghum flour. Traditional fermentation in Kisra bread is desirable because of their role in taste and flavour of the final product. In conclusion, sorghum flour and its fermented foods can be affected by fermentation and the method of preparation. A reduction of some nutritional parameters (carbohydrate and oil) and an increase in others (protein) were observed. In spite of the losses in some nutritional compounds, the fermented sorghumbase items, kissra, was found to have obvious nutritious qualities.

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Chapter 27
Utilization of Gum Arabic as a Thickener and Stabilizer in Production of the Fermented Milk *Zabady*

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27.1 Introduction

Acacia gum is a characteristic agricultural resource from the gum belt region of Africa, i.e., countries geographically going from east to west: from Sudan, Somalia, Eritrea, and Ethiopia to Chad, Central African Republic, Mali, Niger, and farther west up to Nigeria, Senegal, and even Mauritania (Hamad et al. 2013; Mohammed 2011). Financially, acacia gum mainly comes from Sudan, Chad, and Nigeria. Gums are polysaccharides, that are similar to sugar units making up a large molecule. They are bland in taste, odour less and tasteless. They may have a nutritional quality besides the primary function but they certainly help in digestion and might be utilized as laxatives too.

Gum Arabic speaks to about 12% of Sudan's GDP; the country delivers more than 80% of the absolute world gum Arabic (Abdulgadir 2013; Forman 2012). Gum Arabic Arabic assumes an essential function in the country's economy. Added to that, it is viewed as a steady kind of revenue for provincial occupants, particularly during periods of yield disappointment (Abdelnour 1999). Being the primary part of conventional rain-fed farming, *A. Senegal* is acknowledged as momentous due to its contribution to household income and the country's foreign trade profit (Pretzsch et al. 2014).

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Fig. 27.1 Gum Arabic pieces and powder. (Source: https://commons.wikimedia.org/)

Gum Arabic is extensively utilized for current purposes as a stabilizer, a thickener, an emulsifier, and, less significantly, in textiles, pottery creation, lithography, and in the cosmetic and pharmaceutical industries. In the food industry, GA is, on an essential level, utilized in confections, heated merchandise, dairy, refreshments, and as a microencapsulating agent (Sulieman 2018; Montenegro et al. 2012) (Fig. 27.1).

Gums are essential ingredients in production of food emulsifiers, food additives, food thickeners and many other products. The main reason for adding a gum or hydrocolloid to food commodities is to enhance its overall quality (Panda 2016). Similarly stabilizers are an indispensable substance in food items when added to the food items, they smoothens uniform nature and hold the flavouring compounds in dispersion.

Zabady (yoghurt) is the coherent or stirred product obtained from natural milk or milk recovered from condensed or dried milk or their mixtures, sweetened and thermally processed and frosted by the action of pure cultures of bacteria producing lactic acid and added to parts, juices or natural fruit concentrates. The Sudanese

Standards and Standards Organization (2007 CE) also defines that yogurt is the product of the curd of milk, raw, recycled (powdered milk), pasteurized or sterilized, obtained from acid fermentation. Lactic acid is produced by the influence of *Lactobacillus bulgarcius* and coccus *Streptococcus thermophilus* on milk.

Zabady fermented milk is not a truly indigenous food of the Sudan. The product is recognized to urban populations and is unknown to the older generations of rural communities or to those of the pastoral sector (Dirar 1993). *Zabady* contain 2.8% protein, 3.1% fat, 2.7% lactose, 0.7% ash, 0.3% ethanol, 10.9% total solids, 1.9% total acidity (as lactic acid), 0.2% volatile fatty acids and the product had a pH of 3.6 (Sulieman 2001; Abdelgadir et al. 1997).

Many of the traditionally food products in Sudan, particularly, fermented milk products such as *robe* and *zabady* require some kind of stabilization at some point during production, transportation, storage and serving. This is because the whey separates from the curd after a short period of its preparation and storage, this can result in deterioration of its overall quality. Therefore, the current study was initiated to use Gum Arabic as a thickner and stabilizer in production of the Sudanese fermented milk product *zabady*and to evaluate its chemical composition as well as its sensory evaluation.

27.2 Materials and Methods

Fresh cow's milk was obtained from a local farm at Wadmedani city, Sudan immediately after milking operation during April 2018, transported in sterilized container to the dairy laboratory of the Department of Food Science and Technology, University of Gezira for further processing and analysis.

27.2.1 Zabady *Preparation*

Fresh milk sample was collected immediately after milking in plastic containers and was inoculated with starter (3 ml) of previously prepared *zabady*. Then the product was incubated over night at 37 °C and cooled to 20 °C. Gum Arabic powder was then added at 5%, 7.5% and 10% concentrations and stored at a refrigerator for 1, 7, and 14 days (Fig. 27.2).

27.2.2 Methodology

The chemical analyses were carried out to determine the pH values and the contents of protein, total soluble solids (TSS), moisture, ash, lactose, fat and titratable acidity (TA) of *Zabady* according to AOAC (2010) methods.

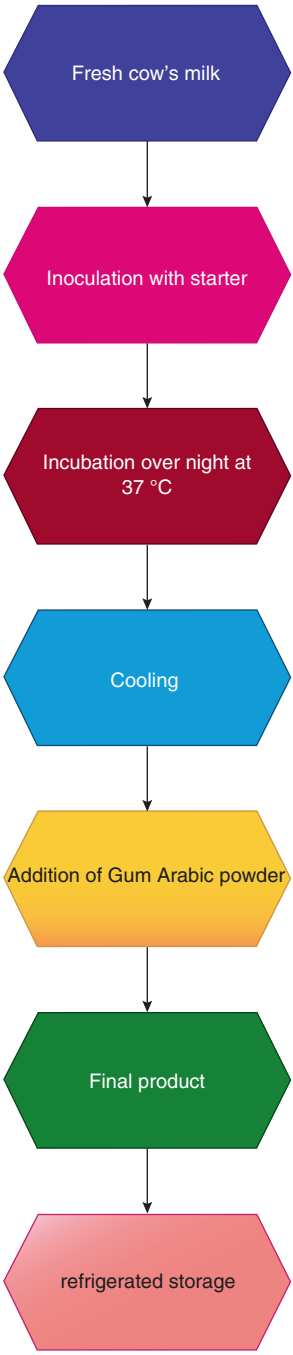

Fig. 27.2 Flow chart of Gum-Zabady preparation

The whey separation (Susceptibility to syneresis) from *zabady* samples was evaluated according to the drainage test described by Modler et al. (1983). 25 ml sample were taken into filter paper with a funnel, and then put in scale cylinder for 2 h at 6°C to measure the filtered whey.

27.2.3 Microbiology of Zabady Samples

Microbiological analyses were conducted in order to determine the total viable count using plate count agar, coliform count using MacConkey agar and yeast and mould agar using potato dextrose agar for *zabady* samples.

For detection of salmonella, 10 grams of sample were weighed aseptically and mixed well with 100 ml sterile nutrient broth. This was incubated at 37 °C for 24 h. Then 10 ml were drawn aseptically, added to 100 ml selenite broth, and incubated at 37 ° C for 24 h. Then with a loopful, streaking was done on dried Bismuth sulphite agar plates. The plates were then incubated at 37 °C for 72 h. Black metallic sheen discrete colonies indicated the presence of salmonella. A confirmatory test was carried out by taking a discrete black sheen colony and subculturing it in a Triple sugar iron agar tubes. Production of a black colour at the bottom of the tube confirms the presence of *Salmonella*.

27.2.4 Sensory Evaluation of Zabady

All types of *zabady* were subjected to sensory evaluation using 10 panelists at the first day of storage, the storage temperature was 6 C. The panelists were asked to rank the samples for color and appearance, texture, flavor and overall acceptability using 9 points hedonic scale with 1 as extremely bad score and 9 as the excellent, for these evaluation a special testing area was used so that distractions can be minimized and conditions can be controlled.

27.2.5 Statistical Analysis

All scores of the sensory evaluation were analyzed by the analysis of variance (ANOVA), to determine whether there were significant differences between means for each variable, the least significant difference (LSD) test was used.

27.3 Results and Discussion

Zabady (yoghurt) is the coherent or stirred product obtained from natural milk. The general requirements for proprer zabady include:

1. The raw materials used in the manufacture of the product shall comply with the standard specifications of each of them.
2. It is natural in its characteristics in terms of appearance, taste and smell.
3. The texture shall be homogeneous, free of gaseous gaps resulting from microbial contamination.
4. It is permissible to add health approved flavorings, aroma, and texture stabilizers.
5. The sugar used is sucrose.
6. It is not permissible to use artificial colors and sweeteners.
7. It is permissible to add natural food coloring materials.
8. The packages shall not be inflated.
9. The final product packages shall be kept at a temperature not exceeding 5 °C.
10. The product is not thermally treated after the natural fermentation resulting from the addition of the initiator.
11. It is manufactured in licensed stores, and the workers in its manufacture are subject to continuous health supervision.

In the present study, fermented cow milk (*zabady*) supplemented with various levels of gum Arabia product was prepared at laboratory level. The quality and safety of the product was assessed using chemical, microbioogical and sensory methods.

27.3.1 Chemical Analysis

The data in Table 27.1 shows the impact of storage on the control *zabady* (CZ) compared to *zabady* samples supplemented with 5%, 7.5% and 10% levels of gum powder. The average pH decreased during fermentation, the average pH of CZ after one day of storage was 4.04 which was relatively higher than that reported by Hamad et al. (2013) who reported a value of 3.90 and lower than that of yoghurt (4.4%) as reported by Mahgoub (2010), however, *zabady* supplemented with 7.5% and 10% gum powder had pH values of 4.11, 4.15, respectively. The reduction in pH continued until the 7th day of storage in the CZ sample (3.66), and slightly increased at the 15th day of storage. This result was slightly lower than that reported by Hamad et al. (2013) who reported a value 3.78, and closely related to those of *zabady* samples supplemented with 5%, 7.5% and 10% gum powder which averaged 3.64, 3.61 and 3.68 respectively. However, after 15 days of storage, the pH of control *zabady* gradually increased with an average of 3.75, and this result was slightly lower than that reported by Mahgoub (2010) in control yoghurt sample at 15 days of storage which was 4.0 ± 0.06%.

Table 27.1 Chemical composition of control *zabady* and *zabady* samples supplemented with gum powder during storage period

Zabady/storage period		pH	Acidity%	Protein%	Moisture%	TSS%	Lactose%	Ash%	Fat%
A	1 day	4.04	1.82	2.95	90.13	8.15	4.41	0.77	3.44
	7 days	3.66	2.62	2.23	91.77	8.21	4.18	0.55	3.29
	15 days	3.75	2.21	2.04	91.97	8.01	3.96	0.54	3.11
B	1 day	4.01	1.68	2.56	88.60	12.11	4.82	0.90	3.28
	7 days	3.64	2. 50	2.05	88.04	11.93	4.35	0.82	3.11
	15 days	3.93	2.06	1.94	88.38	10.93	3.90	0.67	3.08
C	1 day	4.11	1.58	2.37	85.72	14.26	4.73	0.91	3.32
	7 days	3.61	2.53	1.97	85.86	12.33	4.28	0.82	3.22
	15 days	3.95	1.89	1.90	86.86	11.71	3.86	0.75	3.37
D	1 day	4.15	1.50	2.33	83.33	16.12	4.78	0.99	3.28
	7 days	3.68	2.55	1.88	83.95	16.53	4.28	0.96	3.30
	15 days	3.96	2.09	1.86	84.01	15.96	3.83	0.82	3.10

A: control *zabady*; B: *zabady* with 5% gum powder; C *zabady* with 7.5% gum powder; D: *zabady* with 7.5% gum powder

Titrable acidity of *zabady* (expressed as lactic acid%), increased by fermentation, the rate of increase in the CR sample after one day of storage was 1.82%, which was lower than that reported by Abbas (2005) who reported a value of 1.50% titrable acidity in cows traditional yoghurt. However, CZ sample had higher titrable acidity compared with zabady samples supplemented with 5%, 7.5% and 10% gum powder which averaged 1.68%, 1.58% and 1.50%, respectively. The increase in acidity continued until the 7th day of storage in CZ sample with an average of 2.62%. The increase was also observed in the other zabady supplemented with 5%, 7.5%, and 10% gum powder with an average of 2.50%, 2.53% and 2.55%, respectively. In 15th day of storage, the titrable acidity values started to decrease in the CZ with an average of 2.21%. In all zabady samples there were an increase in acidity and decrease in pH values until 7 days of storage time, however, after 15 days of storage the acidity slightly decreased and pH values increased and this could be attributed to the action of microorganisms dominant in *zabady*. Taste defects are common in *zabady*, and the most dangerous is the lack of flavor and aroma and lack of taste, what is required for *zabady* when the acidity reaches 85.0%, while an increase in acidity over 95.0% is accompanied by It has va pungent taste, and the deficiency or need for taste and flavor is due to the use of strains of the starter *Lactobacillus balgaricus* that produces little taste and aroma (Al-Hajrawy 1987). The tart, acidic taste results from increasing the incubation time, increasing the amount of starter, or leaving the yogurt without ʒooling, the non-acidic taste results from the use of an inactive starter or insufficient incubation in terms of Temperature and incubation time (Sulieman 2001).

The moisture content in CZ after one day of storage was 90.13% and this value was higher than those found in *zabady* supplemented with 5%, 7.5% and 10% gum powder which were 88.60%, 85.72% and 83.33%, respectively, and higher than that reported by Mahgoub (2010) in control yoghurt which was 84.9% ± 0.71%. The moisture content of CZ increased during storage period. The increase of moisture content may be due to high free water content of zabady. Similarly the moisture content of GZ continued to increase during storage, and after 15 days the moisture values reached 88.38%, 86.86% and 84.01% for *zabady* supplemented with 5%, 7.5% and 10% gum powder, respectively.

The total solids (TSS) content of control *zabady* after one day of storage was 8.15%, this value is lower than that reported by Mahgoub (2010) for yoghurt (15.1% + 0.05%). The TSS of *zabady* supplemented with gum powder which ranged 12.11–16.12%. The TSS of CZ slightly decreased after 7 and 15 days of storage, this reduction could be attributed to the higher moisture content as well as the fermenting of lactose to lactic acid. In other supplemented samples, TSS% continued to decrease during storage time to and reached 10.93%, 11.71% and 15.96% after 15 days of storage of *zabady* supplemented with 5%, 7.5% and 10% gum powder, respectively.

The protein content of CZ *zabady* after one day of storage has an average of 2.95%, This value was slightly higher than those of *zabady* samples supplemented with 5%, 7.5% and 10% gum powder which were 2.56%, 2.37% and 2.33%, respectively. Slight decrease in protein contents of the various *zabady* samples

supplemented with various levels of gum powder during 7 days and 15 days. The decrease in protein content might be attributed to the presence of microorganisms gum *zabady* samples which may hydrolyze proteins to amino acids and utilize them as nutrients.

The ash content of CZ sample after one day of storage was 0.77%, this value was lower than the values of *zabady* samples supplemented with 5%, 7.5%, and 10% gum powder which averaged 0.90%, 0.91% and 0.99%, respectively. This ash value was higher than the value of traditional yoghurt (0.66% as reported by Abbas (2005). However, after 7 days of storage ash content of CZ sample decreased to 0.65% and the reduction continued until the 15th day of storage with an average of 0.54%. This reduction could be due to the effect of microorganisms on some minerals. On the other hand the ash content of *CZ* supplemented with 5%, 7.5% and 10% gum powder decreased after 7 days with an average of 0.82%, 0.82% and 0.96%, respectively. This decrease continued after 15 days of storage to 0.67%, 0.75% and 0.82%, respectively. However, the ash content of *Robe* samples increased with increasing contents of added gum powder, this may be attributed to the presence of some minerals in the gum with relativley high amounts.

The lactose content of CZ after one day of storage was 4.41% which was lower than those of zabady supplemented with 5%, 7.5% and 10% gum powder that averaged 4.82%, 4.73% and 4.78%, respectively. The increase of lactose content in the gum *zabady* samples could be attributed to the presence of polysaccharides in the gum. Lactose content of CZ decreased after 7th day to 4.18%. Moreover, the value of lactose content of *zabady* supplemented with 5%, 7.5% and 10% gum powder also decreased after 7 days of storage with an average of 4.37%, 4.28% and 4.28%, respectively. This decrease in lactose content continued until the 15th day of storage and reached 3.95% in the control *zabady* sample and 3.90%, 3.86% and 3.83% in *zabady* supplemented with 5%,7.5% and 10% gum powder, respectively. In all *zabady* samples, lactose content decreased with an increase of storage period, and this might be due to conversion of lactose to lactic acid.

The fat content of CZ after one day of storage was 3.44%, this value was slightly higher than those of *zabady* supplemented with 5%, 7.5% and 10% which averaged 3.28%, 3.32% and 0.80%, respectively. The fat content of CZ sample highly decreased after 7 days of storage with an average of 3.29%, moreover, fat content slightly decreaesd in *zabady* supplemented with various levels of gum powder. This decrease continued until the 15th day of storage in the CZ to 3.10%. The reduction of fat content during storage period could be attributed to the action of microorganisms.

The amount of whey separated (Fig. 27.3) after one day of storage from the CZ sample averaged 4.12 ml, this amount was higher than those of *zabady* supplemented with 5%, 7.5% and 10% which were 2.81 ml, 1.32 ml and 0.93 ml, respectivel. However, storage for 7 days and 15 days, the amount of whey increased to 6.00 ml and 6.95 ml in CZ, respectively. This increase in whey separation might be attributed to free water and reduction of pH. On the other hand, the amount of whey decreased during storage of *zabady* supplemented with 5%, 7.5% and 10% gum powder to 3.95 ml, 2.50 ml and 1.05 ml, respectively. The reduction of of whey

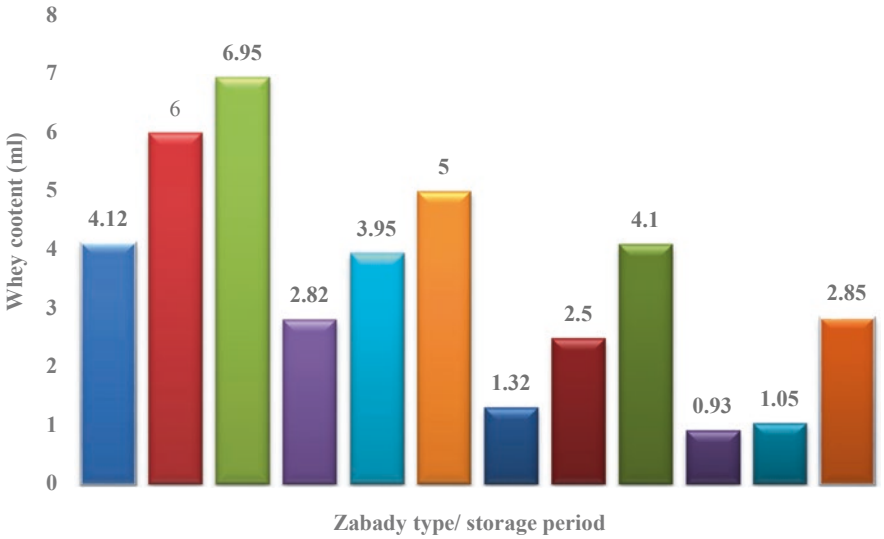

Fig. 27.3 Whey separation (ml) of control *zabady* and *zabady* samples supplemented with gum powder during storage period. A: control *zabady*; B: *zabady* with 5% gum powder; C *zabady* with 7.5% gum powder; D: *zabady* with 7.5% gum powder

separation could atrributed to presence of Gum arabic which is known to be a significant thickenening agaent, and it has high water holding capacity (Sulieman 2018). According to Bai et al. (2016) and Yang et al. (2013), the capacity tests indicated that the GA–balanced out emulsions had great dependability to bead accumulation and gravitational partition fundamentally due to less oil drop estimate as well as high emulsion thickness. A frail curd is a genuine imperfection in yogurt produced using ordinary milk and milk with low substance of complete solids or delivered if the milk is in the water toward the beginning of the production season according to Al-Hajrawy (1987). The shortcoming may be result from not heating up the milk for a while, or inadequate or lacking smoking (Sulieman 2001), and sometimes pungent taste may happen in yogurt, it starts from the presence of sodium chloride in crude utilized during manufacture of yoghurt (Al-Khouli 1999). The reasons for this marvel are the utilization of warmed milk, which prompts calcium precipitation or a lopsidedness of milk salts, particularly calcium. The delicacy of the curd likewise results from the presence of antibiotic and insecticidal buildups, and hurtful pesticides in milk, which influences the development of the initiator.

In their study on yoghurt developed from concentrated whey, Rashid et al. (2019) concluded that WHC was increased as a result of protein denaturation in yoghurt products. The whey proteins denaturation increased the gelling properties with adequate heat treatment and increased the surface area which allowed increased water retention in the yoghurt matrix Akhtar and Dickinson (2003).

The statistical analysis showed that there was no significant difference ($P > 0.05$) in pH, acidity, lactose and fat between the control *zabady* and gum *zabady* samples.

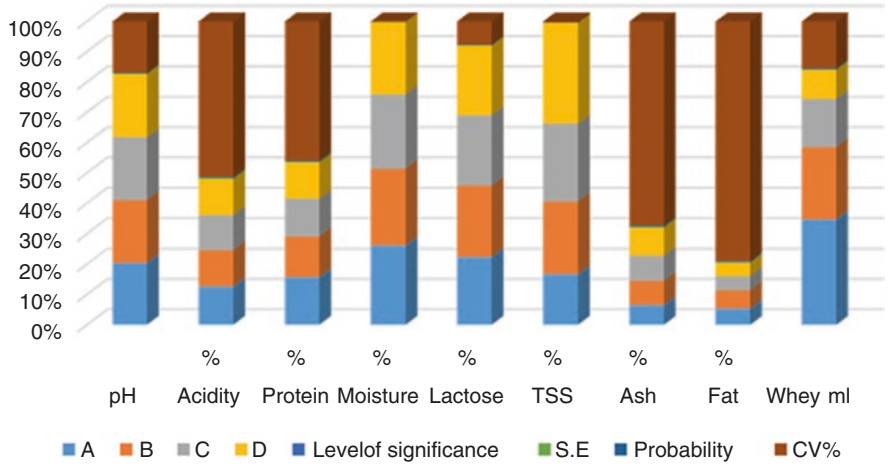

Fig. 27.4 Statistical analysis of chemical composition of control *Zabady* and *Zabady* samples supplemented with Gum powder. **Note: A:** control *Zabady*; **B:** *Zabady* with 5% gum powder; **C:** *Zabady* with 7.5% gum powder; **D:** *Zabady* with 10% gum powder; ****** highly significant differences; and **NS**: no significant differences

However, there were highly significant differences ($P < 0.01$) in protein, moisture, TSS, ash and whey separation between control *zabady*samples and gum *zabady* samples (Fig. 27.4).

*27.3.2 Microbioly of Z*abady

Normally, microbial quality tests are done for fermented milk to protect the consumer from exposure to any hazards, hygienic and ensure that the product is not exposed to microbiological spoilage during the shelf life. The microbiological characteristics of raw cow milk and *zabady* samples are presented in Table 27.2. The total viable count (TVC), yeast and mould counts increased throughout storage period, and increased gradually during storage for 7 days and 15 days. Moreover, all samples were devoid of salmonella cells, while coliform bacteria was detected only at the first day of storage. At the first day of *zabady* storage, the TVC was higher in control *zabady* (6.8×10^7 c.f.u/ml) and slightly increased to 5.1×10^8 and 8.8×10^8 c.f.u/ml during storage for 7 day and 15 days, receptively.

After 15 days of storage, the TVC continued increasing in all *zabady* samples produced from raw milk or milk supplemented with gum powder. It seems that addition of gum powder slightly decreased the microbial load of *zabady* product, and the effect during storage.

Table 27.2 Microbiological characteristics (c.f.u/ml) of raw milk and *zabady* samples supplemented with gum powder during storage period

Sample	Storage period	Total viable count (cfu/ml)	Coliforms (cfu/ml)	Yeast and mould (cfu/ml)	Salmonella (cfu/ml)
A	1 day	6.8×10^7	1.1×10^3	7.2×10^2	Nil
	7 days	5.1×10^8	Nil	1.2×10^3	Nil
	15 days	8.8×10^8	Nil	4.6×10^3	Nil
B	1 day	5.2×10^7	2.1×10^3	6.2×10^2	Nil
	7 days	4.1×10^8	Nil	8.1×10^3	Nil
	15 days	5.8×10^8	Nil	4.0×10^4	Nil
C	1 day	4.8×10^6	1.4×10^3	6.8×10^2	Nil
	7 days	6.1×10^7	Nil	4.5×10^3	Nil
	15 days	8.8×10^7	Nil	1.2×10^4	Nil
D	1 day	7.7×10^6	2.2×10^2	6.2×10^3	Nil
	7 days	3.1×10^7	Nil	8.7×10^3	Nil
	15 days	4.5×10^7	Nil	7.1×10^4	Nil

A: control *Zabady* sample; B: *Zabady* with 5% gum powder; C: *Zabady* with 7.5% gum powder; D: *Zabady* with 10% gum powder

27.3.3 Sensory Evaluation

The sensory assessment is used to ascertain the natural qualities of the product and the degree of its acceptability in terms of taste, flavor, texture and general appearance and it is done through the main senses of the human being. Or in another sense: it is an evaluation of many characteristics in which the senses are used and translated by the nervous system to give a certain impression and a close indicator that expresses the quality of the product and the acceptance of the consumer.

The result of sensory evaluation (Table 27.3) indicates that there were no significant differences ($P < 0.05$ in flavor between the control *zabady* and gum *zabady* samples during the entire storage time. And there was significant difference ($P < 0.05$) between the samples in the colour at the first day and 7th days of storage since the panelists mostly preferred white color which exists in control *zabady* sample. However, there was no significant difference between control *zabady* and gum *zabady* samples in the color at the 15th day of storage. There was a highly significant difference ($P < 0.01$) in the texture between control *zabady* and the other *zabady* samples at 1 day and 7 days of storage. However, there was significant difference ($P < 0.05$) between the control sample and gum *zabady* samples in the texture at the 15th day of storage. Panelists gave high scores of texture to *Robe* prepared using 10% gum powder. There were significant differences ($P < 0.05$) in overall acceptability between control and other samples at the first and 7th day of storage.

Table 27.3 Mean scores for sensory evaluation characteristics of gum zabady during sotrage

	1 day				7 day				15 days			
Sample	Color	Texture	Flavor	Overall	Color	Texture	Flavor	Overall	Color	Texture	Flavor	Overall
A	7.62 a	5.25 a	6.75 a	5.25 a	7.75 a	4.38 a	7.12 a	5.50 a	7.00 a	5.12 ac	6.88 a	3.75 a
B	6.62 ab	6.88 b	6.50 a	7.12 b	6.62 a	6.75 b	6.38 a	6.25 ab	7.00 a	7.00 b	7.00 a	6.50 bd
C	6.12 abc	7.38 bc	6.62 a	7.38 b	6.12 ab	7.25 b	7.00 a	7.62 b	6.75 a	6.38 bc	5.75 a	8.00 c
D	4.75 c	8.25 c	6.62 a	7.88 b	5.38 b	7.75 b	7.62 a	7.50 b	6.50 a	7.12 b	5.62 a	7.50 cd
Level of significance	*	**	NS	*	*	**	NS	*	NS	*	NS	**
Probability	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

A: control *zabady* sample; B: *zabady* with 5% gum powder; C: *zabady* with 7.5% gum powder; D: *zabady* with 10% gum powder
Means are based on a point scale (9 is excellent and 1 is extremely bad)
a, b and c means in he column with different super scripts are significant different ($P > 0.05$) or highly significant ($P > 0.01$)
a, b and c means in the column with the same super scripts are not significant different ($P < 0.05$)
*Significant differences
**Highly significant differences
NS, No significant differences

27.4 Conclusion

In the present study quality and saftey of *zabady* produced using gum powder with three levels (5%.7.5% and 10%) was investigated, in addition; control *zabady* was prepared without addition of gum. All samples were stored at 6 °C for 1,7 and 15 days, the chemical analysis showed that there were decreases in protein, moisture, fat, acidity and whey separation for *zabady* with added gum powder, and increases in contents of ash, lactose, T.S.S and pH. After 7 days of storage most of the chemical components of *Robe* gradually decreased and the reduction continued until 15th day of storage. The microbiological analysis showed that The TVC, yeast and mould counts increased throughout storage period. Moreover, addition of gum powder slightly decreased the microbial load of *zabady* product, and the effect during storage.

The sensory evaluation of *zabady* produced using gum powder showed good results regarding to texture and overall acceptability than that produced without addition of gum powder (control yoghurt).

It is highly recommended focus on the level of safeguarding of the product in the centers of sale and dissemination. Leading investigations that incorporate all important tests to decide the quality and congruity of the zabady with the specifications and standards.

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Chapter 28
Bioactive Components of Fermented Food Products: Phytochemicals, Phytosterol and Vitamins

Haroon Elrasheid Tahir, Zou Xiaobo, Gustav Komla Mahunu, and Abdalbasit Adam Mariod

28.1 Introduction

Cereals, legumes, and oilseeds are a valuable source of proteins, carbohydrates, oil, dietary fiber, vitamins, carotenoids, and polyphenol compounds. In Africa, there is great interest in using indigenous cereals and legumes for value-added food products (Uzogara et al. 1990; Taylor and Duodu 2015; Niveditha and Sridhar 2017). Fermentation is the earliest technology for preserving food, enhances nutritional value, and improves its sensory properties and shelf life. It can be defined as a desirable process of biochemical modification of raw food substances caused by the activities of microorganisms and their enzymes (Lyumugabe and Bajyana Songa 2019). African countries produce a wide variety of their own fermented foods. Most of them produced from vegetables, cereals, legumes, and seeds. Most of the fermented food produced in African countries is prepared by natural or spontaneous fermentation. Women in households or at a commercial small-scale level commonly accomplish this procedure. Based on the type of product, such fermentation might be only lactic acid bacteria (LAB) with *Lactobacillus spp Leuconostoc* spp., *Pediococcus* spp., and *Weissella* spp. as the major species involved in the fermentation or both LAB and alcoholic fermentation for the making of alcoholic beverages

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(Adebiyi et al. 2018; Adebo et al. 2018a, b, c; Mahgoub 2019). During the fermentation process, the production of lactate and acetate decreases the pH value and limits the growth of spoilage microorganisms in the final products. Furthermore, the metabolism of lactic acid bacteria may produce biologically active compounds, including phenolic compounds and vitamins and bioactive peptides produced by proteolysis (Tamang et al. 2020). Yeast fermentation also can affect the antioxidant capacity of fermented food products through an increase in the phenolic contents (Wang et al. 2014).

There many scientific articles, which demonstrated that Africa's fermented condiments, bread, and beverages, are foods with high nutritional values. See, for example, (Taylor and Duodu 2015; Adebo et al. 2019; Olasupo and Okorie 2019). The use of vegetable and legumes proteins as a condiment or meat substitute is widely used in African countries, mainly in rural communities. In African countries, some fermented legumes, vegetables, and oilseeds with considerable bioactive compounds include Iru *or Dawadawa* from locust bean (*Parkia biglobosa*), Dawadawa from roselle (*Hibiscus sabdariffa*) or Bambara groundnut (*Vigna subterranea*), *Ugba* from African oil bean seed (Pentaclethra macrophylla) and Fururndu from roselle seed. Tables 28.1 and 28.2 present the list of African fermented food rich in biologically active materials. Besides, African fermented foods consist of a wide range of cereals-based products (e.g. Injera, Hulu-Mur, *kisra*, *Ben-saalga*, t*chouk-outou, ting* and Ogi) and fruits (e.g. Zoborodo or *Sobolo*).

28.2 Major Bioactive Compounds in African Fermented Foods

During the fermentation process, foods are enriched with bioactive components, improving the safety and quality of the final products. For instances, cereal-based lactic acid bacteria (LAB) fermentation improves the concentration of folates, soluble dietary fibers, polyphenol compounds, vitamin B groups showing health benefits and improve protein digestibility (Bvochora et al. 1999; Adebo et al. 2018a, b; Saubade et al. 2018; Tamene et al. 2019; Bationo et al. 2020). Bioactive compounds in African fermented could play an important role against cardiovascular diseases, which are accountable for mortality globally; these compounds appear to have antimicrobial activity against pathogens. Furthermore, they are capable of scavenging reactive oxygen species, dangerous species that harm DNA, proteins, lipids, and carbohydrates creating many diseases such as diabetes, inflammation and cancer (Aliya and Geervani 1981; Taylor and Duodu 2015; Erukainure et al. 2019a, b; Şanlier et al. 2019). According to existent bioactive compounds, fermentation could be considered as an effective biotechnological process to produce functional foods from plant origin. Information on the effects of fermentation on the vitamin content and phytochemical compounds of African fermented food scarce, especially for individual polyphenol compounds (Table 28.1). The bioactive content of African

Table 28.1 Bioactive components in various African fermented foods

Products	Main material	Compounds/concentrations	Country	References
Ting (a Southern African food)	Whole grain sorghum (Sorghum bicolor L.)	Total phenolic (8.11–46.1 mg GAE/g); total flavonoid content (7.53–40.9 mg CE/g); tannin content (0.41–14.1 mg CE/g)	South Africa	Adebo et al. (2018a, b)
Iru	Parkia biglobosa seeds (African locust beans)	Total phenolic (0.71 mg/g); free phenols (0.61 mg/g); bound phenols (0.03 mg/g);	Nigeria	Oboh et al. (2008)
Wine-like alcoholic drink	Coca juice Roselle juice	Polyphenol (962 mg/l,)	Cameroon	Darman et al. (2011)
Ting (a Southern African food)	Sorghum (Sorghum bicolor L.)	Gallic acid (1.50 μg/g); catechin (14.94 μg/g); vanillin (trace); quercetin (1.94 μg/g)	South Africa	Adebo et al. (2018a. b, c)
Dawadawa	Bambara groundnut (Vigna subterranea)	Quinic acid (0.12–3.09 μg/g); lamerioside (0.01–1.55 μg/g); catechin O-glucoside(0.01–.030 μg/g); caffeic acid derivative (0.05–0.16 μg/g); lalioside (0.0–0.13 μg/g); medioresinol (0.03–0.80 μg/g); dihydro oleuropein (0.02–0.07 μg/g); quercetin-3-O-galactoside-7-O-rhamnoside (0.01–0.02 μg/g); caffeic acid derivative (0.46–0.56 μg/g); phenethyl-β-primeveroside (0.02–0.23 μg/g)	South Africa	Adebiyi et al. (2019)
Injera	Whole-grain cereal (tef)	Folate content (14.3 μg/100 g)	Ethiopia	Tamene et al. (2019)
Hulu-Mur	Feterita sorghum	Total phenolic (305.17 mg GAE/g)	Sudan	Salih et al. (2020)
Condiment	Bambara groundnut (Vigna subterranea L. Verdc)	Total phenol content (550 mg/100 g); total flavonoid (64 mg/100); nonflavonoid content (486 mg/100 g)	Nigeria.	Ademiluyi and Oboh (2011)
Kisra	*Sorghum* cultivars (*Tabat* and *Wad Ahmed*)	Total phenol (7.23–18.41 mg/g); flavonoids content (0.34–3.52 mg/g); tannins (0.046–0.152 mg/g)	Sudan	Zaroug et al. (2014)
Kisra bread	*Sorghum cultivars dabar and fetarita,*	Riboflavin (1.24–1.44 μg/g); thiamine (2.72–3.61 μg/g)	Sudan	Mahgoub et al. (1999a, b)
Hulu-Mur	*Dabar and Fetarita*	Riboflavin (0.29–2.82 μg/g); thiamine (1.88–4.45 μg/g)	Sudan	Mahgoub et al. (1999a, b)

(continued)

Table 28.1 (continued)

Products	Main material	Compounds/concentrations	Country	References
Ogi cakes	Maize	Total phenol (144.50–152.63 GAE mg/g); total flavonoid content (114.64–168.11 mg QUE/100 g,);	Nigeria	Olaniran and Abiose (2018)
Ogi cakes	Sorghum	Total phenol (171.50–185.75 GAE mg/g); total flavonoid content (150.70–198.83 mg QUE/100 g)	Nigeria	Olaniran and Abiose (2018)
Fermented porridges	Whole Sorghum [*Sorghum bicolor* (L.) Moench]	Tannin content (2.0–2.4 CE/g)	South Africa	Dlamini et al. (2007)
Akassa	Gelatinized dough, corn	Folate content (1.8–3.1 µg/100 g FW)	Burkina Faso	Bationo et al. (2020)
Doncounou	Gelatinized dough, corn)	Folate content (3.6–4.8 µg/100 g FW)	Burkina Faso	Bationo et al. (2020)
Kaffa	(gelatinized dough, sorghum)	Folate content (3.9–6.5 µg/100 g FW)	Burkina Faso	Bationo et al. (2020)
Massa	Batter fritters, pearl-millet	Folate content (7.2–12.6 µg/100 g FW)	Burkina Faso	Bationo et al. (2020)
Fura	(dumplings, pearl-millet)	Folate content(16.2–29.6 µg/100 g FW)	Burkina Faso	Bationo et al. (2020)
Ben-kida	Porridge, pearl-millet)	Folate content (1.7–3.3 µg/100 g FW)	Burkina Faso	Bationo et al. (2020)
Ben-saalga	(porridge, pearl-millet)	Folate content (1.9–2.9 µg/100 g FW)	Burkina Faso	Bationo et al. (2020)
Ben-saalga	pearl-millet grains (Pennisetum glaucum)	Folate content (2.2 µg/100 g FW)	Burkina-Faso.	Saubade et al. (2018)
Kombucha beverage	Mustard (B. tournefortii) leaves	Total phenol (175–270 GAE/g dw)	Tunisia	Rahmani et al. (2019)
Ugba	Oil bean seeds (*Pentaclethra macrophylla*)	Thiamin (0.07 mg/100 g); riboflavin (0.30 mg/100 g); niacin (0.30 mg/100 g)	Nigeria	Olasupo et al. (2016)
Fermented porridges	Sorghum Macia, NK 283, Red Swazi, NS 5511 and Framida	Total phenols (3.3–9.1 mg CE/g)	South Africa	Dlamini et al. (2007)
Fermented Zobo drink	Roselle (Hibiscus Sabdariffa)	Vitamin C (0.36–0.63 mg/mL)	Nigeria	Nwafor and Akpomie (2014)

(continued)

Table 28.1 (continued)

Products	Main material	Compounds/concentrations	Country	References
Fururndu	**Roselle (*Hibiscus sabdariffa* L.) Seed:**	Total phenols (884.33–901.33 mg/100 g);	Sudan	Yagoub et al. (2004), Omer and Yagoub (2007)
Raffia palm	Raffia palm	Total phenols (0.3–1.5 μg/g)	Nigeria	Erukainure et al. (2019a, b)

Table 28.2 Bioactive metabolite identified in LAB fermented ting sample

Classes	Metabolites name
Phenols	Phenol
	Guaiacol
	p-Ethylphenol
	p-Ethylguaiacol
	Phenol, 2,6-dimethoxy-
	2,4-Di-tert-butylphenol
	Butylated Hydroxytoluene
	Phenol, 2-(2-benzoxazolyl)-
	Phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl
	3,3′-(*p*-phenylenedioxy)diphenol
Phytosterols	Campesterol
	Stigmasterol
	ç-Sitosterol
	Stigmasta-5,24(28)-dien-3-ol, (3á,24Z)-
Terpenes/Terpenoids	Eucalyptol
	Supraene
	Squalene
	Stigmasta-3,5-diene
Vitamins	ç-tocopherol
	Dl-à-tocopherol

Source: data adapted from (Adebo et al. 2019; Kewuyemi et al. 2020)

fermented foods can be classified into two groups i.e. polyphenol compounds and vitamins, which are discussed in a comparative theme over the next subdivisions with their concentrations, are indicated in Table 28.1. In Africa, the trend toward fermented food with high nutritional value, rich in health-promoting compounds, with higher sensorial properties have been growing with the consciousness of consumers. For this reason, the present chapter is written considering the literature regarding traditional African fermented food (e.g. condiments, bread, porridges, and beverages) with reputed biological active materials.

28.2.1 Phenols and Terpene/Terpenoid Compounds

Polyphenols are a large group of bioactive compounds present in the plant, which show various biological activities (Fraga et al. 2019). They include various chemical structures; in fact, they could range from simple, small molecules as phenolic acids to complex compounds with a high molecular mass such as tannins. Polyphenols are described as strong antioxidants; they may have a beneficial effect on the prevention of non-communicable diseases. Being extant in plant origin food; they are dietary natural compounds, and the nutritionists recommend their daily intake to have positive impacts on health (Liburdi et al. 2020). Unfortunately, these health benefits are sometimes reduced by their lower bioavailability, which depends on the chemical structure of these compounds. In plants, phenolic compounds are present as glycosides, esters, and covalently bound to cell wall structural components such as cellulose, hemicellulose, lignin, pectin, and proteins (Liburdi et al. 2020).

Sorghum is an important cereal crop in Africa and the main source of food for millions of people. Similar to other cereal produces, it is transformed into an edible formula using numerous processing procedures with fermentation being the most technique known to enhance nutritional values, shelf life, the bioavailability of nutrients, palatability, functional components, and consumer appeal (Taylor and Duodu 2015). A Southern African traditional food fermented (Ting) is one of the richest sources of polyphenol compounds (Table 28.1). With regards to optimization of fermentation conditions for ting production, (Adebo et al. 2018a, b, c) reported that the optimum conditions for the production of bioactive-rich Ting produced from Sorghum (*Sorghum bicolor* L.; cultivar Titan) was 34 °C for 24 h. At these parameters, the highest phenolic (16.06 mg GAE/g), tannin (9.97 mg CE/g), and flavonoid contents (8.70 mg CE/g) were released. The same authors investigated the effect of fermentation conditions on physicochemical properties, bioactive components, and microstructure of Ting from whole grain sorghum (*Sorghum bicolor* L.; Avenger cultivar (Adebo et al. 2018a, b, c)). The optimum fermentation parameters were 28 °C for 72 h and at these conditions, higher contents of total phenolic (46.1 mg GAE/g), total flavonoid content (40.9 mg CE/g), and tannin were achieved. These conditions also showed considerable concentrations of catechin (12.4 μg/g), gallic acid (0.75 μg/g), and quercetin (0.61 μg/g).

A study by (Adebo et al. 2018a, b, c) examined the effect of fermentation by *Lactobacillus fermentum* strains (*Lactobacillus fermentum* FUA 3165 and *Lactobacillus fermentum* FUA 3321) individually and in combination on the properties of Ting made from low tannin and high tannin whole grain sorghum. Both single and combined starter cultures obtained good results with significantly lower, tannin content (0.41–2.83 mg CE/g), total phenolic content (8.11–32.13 mg GAE/g) and flavonoid content (7.53–26.38 mg CE/g). The lower phenolic compounds could be attributed to the degradation and hydrolysis during the fermentation process. Another possible reason for lower content is oxidation and condensation reactions of polyphenols forming different compounds (Taylor and Duodu 2015). The

phenolic profiles indicated that Ting samples from high tannin sorghum with Lactobacillus fermentum FUA 3321 had a significantly higher content of catechin and quercetin than the spontaneously fermented ones. Similar behavior was observed for the product produced using low tannin sorghum as illustrated in Fig. 28.1. The slight increase in the content of flavonoids and phenolic acid

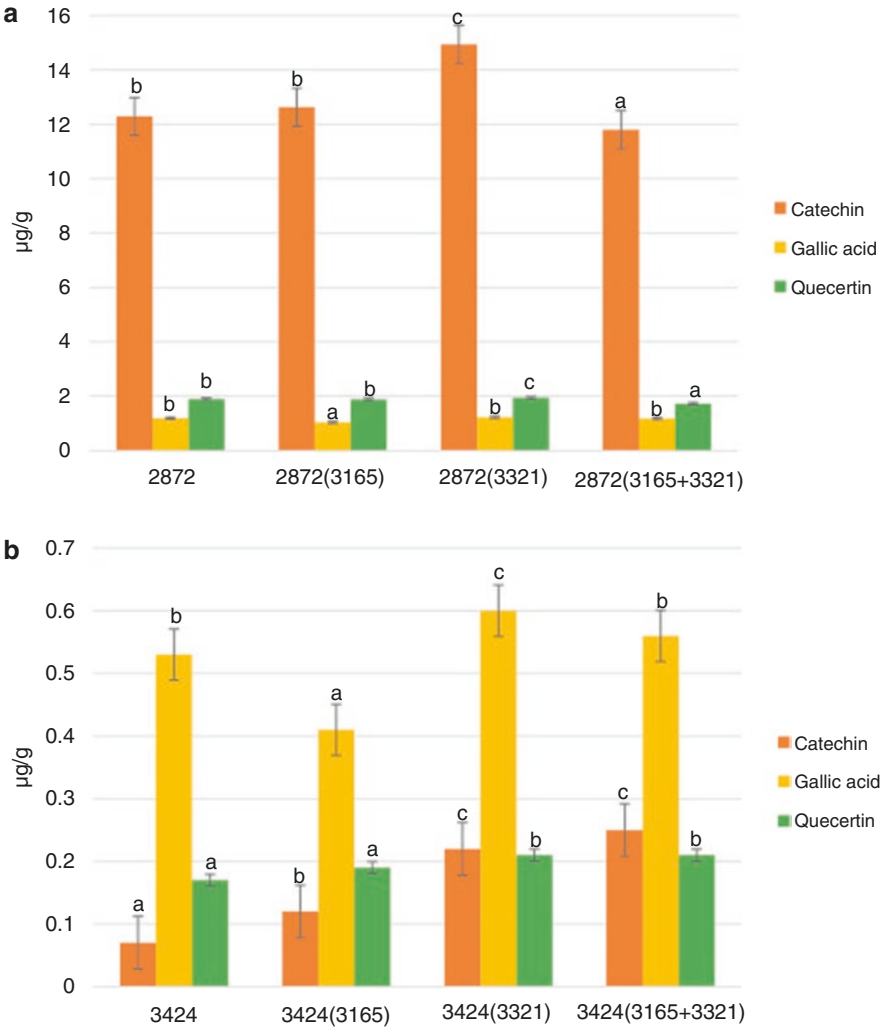

Fig. 28.1 Effect of fermentation by L. fermentum strains on phenolic compounds of ting from whole grain sorghum. (**a**) Ting samples obtained from the LT sorghum type; (**b**) Ting samples obtained from the LT sorghum type. 2872 – naturally fermented ting from HT-sorghum; 3424 – naturally fermented ting from LT-sorghum type; (3165) – fermentation with L. fermentum FUA 3165; (3321) – fermentation with L. fermentum FUA 3321; (3165 + 3321) – fermentation with L. fermentum FUA 3165 and L. fermentum FUA 3321

indicated a better release of these compounds in LAB-fermented *ting* (Adebo et al. 2018a, b, c).

Qualitative analysis of metabolite profile of whole red sorghum grain "ting" obtained using L. fermentum FUA 3165 and L. fermentum FUA 3321 showed that L. *fermentum* FUA 3321 is found to be the most suitable choice for *ting* producing rich in phenolic compounds, phytosterols, terpenoids and vitamins accounted for biological activities (Adebo et al. 2019; Kewuyemi et al. 2020). Table 28.2 shows the major bioactive compounds found in LAB fermented ting as determined by gas chromatography-mass spectrometry (GC-MS). Earlier studies have demonstrated the antioxidant activities for many lignin-related methoxyphenols (Barclay et al. 1997; Kjällstrand and Petersson 2001). Both p-Ethylphenol and p-Ethylguaiacol are small antioxidants in fermented food (Li et al. 2018). P-Ethylguaiacol was derived from lignin pyrolysis while p-Ethylphenol was produced from lignin glycoside during the fermentation process (Lee et al. 2006; Yanfang and Wenyi 2009). A previous study showed that p-Ethylguaiacol has antioxidant activity equivalent to 22.3 mol/mol Trolox C by the crocin bleaching inhibition method (Bortolomeazzi et al. 2007). (Bortolomeazzi et al. 2007) observed that p-Ethylphenol had good DPPH radical scavenging activity. Many authors have reported the antioxidant activity of phenols identified in ting samples (Table 28.2) (Kjällstrand and Petersson 2001; Bortolomeazzi et al. 2007; Gallardo-Chacón and Karbowiak 2015; Li et al. 2018). Terpenoids represent a large group of bioactive compounds with medicinal properties, antifungal and bacterial activity against resistant pathogens (Dunkić et al. 2010; Zacchino et al. 2017; Mahizan et al. 2019). The terpene/terpenoid presence n ting samples can be categorized as monoterpene (eucalyptol) and triterpenes (supraene, squalene, and stigmasta-3, 5-diene) class (Table 28.2). The diverse metabolites identified indicated the rich composition of sorghum and the processed product (i.e. ting) and the need for future experiments to quantify these metabolites.

Salih et al. (2020) investigated the effect of fermentation on phytochemicals of Hulu-Mur (A fermented Sorghum bicolor Grain product). It is very popular in Sudan and has a strong taste and characteristic aroma. The results indicated the existence of polyphenols, flavonoids and terpenoids. Flavonoid mainly accumulated in the ethyl acetate fraction in all extracts before and after fermentation. The fermentation and cooking for the sorghum grain increased the total phenol (305.17 mg GAE/g) in the final product.

Zaroug et al. (2014) evaluated the total phenols, total flavonoids, and total tannins antioxidant activity of Sudanese traditional bread (also spelled *kisra*) known as kisra rhaheeefa, is a popular thin fermented bread prepared by sorghum flour) made from two Sorghum cultivars (Tabat and Wad Ahmed). The study showed that fermentation (up to 24 h) and backing processing have enhanced the bioactive compounds of Kisra prepared from both cultivars and consequently contributed significantly to the health benefits associated with sorghum-based foods consumption. In a study by Dlamini et al. (2007), the products investigated included fermented porridges and extrudates prepared from the whole and decorticated tannin and non-tannin sorghums. In the cooked, ready-to-eat whole-grain products, the porridges had higher phenolic compounds than the extrudates.

Ogi cake is a Nigerian traditional food prepared from produced from sorghum and quality protein maize. The study of Olaniran and Abiose (2018) showed that the Ogi flours enhance with garlic and ginger showed good nutritional values and have the potential to be healthy food.

Mahewu is a traditional non-alcoholic beverage popular among the Bantu people of Southern Africa where it often plays an important role in their diet (Schweigart and Fellingham 1963). In Zimbabwe, a Mahewu is fermented thin, gritty gruel that can be produced by maize, sorghum, and millet (Bvochora et al. 1999). Bvochora et al. (1999) evaluated the effect of fermentation processes on proanthocyanidins and phenolic compounds in four sorghum cultivars (Sorghum bicolor (L) Moench) during the preparation of Mahewu, a non-alcoholic beverage. The study revealed that fermentation decreased proanthocyanidins while the phenolic compounds increased. Tchoukoutou is a traditional opaque sorghum beer in Western African countries. It is known as tchoukoutou in Benin, dolo in Burkina-Faso, pito in Ghana, and burukutu or otika in Nigeria (Motlhanka et al. 2018). Compared to European lager beers, African traditional beverages are rich in calories vitamins, including thiamine, folic acid, riboflavin, and nicotinic acid (Tokpohozin et al. 2016). The available literature on the fermentation of Opaque sorghum has documented both decreases of phytate and increase in the total phenolic compounds (Kayodé et al. 2007). The increase in the level of reactive phenolic groups could be attributed to the germination and fermentation processes as well as from a shift in dry matter composition. Kombucha is a refreshing drink produced by fermentation of African mustard (B. tournefortii) leaves, for about14 days with indigenous bacteria (Acetobacter and Gluconobacter) and yeasts (Saccharomyces spp. and non-Saccharomyces spp) (Malbaša et al. 2015). A previous study showed that kombucha fermentation significantly increased total phenolic content, with the highest amounts in the ethyl acetate extract (Rahmani et al. 2019).

In Africa, the yeast fermentation of Raffia palm (*Raphia hookeri*) produces palm wine. It is an alcoholic beverage that popular in West Africa. It has different names based on production regions, it is named “mimbo” in Cameroon, “nsafufuo” in Ghana, and “emu” in Nigeria (Liburdi et al. 2020). It is rich in vitamin C, nicotinic acid, and thiamine (Erukainure et al. 2019a, b). Short-term open-air fermentation (24 and 48 h) of Raffia palm did not show significant improvements in phenolic compounds wine (Erukainure et al. 2019a, b). The increased enzyme inhibitory activity with fermentation up 48 h indicated that fermentation of Raffia palm wine might improve glycemic control. The authors suggested further researches to quantify products of fermented palm wine. In another study, Oboh and Okhai (2012) analyzed the phenolic compound in Raphia palm wine and local palm wine and found that palm has a higher content of phenols and flavonoids than those in local palm wine. In dolo samples (traditionally fermented sorghum beer in Burkina Faso), the average concentration of phenols and proanthocyanidins was 506 μg GAE/ml of dolo and 45 μg APE/ml of dolo, respectively (Abdoul-latif et al. 2012).

Dawadawa is the Hausa name for the fermented African locust beans, of Parkia *biglobosa,* Bambara groundnuts (BGN) (*Vigna subterranea*) and fermented *Hibiscus sabdariffa* seeds, and it is used commonly as condiments in the savannah

regions of West Africa. The name of the seasoning agents different from one region to another. For examples, in Burkina Faso known as Bikalga (Parkouda et al. 2008), Mari-Bi in Niger (Rabiou et al. 2019), Mbuja in Cameroon (Bouba Adji et al. 2007; Mohamadou et al. 2009), dawadawa and Iru in Nigeria (Oboh et al. 2008; Ibrahim et al. 2018), Fururndu in Sudan (Abu El Gasim and Mohammed 2008) and datou in Mali (Tounkara et al. 2011).

According to (Oboh et al. 2008) study, the fermentation process increases free phenolic content and decreases bound phenolic content of Iru samples (fermented African Locust Beans). Results demonstrated that fermentation increase the free phenolic and antioxidant activity of Iru samples, thus the consumption of fermented African locust might offer a rich source of dietary antioxidant compounds that could enhancement of dietary antioxidants during fermentation of African Locust Beans. In a study, (Adebiyi et al. 2019) investigated the nutritional value, anti-nutrients and phenolic composition of raw, hulled, and dehulled Bambara groundnuts (BGN) (*Vigna subterranea*) and their derived Dawdawa products. The results showed that both the raw sample and dawadawa contain important nutrients and phenolic compounds. The existence of higher phenolic, especially in the hulled dawadawa samples could be contributed to higher antioxidant activities, vital desirability from a health perspective. Similar higher phenolic and flavonoids were observed by Ademiluyi and Oboh (2013) when Bambara groundnut seed was used to produce a condiment, thus this condiment could serve as a cheap functional food for low-income families in Africa. Previous studies showed that fermentation reduced Total polyphenols and phytic acid in Fururndu made from sprouted of Roselle (Hibiscus sabdariffa L.) seeds (Yagoub et al. 2004; Omer and Yagoub 2007). From the above-mentioned studies, it clearly, there is need for further study to investigate the effect of fermentation on the individual phenolic compound presence in African fermented foods.

28.2.2 Vitamins

Vitamins are organic compounds that play an important role in the physiological functions of mammalian cells. A few studies on the effect of fermentation on vitamins of African fermented condiments, bread and beverages are reported in the literature (Tables 28.1 and 28.2). Available literature showed that African fermented foods various water-soluble vitamins including, vitamin C, thiamine, riboflavin, niacin, and folate (Fig. 28.2). These vitamins are not lost during the fermentation of foods because of the fact that the fermentation liquid comprises part of the food product. Recently, the used of fermented condiments have become common in the diet of many peoples. Apart from the fact that those flavoring agents improve the level of vitamins (Table 28.1). In Africa, legumes, oil bean seeds and cereals are widely used for the preparation of condiments rich in vitamins. Ugba is a flavoring agent produced by alkaline fermentation of oil bean seeds (*Pentaclethra macrophylla*). It is very popular for many low-income families particularly Ibos in the

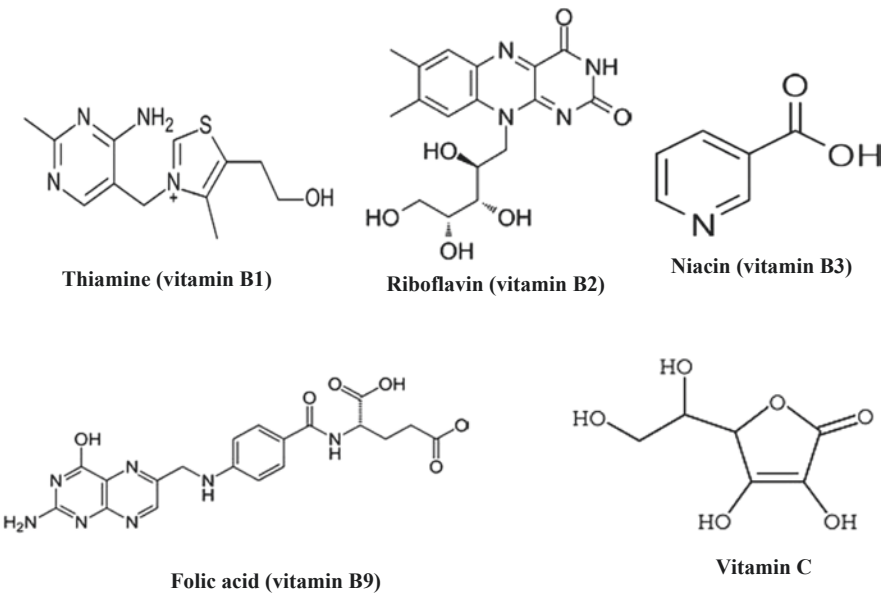

Fig. 28.2 B1, B2, B3, B9, and vitamins C

eastern part of Nigeria. Available information indicated that Ugba samples contained a considerable amount of Thiamin niacin and riboflavin (Olasupo et al. 2016).

In a study by Bationo et al. (2020), the total folate in cereal-based fermented foods that are frequently consumed by young children in West Africa was evaluated. Seven kinds including gelatinized doughs, porridges, dumplings, and fritters, made from corn, sorghum, or pearl millet were prepared according to the traditional methods used in Burkina Faso (Table 28.1). Their total folate content ranged from 1.8 to 31.3 μg/100 g on a fresh weight basis. Folate bioaccessibility, measured using a static in vitro digestion model, reached 23% to 81%. This study introduced new information on the effect of traditional processing on the folate level of African cereal-based fermented food (ACBFF) and the first data on folate bioaccessibility in West African CBFF. Another study was conducted by (Saubade et al. 2018) on the effect of fermentation and other processing steps on the folate content of Ben-saalga a traditional ACBFF. The results showed the fermentation process had no significant effect on folate content whatever the duration and the process employed. Thus, other processing such as germination of grains or incorporation of natural sources of antioxidants and vitamins need to be studied.

Fermentation of cereal-based products has been found to improve the level of most of these B-group vitamins. Mahgoub et al. (1999a, b) examined the effect of germination and fermentation on the levels of thiamine, riboflavin in Sudanese Kisra and Hulu-mur drinks produced from the two sorghum cultivars Dabar and Fetarita, consecutively. Fermentation of Kisra improved riboflavin but reduced thiamine content, while fermentation of Hulu-mur decreased the content of both

vitamins. Germination of Fetarita grains for 6 days results in an increase of riboflavin (700%) and a decreased in thiamine (42%). Besides, the authors observed that baking of Kisra has a slightly negative effect on the thiamine, while Hulu-mur baking results in the decrease of both thiamine and riboflavin. Injera is an Ethiopian fermented flatbread preferably prepared from whole-grain cereal (tef) (Tamene et al. 2019). The fermentation process can improve or reduce the levels (60–148%)) of folate of Injera while baking resulted in folate losses (52.8%.). the authors suggested that the folate content of Tef Injera (14.3 μg/100 g) can contribute up to 10% of the recommended nutrient intake of folate for children aged 1–3 and women of reproductive age. Tocopherols and other antioxidant compounds react as scavengers of peroxide radicals, thus inhibiting extensive peroxidation of lipids and other essential biomolecules (Barclay et al. 1990; Niki 1997). Ting samples showed a considerable concentration of tocopherols as presented in Table 28.2.

Zobo also called Zoborodo and Sobolo is a Nigerian non-alcoholic drink prepared from the roselle (*Hibiscus sabdariffa*) calyces. Fermentation with *Saccharomyces cerevisiae* and *Aspergillus niger* increased the concentration of vitamin C (Nwafor and Akpomie 2014). Tella is one of the several, native fermented beverages, traditionally produced and consumed in Ethiopia. Considerable concentration of Niacin(0.02–0.05 mg/100 ml) and folate (0.09–0.094mcg/100 ml) were reported vacuum-filtered and pasteurized tella samples (Tekle et al. 2019).(Oboh and Okhai 2012) investigated the vitamin C in Nigerian Raphia palm wine and local palm wine. Raphia palm wine had higher Vitamin C (0.35 ± 0.04 mg/ml ascorbic acid equivalent) as compared with local palm wine (0.29 ± 0.02 mg/ml ascorbic acid equivalent).

28.3 Conclusion

African ferment foods contain many kinds of bioactive components and fermented cereals, legumes, and oilseeds have been utilized to promote health in African countries. Microbial enzymes improve the levels of polyphenols and vitamins significantly during the fermentation of bread, porridges, condiments, and beverages. Thus, fermentation technology has been found to improve the levels of biologically active compounds in traditional African foods. With the fermentation process, the concentrations of many vitamins such as Thiamine (vitamin B1), vitamin B2 (riboflavin), Niacin (vitamin B3), vitamin B9 (folate), and Vitamin C in foods are increased. Generally, the present chapter show with great strength that fermented cereals, legumes, and oilseeds can be used as dietary sources of polyphenols and vitamins. However, there is a need for comprehensive studies for the determination of the phenolic profile of African fermented foods to provide valuable information to nutritionists and consumers.

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Chapter 29
Fermented Millet for Porridge Production: A Model for Improved Gastrointestinal Health

Afoakwah A. Newlove, Gustav Komla Mahunu, and Haroon Elrasheid Tahir

29.1 Introduction

Modern lifestyle has greatly influenced the choice of food consumed by people. The nature and demand of work and the need for wealth creation have made people become time conscious, thus making people adapt to the consumption of processed convenient foods than spending long hours in the kitchen preparing food. The need for breakfast food such as porridge is an essential diet for numerous nutritional and health benefits.

Porridge is mostly consumed as a complementary food for children above the age of two in Sub—Sahara Africa, providing nourishment to the sick and as a breakfast staple for adults (Onyango and Wanjala 2018; Rhim et al. 2011). The main ingredients used in the preparation can be grouped into two types; those from humid areas such as maize, finger and pearl millets as well as sorghum, then those from starchy tuber crops such as cassava, potato, or plantain (Onyango and Wanjala 2018; Thaoge et al. 2003). Moreover, according to Šimurina et al. (2018), two types of porridge can be classified based on its consistency: thin and thick porridge. Thick porridge contains less water and tends to be solid-like, whereas thin porridge is more liquid-like and can be sipped like a drink or soup. Half of the nutrients in porridge are carbohydrates (50–60%), followed by protein, which contains about 5–10% from porridge prepared with cereals such as millet (Jackson and Gradmann 2018; Om 2019).

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In recent times, cereal grains has become a significant global source of food for humans (Rathore et al. 2016), while in the past five decades, cereal productivity has doubled. Millet covers a total percent of 11.4 area used for cereals and a percent of 4.1 of the cereal supply (FAOSTAT 2008). Millet can survive under challenging climatic conditions such as less rainfall, no fertilizers, or other stressful biological situation.

There are numerous kinds of millets, they are named as Pearl millet (*Pennisetum glaucum* accounts for 40% of global production), Foxtail millet (*Setaria italica*), Proso-millet (*Panicum miliaceum*), and Finger millet (*Eleusine coracana*). Major seeds are produced by pearl millet, and it is usually consumed by humans (ICRISAT 2007). Barnyard millets (*Echinochloa spp*.) are minor millets. Kodo-millet (*Paspalum scrobiculatum*), Little-millet (*Panicum sumatrense*), Guinea millet (*Brachiaria deflexa* or *Urochloa deflexa*), Browntop millet (*Urochloa ramose* or *Brachiaria ramosa* or *Panicum ramosum*), Teff (*Eragrostitef*) and Fonio (*Digitaria exilis*) are also frequently named millets (ICRISAT 2007; FAO 2009).

Millets are served in many parts of Africa as a primary food component in local foods and beverages, such as fermented or unfermented-bread, porridges, and snack. Millets provides energy proteins, minerals, and vitamins in deprived communities and hard-working regions such as Northern Ghana. Moreover, millets have nutraceutical properties, such as reducing cancer risk, lower blood pressures, cholesterol issues, fat absorption, cardiac disease, gastric issues, and gastrointestinal bulk. They also provide nutritional and positive health care benefits (Rathore et al. 2016). A previous study has shown that primary fermentation of millet grain has been used for several processes, including the preparation of porridge with an innumerable groups of microorganisms, chiefly among them are starch degrading ones, and lactic- acid bacteria (Thirumangaimannan and Gurumurthy 2013).

The fermentation of millet-based porridges is a good alternative for other carbohydrate-rich cereal products with improved digestibility, enhancing gastrointestinal health in all persons. Fermentation of cereal grains is a low-cost food preservation method and an old-styled tradition, which is practiced within indigenous African communities and in most undeveloped countries (Blandino et al. 2003). It enhances the nutritional adequacy and, assimilation of raw-products, again it improves the sensory features of foods, that has been fermented (Borresen et al. 2012; Blandino et al. 2003). Mostly, the micro-organisms may form part of the diet or may be included as a starter culture before pretreating or during the cooking of the product (Mokoena et al. 2016). The usage of lactic acid bacteria (LAB) increases the acid value and decrease the pH substrate, thus hindering many disease causative agents called pathogens (Charalampopulos et al. 2002). Many LAB are utilized as probiotics, well-defined as “live microorganisms, which may confer a health benefit to the host” (FAO/WHO 2002).

This chapter explores fermentation procedures of millet, the methods employed for the preparation of fermented millet porridge, nutritional composition of millet and millet porridge and the significance of fermented porridge on gastrointestinal health.

29.2 Food Crops Used for Porridge

The type of ingredients and preparation of porridge differ from household to household and from one country to another. Oats are used to prepare porridge in the North of Europe and North of America (Šimurina et al. 2018), while rice is the primary ingredient of traditional Korea porridges (Rhim et al. 2011). Moreover, porridges in Sub-Saharan Africa is made from tropical cereals and root crops (Akande et al. 2017; Onyango and Wanjala 2018; Taylor and Emmambux 2008).

29.3 Production and Consumption of Millet

Globally, the area sown for the production of millet is about 38 million hectares, which have maintained relative stability in the last two decades. Its production and yield have risen to about 10% in the past two decades and have remained unchanged. Currently, the worldwide production of millet is about 28 million, with a projected mean yield of 0.75 t/ha. The estimated output trend in two leading producers, Africa and Asia, has indicated an essential difference at the regional production (Figs. 29.1a and 29.1b). The production trends in China have doubled in recent time, so as the consumption irrespective of the past decline in the past few decades. This was due to thorough scientific research work. However, the production trend of millet in Africa has rather seen a boost than a fall, as there is a rise in the production of millet from 8 million to 11 million tons in the past decades. Moreover, the consumption of millet in different countries has been influenced by the socio-economic status (FAO 2001).

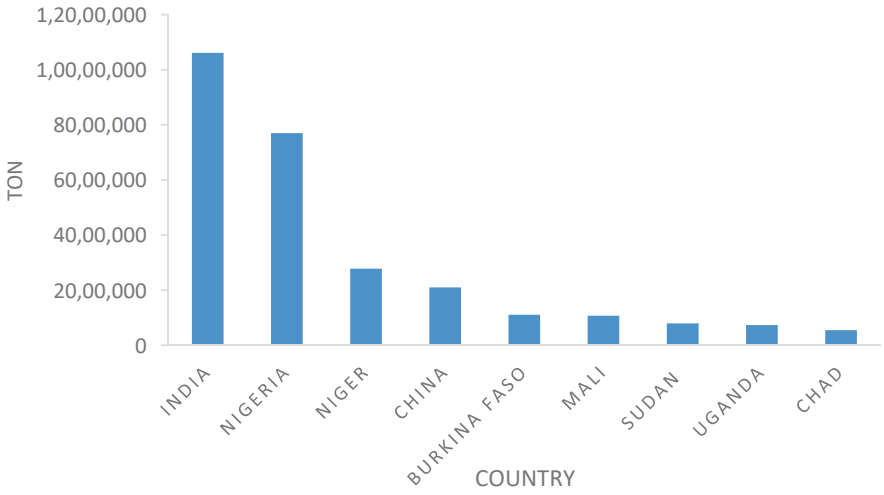

Fig. 29.1a Global millet production in tons, 2007: (Source: FAO 2009)

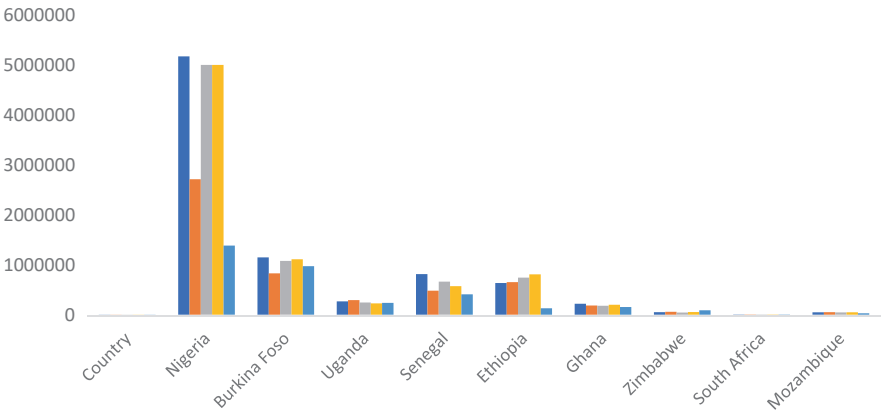

Fig. 29.1b Africa millet production in thousands per tons

29.4 Nutritional Value of Millet

Millets are rich in nutrients such as protein, calcium, and dietary fiber; again, it contains polyphenols (Devi et al. 2011). Table 29.1a signifies amino acid concentration in different kinds of millets. Millets are noted to own significant quantities of essential-amino acids, mostly the sulphur type amino acids, for example, methionine and cysteine. Besides, they contain superior fat content than sorghum, maize and rice. However, millets are inadequate in lysine and tryptophan concentration and may differ based on the type of cultivar, but several kinds of cereal-grains contain vitamins, essential amino acids, and minerals (Devi et al. 2011; FAO 2009).

Whole cereal grains are outstanding source of fiber (Table 29.1b). However, foods processed using grains have noticeable differences in the quantity and kind of dietary fiber component (Shukla and Srivastava 2014). The concentration of dietary fiber in cereal products differs based on the extent of milling. Finger millet has a higher carbohydrate concentration (Table 29.1b) compared to other cereal-carbohydrate (Devi et al. 2011). Millets are sources of minerals, vitamins and fatty acid (Tables 29.1c and 29.1d). Magnesium can decrease or act against headaches and heart attacks, but phosphorus is a vital component of the energy- precursor named adenosine triphosphate in the human body (Badau et al. 2005; Liang et al. 2010; Devi et al. 2011). However, polyphenols in cereals comprises of phenolic acids and tannins, but flavonoids are in minor quantities; they are known to be antioxidant and are responsible for the defense of the body immune network (Chandrasekara and Shahidi 2010; Devi et al. 2011).

Table 29.1a Amino acid profiles of diverse millet grains

Amino acids (g/100 g)	Foxtail millet defatted powder	Proso- millet hulls removed	Pear-millet	Finger-millet
Isoleucine	4.59	4.1	5.1	4.3
Leucine	13.60	12.2	14.1	10.8
Lysine	1.59	1.5	0.5	2.2
Methionine	3.06	2.2	1.0	2.9
Phenylalanine	6.27	5.5	7.6	6.0
Threonine	3.68	3.0	3.3	4.3
Valine	5.81	5.4	4.2	6.3
Histidine	2.11	2.1	1.7	2.3
Tryptophan	NA	0.8	1.2	NA
Alanine	9.30	10.9	8.1	6.1
Arginine	3.00	3.2	0.9	3.4
Aspartic acid	7.71	6.2	6.2	5.7
Cystine	0.45	NA	0.8	NA
Glutamic acid	22.00	21.3	22.8	23.2
Glycine	2.91	2.1	0.7	3.3
Serine	4.56	6.3	5.4	5.3
Tyrosine	2.44	4.0	2.7	3.6
Proline	5.54	7.3	8.2	9.9

Amadou et al. (2013), Kamara et al. (2009), Bagdi et al. (2011), Saldivar (2003) and Devi et al. (2011)

Table 29.1b Proximate composition of some millet varieties

Component g/ 100 g dry basis	Foxtail millet powder	Fonio	Proso millet	Peal millet	Finger millet
Protein	11.50	9–11	11.58	14.8	8.2
Ash	0.47	1–1.1	NA	1.68	2.7
Total CHO[a]	75.2	84–86	80.1	59.8	83.3
Crude Fiber	NA	NA	0.7	12.19	3.5

Source: NA (Not Available)
[a]CHO (Carbohydrate)
Amadou et al. (2013), Kamara et al. (2009), Vodouhe et al. (2003), Bagdi et al. (2011), Taylor et al. (2010), Devi et al. (2011) and Dayakar et al. (2017)

29.5 Useful Microbes for Millet Fermentation

LAB are the organisms needed for cereals-fermentation because of their beneficial role such food preservation, improvement of nutritional value, detoxification, flavour and aroma production. The predominant types are Lactobacillus, Lactococcus, Leuconostoc and Pedicoccus (Salovaara 2004). Other microbes include Corynebacterium, Saccharomyces cerevisiae and Streptococcus. Also, species of mould (Aspergillus, Penicillum, Fusarium and Cladosporium) may be used.

Table 29.1c Mineral content in millet varieties

Nutrients g/100 g	Foxtail millet	Kodo millet	Barnyard millet	Pearl millet	Finger millet
Phosphorus	290	188	280	296	130–250.0
Potassium	250	144	–	307	430–490
Magnesium	81	147–228	82	137	78–201
Calcium	31	27	20–22	42	398.0
Sodium	4.6	4.6		10.9	49.0
Zinc	2.4	0.7	3.0	3.1	2.3
Iron	2.8	0.5–5.0	5.0–18.6	8.0	3.3–14.89
Manganese	0.60	1.10–3.3	0.96	1.15	17.61–48.43
Copper	2.4	1.60	0.60	1.06	0.47

Ramashia et al. (2019), Ravindran (1991), Hassan et al. (2021) and Dayakar et al. (2017)

Table 29.1d Vitamins and essential oil found in finger Millet

Vitamins	mg/100	Fatty acid	g/100 g
Vit A (Retinol)	6.0	Palmitic	21.1–24.7
Vit B1 (Thiamine)	0.2–0.48	Oleic acid	49.8
Vit B2 (Riboflavin)	0.12	Linoleic acid	24.2
Niacin	1.0–1.30	Linolenic acid	1.3–4.40
Vit C (Ascorbic acid)	0.0–1.0	–	–

Hassan et al. (2021), Ramashia et al. (2019) and Dayakar et al. (2017)

29.6 Technique for Millet Fermentation

According to Thirumangaimannan and Gurumurthy (2013); the following are the basic techniques used in the fermentation of millet in millet-based porridge.

29.6.1 Natural Fermentation by Mixing Millets and Water in a 1:2 Ratio of Millet: Water

According to Stefano et al. (2017).

29.6.2 Millet Fermentation Using Water

Add 1 L of water to a hulled millet at different concentrations of 4%, 6%, 7%, 8%, and 10%. Heat the mixture up to 90–95 °C for 60 min. Optionally, add sugar or honey during the last 5 min of pre-treatment. Afterwards cool the mixture to 40 °C then bacterial cultures are added to the mixture.

29.6.3 Milk-Based Fermented Millet

Add 1 L of (3.25%) homogenized milk or 50% water and 50% milk to a hulled millet with different concentrations of 3%, 4%, 6%, 7%, 9%, and 10%. Then, heat the mixture to 85–90 °C for 30–60 min. Optionally, add sugar or honey at the last 5 min of pre-treatment. Subsequently, cool to 40 °C. Then, add bacterial cultures to the mixture.

29.6.4 Flour-Based Fermented Millet

Prepare 1 L of milk, water, or 50%: 50% milk and water respectively. Mix 200 ml milk, water, or 50% (water): 50% (milk) in a bowl containing 152 g of millet flour. Heat the remaining 800 mL milk until it boils. Once it boils, add the wet-flour mixture and stir for 15 min. After the pre-treatment, cool the mixture to 40 °C, finally add bacterial cultures to the mixture.

29.6.5 Dried Fermented Millet

Mix water and millet in a proportion of 2:1 respectively, and then heat to boil. Cool the mixture and let it to simmer under reduce heat until there is water uptake by the millet. After this, cool the mixture at a temperature of 40 °C, then add bacterial cultures to the mixture.

Other alternative techniques have been proposed by Osman (2010).

29.6.6 Traditional Fermentation with Lohoh Preparation

A proportion of 1:2 pearl millet flour and water are combined to make a dough; it is followed by incubation at 30 °C for 24 h using a sanitized covered flask. Before the fermentation starts, starter culture (5% inoculate) is then added to the dough.

29.7 Production of Fermented Millet Porridge

29.7.1 Fermented Millet Porridge in Africa

Millet is commonly used in Africa as a basis of porridge. In West Africa, fermented millet, sorghum, or maize is processed as a traditional gruel or porridge, known as *Ogi* (Blandino et al. 2003) *Ogi* as a weaning meal/ food used to wean infants. This is prepared when the grain is soaked (24–72 h) in water. It is then wet milled, and

sieved to eliminate the bran. The eliminated bran may be further undergo fermentation of 2–3 days to produce Ogi (Adebiyi et al. 2018).

In Ghana millet porridge called Hausa *koko*, produced by steeping the pearl millet overnight it is wet milled the with ginger, chilli pepper, black pepper, and clove. Water is added and sieved. Fermentation and sedimentation are allowed to take place for 2–3 h. After fermentation, the upper layer is heated within 1–2 h. Finally, the solidified sediment is added until a perfect uniformity is attained (Lei and Jakobsen 2004; Lei et al. 2006).

Botswana and Southern Africa also have a porridge from fermented maize, sorghum, or pearl millet, known as *bogobe* (*ting*) (Jackson et al. 2013). Similarly, Burkina Faso also has Dèguè (tchobal), prepared by hulling, wet grinding, and steam cooking to obtained gelatinous texture, then fermentation for 24 h is done (Abriouel et al. 2006; Hama et al. 2009).

29.8 General Processes for the Production of Fermented Millet Porridge

In general, the production processes of fermented millet porridge involve soaking process of the grain to soften the grain texture, wet milling, sieving, fermentation using lactic acid bacteria, sedimentation, then cooking (Abriouel et al. 2006; Hama et al. 2009). (Fig. 29.2).

29.9 Nutritional Value of Fermented Millet Porridge

Millets are rich in nutrients (Tables 29.1a, 29.1b, 29.1c, and 29.1d) (Kaur et al. 2012). According Onweluzo and Nwabugwu (2009) the percentage of carbohydrate and protein in millets is about 67% and 12% respectively. Subastri et al. (2015a, b), indicated that the main macronutrients of fermented (finger millet) porridge (koozh) are protein, carbohydrate, glycoprotein, and amino acids, but non-fermented millet porridge contains higher essential amino acids. This may be due to protein converting into peptides and amino-acids (Nkhata et al. 2018; Saleh et al. 2013; Subastri et al. 2015a, b). However, finger millets have higher anti-nutrient (phytates and tannins) concentration, which can decrease nutrient bioavailability. The content of these anti-nutrients can be reduced by utilizing germination and fermentation procedure as a food processing strategy (Makokha et al. 2002; Thuita 2010).

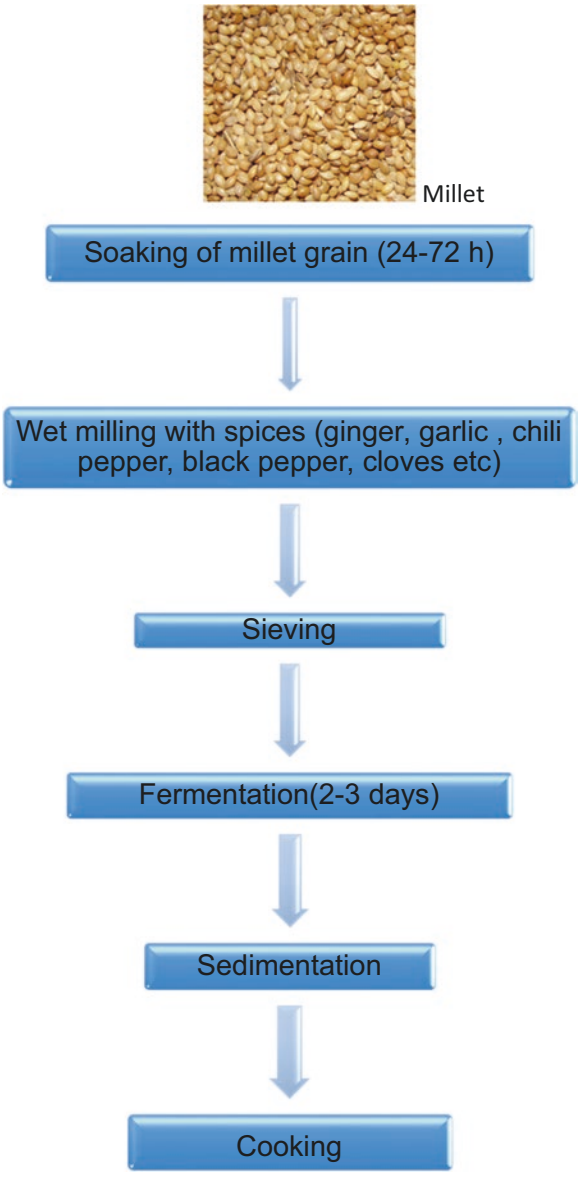

Fig. 29.2 The general procedure for the preparation of millet porridge

29.10 Importance of Fermented Millet Porridge

29.10.1 Potential Food/Nutrient Resource for Growing Population

When cereals are fermented, a higher mineral content, and a lower fat value are obtained, as compared to that of dairy-based counterparts. Fermented cereal based (millet) porridges deliver plant based functional-nutrients, such as fiber, vitamins, minerals, flavonoids and phenolic compounds, which might influence inflammation, oxidative stress, high blood sugar and unusual cell growth in humans (Wang et al. 2013). Producing a uncontaminated porridge from fermented grain (cereal) with cultures of probiotic, may prevent diarrhea and undernourishment caused by un-hygenic traditional-porridges used as a weaning food for children. This may decrease mortalities and improve well-being of consumers (Motarjemi et al. 1993), described an exciting intervention research utilizing fermented probiotic millet product for treating diarrhea in children. An African spontaneous fermented millet porridge called millet-*koko* was explained by Lei and Jacobsen (2004) as a possible probiotic millet-porridge, as well as *Mangisi, Kunu-zaki* and *Uji* a tinny, fermented porridge made from lactic acid (Amadou et al. 2011), also the high nutritional value of millets and its ability to grow in harsh climatic conditions makes it a perfect food source for developing or under developing countries with scarcity of water (Li et al. 2008). As the number of populations rises, it is crucial to find a substitute for staples to make sure the increase of food production is in accordance with the increase of population (Rathore et al. 2016).

29.10.2 Suitable Diet for People with Celiac Disease and Type 1 Diabetes

Patients with celiac disease are counselled to consume a gluten-free strict diet to curtail acute malabsorption, diarrhea, and folate deficiency (Hill et al. 2005; Scaramuzza 2013). Whereas persons having type 1 diabetes are requested to add low-glycemic index food to keep postprandial blood glucose-levels in order (Barclay et al. 2010; Scaramuzza 2013). For instance, Finger millet-grains are gluten-free, and can digest effortlessly. Low glycemic-index of Finger millet grains makes it a fit carbohydrate-containing food choice for celiac disease and type 1 diabetic (Ramashia et al. 2019) patients.

29.10.3 Contributes to the Lessening of Chronic Disease

Millets contain high amount of dietary fiber, carbohydrates, iron, calcium, magnesium, and phosphorus. Consuming this nutrient can decrease the chance of chronic disease, such as ischemic strokes, cancer, and heart disease (Kaur et al. 2012).

29.10.4 Contributes to Gastrointestinal Health

Fermented millet porridge may have an association linking microbial concentration and perfection of the gut microbiota, that is noted to be accountable for the health of people. While it is sometimes uncertain on the functional peculiarity fond in fermented millet porridge that confer farther the underlying nutrition of non-fermented millet porridges, there is corroboration that, some fermented foods give useful results thru direct microbial/probiotic mechanism and indirect way via the process of producing metabolites and a daedal protein breakdown. Fermented-millet porridge may have a link with 'beneficial-bacteria' termed probiotics (Kalui et al. 2010). Probiotics are valuable bacteria that positively modify the intestinal micro-flora, impede the growth of detrimental bacterial, help in digestion, enhance the immune system function and improve resistance to infection (Kalui et al. 2010). Individuals with healthy intestinal colonies made of beneficial bacteria may have the ability to fight disease causing bacteria (Kalui et al. 2010).

29.11 Conclusion

Fermented millet porridge is an essential gluten free breakfast meal suitable for consumption for millions of people of diverse cultural background. Children can rely on it as a complementary food for improving growth as adults and the sick enjoy it as a form of refreshment and a nourishment. The innumerable methods employed for the fermentation process and the preparation of the porridge have helped to improve the utilization of millet and its nutritional benefits. Additionally, the process has assisted in significantly reducing the enzyme inhibitors and phytic acid. The above indications can prove the huge positive impact of fermented millet on the gastrointestinal- health of both adults, the aged and children.

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Chapter 30
Fermented African Cereal Products

Maurice Tibiru Apaliya, Richard Osae, Emmanuel Kwaw, Gustav Komla Mahunu, Mildred Osei-Kwarteng, and Joseph Kwabena Ahima

30.1 Introduction

Fermentation is one of the oldest and most economical methods of producing and preserving food. Fermented foods are produced world-wide using various manufacturing techniques, raw materials and microorganisms. Since the beginning of human civilisation there has been an intimate companionship between the human being, his fare and the fermentative activities of microorganisms. These fermentative activities have been utilised in the production of fermented foods and beverages, which are defined as those products that have been subordinated to the effect of microorganisms or enzymes to cause desirable biochemical changes. The microorganisms responsible for the fermentation may be the microflora indigenously present on the substrate, or they may be added as starter cultures (Blandino et al. 2003). African countries require food processing technologies that will meet the challenges of the peculiar food security problems in the continent. Such a technology should be low-cost to be affordable by the poor sectors of the community and should be able to

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address the problems of food spoilage and foodborne diseases which are prevalent in the continent. Fermentation is one important food processing technology that meets these challenges. Fermentation is indigenous to the African cultures and has been used in many of these countries for centuries (Holzapfel 2002). Fermentation is recognized as a natural way to preserve and safeguard foods and beverages, enhancing the nutritional value, improving the digestibility, destroy undesirable components, and inhibit undesirable microorganisms (Marshall and Mejia 2011). The beneficial effect of fermented African cereal products on health is due to their excellent functional and nutritional properties assisted by fermenting microorganisms, and novel bioactive compounds released. The fermentation process and the resulting fermented products have recently attracted scientific interest as consumers are becoming aware of the possible positive role diet can play in disease risk management and perhaps because of their increasing interest in the relations between food and quality of life. In addition, microorganisms contributing to the fermentation process have recently been associated with many health benefits, and so these microorganisms have become another focus of attention (Achi and Ukwuru 2015). Africa has an age-old history of production of traditional fermented cereal foods and is perhaps the continent with the richest variety of lactic acid fermented foods (Franz et al. 2014). These cereal fermented foods have large impact on the nutrition, health and socioeconomic of the people of the continent, often plagued by war, drought, famine and disease. Although the African cultural arena is associated with a great diversity of cereals fermented foods, these are usually based on vegetable, protein, cereal or starchy root fermentations and, with some exceptions, less on dairy products, which are dominant in northern Europe and North America (Franz et al. 2014). For Africans, the importance of traditional fermented cereal food lies in providing improved flavors to existing staples (e.g. cereals and root crops), and as a cheap way of food preservation and an enhancement of the nutritional quality and digestibility of the raw products (Tamang and Kailasapathy 2010). Frequently, fermented African cereal foods are considered to have health benefits, and in many regions they are believed to aid in the control of some diseases, in particular intestinal disorders (Mathara et al. 2004). In African civilizations, fermented cereal food still plays a major role in combating food spoilage and foodborne diseases that are prevalent in many of its resource disadvantaged regions. A study conducted on the beliefs and consumption patterns of fermented food among mothers of children under years of age and health workers in rural and urban Kenya showed that the majority (83%) of rural mothers reported that their families regularly consumed fermented food and over half (66%) gave their young children fermented food (Anukam and Reid 2009). The use of isolated strains during cereal dough fermentation was reported to (1) minimise dry matter loss, (2) enhance control over the fermentation step, (3) enhance acid production or reduction in pH levels, (4) contribute to aroma and taste formation, as well as increase the overall acceptability of the product and (5) enhance the nutritional quality of the product through the formation of preservative compounds or a reduction in mycotoxins, such as aflatoxins and fumonisins (Agarry et al. 2010; Ekwem 2014). This is clear indication that fermented foods constitute the majority of diets of Africans. The traditional

cereal-based foods that are consumed in West Africa are processed by the natural fermentation of maize, sorghum and/or millet and are particularly important as weaning foods for infants and as dietary staples for adults (Soro-Yao et al. 2014). In terms of texture, the fermented cereal foods are either liquid (porridge or gruel), stiff gels (solid) or dry (fried or steam-cooked granulated products). The fermentation process is often carried out on small or household scales and are characterized by the use of simple, non-sterile equipment, random or natural inoculums, unregulated conditions, sensory fluctuations, poor durability and unattractive packaging of the processed products (Olotu et al. 2009). According to (Soro-Yao et al. 2014), the modern large-scale production of fermented cereal-based foods is almost entirely dependent on the use of defined strains of microorganisms, which could replace the undefined strain mixtures traditionally used for the manufacture of these products. The purpose of this chapter is to highlight the common fermented African cereal products and the microorganisms that are used to ferment such products. In addition, LAB that are commonly used to ferment African cereal products have also been reviewed.

30.2 Nature of Cereal Fermentation

Cereals are globally number one as food crops as well as substrates for fermentation. Well known products obtained from cereals are beer, sake, spirits, malt vinegar, and baked foods made from doughs leavened by yeasts or sourdough. When considering the multitude of foods made from cereals in Africa, one has to recognize that, their greater part has been subjected to fermentation processes taking place at least at one step of their generation. In fact, microorganisms and enzymes activities are important in cereal fermentation. Like other fermentation process, the understanding of the microbial ecology of cereal fermentations needs the knowledge of the fermentation substrates, such as the grains or seeds of the various cereal plants, as well as the products obtained thereof. In cereal fermentations endogenous enzymes, bacteria, yeast and moulds play roles either singularly or in combination, and contribute to the creation of a great variety of products (Hammes et al. 2005). The greater part thereof is subjected to fermentation and its volume surpasses by far that of all other fermented foods such as those made from milk (cheese and yoghurt), meat (fermented sausages), fish (fish sauce), soy (soy sauce), olives (fermented), or cabbage (sauerkraut). In many of those processes, cereal grains, after cleaning, are soaked in water for few days during which a succession of naturally occurring microorganisms will result in a population dominated by LAB. In such fermentations, endogenous grain amylases generate fermentable sugars that serve as a source of energy for the lactic acid bacteria. Titratable acidity, after fermentation among cereals varied markedly (0.84–1.46%) (Stahl 2014). More acid was produced at 37 °C than at 22–25 °C. Similar observations are also reported for sorghum (Stahl 2014). Cereals are deficient in lysine, but are rich in cysteine and methionine and therefore often combine with legume which are rich in lysine but deficient in

Table 30.1 Fermented African Cereal products

Country	Product	Substrate	Form of use	Microorganisms
Kenia, Uganda, Tanganyika	Uji	Maize. Sorghum, millet	Porridge as a staple	*L. mesenteriodes, L. platarum*
Zimbabwe	Tobwa	Maize	Non-alcoholic drink	LAB
Ghana	Banku	Maize	Dough as staple	LAB, moulds
Nigeria, West Africa	Ogi	Maize, sorghum or millet	Paste as staple. For breakfast or weaning food for babies	*L. plantarum, S. cerevisiae, C. mycoderma, Corynebacterium, Aerobacter, Rhodotorula, Cephalosporium*, *Fusarium, Aspergillus* and *Penicillium*
South Africa	Mawe	Maize	Basis for preparation of many dishes	LAB, yeast
South Africa	Mahewu	Maize	Solid staple	*Streptococcus lactis*
Sudan	Kisra	Sorghum	Staple as bread	Unknown
Ghana	Kenkey	Maize	Mush, steamed eaten with vegetables	*L. fermentum, L. reuteri, Candida, Saccharomyces, Penicillium, Aspergillus and Fusarium*
Ethiopia	Injera	Sorghum, tef, maise or wheat	Bread-like staple	*C. guilliermondii*
Egypt	Kishk	Wheat and milk	Solid, dried balls	*L. plantarum, L. brevis, L. casei, Bacillus*

sulphur containing amino acids (Iqbal et al. 2006). A range of indigenous fermented African Cereal products prepared from cereals are listed in Table 30.1. The flow chart for the preparation of cereal based products such as *Ogi, Kenkey* and *Mawe* is shown in Fig. 30.1.

30.3 Cereal-Based Fermented Beverages

Traditionally fermented beverages significantly contribute to food security in Africa. The major cereal grains used for making African non-alcoholic and alcoholic cereal-based beverages are sorghum (*Sorghum bicolour* (L.) Moench), pearl millet (*Pennisetum glaucum* (L.)), finger millet (*Eleusine coracana*) and maize (*Zea mays* (L.)) (McFarland 2015). These beverages are produced through fermentation of germinated cereals such as maize, millet and sorghum. Several studies have dealt extensively into the type of beverages found in African countries, the substrate and type of microorganisms used in the production of such beverages (Blandino et al. 2003; Nyanzi and Jooste 2012). Traditionally, cereal based beverages (non-alcoholic and alcoholic) in Africa are prepared through spontaneous fermentation.

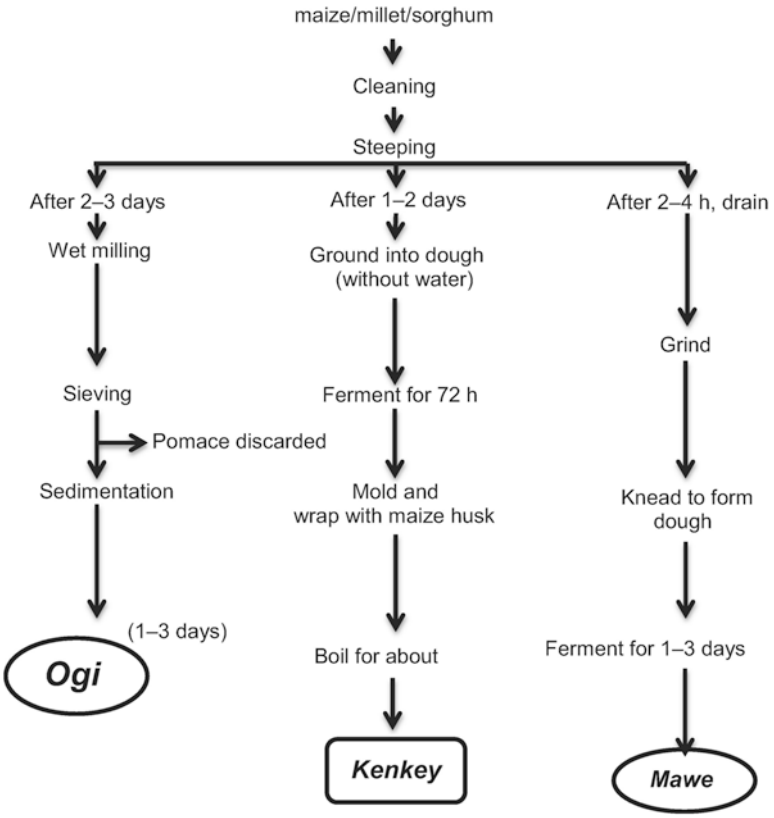

Fig. 30.1 A flow chart indicating how *ogi, kenkey* and *mawe* are prepared

Lactobacillus spp. and yeasts are mainly involved in this kind of fermentation (Aka et al. 2014). Lactic acid fermentation produces non-alcoholic beverages whereas lactic acid and alcoholic fermentations give rise to alcoholic beverages (Kohajdová 2017).

Cereal beverages are important to individuals, families and the society as a whole. Socially, when served; they show a gesture of hospitality, friendliness and also to strengthen amicable relationships between individuals (Aka et al. 2014). They are also consumed in farm work, ceremonies such as marriage and funerals and as supplement food; weaning food for babies (health giving drinks). The beverages also improve lactation in nourishing mothers and prevent coronary diseases and cancer. They are consumed by all social classes. Non-alcoholic beverages are guzzled by all age groups; infants, children, pregnant women, the old and sick people. Alcoholic beverages are mainly consumed by men (Kalui et al. 2010). They provide energy in the diet (starch, sugars, proteins) and valuable nutrients such as B-group vitamins; thiamine, niacin and riboflavin as well as vitamin C (particularly in sorghum beverages). They are also sources of minerals like iron, calcium,

potassium, magnesium, manganese, phosphorus and copper (Kohajdová 2017). Fermentation improves palatability, aroma, flavor and taste to cereal beverages as a result of production of organic acids and volatile compounds such as lactic acid, acetic acid, propionic acid and acetaldehyde during the process (Setta et al. 2020). Economically, the sale of fermented cereal based alcoholic and non-alcoholic beverages provide income to households particularly women and men who deal in them. Setta et al. (2020) reported that African fermented cereal beverages have prolonged shelf-lives because fermentation produces organic acids such as butyric, propionic, lactic as well as acetic acids which lower their pH to below 4 and consequently inducing inhibitory effects to growth and proliferation of both spoilage and pathogenic microbes. Beverages are primarily made with water, though milk may be used as well and additives added to improve the overall quality of the beverage (Fernandesa et al. 2021). Table 30.2 shows some African fermented beverages.

30.4 Fermented Probiotic Cereal Foods

There are some new cereal-based fermented foods that are considered as probiotic products. Some investigations have revealed that traditional African fermented cereal-based beverages are potential probiotic carriers because of the probiotic *Lactobacillus* spp. and yeasts which are involved in the fermentation of such products (Setta et al. 2020). Increased awareness of consumer health and interest in employing functional foods to achieve a healthy lifestyle has resulted in the demand for food products with versatile health-benefiting properties. Cereal-based probiotic foods have debuted only recently (Dornblaser 2007). Cereal and cereal component-based foods offer opportunities to include probiotics, prebiotics, and fiber in human diet. Whole grains consumption has been correlated to health benefits including reduction in type 2 diabetes risk, cardio vascular disease, obesity, and certain types of cancers (Clemens and Pressman 2006). The bran and germ of cereals supply the majority of the biologically active components. Were such cereal components employed as probiotic carriers, they would additionally benefit the consumer by bringing in healthful bioactive components and fibers in the food system along with the probiotic microorganisms. Further, grain based probiotic carriers resist gastric acids enabling probiotic organisms to be delivered to lower intestine with less loss in viability (Lamsal and Faubion 2009). The role of cereals or cereal components as probiotic growth media and carriers have not been fully addressed. When various cereal components such as water-soluble and insoluble β-glucan and arabinoxylans, oligosaccharides, and resistant starch etc. are utilized to grow probiotic microorganisms, it is possible to realize the beneficial effect of both the probiotics and prebiotic effects. In Africa, whole grain or grain components are fermented to produce beverages, gruels, and porridges. Other traditional cereal-based fermented foods has been modified to aid the control of some diseases (Achi and Ukwuru 2015). An improved ogi named Dogik has been developed using a lactic acid starter with antimicrobial activities against some diarrhoeagenic bacteria (Nyanzi and Jooste 2012).

Table 30.2 Fermented African beverages

Country	Product	Substrate	Beverage	Microorganism
Ghana	Koko	Maize	Porridge as staple	*Enterobacter clocae, Acinetobacter., Lactobacillus platarum, L. brevis*
South Africa	Kaffir beer	Kaffir corn	Alcoholic drink	Yeasts, LAB
Zimbabwe	Kachasu	Maize	Alcoholic beverage	Yeasts
Sudan	Nasha	Sorghum	Porridge as a snack	*Streptococcus, Lactobacillus, Candida, Saccharomyces cerevisiae*
Zambia	Munkoyo	Kaffir corn, millet or maize plus roots of munkoyo	Liquid drink	Unknown
Sudan	Merissa	Sorghum and millet	Alcoholic drink	*Saccharomyces*
Ethiopia	Talla	Sorghum	Alcoholic drink	Unknown
South Africa	Sorghum beer	Sorghum, maize	Liquid drink	LAB, yeast
Zimbabwe	Mangisi	Millet	Sweet-sour non-alcoholic drink	Unknown
Nigeria	Otika	sorghum	Alcoholic beverage	Unknown
Zimbabwe	Ilambazi lokubilisa	Maize	Porridge as weaning food	LAB, yeasts and moulds
Nigeria	Kwunu-Zaki	Millet	Paste used as breakfast dish	LAB, yeasts
Zimbabwe	Doro	Finger millet malt	Colloidal thick alcoholic drink	Yeasts and bacteria
Nigeria	Dalaki	Millet	Thick porridge	Unknown
Zimbabwe	Chikokivana	Maize and millet	Alcoholic beverage	*S. cerevisiae*
Nigeria, Ghana	Busaa	Maize	Alcoholic beverage	*L. helveticus, L. salivarus, L. casei, L. brevis, L. plantarum, L. buchneri, S. cerevisiae, P. damnosus*
Nigeria	Seketeh	Maize	Alcoholic beverage	*S. cerevisiae, S. chevalieri, S. elegans, L. plantarum, L. lactis, B. subtilis, A. niger, A. flavus, Mucor rouxii*
Nigeria, Ghana	Pito	Maize, sorghum	Alcoholic dark brown drink	*G. candidum, Lactobacillus, Candida*
Zimbabwe	Mutwiwa	Maize	Porridge	LAB, bacteria and moulds
Egypt	Busa	Rice or millet	Liquid drink	*Lactobacillus, Saccharomyces*

(continued)

Table 30.2 (continued)

Country	Product	Substrate	Beverage	Microorganism
Nigeria, Benin, Ghana	Burukutu	Sorghum	Alcoholic beverage of vinegar-like flavou	*Saccharomyces cerevisiae, S. chavelieri, Leuconostoc mesenteroides, Candida, Acetobacter*
Egypt	Bouza	Wheat	Thick, acidic	Unknown
Botswana	Bogobe	Sorghum	Soft porridge staple	Unknown

Development of fermented cereal based probiotic beverages from traditional African fermented cereal beverages will provide accessible, low cost and acceptable probiotic products especially to rural people. Traditional African fermented millet-based beverages with probiotic potential because of some probiotics they contain include Koko (West Africa), Mangisi (Zimbabwe and Uganda), Uji (East Africa), Burukutu and pito (Togo, Benin), Kunun zaki (Nigeria, Niger, Tchad), Ogi (West Africa), Ben-saalga (Burkina Faso), Togwa (Tanzania). Other studies with positive results on development of fermented cereal-based probiotic beverages include those by (Di Stefano et al. 2017; Ogunremi et al. 2015; Salmerón 2017).

Fermentation of cereals reduces/removes antinutritional factors and mycotoxins from them and thus provides essential minerals and vitamins to beverage consumers (Nyamete et al. 2016; Nyanzi and Jooste 2012). The probiotics they contain produce metabolites such as organic acids and bacteriocins which act as biopreservatives for the beverage. Consequently, millions of Africans will derive health benefits from the health-giving microorganisms. Cereals make up the larger portion of staple diet for majority of people in Africa and other developing countries and thus there is justification for more researches on the use of spontaneously fermented cereal beverages as probiotics carriers. Moreover, it is known that 60% of the diet in developing countries is prepared from fermented cereals (Waters et al. 2015). Probiotics have a great potential for improving nutrition, improving the immune system, soothing intestinal disorders, optimizing gut ecology, and promoting overall health because of their ability to compete with pathogens for adhesion sites, to antagonize pathogens, or to modulate the host's immune response (Bonifait et al. 2009; Kore et al. 2012).

30.5 Lactic Acid Bacteria in Fermented African Cereal Products

According to Aguirre and Collins (1993), the term LAB is used to describe a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, usually non-motile, that utilize carbohydrates fermentatively and form lactic acid. LAB comprise a significant component of the human gut flora and have several beneficial roles in the gastrointestinal tract (Mokoena et al. 2016). Lactic acid bacteria (LAB)

are the primary microorganisms used to ferment maize, sorghum or millet-based foods that are processed in West Africa. Fermentation contributes to desirable changes in taste, flavour, acidity, digestibility and texture in gruels (baca, ogi, dalaki), doughs (banku, agidi, komé) or steam-cooked granulated products (ciacry, arraw, dégué) (Soro-Yao et al. 2014). Fermentation of cereal-based beverages, as well as other food substrates, by LAB has been shown to improve protein digestibility (Taylor and Taylor 2002), increase nutritional bioavailability of minerals and other micronutrients (Agarry et al. 2010; Greffeuille et al. 2011) prolong shelflife, (Gupta et al. 2010), and enhance organoleptic qualities (Luana et al. 2014; Peyer et al. 2015). Lactic acid fermentation contributes towards the safety, nutritional value, shelflife and acceptability of a wide range of cereal based foods (Vieco-Saiz et al. 2019). Fermented traditional foods and beverages in developing countries constitute one of the main dietary components, with some being used as light meals or refreshments (Mokoena et al. 2016). LAB isolated from various fermented foods produce organic acids and a high diversity of antimicrobial agents, which are responsible for the upkeep of quality and the palatability of fermented foods. According to (Mokoena et al. 2016) LAB are used in African communities to produce a variety of fermented foods which include cereal-based fermented porridges, beverages, fermented fruits and vegetables (including roots or tubers), fermented milks, and fermented meats. *Amasi* is produced by using specific LAB such as *Lactobacillus delbrueckii* and *Streptococcus* spp. *Amahewu* is produced with LAB starter culture, whereas umqombothi is produced from maize or sorghum via wild yeast fermentation, and LAB from malted sorghum adjuncts play a limited role (Chelule et al. 2010). Sour porridge is maize or sorghum, which is fermented using mainly LAB to improve and develop palatability, flavor, and nutrition (Abdelgadir et al. 1998).

30.6 Starter Culture Used in Fermentation of Cereal Products

The genera *Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Pediococcus,* and *Weissella* are naturally found on the surface of grains and in the surrounding environment (Guyot 2012). The composition of the microbial community in a fermented food product largely determines key product properties (Macori and Cotter 2018). Spontaneous fermentation is the process used in making all African traditional fermented cereal beverages and the procedure of how to make such beverages has been handed down from one generation to another. Since starter cultures are not used such fermentation is uncontrolled and thus the quality and stability of the products are compromised (Guyot 2012). Investigations made have so far revealed that LAB and yeasts are the major groups of microbes in fermented cereal beverages (Aka et al. 2014; Guyot 2012). Successful use of various species of LAB and yeasts as pure starter cultures and co-cultures to produce traditional

fermented cereal products have been reported by (Aka et al. 2014). *Pito* produced from (Nigeria, Ghana) and opaque sorghum beer *tchoukoutou* (Benin, Togo) are drinks in which the co-culture has been used. It has also been observed that production process of enturire; an alcoholic sorghum-and honey-based beverage produced in Uganda can be modified and shortened by using pure starter cultures instead of spontaneous fermentation (Mukisa et al. 2017). Based on the above studies; Africa needs to develop pure starter cultures which contain efficacious microbial strain that can lead to control and optimization of the fermentation process so that beverages produced are of high organoleptic quality and microbial stability. It is thus obvious that selection of an appropriate strain or strains for a particular fermented product is paramount as a first step in a fermentation process which is controllable, predictable and efficient.

30.7 Biochemical Changes During Cereal Fermentation

Cereal grains are considered to be one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fibre for people all over the world. However, the nutritional quality of cereals and the sensorial properties of their products are sometimes inferior or poor in comparison with milk and milk products. A number of methods have been employed, to ameliorate the nutritional qualities of cereals some of which include processing technologies such as cooking, sprouting, milling and fermentation, although probably the best one is fermentation (Mattila-Sandholm et al. 1999). In general, natural fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly and oligosaccharides (Blandino et al. 2003). Certain amino acids may be synthesised and the availability of B group vitamins may be improved. Fermentation also provides optimum pH conditions for enzymatic degradation of phytate which is present in cereals in the form of complexes with polivalent cations such as iron, zinc, calcium, magnesium and proteins (Poutanen et al. 2009). Such a reduction in phytate may increase the amount of soluble iron, zinc and calcium several folds. Fermentation significantly improves the protein quality as well as the level of lysine in maize, millet, sorghum, and other cereals (Hamad and Fields 1979). It appears that the effect of fermentation on the nutritive value of foods is variable, although the evidence for improvements is substantial. Fermentation also leads to a general improvement in the shelflife, texture, taste and aroma of the final product. During cereal fermentations several volatile compounds are formed, which contribute to a complex blend of flavours in the products. The presence of aromas representative of diacetyl acetic acid and butyric acid make fermented cereal-based products more appetizing (Table 30.3). Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, corn or sorghum) are well known in many parts of the world. Some are utilized as colorants, spices, beverages and breakfast or light meal foods, while a few of them are used as main foods in the diet. The microbiology of many of these products is quite complex and unknown. In most of these products the

Table 30.3 Compounds formed during cereal fermentation (Campbell-Platt 1994)

Organic acids		Alcohol	Aldehydes and ketones	Carbonyl compounds
Butyric	Heptanoic	Ethanol	Acetaldehyde	Furfural
Succinic	Isovaleric	n-Propanol	Formaldehyde	Methional
Formic	Propionic	Isobutanol	Isovaleraldehyde	Glyoxal
Valeric	n-Butyric	Amy alcohol	n-Valderaldehyde	3-Methyl butanal
Caproic	Isobutyric	Isoamyl alcohol	2-Methyl butanol	2-Methyl Butanal
Lactic	Caprylic	2,3-Butanediol	n-Hexaldehyde	Hydroxymethyl furfural
Acetic	Isocaproic	β-Phenylethyl alcohol	Acetone	
Capric	Pleargonic		Propionaldehyde	
Pyruvic	Levulinic		Isobutyraldehyde	
Plamitic	Myristic		Methylethyl ketone	
Crotonic	Hydrocinnamic		2-Butanone	
Itaconic	Benzylic		Diacetyl	
Lauric			Acetoin	

fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi. Some microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant flora during the course of the fermentation (Steinkraus 1998). The type of bacterial flora developed in each fermented food depends on the water activity, pH, salt concentration, temperature and the composition of the food matrix.

30.8 Nutritional and Sensory Characteristics of African Fermented Foods

Fermentation may improve the nutritional value, sensory attributes and functional qualities of cereals (Navarrete-Bolaños 2012). The nutritional and sensory quality characteristics of these beverages are closely linked to the different microorganisms they contain. Regarding the sensory characteristics of cereal-based products, usually hetero-fermentative metabolism, synthesis of aroma compounds or their precursors, and release of free amino acids through proteolysis are considered important (Gobbetti et al. 2005).

30.9 Conclusion

The value of fermented African cereal products cannot be overemphasized, as they have always played a vital role in diet and nutrition. The benefits associated with African cereal products, such as increased shelflife, palatability, and nutritional value, suggest the importance of these foods. The probiotic effects of various fermented African cereal foods and their associated microbial cultures look promising and warrant further research. Fermentation of African cereal products of probiotic LAB from traditional foods can be a suitable alternative source of antimicrobial agents, incorporated in the fight against emerging antibiotic-resistant microorganisms. Probiotic bacteria have a potential in solving current and emerging lifestyle diseases. The consumption of fermented African cereal foods is part of African culture and will continue to be so. There is therefore the need to conduct further research on the use of novel fermentation technologies aimed at improving the quality of fermented cereal African products in order to derive maximum benefit from them.

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Chapter 31
Fermentation of Cocoa Bean

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31.1 Introduction

Although the quality characteristic of the cocoa bean is determined primarily by genetic factors. Fermentation processes and agronomic practices can modify the innate flavor precursors of the cocoa bean. The development of this flavor precursors involves the activity of various microorganisms and enzymes on proteins, carbohydrates and polyphenols in the cocoa beans. Depending on the producing country, different fermentation techniques are employed in fermenting cocoa bean, with all aimed at removing the bean from the pulp. This results in fermented dried beans with varying compositional profile.

Several studies have profiled the influence of processing techniques, time of fermentation and pod storage on the antioxidant capacity, macro- and micronutrients densities, amino acid profile pH and titratable acid of fermented dried cocoa beans (Afoakwa et al. 2012, 2013; Camu et al. 2007; Fang et al. 2020; Lefeber et al. 2012; Sulaiman and Yang 2015). This chapter explore conventional and modern research

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outcomes in the concepts and methods of cocoa bean fermentation, as well as their effects in compositional profile of fermented dried cocoa beans.

Cocoa tree (*Theobroma cacao* L.) is an economical viable agricultural commodity supporting several economies either directly through export of raw cocoa bean (mostly developing countries) or indirectly through import/export of processed cocoa products such as chocolate. For optimum growth and development, the tree requires high temperatures with humid environment. As a result, it grows well in the hot and humid regions of Africa, South and Central America, and Asia. There are three classes of *T. cacao*, Criollo, Forastero and Trinitario which is a hybrid from Criollo and Forastero. Matured and ripe pod from criollo is characterized with long pod with yellow or red color. Although this variety yield less compare to the others, they produce bean with much better chocolate characteristics. They are largely grown in America. On the other hand, Cocoa pod from Forastero is characterized with short pod with smooth yellow skin. They are commonly used in commercial production due to their generic resistance to diseases. This variety is mostly grown in Africa. The major economical part of *T. cacao* is the cocoa bean.

Cocoa production plays a significant role in the social and economic development of many rural farmers especially West Africa which is known to be the production hub of cocoa. It is one of the key drivers in the African's gross domestic product (GDP). As a cash crop it provides livelihood to about 40–50 million people worldwide. In Ghana about 800,000 people depend on cocoa for livelihood. Although several studies seek to suggest that African economy may report a major downturn due to decline in cocoa production, resulting from current conditions (inadequate technology usage and infrastructure) of cocoa farmers, Ghana stand a chance to gain from this draw backs due to the fermentation techniques employed in cocoa processes as well as its market structure.

This chapter review highlights the changes in fermentation conditions, which has significant effect on physical and chemical properties of the cocoa beans. It also considers how the fermentation conditions facilitate color change of cocoa bean to the desired chocolate color. Fermentation impacts significantly on precursor compounds for aroma and flavor characteristics of chocolate.

31.2 Nutritional Value and Health Benefits of Fermented Cocoa Beans

The cocoa beans are enclosed in a white sweet pulp, when the pods are fresh. Due to the rich polyphenolic content, seeds appear reddish brown outside and dark brown inside. The pulp is sweet scented and mucilaginous; made up of spongy parenchymatous cells with a sap rich in carbohydrates. The pulp as well as the beans make up about $^{1}/_{3}$ to half of the total pod weight (125–200 g). With regards to food, the beans and pulp from a single pod contain sufficiently, half the daily requirement (450–700 calories) of a small person. About 100 g of cocoa powder can provide

10–20% of the energy needs of a young adult (Rosenberg et al. 2004). Cocoa powder contribute substantial amounts of vitamin and mineral needs a human being (Raw 2012). The seeds of cacao have fat (about 40–50%) and protein (14–18%) but the amount present depends on the time of harvest. On a per unit energy basis (e.g., expressed as an amount per calories), cocoa has adequate vitamins, even though cocoa is not an optimal vitamin source as a single food. Nevertheless, it is an excellent food source of essential minerals; 50–100 g of processed cocoa powder can easily meet the daily human requirements of most of the essential trace minerals or elements (Haytowitz 2015). The protein content in cacao is satisfactory on a caloric or energy basis, although the protein quality is slightly marginal as the concentration of two essential amino acids (methionine and cysteine) is too low on its own to meet human needs (Maleyki and Ismail 2010).

The complex composition of cocoa beans makes it commercially important raw material. Various parts of the cocoa bean exhibit diverse activities with their corresponding physiological effects. Therefore, the complete understanding of this information is important for successful use of the beans. The fatty constituents of the beans direct their nutritional value and application in various food and non-food products. The content of xanthine alkaloids in the bean produces the diuretic effect, which excites the central nervous system, as well as stimulate the cardiovascular activity (Rusconi and Conti 2010). Rich polyphenol content in cocoa beans is due to antioxidant properties and effect on reactive oxygen species (ROS) (Andújar et al. 2012). The quantity of catechins are considered very important for healthy properties of cocoa and related products (Caprioli et al. 2016). The volatile constituents of cocoa powder are very important determinant of flavor profiles of the final product (Li et al. 2012). Also the activities and effects of other substances including simple organic acids, and gamma-aminobutyric acid (GABA) make cocoa beans potential candidate in the nutraceuticals and food supplements aside chocolate as the primary product (Ackar et al. 2013).

Cocoa bean is composed of two cotyledons and an embryo enclosed by a seed coat. From the nutritional perspective, the cocoa beans are made up of mainly water (32–39%), total fat (30–32%, of which 65% is saturated fat), proteins (8–10%), cellulose (2–3%), starch (4–6%), pentoses (4–6%), sucrose (2–3%), polyphenols (5–6%), organic acids, mainly citric, oxalic, and malic (1%), theobromine (1–3%) and caffeine (0.2–0. 1%) (de Melo Pereira et al. 2016). This chemical composition of cocoa beans and pulp can be considered an appropriate medium for microbial growth (Ozturk and Young 2017). Once the pulp and the cocoa beans are removed from the pod (mechanically or manually), they interact with a vast variety of microorganisms from the environment like the tools used to open them or worker's hands.

Over the past few decades, with the development in the rapid use of advanced biological tools (engineering and biotechnology) in food product development particularly fermentation, the cocoa processing sector has developed innovative application prospects, with extensive economic and social impact. The processing of raw cocoa bean into cocoa mast such as cocoa liquor, cocoa butter, cocoa cake and cocoa powder consist of a number of stages (Fig. 31.1). Among the cocoa producing countries, Ghana cocoa beans are well fermented, with high flavored beans

Fig. 31.1 Flow chart of cocoa from the tree through typical traditional fermentation produce and drying process to the cocoa bean products

compared to the others (less fermented and of lower quality due to their bitterness and low cocoa flavor).

31.3 Fermentation

Fermentation seeks to liquefy and degrade the residual mucilage layer/pulp; it kills the bean; and initiates the development of aroma, flavor and color. The best type of cocoa fermentation method mostly practiced in various countries have been reported; heaps (e.g. Ghana, and Ivory Coast) (Daniel et al. 2009; Samagaci et al. 2016), baskets (e.g. Nigeria and Ghana) (de Melo Pereira et al. 2016), boxes (e.g. Brazil and Malaysia) (Arana-Sánchez et al. 2015; Papalexandratou et al. 2013; Papalexandratou et al. 2011b), trays (Ghana)(Crafack et al. 2014; Crafack et al. 2013; Kostinek et al. 2008), sacks (Ecuador) (Schwan and Wheals 2004), platform (Papalexandratou et al. 2011a, b). The process of fermentation is driven by a complex action of microbial interactions.

31.4 Effect of Fermentation on Product Quality

31.4.1 Volatile Compounds

Aromatic profile of cocoa beans is a key player in the classification of cocoa bean. The methods and time of fermentation are also critical factors that combine with the genotypes, agroclimatic conditions, drying and industrialization processes to influence volatile compounds in cocoa bean. It was reported that cocoa beans fermentation for 24 h or lower, produces high concentrations of flavour precursor compounds:

2-methylpropanal, 2,3-butanedione(diacetyl)/2-pentanone, 2-pentanol, methyl acetate, 2-heptanone,2-pentyl propanoate, 1-pentanol, 2/3-methylbutanal, tetrahydro-2-methyl furan, 2-methyl-1-propanol, and ethyl acetate (Afoakwa et al. 2015). Whereas 72 h or above cocoa bean fermentation produces higher concentrations of propanoic acid, linalool oxide, 3-hydroxy-2-butanone, 2-methyl propanoic acid; these are important odor-active compounds of the cocoa bean. The complex interactions noted between polyphenols to constitute high molecular weight tannins and their interactions with protein actually influence the total quality of fermented cocoa beans for chocolate production. During cocoa beans fermentation, polyphenols diffuse with cell liquids from their storage cells and they are oxidized enzymatically through the polyphenol oxidase toward condensed high molecules mostly insoluble tannins. Evidentially, increased pod storage and increased in fermentation time, decreased sharply the total polyphenol content (Afoakwa et al. 2012).

Postharvest handling practices of fresh cocoa beans such as pod storage, enzymatic and mechanical depulping have effect on the acidity profile of fermented cocoa beans. Fermentation helps obtain desirable characteristics; to cure the astringency, unpleasant taste and flavor of the fresh cocoa beans. Through fermentation, bitter and astringent taste, considered undesirable are transformed into durable qualities like fruity, floral and cocoa flavors (Afoakwa et al. 2015). The mechanism of producing acceptable flavour is that the phenolic compounds are oxidized and polymerized to tannins (insoluble high molecular-weight compounds) leading to a significant reduction of its concentration (Misnawi 2008). This implies that fermentation involves complex chemical and biochemical changes to obtain quality flavor. Flavor precursors (free amino acids and peptides) that occur through degradation of protein enzymes (Afoakwa et al. 2013; Krähmer et al. 2015). Fermentation also contributes to increased alcohols, organic acids, esters and aldehydes (Magi et al. 2012). Furthermore, Sulaiman and Yang (2015) discussed color changes during fermentation. It was found that increase of pod storage earlier to fermentation enables color change, which further explains the production of the best chocolate color cocoa bean by small holder farmers in Africa. The general conclusion was that time and method of fermentation are important consideration with regards to qualities of flavor compounds and flavor precursors (Kongor et al. 2016).

31.4.2 Proximate and Micronutrients Densities of Cocoa Bean

The length of pod storage is very key among postharvest management of cocoa bean processing into fermented dried bean. Regardless of the pod storage method, the extent of storage has significant effect on the macro- and micronutrients profile of fermented dried cocoa beans. Afoakwa et al. (2013) reported that the extension of pod storage time before fermenting did significantly reduced ash, protein and fat contents of bean as well as micronutrient levels (Table 31.1).

Table 31.1 Effect of pod storage (pulp pre-conditioning) and fermentation on proximate composition of cocoa beans

Pod storage (Days) before fermentation	Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
0	Unfermented	4.2 ± 0.02	21.6 ± 0.83	55.2 ± 0.10	3.5 ± 0.11	15.5 ± 0.63
	Fermented	4.0 ± 0.02	18.8 ± 0.56	53.4 ± 0.63	2.8 ± 0.07	21.0 ± 0.08
7	Unfermented	4.4 ± 0.04	20.8 ± 0.05	53.3 ± 1.5	2.9 ± 0.05	18.6 ± 0.72
	Fermented	4.3 ± 0.09	18.2 ± 0.13	52.2 ± 0.05	2.3 ± 0.04	23.1 ± 0.54
14	Unfermented	4.2 ± 0.02	19.7 ± 0.06	52.5 ± 0.04	3.1 ± 0.01	20.5 ± 0.24
	Fermented	4.5 ± 0.03	17.6 ± 0.60	50.5 ± 0.15	2.7 ± 0.18	24.7 ± 0.31
21	Unfermented	4.9 ± 0.01	20.4 ± 0.48	52.3 ± 0.07	3.3 ± 0.05	19.1 ± 0.09
	Fermented	3.8 ± 0.04	17.9 ± 0.07	50.4 ± 0.05	2.9 ± 0.09	24.9 ± 0.11

Modified from Afoakwa et al. (2013)

31.4.3 Antioxidant Activities

Generally, fermentation has the potential to impact on the bioavailability of antioxidant compounds including ABTS or DPPH in food products. More so, the level and rate of scavenging ability would somewhat as well influence the microorganisms acting in fermentation. Several studies assessed the effect of fermentation rates on the phenolic concentration and antioxidant activity of fermented cocoa. The results showed that the increasing fermentation time decreased the antioxidant potential of the cocoa beans. Fang et al. (2020) relied on ABTS and DPPH as guides in the study of scavenging activity of fermented cocoa beans. Here, same authors indicated that scavenging ability of cocoa bean to ABTS and DPPH decreased with constant fermentation of 7 days. Suazo et al. (2014) also mentioned that a significant decrease was observed in phenolic content (from 115 ± 2 at the start point to 43 ± 1 mg/g) and antioxidant activity (from 709 ± 17 at the start point to 124 ± 4 μM) of fermented cocoa beans.

31.5 Selection Criteria for Cocoa Starter Culture

According to Vinicius De Melo Pereira et al. (2020) starter cultures are selected microbial preparations that are applied to increase the efficacy of the processes of fermentation. Several microbial cultures have been used in the food industry to ensure the production of and high-quality commodities. Also, numerous studies have been designed for microbial starter culture and the evidence is starter cultures are practical options to obtain a reliable fermentation process and therefore desirable characteristics of chocolate. The basis of selecting and isolating functional microorganisms from natural cocoa bean fermentation is due to their inherent ability to yield aromatic volatiles compounds. Apparently, fermentation of

cocoa beans is still natural, often under uncontrolled *in situ* farm (on-farm) environments. Therefore, in order to improve the excellent characteristics of the fermented dried cocoa beans and the cocoa bean product. Microorganisms (including yeast, lactic acid bacteria and acetic acid bacteria) constitute the major categories that occurs in natural fermentation of cocoa beans (Table 31.2). It was reported that microbial diversity of cocoa bean fermentation vary with location and processing conditions; *Hanseniaspora*, *Pichia kudriavzevii* and *Saccharomyces cerevisiae* are the most frequent yeast species isolated from natural fermentation of cocoa bean (Daniel et al. 2009; Papalexandratou and De Vuyst 2011). As indicated earlier, the categories of microorganisms occur at the different phases of fermentation process. At the early stages (between 1 and 3 days) of fermentation yeast and lactic acid bacteria are the predominant occurring microorganisms (Schwan and Wheals 2004). Followed by the start of acetic acid production, which results in the decline of the growth and development of yeast with corresponding increased temperature up to 45 °C (Papalexandratou and De Vuyst 2011; Schwan and Wheals 2004).

The description of the fermentation process constitutes the foundation for manipulation and selection of the microorganism's composition in the fermentation of cocoa beans. The ability of yeast strain-starter cultures for instance, secrete enzymes that degrade pulp in the early stages of cocoa bean fermentation; is essential factor considered for increasing aeration of fermented mass, growth of aerobic acetic acid bacteria and followed by declining acid development. Although, yeast strains may occur at different concentrations in the fermenting media, their enzymatic activities still vary. *Kluyveromyces marxianus* KM16-6, K. *marxianus CCT* 3172 and *K. kluyveti* S13Y4 were three yeast stains that were isolated from the natural cocoa fermentation in Ghana in line with their capacity to produce polygalacturonase (PG) (Crafack et al. 2013). The results showed that at temperature between 20 and 60 °C, PG activity on KM16-6 increased by 25%. In another study, an indigenous yeast strain *Pichia kluyveri* of natural cocoa fermentations were isolated from the Ghana and Brazilian cocoa, where co-fermentation of this yeast increases varietal thiol concentration of fermented products.

Bacterial culture selection for the fermentation of cocoa bean is a significant consideration for their capacity to breakdown critic acid (LAB) and to oxidize ethanol into acetic acid (AAB). For proteolysis of seed proteins initiation and flavor precursors formation to occur, the oxidation of ethanol into acetic acid is required. Lefeber et al. (2010) reported that *L. fermentum* 222 strains compared to *L. plantarum* 80, exhibited higher ability for acetic acid production with low ethanol; although strains of *L. fermentum* 222 displayed low glucose and fructose consumption.

The application of genetically modified microorganisms in cocoa bean fermentation has been novel with success but extensive studies to improve traditional methods are still needed. The use of a hybrid strain of *K. marxianus* has been successful and it has been recommended due to the following reasons; ability to

Table 31.2 Stages of the fermentation process and their respective characteristic effects on fermented cocoa beans

Stages/ period	Microorganism	Microbial activity and Characteristic Effect	References
Stages 1	Anaerobic yeasts of the Saccharomycetaceae family, including Hanseniaspora, Saccharomyces, Kluyveromyces and Pichia	Production of ethanol, are favored by the higher concentration of glucose and citric acid and the low availability of oxygen. Production of other compounds such as carbon dioxide, organic acids (acetic and succinic acid) and glycerol. Degradation of pectin in the cocoa and production of significant amount of aroma compounds precursors (such as higher alcohols and esters) for the development of chocolate aroma profile.	Camu et al. (2008), Crafack et al. (2013), Dujon and Louis (2017), Papalexandratou and De Vuyst (2011) and Papalexandratou et al. (2013)
Stages 2	Lactic acid bacteria including *Lactobacillus fermentum*	Increased LAB populations and decreased yeasts resulted in increased lactic acid concentrations. LAB mainly *Lactobacillus fermentum* is heterofermentative: produce volatile compounds such as diacetyl, acetoin and 2,3-butanediol to sustain bacterial growth and permit a slight increase of pH in cocoa	Camu et al. (2008), Lefeber et al. (2010) and Papalexandratou et al. (2013)
Stages 3	Acetic acid bacteria including *Bacillus subtilis, B. pumilus* and *B. fusiformis*	Decreased LAB population coupled with concurrent increased AAB population, lead to simultaneous oxidation of yeasts-produced ethanol and the conversion of LAB-lactic acid to acetic acid and acetoin. Afterward, acetic acid is overoxidized to CO_2 and H_2O. The death of the embryo is attributed to increased temperature, decreased pH (from 6.5 to 4.8) and the diffusion of acetic acid and ethanol to the cocoa bean. Production of pectin lyase to participate in cocoa fermentation. Production of other pectinolytic enzymes such as polygalacturonase. Production of short-chain fatty acids leading to potential release off-flavour on fermented cocoa beans.	Camu et al. (2008), Lefeber et al. (2012) and Schwan and Wheals (2004)

increase pectinolytic activity for improved pulp drainage, increased aeration, hastened seed protein degradation and reduce cotyledon acidity for production of chocolates with a higher acceptability compared to a natural fermented process (Leal Jr et al. 2008).

31.5.1 Mycotoxin Contamination of Cocoa Beans During Fermentation

It was recommended that fermentation of cocoa beans must not exceed 7 days, since this could contribute to fungal proliferation (Commission 2013). Accumulation of ochratoxin A (OTA) could occur at postharvest and during fermentation in particular, where damaged and infected harvested pods from the field are not carefully identified, separated and discarded (Copetti et al. 2014). Therefore, mycotoxins formation is possible during fermentation; where, aflatoxin-producing species (*Aspergillus flavus* and *A. parasiticus*) were found in cocoa bean samples from fermentation as well as OTA-producing species (*A. niger* and *A. carbonarius*) (Copetti et al. 2010). As a matter of fact, as fermentation ends toxigenic-producing fungi can grow; hence, drying of cocoa beans should commence immediately (Copetti et al. 2011).

31.6 Improving Conditions for Fermentation

The combination of multifaceted approaches has proven capable of identifying individual strains and ensuing the evaluation of the potential of yeasts and bacteria respectively, as starter cultures to perform cocoa beans fermentation. More so, the environment and/or agricultural practices of the cocoa producing region have significant effect the success of the starter culture being applied (Mota-Gutierrez et al. 2018). In other words, efficient fermentation microbial diversity in the process must be grouped according to the various regions of cocoa production. Therefore, research work develop mixed starter cultures that are appropriate to conduct the fermentation process and can survive diverse conditions and finally attain quality fermented cocoa bean that can have significant value on the global market (Figueroa-Hernández et al. 2019).

However, starter cultures alone cannot be relied on to standardise the entire fermentation process (Figueroa-Hernández et al. 2019). Essentially, the design and construction of an entire fermentation architecture has influence on the specific environment, which also has significant effect the quality of fermented cocoa beans. Here, the controlled environment helps to the shorten time of fermentation and also prevent microbial contamination. So, the understanding of a well-organized and constructed fermentation structure will ensure the calibration, mechanization and

reproducibility of the cocoa bean fermentation process. For instance, stainless-steel bioreactors are innovative models proposed for undertaking the controlled fermentation (de Melo Pereira et al. 2013). Furthermore, the specific amount of agitation, coupled with suitable temperature and pH control succeeding successive microbial activities are the other conditions that must be considered very important. Also, it is important to adopt a mechanism for the exclusion of waste from the system. In all, a well-designed starter culture alongside the right fermentation system will assist the producer to regulate the fermentation process. Ultimately, the various diversity of microbial species and their corresponding interactions in fermented cocoa beans (within a controlled fermentation system) will yield high-quality cocoa products, especially chocolate (Figueroa-Hernández et al. 2019).

31.7 Conclusion

In conclusion, the available information points to fermentation as an essential step to develop precursor compounds for aroma and flavor characteristics of chocolate and other cocoa products, without comprising the cocoa beans to germination. The role of microorganisms is very essential in the processing of cocoa bean. The attributes of microorganisms found in fermentation and the effect of starter culture in fermented cocoa beans have been captured in this chapter. Different microorganisms have been found to be responsible for the three phases of the fermentation process with their corresponding activities and effect. Cocoa beans have high nutritional value and health benefits against several ailments. There are various criteria for the selection of cocoa starter cultures. The starter cultures control fermentation with the resultant effect on final product quality and causes potential decontamination of mycotoxins.

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Chapter 32
Fermentation of *Parkia biglobosa* Seeds: Effect of Temperature Conditions on Bioactive, Nutritive and Antioxidant Parameters

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32.1 Introduction

Fermentation of food is an important traditional practice of adding desirable properties including flavors to staples such as cereals, legumes, root crops, and many others (Odunfa and Oyewole 1998; Blandino et al. 2003; Chelule et al. 2010; Franz et al. 2014). Application of fermentation process is inexpensive technique to preserve food in addition to improving the nutrient quality and digestibility of raw products that might contain undesirable traits (Olasupo et al. 2016; Atere et al. 2020). Besides, it's a cheap way of reducing or decontaminating mycotoxin in foods and at the same time enhancing the food constituents (Adebiyi et al. 2019). According to (Mathara et al. 2004; Franz et al. 2014), whiles food that have been fermented are branded as healthy, in other regions it is used as a way of disease control such as intestinal ailments. Traditional fermented foods are local dietary preferences characteristic of regions with long standing food cultures.

Parkia biglobosa (common name: African locust beans) seeds are typically fermented products common in the Western Africa and other sub-Saharan African countries, where the tree grows naturally (Nyadanu et al. 2017). The tree is native to Africa but includes other growing countries, it represents an important economic tree and seeds extraction from the fruits has a long tradition (Odunfa 1985b). The

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seeds outside the pods have poor storage life without refrigeration. They quickly deteriorate shortly after extraction from the pods perhaps due to the high protein content. Therefore, the fermentation of the dried seeds helps to store the seeds for more than a year outside cold storage with even better flavor and other nutrient properties. Dried fermented seeds of *Parkia biglobosa* preserve well, improve tastiness, nutritional profile and impute desirable organoleptic characteristics that influence flavor, texture, and aroma (Omodara and Aderibigbe 2018). The microbiology and biochemistry aspects of fermentation are well documented (Azokpota et al. 2006; Bukar and Balarabe 2019).

Again, there are several factors that control fermentation processes, which ultimately determine the quality characteristics of the fermented product. Temperature (Xu et al. 2010), pH (Huang et al. 1986; Jain and Pundir 2011), and oxygen availability affect the microbial system during fermentation. However, temperature conditions largely influence the extent to which complex chemical and biochemical changes occur during fermentation. With increasing demand for high quality fermented *Parkia biglobosa*, in-depth understanding of temperature conditions as contributing factor to fermentation attribute would have substantial commercial implications.

This chapter seeks to make comprehensive documentation and discussion of research findings to enable a better understanding of the effect of temperature conditions during fermentation on bioactive, nutritive and antioxidant parameters of *Parkia biglobosa* seeds.

32.2 Process of *Parkia biglobosa* Fermentation

"Dawadawa" (locally known in Ghana) or *Iru* (called in Nigeria) is a final product of alkaline fermentation of African locust seeds, commonly used as food seasoning and condiment in West Africa. In this chapter the final fermented product will be referred to as "Dawadawa". It is a good source of protein supplement for a protein deficient country and low-income earners who otherwise might not afford animal protein. Fermentation results in the breakdown of complex organic substances into smaller ones through the action of catalysis.

The fermentation of *P. biglobosa* seeds is mostly carried out by women either individually or in small groups (Odunfa 1985b). The process includes depulping of pods deemed suitable then brought to boil, dehulling, and finally the fermentation of the seeds (Odunfa 1985a; Odunfa and Oyewole 1998). The traditional fermentation of *P. biglobosa* seeds begins with washing and then boiling the raw bean seeds for about 24 h until it softens so that the coat can be removed easily. Water is added frequently. The boil seeds still quite firm are then mixed with wood ash and pounded and washed several times to remove seedcoats. The cotyledons are boiled for another 2–3 h until they become softer. The water is drained using a sieve and the seeds are spread wide in a flat basket out to cool before being packed into earthenware pots, covered, and left to undergo fermentation for 3–5 days. Fermentation

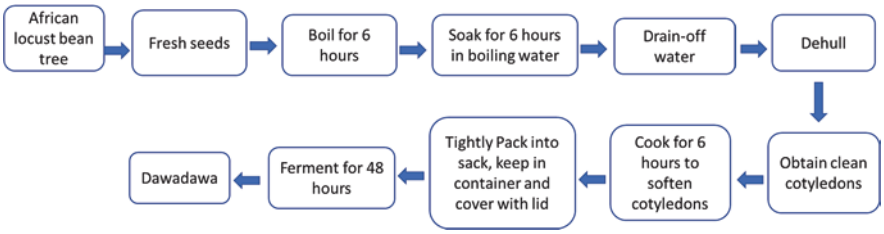

Fig. 32.1 Fermentation process of African locust beans

time vary according to preference for flavour, taste and texture (Odunfa 1985b; Odunfa and Oyewole 1986). The detailed procedure for processing raw African locust beans is illustrated in Fig. 32.1.

Several studies have identified several microbial species involved in the fermentation of African locust bean seeds (Ouoba et al. 2003; Uaboi-Egbenni et al. 2009; Adewumi et al. 2013). *Bacillus* spp. (Ogbadu and Okagbue 1988; Ouoba et al. 2003) is the main microorganism found to be involved in the fermentation of most legumes including African locust bean with *Bacillus subtilis* as the predominant microorganism (Odunfa and Oyewole 1986). Irrespective of the technique adopted, temperature activity is critical to initiate, maintain or improve fermentation quality.

32.3 Factors That Affect Fermentation of African Locust Bean

The traditional fermentation of African locust bean seeds is carried out according to indigenous knowledge (Odunfa 1985a) (Fig. 32.1). The chemical, physical, and nutritional properties of the African locust bean seeds transform immediately after fermentation; this is because the raw African locust beans are nutritionally deficient and unpalatable (Azokpota et al. 2006). Fermentation of African locust bean seeds is a traditional art that is practiced in rural areas with basic utensils that is likely to compromise hygiene of the entire process and final product (Odunfa and Oyewole 1998). Here, there is no standard fermentation process, therefore every producer depends on the culture and traditions in the region; this introduces variation in the quality of dawadawa (Modupe et al. 2016). In other words, to a large extent the fermentation techniques are small-scale and household basis, characterized by simple non-sterile equipment, chance or natural inoculums, unregulated temperature conditions, sensory fluctuations, poor durability, and unattractive packaging of the final products compromising quality and uniformity.

Temperature is a major factor affecting the quality of fermentation products. This has been shown for both traditional and commercial fermentation products. In a study, tea fermented at 15 °C, 25 °C and 30 °C revealed that those fermented at 15 °C resulted in the development of desirable theaflavin class of pigments while

those at the higher temperatures resulted in non-dialysable material (Cloughley 1980). Another study showed that higher temperature negatively affected the fermentation rate, and composition of microbial community in a traditionally fermented milk product "mabisi" (Moonga et al. 2021). Temperature has also been found to affect the antioxidant activity of various fermented products (Huang et al. 2011). When it comes to fermented *Parkia biglobosa*, few studies have scantily touched on the temperature effect on Dawadawa which is the fermented product of *Parkia biglobosa* seeds. The temperature that has been studied ranges from 20 to 70 °C. These temperatures were seen to exert different properties on the final product. Protein content in fermented *Parkia biglobosa* under varying temperatures were found that the optimum protein content was 52.7% after 3 days of fermentation at 40 °C while fat, carbohydrate and crude fibre were not affected much by the different temperatures (Ojewumi et al. 2016). In another study, the optimal protein content of 37% was achieved after 3 days of fermentation at 30 °C (Mohammed 2020). Again, for nutritional contents, temperatures 40 °C and 50 °C gave the highest nutritional content after 3 days of fermentation with acceptable end products while temperatures above 50 °C resulted in poor nutritional content with unacceptable end products (Ojewumi et al. 2018). In a study with lower temperatures of 20–30, it was found that there were no significant differences in the nutritional component comparing the temperatures under study (Mohammed 2020).

32.4 Effect of Temperature on Quality of Fermented *Parkia biglobosa*

32.4.1 Effect of Temperature on Bioactive Content During Fermentation

Secondary metabolites comprise of bioactive constituents of plants, which form the active component of many medicinal preparations. Terpenes, alkaloids, flavonoids and phenolic compounds are the most important of these bioactive constituents of plants (Usunobun et al. 2015; Edrah et al. 2016). Medicinal extracts containing these compounds (polyphenols, flavones, alkaloids, terpenes, flavonoids and tannins) are alternative therapeutics, which are being sought in treatment of cancer in recent times. Plants constitute a rich source of chemical constituents with promising medicinal properties for the sustenance and improvement of human health (Moghadamtousi et al. 2013). More so, various phytochemical molecules including secondary metabolites are present in these chemical constituents.

In a study where African locust beans seeds were analysed after 5 days of fermentation at 34 °C, phytochemicals present were alkaloid, saponins and terpenes. Alkaloids demonstrate muscle relaxant property and can have analgesic, antispasmodic and bactericidal effects (Okwu 2004). Saponins present in the fermented

samples qualifies it for the treatment of inflammations. It was also reported that saponins have the potency to precipitate and coagulate red blood cells. Some of the qualities of saponins are foam formation in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Nath et al. 2015). Also, in nature, saponins appear to act as antibiotics for the protection of plants from microorganisms, whereas, they have the capacity to fight cancer and infection in humans (Adunola et al. 2015).

Similarly, fermented samples of African locust beans seeds exhibited high amount of flavonoid (+++). Flavonoids have enormous range of bioactive compounds, being used in traditional medicine and also have antioxidant and antiproliferative effects especially against health disorders including chronic inflammatory and allergic diseases, breast cancer and coronary artery disease (Ochwang'I et al. 2016). Flavonoids are also effective water-soluble antioxidants and free radical scavengers, which prevent the damage of oxidative cell and have strong anticancer activity (Okwu and Josiah 2006). Tannins was also noticed in dawadawa though in minimal quantity (++) (Osuntokun et al. 2020). According to Osuntokun et al. (2020), tannins are astringent in taste and aid the healing of wounds and swollen mucous membrane. Through microbial metabolism, tannins reduce the bacterial production by blocking key enzymes. Therefore, the "Dawadawa" product could act as potent antimicrobial drug (Pradeepa et al. 2016). indicated that tannins also inhibit the synthesis of protein.

High quantity of terpenoids is found in "Dawadawa". The terpenoids act as antifungal and antibacterial; they disrupt the membrane and also inhibit bacterial cell or fungus (Amin and Thakur 2014). "Dawadawa" contain minimal proportion (++) of steroids. The presence of steroids in plants exhibit analgesic properties and they are responsible for central nervous system activities (Olatunde and Dikwa 2014). It also has high quantity of glycosides which are beneficial in several ways including reducing inflammation, protecting against endotoxemia and may be used to support cardiac treatment of congestive heart failure (Amin and Thakur 2014).

32.4.2 Effect of Temperature on Nutritive Parameters During Fermentation

The effect of temperature difference on the nutritional composition as investigated established the optimal temperature that yielded the best nutrient composition for fermentation quality of "Dawadawa". The results of proximate analysis of fermented African locust beans seeds indicated the percentages of constituents as follows: moisture content (33.9 ± 0.05), ash content (4.55 ± 0.02), total protein (30.5 ± 0.20), crude lipid (20.91 ± 0.02), carbohydrates (24.2 ± 4.03) and crude fibre composition (5.94 ± 0.01). A study indicated that fermented locust beans (except ash) the nutrient components were much higher than those of raw beans (Ijarotimi

and Keshinro 2012). Other studies indicated that moisture content was for raw seeds (68%) and fermented (76%), fat in raw (17.33%) and fermented (19.33%). Furthermore, it was reported that temperature influenced the protein content; thus, at the third day of fermentation 40 °C gave protein content of 52.7% and at 70 °C it was 25.6%. Evidentially, higher temperature produced decline in protein (Ojewumi et al. 2018). Therefore, it is justified to reason that higher fermentation temperature denatures nutritional values and lower temperature supports higher accumulation of nutrients (Ojewumi et al. 2018).

"Dawadawa" with high moisture content mostly increases perishability because it enhances microbial activities. Therefore, processing method must ensure low water activity in the paste. *Parkia biglobosa* seeds are rich in protein and eventually dawadawa also has high protein content representing a good source of protein. *P. biglobosa* fruit is in low crude fibre. Mostly, the fibre helps to prevent a number of diseases by reducing the cholesterol level. The average daily intake of dawadawa per capital among some Hausas in Northern Nigeria is 1.4% of daily calories and 5% of protein (Onofiok et al. 1996). It was found that dawadawa regular protein intake per capital is high than poultry but lower compared to beef (Odunfa and Oyewole 1998; Oyewole and Isah 2012).

32.4.3 Effect of Temperature on Antioxidant Activities during Fermentation

Antioxidants (vitamins, carotenoids) including are usually the free radicals reducing and neutralizing agents, which rummage reactive oxygen molecules or species (ROS) and inhibit their initiated chain reactions (Singh and Sharma 2018). Natural antioxidants show the potential to protect DNA/RNA and other major biomolecules from radiation mediated damage (Singh and Sharma 2018). Plant products aid in chelating heavy metals and protect DNA from oxidative damage. In cells, reactive oxygen species are strictly regulated by antioxidant defense systems, which mainly comprise of glutathione peroxidase, catalase, vitamin C and E, dismutation of superoxide enzyme and glutathione reductase (Weng et al. 2018) and when the formation of free radicals overwhelms the antioxidant defense system, oxidative stress is induced. According to de Oliveira et al. (2018), exogenous and endogenous antioxidants promote the inactivation of free radicals, preventing the oxidative stress and their consequences. Antioxidants, in reference to their mechanisms of action, can be categorized into primary antioxidants, free radical scavengers, enzymatic antioxidants, chelating agents and mixed antioxidants (de Oliveira et al. 2018).

32.5 Conclusion

Parkia biglobosa fermentation requires milder temperatures to enhance its high nutritional value. Fermentation causes substantial reduction in the amount of antinutritional factors such as phytate and oxalate found in the seeds. These factors are generally present in leguminous foods, which are important components of the diet of a large section of the world's population, and particularly of populations in developing countries. Ways and techniques are necessary to decrease the content of antinutritional factors in food. Dawadawa has several health benefits but nutritional constituents during fermentation are influenced by the temperature. Thus, it is important that traditional ways of maintaining the temperature is introduced to women involved in this traditional act to preserve the nutritional qualities in this important condiment.

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Chapter 33
Improvement of Indigenous Fermentation Technologies for Certain Ghanaian Fermented Foods

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33.1 Introduction

In West Africa, as much as 50% of the perishable food commodities (fruits, vegetables, roots and tubers) and 30% of food grains (maize, sorghum, millet, rice and cowpeas) are lost after harvest (REF……). Several factors including ineffective or unsuitable food processing technologies, poor harvesting and ineffective postharvest management practices, poor roads, poor market practices and insufficient or total lack of storage facilities, packing houses and market infrastructures contribute to the significantly high post-harvest food losses in West African countries including Ghana (FNIFST 2008). In Ghana, indigenous food preservation methods have been used to prevent the rapid deterioration of commodities and to facilitate their effective distribution beyond the known production areas. Often the same processor and within processors, there are differences in traditional foods produced.

Indigenous fermentation has been valued throughout history for its preservative, health, nutritional effects. However, the concept and optimum conditions for indigenous fermentation technologies (IFT) in Africa are still less developed and they performed base on spontaneous uncontrolled environmental conditions (Sanni 1993). Various countries and regions have diverse fermentation procedures for

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relatively similar indigenous foods (Abodjo Kakou et al. 2010; Ramos et al. 2015). The methods of fermentation basically determine the microbial and biochemical profile, as well as the sensory characteristics of these indigenous fermented foods; which are difficult to maintain under spontaneous fermentations. Recent improvement in indigenous fermentation processes has helped reduce certain factors including fermentation time, enhanced bio-availability of nutrients, degrading toxic compounds as well as identification of functional properties such as probiotics. (Ashaolu 2019; De Roos and De Vuyst 2018).

Fermented foods constitute about 40% of the food consumed in Ghana (Dzikunoo et al. 2021). Most Ghanaian foods are high in carbohydrates and easily get converted into sugars through fermentation process. Also, fermentation offers food a strident and acrid taste; this could signal that the food probably of deteriorated. In Ghana, fermented foods are produced mostly by households or community level and rely on spontaneous methods of inoculation; where fermentation process mostly not controlled. This chapter attempts to highlight the advances of indigenous fermentation technologies for selected Ghanaian Fermented food; thus, including the significance, benefits, advantages and limitations. It also reviewed the effect of indigenous fermentation technologies on nutrient values and functional properties of fermented foods.

33.2 Some Indigenous Fermented Foods in Ghana

In Ghana, a range of indigenous fermented foods and beverages are produced from different raw materials from cereals (maize, rice), legumes (sorghum, millet, groundnut, soybean), roots tubers (cassava) and vegetables (roselle calyx), using different spontaneous fermentation techniques (Simatende et al. 2015) fermented foods and beverages consumed across Africa include koko from millet/maize, koshei from cowpea/beans, agbele kaklo from cassava, sobolo from roselle calyx and zumkom from millet and dawadawa among others foods (Table 33.1). Notwithstanding the extensive consumption of these local fermented foods, they are generally ethnic-based and some are used for festivals.

Momone is a delicacy in Ghana and it's used as condiment in traditional soups and stews. Some main fermented fish products (Catfish, barraccuda, seabream, threadfin, croaker, grouper, bonito, mackerel, herrings, squid, octopus, bumper, snapper, ribbon fish) are salted and fermented (overnight to 3 days) by various microorganisms (*Bacillus subtilis, B. licheniformis, B. megaterium, B. cereux, B. mycoides, Micrococcus luteus., Staphylococcus* spp., *Lactobacillus, Pseudomonas, Pediococcus, Klebsiella, Debaryomyces, Hansenula* and *Aspergillus*) in their production (Anihouvi et al. 2012; Sanni et al. 2002).

In general, fermented food products have been extensively used but their traditional processing methods are labour intensive and time consuming. In order to overcome these limitations, several processing parameters have been improved such

Table 33.1 Some selected local fermented foods and beverages commonly consumed in Ghana

Product	Substrates	Functional microflora	Type of fermentation	Description and usage
Banku	Maize, cassava	Yeasts, LAB	SSF, N	Dumpling; staple
Kenkey	Maize, cassava	Yeast, LAB	SSF, N	Sour dumpling, cooked, staple
Agidi	Maize, sorghum, millet	*Pediococccus acidolactic, L. plantarum, L. acidophilus, Leuconostoc, Streptococcus, Bacillus*		Sour, cooked staple
Koko	Pearl millet, maize	Bacteria (*Weissella confusa, LAB fermentum, LAB. salivarius*: Yeasts, LAB; Uncharacterised fungal component		Sour water, cooked staple
Pito	Sorghum	LAB, yeast	SmF, N	Sour gruel; staple
Dawadawa	African locust bean, soya bean	Bacteria	SSF, N	Pungent smile, flavoring agent; stew and soap
Palm wine	Coconut sap	Yeast, LAB	SmF	Sweet-sour
TZ (Tuo zaafi)	Maize, cassava	LAB, yeast	SSF	Dumpling; staple
Akasa	Maize	Yeast, LAB	SmF	Gruel
Fermented fish (Momone, Koobi, Kako, Ewule)	Catfish, barraccuda, seabream, threadfin, croaker, grouper, bonito, mackerel, herrings, squid, octopus, bumper, snapper, ribbon fish			

Lactobacillus (LAB); SSF

as size reduction, incorporation of starter cultures and reduced dimension of mass. Nout et al. (1995) conducted studies on traditional fermentation process in kenkey production to their improved processing technique; results showed that pre-cracking maize kernels reduce soaking (40% moisture content) time of maize from 48 to 10 h, while incorporating "aflata", a gelatinized maize paste serving as intermediate for fermentation, shorten fermentation time from 2–3 days to 12 h. However, the relationship between the fermentation condition and quantity of the aflata has not

been clearly indicated. Changing dimensions of kenkey mass from 10–15 cm diameter balls to 6 cm diameter balls decreased cooking time from 2 h to 35 min. Similarly, coarse dry-milled maize enhanced hydration capacity of maize (0.63 ml/g in 1 h) than whole grain (0.5 ml/g in 3 days). Also, adjusting water temperature up to 60 °C before soaking maize reduced peptidase and carbohydrase activity except β-galactosidase and α-mannosidase, pasting viscosity was increased (Halm et al. 2004; Nche et al. 1996).

The high accumulations of biogenic amines mainly histamine have been detected in fermented fish products (Prester 2011). Decarboxylase enzymes are found in bacteria contaminated foods; then the decarboxylated free amino acids produce biogenic amines (Biji et al. 2016; Özogul and Özogul 2019). Also, decarboxylation of histidine produces histamine as derivative while lysine is will produce cadaverine and putrescine (Ben-Gigirey et al. 1999). Most amines are heat-stable and some decarboxylases activity persist even after pasteurization. Therefore, cooking will not reduce the extent of amine when it is produced. (Anihouvi et al. 2012). Also, the intake of large quantities of histamine exposes consumer to toxicity. Histamine poisoning has short gestation periods, which starts from minutes to a few hours after intake of high levels of histamine contaminated food, with a corresponding arise in poisoning effects (Anihouvi et al. 2012). More or less frequent symptoms of poisoning effects of histamine contaminated food have been reported (Prester 2016).

Starter culture play significant role in the promotion of traditional process of food fermentation in Ghana. Nout (2009) defined a starter culture as a microbial preparation of large numbers of cells or at least one microorganism added to a raw material to produce a fermented food through the acceleration and direction of its fermentation process. Compared to spontaneous (uncontrolled) fermentation, starter cultures control the highly variable fermentation conditions and introduce uniformity in product quality (e.g., increasing shelf-life). Starter cultures have been used to suppress growth of spoilage microbes' (bacteria) as well as considerable inhibition of volatile basic nitrogen change (Anihouvi et al. 2012). When pH reduces rapidly, the acid denaturation of muscle protein will make food products acquire exclusive lactic acid flavor, increases firmness, texture and tastiness (Yin and Jiang 2001).

The use of amine-negative starter cultures can avert the formation of biogenic amines (Shawish et al. 2017). It was found that adding *Pediococcus pentosaceus* as amine–negative as starter culture was able to decrease the concentrations of histamine, tyramine and cadaverine produced in ripening phase of dry sausages (Bover-Cid et al. 2000). Same starter culture was able to decrease of biogenic amines formation in fermented fish products. The use of starter cultures to control fermentation will also reduce the time of fermentation, enhance inhibition or elimination of food borne pathogens, while improving shelf life and other sensory quality of products (taste, aroma, appearance and texture) (Anihouvi et al. 2012).

33.3 Improved Indigenous Fermentation Technologies

33.3.1 Enhancement of Nutrition

The chemical composition of indigenous fermented foods and beverages is strongly affected by both raw material compositional profile and processing factors, but could differ based on geographical location. Studies on the proximate composition of dawadawa (fermented condiment) produced from Africa locust bean, indicated that fermentation increase protein, fat and moisture levels, which is consistent with decrease in carbohydrate levels (Gernah et al. 2007; Sackle 2013). Protein and fat levels in dawadawa increased from 24.80% to 30.52% and 16.04% to 25.03% respectively in raw African locust bean, when fermented on gmelina; 30.53% and 25.01% when fermented on banana leaves; 25.91% and 20.63% when fermented on jute bag and 28.04% and 21.40% when fermented in polythene bag after 72 h of fermentation (Gernah et al. 2007). In Ghana, protein and fat contents in fermented (72 h) African locust bean increased from 45.82% to 52.33% and 35.12% to 38.52%, respectively while fiber levels declined from 10.77% to 6.77% (Felix and Francis 2019).

To improve the nutritional and digestibility profile of indigenous fermented foods and beverages, addition or substitution of other food crops has been recommended. Dawadawa is one essential indigenous fermented condiment that has seen the improvement in its nutritional composition through addition of other crops such as soya bean and maize. It was reported by Dakwa et al. (2005) that increased protein, isoleucine, leucine, lysine, phenylalanine, arginine and proline was significant with fermentation time after fortifying dawadawa with soya bean. However, fat and ash contents decrease during the 72 h fermentation period. Studies have tried to improve the protein level of kenkey by supplementing fermented dough with 20% white cowpea. Similar author detected 20.5% increase in protein level compared to the whole maize kenkey.

Non-malted cereal-based foods from indigenous crop used in Africa, are limited in high viscosity, poor digestibility coupled with low bioavailable macronutrients. Fermented maize flour and dough are also important indigenous food consumed in nearly all African countries. Various indigenous fermentation methods and food crops have been modified with the objective of improving the digestibility and bioavailability of these local diets. In Ghana, information on the supplementation of fermented maize flour with other food crop mostly legumes are limited. However, some studies have stated that the supplementation of fermented maize flour with protein rich food crops (Tables 33.2, 33.3, and 33.4).

Table 33.2 Amino acid (μg/g) profile of improved fermented soyabean dawadawa and peal millet

Amino acid	Fermenting soybeans dehulled after boiling					Fermenting soybeans dehulled after roasting					Peal millet		
	0 h	24 h	48 h	72 h	Final product	0 h	24 h	48 h	72 h	Final product	0 h	12 h	24 h
Isoleucine	228	227	232	280	276	225	223	230	272	269	4.34	4.28	4.11
Leucine	426	422	434	490	487	422	421	432	466	464	11.34	11.15	10.79
Lysine	338	336	342	400	398	332	331	341	392	390	2.06	1.85	1.78
Methionine	76	72	78	80	78	72	67	62	77	76	1.43	1.53	1.47
Cysteine	67	65	64	66	65	64	63	67	96	94	–	–	–
Phenylalanine	272	267	274	310	310	263	257	270	303	303	5.41	5.31	5.44
Tyrosine	207	205	213	200	198	203	200	192	196	193	2.52	2.54	2.58
Threonine	232	228	248	240	240	210	198	242	230	230	3.68	3.58	3.34
Tryptophane	89	86	89	80	78	85	83	79	77	75	–	–	–
Valine	302	300	312	300	300	280	276	335	289	288	5.41	5.39	5.05
Arginine	262	261	274	350	335	250	247	273	335	333	4.28	3.58	3.28
Histidine	147	144	153	160	157	132	120	154	158	157	2.25	2.16	2.00
Alanine	278	269	264	270	270	268	262	282	268	268	8.53	8.48	8.31
Aspartic	335	322	325	330	328	325	312	310	321	318	7.94	7.87	7.57
Glutamic	257	243	258	270	265	263	242	234	272	267	23.73	23.21	22.54
Glycine	233	229	246	260	256	238	235	242	252	250	2.72	2.60	2.48
Proline	229	218	338	340	335	229	226	234	339	336	4.82	4.59	4.51
Serine	324	318	316	320	320	298	1296	312	318	316	4.56	4.47	4.32

Source: Dakwa et al. (2005) and Osman (2011)

Table 33.3 Influence of indigenous process on the nutritional profile of pearl millet

Time (h)	Protein	Fat	Ash	Carbohydrate	Glucose	Fructose	Maltose
0	15.25 ± 0.21^{ab}	5.77 ± 0.25^{a}	1.80 ± 0.03^{a}	72.63 ± 0.56^{a}	6.83 ± 0.35^{e}	1.17 ± 0.06^{a}	1.50 ± 0.10^{a}
4	14.79 ± 0.10^{ab}	5.65 ± 0.01^{a}	1.57 ± 0.01^{b}	72.24 ± 0.00^{ab}	8.73 ± 0.12^{d}	0.73 ± 0.12^{b}	0.70 ± 0.10^{b}
8	14.99 ± 0.02^{ab}	5.75 ± 0.03^{a}	1.82 ± 0.10^{a}	71.65 ± 0.09^{bc}	9.40 ± 0.14^{c}	1.11 ± 0.12^{a}	ND
12	14.75 ± 0.36^{ab}	5.58 ± 0.06^{a}	1.84 ± 0.00^{a}	71.84 ± 0.01^{b}	10.75 ± 0.17^{b}	1.17 ± 0.06^{a}	ND
16	14.50 ± 0.42^{b}	5.53 ± 0.42^{a}	1.87 ± 0.01^{a}	72.05 ± 0.33^{ab}	11.41 ± 0.12^{a}	1.13 ± 0.16^{a}	ND
20	14.55 ± 0.35^{b}	5.60 ± 0.28^{a}	1.65 ± 0.04^{b}	72.66 ± 0.01^{a}	11.35 ± 0.05^{a}	1.20 ± 0.14^{a}	ND
24	15.35 ± 0.35^{a}	5.79 ± 0.03^{a}	1.79 ± 0.10^{a}	70.97 ± 0.37^{c}	7.30 ± 0.58^{e}	0.64 ± 0.17^{b}	ND

Source: Osman (2011)

Table 33.4 Proximate composition of fortified fermented maize flour

Nutrient	Supplemented crop	Nutrient level (%)		References
		Fermented maize	Fortified maize	
Protein Fats Carbohydrate	Bambara groundnut	18.40 5.40 7.21	21.60 6.60 63.32	Mbata et al. (2009)
Protein Fats Carbohydrate	Soyabean	8.92 4.85 84.31	19.32 7.53 69.43	Ikya (2013)
Protein Fats Carbohydrate	Soyabean, Banana flour	4.50 5.17 80.08	12.28 6.87 71.28	Ezeokeke and Onuoha (2016)
Protein Fats Carbohydrate	Mungbean	7.89 5.18 77.53	9.80 4.56 71.30	Onwurafor et al. (2020)

33.3.2 Enhancement of Food Detoxification and Other Functional Properties

Antinutritional properties such as phytate, oxalic acid and tannin are among the major contributing factors that make application of indigenous fermentation appropriate. Studies have indicated the influence of indigenous fermentation on antinutritional factors indicated, where increase in fermentation time did reduce the rate antinutrients occurrence in food (Ahmed et al. 2020; Buta and Emire 2015; Ogodo et al. 2019). Clearly, adequate time for fermentation has contributed to the enhancement of bio-availability of nutrients especially micronutrients. Another important consideration is the community of the fermenting microbes. This also means that reducing the levels of anti-nutritional factors are controlled by both increasing time of fermentation as well as improving the community in which microbial fermentation occurs. The differences in phytate and tannin concentrations between spontaneous and controlled fermentations have been compared. Chibuike et al. (2019) reported on detection of significantly higher tannins and phytate levels in spontaneous fermentation than controlled fermentation using consortium of lactic acid bacteria. It was also observed that phytic acid, oxalic acid and tannin activities declined by 26.3%, 80.9% and 46.5% respectively after 72 h of fermenting "*kareeb*" seed (Ahmed et al. 2020). Studies on various foods to determine the effect of time of fermentation on antinutritional properties (Table 33.5).

Cassava roots (mostly the edible part) is also rich in cyanide in the form of cyanogenic glucosides, linamarine and lotaustraline in a ratio of 93:7 (Nambisan 1994). The classification of cassava is based on the cyanhydric acid content into three categories as [a] variety that are highly toxic with more than 100 mg HCN/kg of pulp; [b] variety that are moderately toxic with 50–100 mg HCN/kg of pulp; and [c] variety that

Table 33.5 Effect of fermentation time on antinutritional properties in food

Time (hours)	Food crop	Phytate	Tannins	Oxalic acid	Trypsin inhibitor	Amylase inhibitor	References
0	*Kareeb* seed	468 ± 0.04	315 ± 0.02	486 ± 0.05	–	–	Ahmed et al. (2020)
18		445 ± 0.02	189 ± 0.03	333 ± 0.02	–	–	
36		376 ± 0.07	126 ± 0.04	264 ± 0.03	–	–	
54		342 ± 0.09	63 ± 0.01	261 ± 0.07	–	–	
72		345 ± 0.01	61 ± 0.02	260 ± 0.04	–	–	
0	Pearl millet	647.00 ± 27.01	0.010 ± 0.004	–	7.33 ± 0.01	80.16 ± 0.46	Osman (2011)
4		535.85 ± 10.54	0.027 ± 0.002	–	7.03 ± 0.00	56.82 ± 0.45	
8		456.95 ± 2.33	0.036 ± 0.002	–	6.95 ± 0.01	56.48 ± 0.92	
12		348.25 ± 5.87	0.041 ± 0.001	–	6.95 ± 0.03	46.42 ± 0.00	
16		342.45 ± 14.07	0.041 ± 0.002	–	6.90 ± 0.03	47.72 ± 0.00	
20		319.22 ± 7.00	0.030 ± 0.001	–	6.76 ± 0.11	45.29 ± 3.44	
24		10.95 ± 7.00	0.035 ± 0.004	–	6.65 ± 0.04	39.45 ± 2.52	
0	Corn paste	278.7	215.1	–	–	–	Roger et al. (2015)
120		12.4	2.5	–	–	–	

are not toxic with less than 50 mg HCN/kg of pulp. Endogenous or microbial linamarase enzyme hydrolysis the cyanogenic glucosides to release the cyanhydric acid (toxic substance). Cyanogenic glucosides cause the toxicity of unfermented or raw cassava roots (Mlingi et al. 1991). After fermentation the linamarine that is not hydrolysed remain in the cassava roots and can create a health risk for the consumers (Gómez and Valdivieso 1985). Certain number of diseases such as goitre, dwarfism and the tropical ataxic neuropathy are attributed the consumption of non-detoxified cassava products (Kobawila et al. 2005). Cyanhidric acid is poisonous when it is consumed at a quantity of 0.5–3.5 mg per kilogram body weight. Apparently, this is the case in areas where cassava is the major source of carbohydrate (Balagopalan 2002). In Ghana, traditional know-hows have been used to remove cyanhydric acid content in cassava roots to make the utilization suitable. Improving upon the traditional processes are fundamental to obtaining quality and safe products.

33.4 Associated Risks in the Consumption of Fermented Food Products

Some observations posing health threats to Ghanaian consumers have been linked to the following: processing technique itself, the processing environment, waste disposal of the food, the unhygienic condition of processing raw materials and then inappropriate product packaging. Where raw food materials are held on the ground before processing result in microbial contamination of food products. Also, the lack of potable water has compelled processors to use ground water and harvested rain water for the cleaning of raw produce. Often ground water is polluted (with solid or liquid waste); serving as potential sources of microbial and chemical contamination. Locally, fermented Ghanaian food materials (e.g., cassava, maize, millet, fish) are dried on the ground or dirty materials, which also introduce sand or other physical materials into the final food. Also, blow flies as well as other kinds of insects invade especially the unsalted food (e.g., fish, meat products). Food processors choice to apply unapproved substances such as insecticides and pesticides in order to prevent or minimize these problems mentioned above. Indeed, food products are often packaged in different types of receptacles (recycled of traditional containers) before fermentation, storage and transportation to the consumer. However, the use of unhygienic containers become possible sources of products contamination. In cases where technologies and standards are poor, fermented food products could become potential means of transmitting food borne diseases (Anihouvi et al. 2012).

33.5 Conclusion

Diverse use of fermentation technologies to improve the production of certain indigenous fermented foods in Ghana has significant commercial benefits with more innovative advantages. Many fermented foods are produced locally with the expectation that several improvements will be made on the characterization, safety and hygiene of the Ghanaian fermented food products. More important, a detailed procedure for traditional fermentation processes in Ghana has to be established, and the various steps involved should be well characterized in order to ensure reproducible conditions for the production of consistent food quality.

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Chapter 34
The Quality Aspect and Safety of Some Traditional Fermented Product from Sorghum and millet

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34.1 Introduction

Cereals are most important source of food to many populaces in the world, since they are essential in human diet; providing energy and other nutrients for human survival. Sorghum and millet are essential source of food for millions of people, especially people from humid and hot (India, Nigeria, Niger, Ghana etc) areas. They are very important cereals in the absence of wheat, rice, and maize and are most often cultivated in areas where cereals are unable to produce better yields (Adekunle et al. 2012; FAO 2008). Sorghum and millet are general terms that are used for variety of cereals representing seeds from several taxonomically divergent grass types (Adekunle et al. 2012). They are said to be part of the first cultivated crops and have been a staple food ingredient for many years in Europe, Asia and Africa (Baltensperger 1996; Kimber 2000).

Sorghum and millet are important foods in most developing countries, because they are able to grow under adverse weather conditions such as limited rainfall. This has made these grains to gain more attention from developing and underdeveloped countries (Li et al. 2008). In addition, they have many nutritional and medicinal properties that provides health benefits like prevention of cancer, reduction of tumor incidence, prevention of cardiovascular disease, low blood pressure, cholesterol problem, fat absorption rate, heart disease, gastric problems, and also gastrointestinal bulk supply (Gupta et al. 2012; Truswell 2002).

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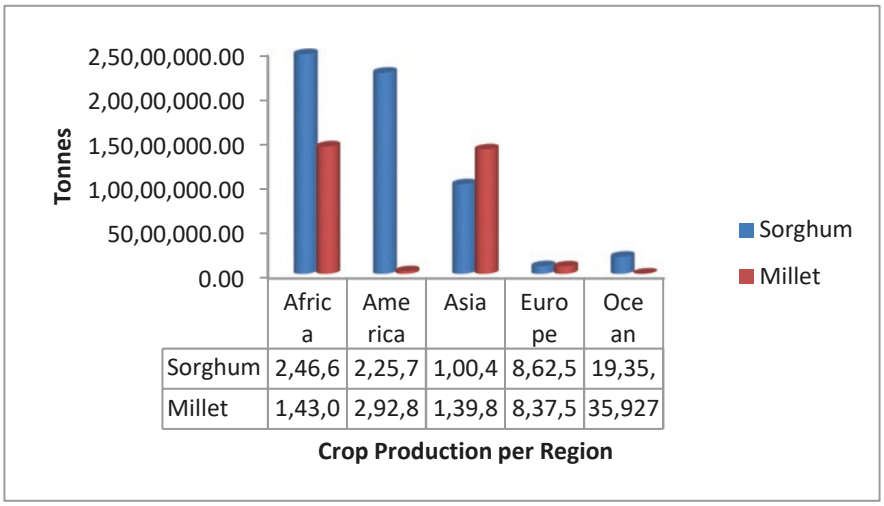
These grains have significant potentials, which has enabled them to be used aa a food and beverage for humans. In developing countries the commercial processing of these grains into value-added food and beverage products is a major concern for economic development (Taylor 2016). Some fermented foods from sorghum and millets grains includes; alcoholic and non-alcoholic beverages, pastries, infant formulas and many more are very useful for human consumption, thus providing the necessary nutrients and phytochemicals. It has been reported that there is an increase living microorganism's in the intestine when fermented food products prepared with probiotic bacteria are consumed. However, there are less information on the quality and safety of traditional fermented food products from sorghum and millet. Therefore, the aim of this chapter was to review the quality aspect and the safety of some fermented food product made from sorghum and millet.

34.2 Millet and Sorghum production

Agriculture remains as the oldest profession that sustains human existence, in the view this without agriculture there will be no life, since there would not be food, clothing and shelter. Sorghum and millet together with other cereals such as maize, rice, etc., are consumed averagely in most homes in Africa, and various other unindustrialized nations around the world, which includes Ghana (FAO 2013). The cultivation of these cereals differs from nation to nation, and this has been as a result of the consumption pattern of humans, as well as the demands from the industries and as a source of revenue generation; but the production is often influenced by soil infertility, subsidies and policies by government and factors related to the environment, which often determines the twelve-monthly production yield. Sorghum and millet were respectively ranked as fifth and sixth cereal crop after wheat, maize, rice and barley in terms of cereals production (FAO 2013). Also, millet can resist pests and diseases, has short growing season, and can thrive produce good yield under drought conditions as compared to major cereals like maize, rice wheat, sorghum and many more (Devi et al. 2011).

Millets are small-seeded with different varieties such as pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum setaceum*), proso millet (*Penicum miliaceum*), foxtail millet (*Setaria italic*), little millet (*Panicum sumatrense*), and barnyard millet (*Echinochloa utilis*) (Bouis 2000; Kaur et al. 2012).

The Food and Agriculture Organization Corporate Statistical Database reported that, the world total production of sorghum and millet grains in the year 2018 were 59,342,103 tonnes and 31,019,370 tonnes. USA and India were the highest producers of sorghum and millet respectively in 2018 with their respective total production of 9,271,070 tonnes and 11,640,000 tonnes (FAOSTAT 2019). In Africa, Nigeria was the leading producer in both crops in the year 2018 with a total production of 6,862,343 tonnes of sorghum and 2,240,744 tonnes of millet (FAOSTAT 2019). It was also reported that Africa was the leading producer of sorghum and millet per



	Africa	America	Asia	Europe	Ocean
Sorghum	2,46,6	2,25,7	1,00,4	8,62,5	19,35,
Millet	1,43,0	2,92,8	1,39,8	8,37,5	35,927

Fig. 34.1 Average production share of sorghum and millet by region (from 2000 to 2018) (FAOSTAT 2019)

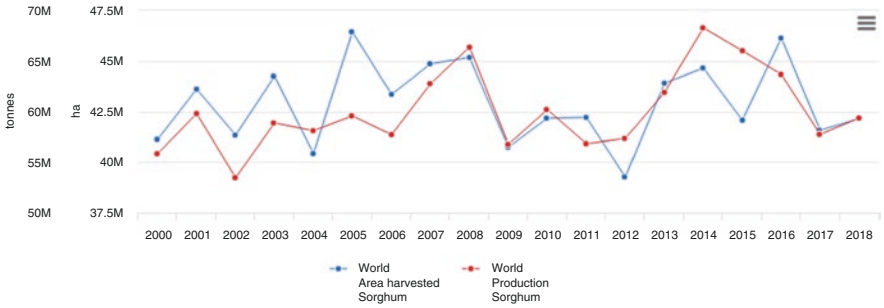


Fig. 34.2 Production/yield quantities of Sorghum in World (from the year 2000 to 2018) (FAOSTAT 2019)

region (World) with a total average production (from 2000 to 2018) being 602,316.42 tonnes and 14, 296,182.95 tonnes respectively (Fig. 34.1).

The data also showed that the highest total global production of sorghum and millet from 2000 to 2018 were achieved in the year 2014 and 2003 respectively (Figs. 34.2 and 34.3) with a total production of 68,277,810 tonnes (sorghum) and 34,820,407 tonnes (millet) (FAOSTAT 2019).

Ghana had a total annual production of sorghum (316,236 tonnes) and millet (181,564 tonnes) in the year 2018 and harvested it highest productions in 2009 with a total production of 350,550 tonnes and 245,550 tonnes respectively for sorghum and millet (FAOSTAT 2019). All the production of sorghum and millet in Ghana is accounted for, by the Northern regions of Ghana, due to the high tolerance of the crop to the dry weather conditions, which prevails in these regions (Wood 2013).

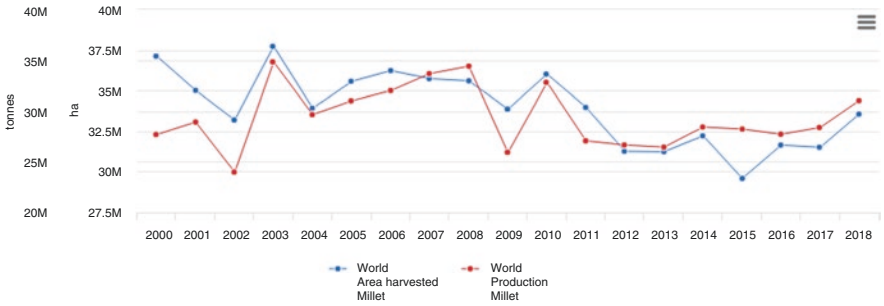


Fig. 34.3 Production/Yield quantities of Millet in the world (from the year 2000 to 2018) (FAOSTAT 2019)

It has been reported that, sorghum may be able to tolerate a higher stress, including water, temperature and salt-stresses (Ejeta and Knoll 2007). The South Africa Department of Agriculture and Forestry (2010) suggested an ideal cultivation time for sorghum starting early November to the late December; with the dates falling on either side of the suggested times regarded as early and late planting, respectively. This suggestion covers the entire sorghum planting areas of South Africa, regardless of climatic variances within these areas.

34.3 Food Products from Sorghum and Millet

Sorghum and millet are important staple crops in Africa that are widely used for many food products. These crops have been utilized in developing nations to resolve problems of food insecurity and malnutrition (Saleh et al. 2013). The grains of these crops are usually converted to many edible forms of products through several processes including soaking, malting, fermentation, germination, milling, roasting, cooking, and many more depending on the product involved. The products from these crops are classified as; beverages (both alcoholic and non-alcoholic), pastries, breakfast foods, infant's foods, and other dishes (Tables 34.1 and 34.2).

34.4 Sorghum and Millet Fermentation

Fermentation can be explained as an aerobic and microbial metabolic process, which uses carbohydrate as a substrate and, thus involve the use of either yeast or bacteria to produce alcoholic or non-alcoholic beverages/products respectively. Fermentation is a method of food processing, which is mostly used on cereal grains whereby the grains become the source of growth for the microorganism. It is a means of food processing and has been applied traditionally and continues to be

Table 34.1 Millet base fermented product from different countries in Africa (Blandino et al. 2003; Osungbaro 2009)

Products	Country
Burikina	Ghana
Busa (liquid drink)	Egypt
Hausa koko (thick porridge)	Ghana
Chikokivana (alcoholic beverage)	Zimbabwe
Dalaki (thick porridge)	Nigeria
Doro (colloidal, alcoholic drink)	Zimbabwe
Kenkey (solid dough)	Nigeria
Kwanu-Zaki (liquid drink)	Nigeria
Ogi (liquid porridge)	Nigeria
Merissa (alcoholic drink)	Sudan
Munkoyo (liquid drink)	Uganda, Tanganyika
Uji (porridge as staple foods)	Egypt, Kenya

Table 34.2 Sorghum base fermented products from different countries in Africa (Ajiboye et al. 2014; Eze and Eleke 2011; Graham et al. 1986; Lemi 2020; Sawadogo-Lingani et al. 2007; Blandino et al. 2003)

Product	Country
Burukutu (alcoholic beverage)	Nigeria, Benin, Ghana
Pito (alcoholic beverage)	Ghana, Nigeria
Orubisi (alcoholic beverage)	Tanzania
Tella (beverage)	Ethiopia
Nasha (Infant food)	Sudan
Khamir (bread)	Sudan
Dolo (alcoholic beverage)	Togo
Bogobe (soft porridge staple)	Botswana
Ogi	Nigeria
Sorghum beer (liquid drink, sour, weak alcoholic drink)	South Africa
Injera (bread-like staple)	Ethiopia
Merissa (alcoholic beverage)	Sudan

used in the production of fermented foods and beverages in most homes, as well as in food industries (Osungbaro 2009).

It increases the starch and protein digestibility, increase protein content, it enhance carbohydrate accessibility, improves organic compound balance, and decrease anti-nutritional factors like tannin and phytic acid (Alka et al. 2012). Previous studies have shown that fermentation improves the palatability, flavor and texture of foods and has shown to increase the concentration of vitamins, protein and minerals (Taylor 2002). The microbial safety and quality of food products, as well as availability of mineral and vitamin B content (thiamine) in food products such as sorghum and millet are enhanced by fermentation process (Mugula et al.

2003). In another report by Léder, (2004) it was observed that, the density of nutrient, the bioavailability and amount of nutrients are increased by fermentation through the breakdown and synthesis of anti-nutritional compounds, pre-digestion of some food components and the uptake of nutrients in the intestine. Fermentation has a positive influence on the health status of humans.

Traditional fermented foods have received scientific attention and plenty of traditional preparations are analyzed for its microbiological, enzymological and biochemical changes (Ayebo and Mutasa 1988). Fermentation induces phytate hydrolysis through the activities of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates, which do not inhibit nonheme iron absorption (Fischer et al. 2014).

Lactic acid bacteria (LAB) fermentation is one of the most widely used traditional method of preparing food in Africa. It is done usually to increase the sensory characteristic (aroma, taste, texture, etc.) of food and improves the quality of food thereby increasing the available of proteins and vitamins present in the food. Some common traditionally fermented foods in West Africa and Africa at large includes; millet and maize porridge, alcoholic and non-alcoholic beverages and dairy products. In addition, LAB fermentation preserves and detoxifies food products thereby increasing the shelf life of the product. Regular use of LAB-fermented foods products is proven to boost the immune system and strengthen up the body of the individuals, in the fight against bacterial infections more especially pathogenic infection. This then support the argument that, LAB fermentation does not only serve as an economic importance, but rather increases human health as well (Masood et al. 2012). Fermentation is usually done by mixing a reasonable amount of water with sorghum or millet flour. The dough or mixture is then allowed to stand between 12–72 hours (depending on the type of product or beverage intended to produce) to ferment at a room temperature (Ranasalva and Visvanathan 2014). The flow chart presented in Fig. 34.3 indicates the steps involved in producing fermented millet flour. Due to fungal growth other contaminating microorganisms on the dough, potassium sorbate or ordinary salt are used to inhibit their activities. Fermentation times differ depending on the product involved (Table 34.3).

Table 34.3 Fermentation time in different product (FAO 2008)

Product	Crop	Fermentation time(hours)
Indera	Sorghum	12–48
Bogobe	Sorghum	24
Injera	Sorghum	12–24
Burungu	Sorghum	48
Porridge	Millet	12–24
Burkina	Millet	12–24

34.5 Nutritional Value of Sorghum and Millet Food Product

As nutritional wellbeing is the driving force for the formation and optimization of human genetic potential, the significant parameter for sustaining human health and full physical wellbeing is conceded to be the nutritional quality of a food (Radhika et al. 2011). It has been reported that over 78% of the sorghum produced in the developing countries, more especially in Africa, are used as food, with about 14% and 7% used as animal feed and other purposes respectively (Batey 2017). It has been well established that, starch and dietary fibre are major components of sorghum, which possibly constitute about 75% of the grain (Awika 2017; Beta et al. 2000). Comprehensive studies by previous authors on sorghum indicated that, the composition of the grain is a good source of energy, protein, carbohydrates, polyunsaturated fatty acids, vitamins, minerals, and some essential amino acids (Awika 2017; Dahlberg et al. 2003). Proximate composition of sorghum grains from previous studies has shown that it contains carbohydrates (54.6%–85.2%), fat (1.3%–10.5%), protein (6.2% to 14.9%), ash (0.9%–4.2%) and fibre (1.4%–19.5%). The variations in these values might possibly be attributed to the growth conditions and genotypes of the grains, as well as other cultivar specific differences.

Millet grain comprises fibres, which are mainly concentrated into the outer layers of the grain. Its endosperm is created of two layers, the aleurone layer the starchy and endosperm. The aleurone layer surrounds the starchy endosperm, with concentrations of lipids, protein, minerals and vitamins. The most important part of the grain is that of the starchy endosperm and consists of floury, corneous zones and peripheral. It is mostly constituted with starch granules within a matrix of protein bodies. The germ of the grain includes the embryo and scutellum, which is found to be rich in proteins, lipids and minerals (Evers and Millar 2002). As presented in Table 34.4, millet is an important source of fibre, fat, protein, which has been established by previous researchers that, it is used to feed many low-income families and provides proper nutrition to those in need. Pearl millet to be precise is the most utilized variety among the millet family globally (Léder 2004).

The dietary fibre present in millet is found to be in the outer layer (pericarp) and is being decreased in quantity to the inside layer (endosperm) of the grain.

Table 34.4 Proximate Composition of Pear, finger millet and sorghum (per 100 g edible portion) (Léder 2004; Muthamilarasan et al. 2015; Patel et al. 2014; Ramashia 2018; Saleh et al. 2013); Shimelis et al. 2009)

Proximate composition	Pear millet	Finger millet	Sorghum
Moisture	12.4	7.15–13.1	12
Protein	11.6–11.8	7.7	10.9
Fat/Lipids	4.8–5.0	1.8	3.2
Ash	2.2–2.3	2.7	1.6
Dietary fiber	11.3	15.0–22.0	2.3
Carbohydrate	67–67.5	75.0–83.3	73
Energy (Kcal)	361–363	–	329

Comparison millet grain to many other cereal grains, it has been established that, the insoluble fibre content of millet (pearl) is 13.5%, whiles the soluble dietary fibre content is found to be 1.45%. The overall dietary fibre of millet is lower than the values of rye and sorghum grains, but has found to be higher than that of wheat (Ragaee et al. 2006).

Fermentation in food products such as sorghum and millet have positive influences on the nutritional compositions with some few exceptions. A study conducted by Ogodo et al. (2019) showed that, as fermentation time of sorghum increases the moisture, ash and the protein content also increases, while, fat, fibre and carbohydrate contents decreased with increasing fermentation time (Table 34.5). The increments in moisture and ash content were attributed to the addition of water to the substrate just before the start of the fermentation and the mineral composition of the substrate respectively. The increase in protein was also attribute to the activities and increasing number of fermenting organisms, since they were in their active site and, therefore had the ability to multiply to further increase the release of proteolytic enzymes. On the other hand, due to the biochemical and physiological changes (energy and lipids utilized) and the breakdown of fatty acids and glycerol to release aromatic compounds during the fermentation may cause the decrease in the fat content. Also, the decreased in fibre and the carbohydrate were attributed to the breakdown and utilization of fibre and sugar components of the sorghum (Ogodo et al. 2019).

In another research carried out by Mbaeyi-Nwaoha and Obetta, (2016) revealed that, there was direct positive correlation between moisture content of millet and increase in fermentation time; as fermentation time increases, moisture content also increases and this was attributed to the absorption of water during the fermentation, while the fibre content showed an inverse correlation as fermentation time increased fibre content decreased. As indicated in Table 34.5, there was an increased in protein concentration with increasing fermentation time except for 48 h fermentation time, likewise similar increments occurred in ash and fat except beyond 24 h fermentation time. The decreased in protein content beyond 36 h fermentation was attributed to

Table 34.5 Proximate composition (%) of fermented sorghum flour using LAB and naturally fermented millet flour (Ogodo et al. 2019; Mbaeyi-Nwaoha and Obetta 2016)

Product	Fermentation Time (h)	Moisture	Ash	Fat	Protein	Fibre	Carbohydrate
Fermented Sorghum flour using LAB	0	9.84	10.32	3.42	10.39	1.96	72.97
	12	10.28	10.40	3.32	11.12	1.32	72.23
	24	10.88	10.68	3.28	11.98	0.84	71.10
	36	10.96	10.92	3.18	13.22	0.65	69.98
	48	11.04	10.98	3.10	13.97	0.54	69.14
Fermented Millet flour (Natural)	0	4.20	1.06	2.50	9.65	3.45	79.19
	12	5.68	1.35	4.60	9.82	3.43	75.14
	24	5.22	1.67	4.96	11.57	2.96	73.65
	36	5.84	1.53	4.82	11.84	2.72	73.36
	48	7.32	1.09	3.77	8.37	1.13	78.56

LAB (Lactic acid bacteria)

the fact that the microorganism had utilized the available nutrients for their activities. Furthermore, values for carbohydrate decreased with increasing fermentation time except for 48 h fermentation time, which showed increment in its value.

34.6 Safety and Quality of Sorghum and Millet for Food Production

Food safety and quality is a major concern of every consumer. However, in preventing food poisoning and achieving safety and quality of food products from sorghum and millet, the application of the HACCP principles is needed. HACCP is a management procedure used to address food safety and quality through the analysis and control of biological, physical and chemical hazards from raw material production, through handling, to processing, distribution and consumption of the finished product. It is a method used in analyzing and identifying potential hazards in a production; where they may occur and how critical these hazards are to consumer safety.

However, the HACCP system establishes control measures that emphasizes on the prevention of such hazards rather than depending on testing the end-product (Sivasankar 2002). Therefore, for quality and safe fermented sorghum and millet food products, the following seven HACCP principles must be ensured; (1) Conduct hazard analysis to identify hazards that may be present in sorghum and millet from harvest through to consumption, (2) Determine Critical Control Points (CCPs) to control the identified hazards, (3) Establish critical limits at each CCP, (4) Establish proper monitoring systems for each identified CCPs, (5) Establish corrective actions that should be taken when critical limits are not met, (6) Establish recordkeeping and documentation's procedure and (7) Establish procedures for verification to ensure HACCP system is working effectively (NACMCF 1998). Previous studies by Edema and Anetor, (2009) and El-Razik et al. (2016) following the HACCP principles in production of fermented Kunun-zaki and Egyptian kishk respectively showed high level of quality and safety of the products.

Quality control refers to the set of activities that are carried out along the seed systems value chain to ensure that seed produced, processed and marketed are of highest quality. Base on the authority of the National Seed Law, seed quality is regulated and must meet established minimum standards. Seed quality is a major concern in all seed production and delivery systems. All seed programmed seeks to produce and supply highest quality seed to increase productivity, resist or tolerate pest and diseases pressure and fulfill the market demand of quality produce (Van Gastel et al. 2002). However, seed quality has five main features; the physical purity, the vigor, the purity of the variety, the genetic content of the seed and the seed health (Minot et al. 2007).

Grain quality evaluation (GQE) usually employs rapid screening techniques to access all qualitative and quantitative parameters to ensure that, the final use quality of sorghum and millet grain are of the acceptable quality and standard. All tests are carried out on healthy whole grains from a representative sample with a minimum

of two replicate tests on each sample (Gomez et al. 1997). Any defects in the grain within the sample being tested should be noted and defective grain discarded. After harvest the grain should be kept in a cold store at 4 °C. It should be aloof from the shop 24 h before testing begins, and lay out on a working bench to equilibrate to temperature and humidity. Several methods of testing are being employed by the grain quality evaluation, since there are variations in various grains.

Millet and sorghum grains are being tested for their quality and safety before they are used for food productions. They are analyzed for their hardness, grain color, endosperm texture, pericarp thickness and the presence or absence of testa. Furthermore, analyses on insect damage, fungal growth (mold), and shriveled or broken kernels, which may affect the quality of the grains, are inspected. Grain color inspection is very important in food processing, since color has an influence on food product. For instance, millet grain milled for porridge should be of white or light color (Gomez et al. 1997).

In addition, milling of sorghum or millet is influenced by the hardness of the grain, thus the harder the grain the higher the milling yields. Also, hardness of these grains also has direct influence on the ability to absorb water, which however affects the activities of enzymes. Another aspect of sorghum and millet quality is regards to the amount of water present in the grain. Moisture content of these grains has an impact on microbial growth especially fungal growth with aflatoxin been the major contaminant. The higher the moisture content (greater than 12%) present in the grains the higher the susceptibility to fungal growth more especially aflatoxin (Gomez et al. 1997).

34.7 Conclusion

The fermentation of sorghum and millet food products and beverages leads to an increase in the protein content, mineral and vitamins. It also enhances digestion and increases the fiber content and contributes to a higher bioavailability of mineral elements. The quality and safety of food products from fermented sorghum and millet products may be dependent upon the strict adherence and application of the Hazard analysis and critical control points (HACCP) principles and the practice of good manufacturing procedures.

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Chapter 35
Utilization of Jerusalem Artichoke (*Helianthus tuberosus* L.) Tuber as a Prebiotic and a Synbiotic

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35.1 Introduction

Jerusale artichoke (JA) (*Helianthus tuberosus* L.) exist as a plant that produces fresh tubers, and it is native to the North Americans. It was made known to Europeans, where varied Latin and other names were given to it. For instance, Kays and Nottingham (2007) gathered together 100 common names of JA including sunchoke, topinambur, woodland, sunflower or earth apple etc. JA has high growth-rate, can endure a layer of ice that forms on the ground, and impoverished soil. It can resist insects that damage crops and can be planted with or without fertilizer (Duke 1983; Slimestad et al. 2010).

Structurally, the JA is large, gangling, and has a branched yellowish flower head. The stems are thick and strong and grows within the range of 100–300 cm high. Its leaves found at the opposite side of the stem grows between 5–10 cm width and 10–20 cm long (Justimenko et al. 1976). The JA has a fibrous root-system, which are hairy and may grow to 127 cm (Ma et al. 2011). The fleshy tubers (roots) are not regular in shape (Fig.35.1) and differ in color (Long et al. 2016). JA as a crop can easily grow because it tolerates different range of temperatures and has the ability to endure pH levels within the range of 4.5 to 8.2 (Ma et al. 2011). Mostly, it is cultivated for its fleshy root, commonly used as a vegetable. JA is used to feed animal (Ma et al. 2011; Swanton and Hamill 1994), however other uses are being explored for development as a functional-food, since it contains soluble-dietary fiber (Panchev et al. 2011; Praznik et al. 2002). The amount of inulin present in JA tuber is important, since it can drop blood glucose concentration, and has the ability to ensure minerals are made available at the site of physiological activity. Also, it

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Fig. 35.1 Plant and tubers of Jerusalem artichoke

enhances the rheological attributes and technological superiority, as well as nutritional value of eatable products (Afoakwah et al. 2015; Niness 1999a; Praznik et al. 2002). Again, it has bioactive compounds, which can be extracted from its leaves, stems and tubers, and may be used in the pharmaceutical and in the meat and meat product industry (Afoakwah et al. 2015; Pan et al. 2009; Tchoné et al. 2006; Yuan et al. 2012). More so, JA tuber is noted as a healthy vegetable utilized to inhibit several ailments (Ahmed et al. 2005).

35.2 Nutritional and Phytochemical Value of Jerusalem Artichoke

JA do not contain starch and saturated fat, but have trace quantities of mono-unsaturated and poly-unsaturated fatty acids such as linoleic (18:2 cis, cis n-6) and α-linoleic acid (18:3 n-3), (Kays and Nottingham, 2007). It is a known source of stored inulin (fructan). The inulin content is within the range of 7–30% fresh weight (fw) and 50% dry weight (dw). Loo et al. (1995), indicated that inulin concentration ranged between 8–21% fw, but Matías et al. (2011) reported 136.75 and 164.78 (mg/g) inulin concentration in JA tubers. Besides, the existing amounts of fermentable polysaccharides, the JA is well-endowed with amino acids; moreover, the tubers are of a high-quality source of vitamins and β-carotene (Kays and Nottingham 2007; Loo et al. 1995). Also, higher quantities of folic acid (13 to 22 μg) have been reported. JA contains several minerals notable among them are K, Ca, Mg, Fe, Cu, P, Mn, Zn, Co, Al, Pb and Zn (Ekholm et al. 2007). It also contains 83.4% water (Ekholm et al. 2007).

Concerning phytochemicals, it has been shown that it contains coumarins (Cabello et al. 1998), polyacetylenic derivatives (Matsuura et al. 1993), and sesquiterpenes (Baba et al. 2005; Miyazawa and Kameoka 1983; Morimoto et al. 1966, Spring 1991). Extracts from JA can terminate the proliferation of microorganisms. Isolated heliangin, a germacrane sesquiterpene lactone from JA foliage, showed in-vitro action when applied on Ehrlich ascites carcinoma cells (Ahmed et al. 2005). Nakagawa et al. (1996), also proved callus of JA has two lectins, which showed hemagglutination biological activity. In a study, Pan et al. (2009) isolated

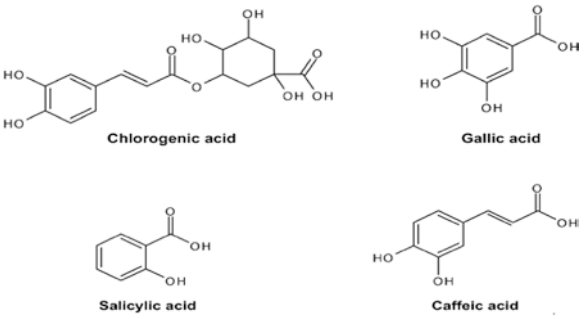

Fig. 35.2 Main phenolic acid in JA tuber

bioactive compounds from JA plant. Also, JA leaves extracts displayed antioxidant and antifungal properties (Hai-wei et al. 2007; Moskovitz et al. 2002; Yuan et al. 2012). JA tubers are composed of phenolic and polyphenolic compounds (Fig. 35.3). These phenolic-compounds (salicylic, chlorogenic-acid caffeic acid and gallic acid) have encouraging physiological abilities like antioxidant, antimutagenic and antitumor capacities (Bach et al. 2013; Ekholm et al. 2007; Tchoné et al. 2006) (Fig. 35.2).

35.3 Chemical Composition and Uses of Jerusalem Artichoke Tuber Inulin

Inulin is the foremost constituent of JA fleshy root-tubers, it also exits in other plants (Table 35.1). Inulin is a D-fructose attached by (2 → 1) β-linkages terminated via D-glucose molecules fused to fructose thru (2 → 1) α-bonds. For inulin to polymerized, it should range between 2–60 with a molecular weight averaged as 5500 Da (Duke 1983; Kalyani Nair et al. 2010; Kelly 2008; Szambelan et al. 2005). The JA fleshy root tubers stores carbohydrate in the form of inulin, while its concentration and polymerization are based on the cultivar, growing environments, harvest time, and how mature the fleshy root tubers are. The fleshy-root tubers can be preserved in cold rooms. During storage inulin concentration may be reduced to form shorter-chain inulin-polymers and sucrose.

Inulin as a natural polysaccharide has varied pharmaceutical and food applications, and can be obtained from JA tubers (Fig.35.3). Inulin is employed as a low energy-sweetening agent capable of forming gels. Generally, it is applied as a sweet substance and fat substitute in processed foods (Meyer et al. 2011). Inulin was utilized in dairy, cereal, bakery and meat products (González-Herrera et al. 2015; Karimi et al. 2015; Kuntz et al. 2013; Rodriguez Furlán et al. 2015; Meyer et al. 2011).

Food usage of inulin has been extensively studied (Barclay et al. 2010; Boeckner et al. 2001; Franck 2002; Kelly 2008; Meyer et al. 2011; Tungland and Meyer 2002). Again, inulin has been utilized in pharmaceutical products as a stabilizer in

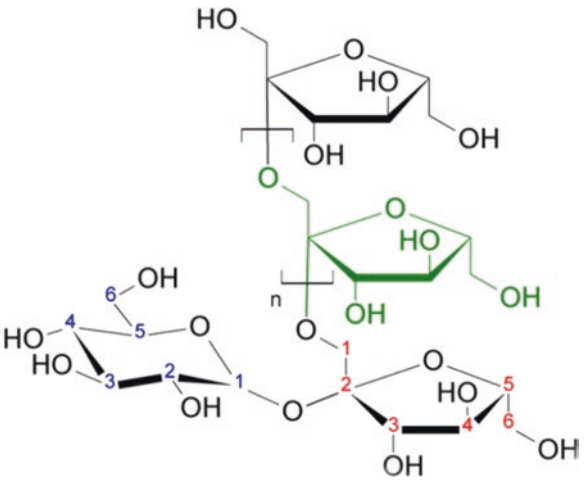

Fig. 35.3 Chemical structure of inulin

Table 35.1 Inulin and fructo-oligosaccharides content of food products

Food sources	Inulin (g/100 g)	Fructo-oligosaccharides (g/100 g)
Jerusalem artichoke tuber (*Helianthus tuberosus*)	16.0–20.0	10–15
Raw onion pulp (*Allium cepa*)	1.1–7.5	2.0–6.0
Asparagus raw (*Asparagus officinalis*)	0–3.0	5.0–10
Chicory root (*Cichoriumintybus*)	35.7–47.6	5.0–10
Barley (raw cereal) (*Hordeumvulgare*)	0.5–1.5	0.5–1.5
Wheat (flour baked) (*Titicum sp.*)	1.0–3.8	1.0–3.8
Garlic (*Allium sativum*)	9.0–16.0	3.0–6.0
Leek (*Allium ampeloprasum*)	3–10	2–5
Banana (*Musa sapientum*)	0.3–0.7	0.3–0.7
Artichoke (*Cynara scolymus*)	3–10	<1
Yacon (*Smallanthus sonchifolius*)	3–19	3–19
Dandelions (*Taraxacum officinale*)	12–15	NA
Rye (*Secale cereale*)	0.5–1.0	0.5–1.0

Gupta and Chaturvedi (2020), Meyer (2009) and Loo et al. (1995)

protein-based products (Hinrichs et al. 2001), as a tool for diagnosis to measure glomerular filtration-rate (Orlando et al. 1998), texture-modification agent, and as a fat substitute in food products, again it is resistant to flow, it is undigestible in people due to the presence (2 → 1) glycosidic bonds, besides it is a sweetener with a lower energy value and fat replacer (Barclay et al. 2010). Colonic-microbes (lactobacilli), have the ability to break inulin bonds, thus enabling inulin biological functions to be realized at the colon. The amorphous nature of inulin coupled with its high transition temperature, and its flexible backbone brands it as a protein

stabilizer (Tonnis et al. 2015) in food (Afoakwah et al. 2015; Rodriguez Furlán et al. 2015). Also, inulin being crystalline in nature makes it a suitable adjuvant for vaccine production (Honda-Okubo et al. 2012).

35.4 Chemical Composition and Uses of Jerusalem Artichoke Tuber Fructo-Oligosaccharides

Fructo-oligosaccharides (FOS) (oligofructan and oligofructose) exist naturally in certain vegetables and fruits (Muir et al. 2009;Ibrahim, 2018). Chemically, FOS is a short chain of fructose polymer made of units of D-fructose. Connected thru β (2–1), and it is undigestible by gastrointestinal enzymes, but can be hydrolyzed by inulin with the help of endoinulinase enzyme through an enzymatic reaction using sucrosetrans-fructosylation with the help of β fructo-furanosidase or fructosyltransferase (De-Sousa et al. 2011). The chemical structures of FOS include 1-kestose, nystose, and 1-β-fructofuranosyl nystose (Fig.35.3).Their fructose-units are connected at β (2–1) glycosidic bonds with the terminal glucose-units connected to fructose unit at the α (1–2) glycosidic-bond.

FOS may be utilized as a sucrose substitute in yogurt, beverages and as a low-calorie sweetener for diabetics. They increase the production of favorable bacteria in human colon, also it can be utilized as soluble-dietary-fibre (Costa, et al. 2015). It has mineral (calcium and magnesium) absorption ability, it lowers blood pressure and it can inhibit the production of reductase enzyme (Coundray et al. 2003). It prevents obesity, it has immune system stimulating ability, it decreases the synthesis of tri-glycerides and fatty acids in the liver, and can reduce blood glucose concentration (Kolida and Gibson 2007) (Fig. 35.4).

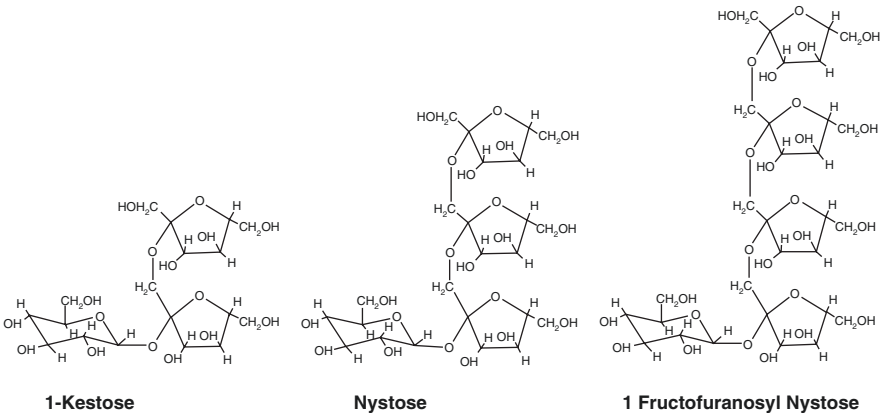

Fig. 35.4 Chemical structure of fructo-oligosaccharides

35.5 Prebiotic Attributes of Jerusalem Artichoke Tuber

Prebiotics are food components that are unable to digest, but has the ability to affect positively the host by stimulating the proliferation of pro-biotic bacteria in the digestive tract, thereby advancing its health enhancing effect. Examples of prebiotics include inulin and oligofructose, they exist in Jerusalem artichoke, banana, onions, garlic, leeks, and wheat, and resistant oligosaccharides including inulin-type fructans. Studies have showed that, inulin has the ability to improve digestive tract functions by boosting fecal frequentness and mass. Also, it decreases fecal pH value. These properties assist in suppressing the output of decaying-substance in the colon (Gibson and Roberfroid 1995; Glenn R Gibson et al. 1995; Kalyani Nair et al. 2010; Niness 1999b; Trowell and Burkitt 1986). Inulin as a prebiotic can invigorate production of valuable bacteria strains in the colon, which has the ability to improve the absorption of vital mineral such as calcium and magnesium and the production of B vitamins (Abrams et al. 2005; Coudray et al. 2003; Franck 2005; Kelly 2008; Levrat et al. 1991; Niness 1999b).

The positive outcome of prebiotics on gut health is not well demonstrated. However, some likely cellular actions have been suggested (1) Prebiotics are noted to bring under control hepatic lipogenic-enzymes, (2) it does produce short chain fatty acid (SCFA) from fermentation of fiber, particularly Butyrate, which is known to change or adjust a histone-tail acetylation and, thus upsurge the availability of several transcriptional gene factors (3) it modulates mucin to grow by proliferating (4) Fructo oligosacharride, and some other prebiotics improves lymphocyte and/or leucocyte-numbers in gut associated-lymphoid tissues and peripheral blood, it improves IgA secretion by the gut associated-lymphoid tissues, which is known to stimulate the phagocytic function of intra-peritoneal macrophages (Schley and Field 2002). Animal study has established that inulin supplementation improved the production of SCFA in the caecum (Birkeland et al. 2020).

Inulin made of β (2,1) linkages (fructose monomers) are not digestible by the intestinal enzymes of humans, thus, making it a good candidate to be incorporated in to food products, which can be used to curb diet-related health conditions (Howard et al. 2015; Kalyani Nair et al. 2010; Kelly 2008; Niness 1999b; Rumessen et al. 1990).

Inulin does not metabolize until it arrives in the large intestine, where it is fermented using colonic-microflora. Thus, intake of inulin has no potential to stimulate insulin production, besides, it has been found that inulin in a foods decreases fat concentration in rats and in humans (Jackson et al. 1999; Kaur et al. 1989; Kaur et al. 1988; Pedersen et al. 1997).

As prebiotic passes thru the gut without being digested by digestive enzymes, they offer useful modifications to the gastro-intestinal tract and other organs. Prebiotics provides nourishment to beneficial bacteria that exist in the gut. Prebiotics and probiotics work together to ensure that fermentable ingredients produce good bacteria to ensure a healthy microbiota. They play an essential role in maintaining healthiness by upholding the balance and diverseness of intestinal bacteria by

multiplying *lactobacilli* and *bifidobacteria.* Because, a healthy gut is linked to various bodily functions, prebiotics and probiotics fights a condition in which part of the body become swollen and painful, also they decrease the risk of diseases.

Higher intakes of prebiotics are linked to benefits including lower risk for cardiovascular disease, good cholesterol concentration, improved gut health, enhanced digestion, reduced stress response, better hormonal balance, higher immune function, lower risk for obesity and weight gain, lower inflammation and autoimmune reactions (Hsu et al. 2004; Nguyen et al. 2007; Parnell and Reimer 2010). Galacto-oligosaccharides (GOS) consumption by humans caused the activity of nitro-reductase involved in creating genotoxic metabolites to decrease. This shows the healthy potentials of prebiotics and probiotics as an agent capable of curbing carcinogenesis (Macfarlane et al. 2006). Prebiotic should possess the following technological properties (1) should be beneficial to the host health, (2) must be able to withstand digestive processes (3) should be able to under-go fermentation by the intestinal microbiota, and (4) be stable to food processing treatments (Kuo, 2013).

35.6 Synbiotic Qualities of Jerusalem Artichoke Tuber Inulin

Vrese and Schrezenmeir (2016) reported that the addition of prebiotics with its beneficial effect to probiotics is termed synbiotics. A synbiotic product has positive outcome on the host by enhancing the growth and/or stimulating the metabolism of a healthy microbes in the intestinal tract. Synbiotics are developed purposely to curb the survival difficulties associated with probiotics. Watson et al. (2013) observed that lactulose, maltodextrin, fructo-oligosaccharides, galacto oligosaccharides, and the galacto-oligosaccharides (9) inulin (1) blends stimulates the growth a of bifidobacteria, while Wichienchot et al. (2010) mixed pitaya oligosaccharides and inulin as a source of carbon for B. bifidum NCIMB 702715 production, and it was clear that inulin had a greater influence on the growth of the bacteria as compared to pitaya oligosaccharides. More so, a blend of B coagulans with inulin, significantly decreased the concentrations of C-reactive protein, but the concentrations of glutathione increased (Panda et al. 2006).

Adding Lactobacillus, Bifidobacterium, and 10% FOS in rats fed containing higher concentration of fat and a lower-fiber diet content prevented intestinal and systemic- inflammation (Delcenserie et al. 2008). Hypercholesterolemic male pigs fed with a synbiotic preparation for 8 weeks revealed a promising hypercholesterolemic activity (Liong et al. 2007).

Lacbobacilli, Bifidobacteria spp., S. boulardii, and B. coagulans are strains used for preparing synbiotic goods, while that of prebiotics comprise of fructooligosaccharide, xyloseoligosaccharide and inulin. The benefits associated with consuming synbiotics are: increase in the levels of lactobacilli and bifidobacterial, stabilization of microbiota of the gut, good-liver performance, enhancement of the immune system, inhibit bacterial translocation and a decrease in the occurrence of nososcomial disease in surgical patients (Zhang et al. 2010).

35.7 Conclusion

Jerusalem artichoke is noted to possess a number of heath properties, which can be ascribe to its salicylic, chlorogenic acid, caffeic acid and gallic acid. It also contains inulin and fructo-oligosaccharides. They have the potential to upsurge the proliferation of favorable bacteria in human colon, thereby preventing obesity, decreasing tri-glycerides and fatty-acids synthesis in the liver and the lowering of blood glucose concentration. The prebiotics ability of inulin together with its beneficial effect on probiotics makes the fleshy root tuber of Jerusalem artichoke a potential agent for the production of synbiotic products.

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Chapter 36
The Nutritional and Therapeutic Benefits of Some Nigerian Fermented Food Products

Beatrice Mofoluwaso Fasogbon, Oluwaseun Hannah Ademuyiwa, and Oluwaseun Peter Bamidele

36.1 Introduction

Fermentation is a common and ancient method of food processing that involves a chemical change in the taste, smell, and texture of food caused by microorganism-generated enzymes actions. Fermentation is a process of converting raw food to edible finished products, with new flavours, texture and extended shelf life. Fermented foods contribute majorly to Nigerian diets, serving as main course meal from roots and tubers such as cassava (*gari, fufu*), beverages from cereals (*kununzaki, fura*), condiments from oilseeds, legumes (*iru, ugba, ogiri*), and other major diets. These food materials are usually not consumed in their raw state; they are fermented to improve nutritional value, organoleptic property and digestibility of the food.

In Africa, many proteinaceous seeds are fermented to make food condiments, but *iru* is known to be one of the most important condiments in Nigeria and many other African countries. This chapter discussed the nutritional and therapeutic benefits of fermented foods, one from each category of fermented starchy foods (*gari*), fermented non-alcoholic beverage (*kunun*), and fermented vegetable protein (*iru*).

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36.2 *GARI*

Cassava (Manihot spp) is a woody shrub cultivated majorly in the tropical and subtropical regions of the world for its edible tuberous roots. It is one of the major sources of carbohydrates in the tropics after rice and maize. It is a major staple in many developing countries, one of which is Nigeria as it provides a basic diet for about 500 million people. Nigeria is the world largest producer of cassava accounting for about 21% of the total world production of the crop (Airaodion et al. 2019).

With carbohydrate content about 40% higher than that of rice and 25% higher than that of maize, cassava is regarded as the cheapest source of calories for both human and animal nutrition. Despite cassava being a rich source of calorie due to its high carbohydrate content, its food value is compromised due to the presence of cyanogenic glycosides. However, these anti-nutritional and toxic constituents can be eliminated via proper processing techniques and technologies (Adepoju et al. 2010). In Nigeria and like other most African countries, 95% of cassava produced is usually consumed as food such as *gari*, *elubo*, *fufu*, bread, *tapioca*, chips and so on (Airaodion et al. 2019; Evans et al. 2013) while 5% is for industrial purposes such as ethanol and starch production.

Gari, been a common sour, starchy, fermented cassava product, is important to millions of people in Nigeria, especially those in the Southern States. *Gari* is dry, crispy, white-creamy granules, made from crushed, fermented, sieved, roasted or fried cassava roots. *Gari* is consumed either by after soaking in water and taken with or without sugar/snacks, or reconstituted to a stiff dough and consumed with various soups. *Gari* involves several methods of production, where the freshly harvested cassava roots are peeled, washed and grated into pulp or mash (Evans et al. 2013; James et al. 2012; Sanni et al. 2009). The mashed cassava roots are allowed to dewater and ferment simultaneously in clean woven polythene sacks for 48 to 72 h (James et al. 2012). These processing steps removes poisonous cyanogenic glycoside from the tuber. The fermentation process of the mashed cassava root start during pressing and is dependent on the autochthonous microbial populations. The most common lactic acid bacteria (LAB) involved in cassava fermentation is *Lactobacillus planatarum* (Petrova et al. 2013). *L. planatarum* is a bacterial lactic acid that converts sugars of low molecular weight into lactic acid and thus contributes to the organoleptic quality and preservation potential of the fermented product (Mora-Villalobos et al. 2020). It synthesizes a large amount of extracellular α-amylase which help to breakdown starch granules (amylose) in cassava to glucose and maltose (Petrova et al. 2013). Glucose generated during the hydrolysis of starch by α-amylase further metabolized to lactate. An increase in fermentation time allows LAB to work on starch conversion to lactic acid, which is responsible for the characteristic sour taste of *Gari* induced after frying/roasting (*gari*fying).

Gari is an energy-dense, widely available and cheap food deficient in several important nutrients such as proteins and vitamins. Because of this deficiency, fortification with other food crops such as cereals, legumes, roots and tubers could improve the nutritional quality of *gari*. Grated *gari* at different ratio has been

combined with soybeans (Osho 2003), melon seed and moringa seed flour (Yetunde Ezinwanyi 2017). During mashing, other root or tuber such as potato can be added to cassava root to improve the nutritional quality of the final product, and to increase utilization of other underutilized roots and tubers. Cassava root and cocoyam have been processed separately (Olatunde et al. 2013), and co-processed at different substitution ratios (Bamidele et al. 2014) before *gari*fying. Mashed sweet potato mixed with cassava gave a good quality of *gari*, acceptable at a certain level of substitution (Ojo et al. 2013). Cassava chips can be frozen (18 ± 2 °C) for one month, and still result in a good quality *gari* (Oyeyinka et al. 2019).

The high fibre content of *gari* benefits digestion. It helps to add bulk to stool for easy movement through the gastrointestinal tract, preventing constipation, intestinal pain, flatulence, bloating and colon cancer (Zekarias et al. 2019). Also, the fibre in *gari* reduces cholesterol in the arteries and blood vessels, thereby reducing the risk of stroke and myocardial infarction, indicating possible cardioprotective ability (Oyabambi et al. 2014). High dietary fibre in *gari* reduces blood cholesterol (Anderson et al. 2010), thus reducing the risk of hypercholesterolemia, a risk factor in atherosclerosis (Trinidad et al. 2013). *Gari* is also rich in potassium, a vasodilator that opens up the blood vessels and arteries making blood flow freely through them to all parts of the body.

Gari has a positive effect on bone and neurological health being a rich source of vitamin K, calcium and iron. These minerals and vitamin play a huge role in protecting the bone mineral density thus preventing the deterioration of the bone as one age, which is associated with conditions such as osteoporosis, osteoarthritis, weakness and lack of flexibility of the bones. Vitamin K has also been implicated in inhibiting the progression of Alzheimer's disease, as it can stimulate neuronal activity in the brain. The antioxidant activity of vitamin K means it can scavenge free radicals which break down brain tissues and keep neural pathways active (Zekarias et al. 2019). The nutritional and health claim of gari as extracted from literature is shown in Fig. 36.1.

36.3 *KUNUN-ZAKI*

Kunun-zaki is a non-alcoholic fermented beverage popularly consumed as breakfast in the Northern part of Nigeria. This beverage is however becoming more widely consumed in the southern part of the country as a refreshing drink any time of the day. The main ingredient in the preparation of *kunun*-zaki is millet, traditionally processed using different methods. Each method involves steeping of grains, wet milling, wet sieving, mixing a larger portion of the boiled slurry with the remaining raw portion. Spices such as ginger, garlic, cloves, black pepper, chilli pepper and tamarind are added to the mixed slurry for flavour, (Olaoye et al. 2016) and allowed to ferment (Agarry et al. 2010), then the beverage is sweetened, bottled and stored under refrigeration condition. The fermentation process involves mainly lactic acid bacteria and yeast, which improves the sensory and nutritional properties of the

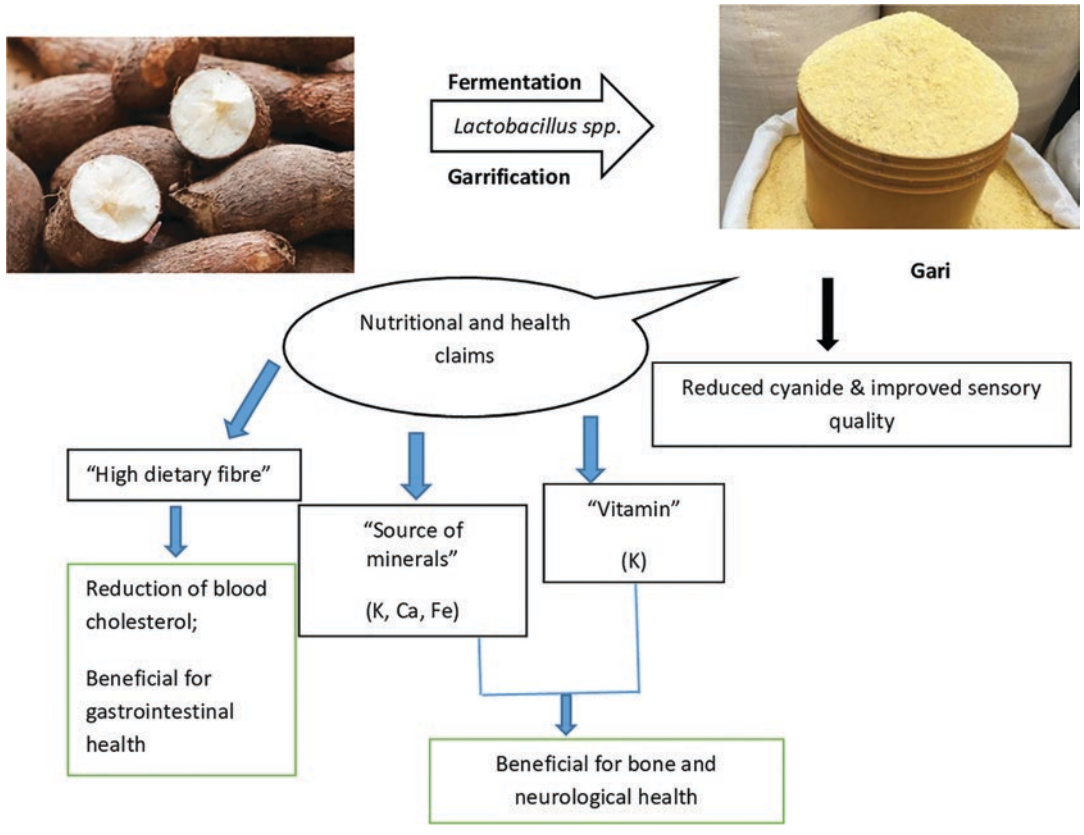

Fig. 36.1 Nutritional and health claim of *gari*

beverage. Several types of cereals (maize, sorghum, wheat, guinea corn, and rice) are sometimes mixed with millet during the preparation of *kunun*-zaki, using different processing methods and ingredients (Agarry et al. 2010; Ogungbemi et al. 2017). However, sorghum has shown to be a better grain in *kunun*-zaki production than maize, based on the result of the nutritional, sensory and shelf life properties (Ogungbemi et al. 2017). In the aim of reducing processing time, a shelf-stable powdered *kunun* has been explored using sorghum and sweet potato (Ndulaka et al. 2014), which will be reconstituted before consumption.

Kunun-zaki have high water content (87–91%), but low in crude protein (3.19–7.86%), fats (0.37–0.75%), ash (0.93–1.20%) and carbohydrates (2.69–5.84%) (Ofudje et al. 2016). *Kunun-tsamiya* (millet, sorghum or rice), *kunun-gyada* (rice, peanut, millet or sorghum), and *kunun-zaki* (millet, sorghum or maize) are different varieties of *kunun* beverage. *Kunun-zaki* is the commonest among other varieties (Ezekiel et al. 2019), enrichment with other food crops (legumes, nuts and tubers) and spices has helped to improve the nutritional quality of this refreshing beverage (Adelekan et al. 2013). The fortification, elongation of shelf-life, improvement of organoleptic and probiotic properties of *kunun-zaki* is achievable by systematically selecting microbial strains (*Lactobacillus plantarum, Lactobacillus fermentum and lactococcus lactis*) as starter cultures for fermentation purpose and/or to augment natural fermentation of this beverage (Adedire et al. 2018; Agarry et al. 2010).

Millet grains, the main ingredient in *kunun* is an alkaline-forming grain that is gluten-free. They are good sources of carbohydrate, fats, proteins, dietary fibre, vitamins, minerals, and some essential fatty acids. Millet proteins are good sources of essential amino acids except for lysine and threonine but are quite high in sulphur-containing amino acids methionine and cysteine. Millets have antioxidant, anti-cancer, and cardioprotective properties (Adedire et al. 2018) due to the presence of vitamins, minerals, essential fatty acids, fibre, as well as resistant starch, β-glucans, flavonoids and other active compounds including lignans and phytosterols (Saleh et al. 2013). Evidence has shown that millet and resultant food products such as *kunun* are protective against several degenerative diseases, good galactagogue, and its high iron content help in blood circulation (Saleh et al. 2013), an illustration of these health and nutritional benefits is shown in Fig. 36.2.

Kunun **as a Probiotic Food** Several probiotics have been isolated from *kunun*, including *Lactobacillus spp, Bifidobacterium spp* and *Lactococcus spp*. These probiotics bring balance to the gut microflora by increasing the number of beneficial organisms while inhibiting the growth of harmful bacteria in the gut, thus repopulating the colon when antibiotics, chemotherapy or disease reduces bacteria levels. Probiotics help to aid digestion and improve bowel movement (Ayo-Omogie and Okorie 2016). Consumption of *kunun* can therefore help to modify the gut immune response, control or modulate the development of certain allergic reactions, modulate the immune system and improve barrier function.

Kunun **as Anti-Diabetic Food** Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia due to dysfunctional carbohydrate, protein, and lipid metabolism (Saleh et al. 2013). Millets possess anti-diabetic property, which

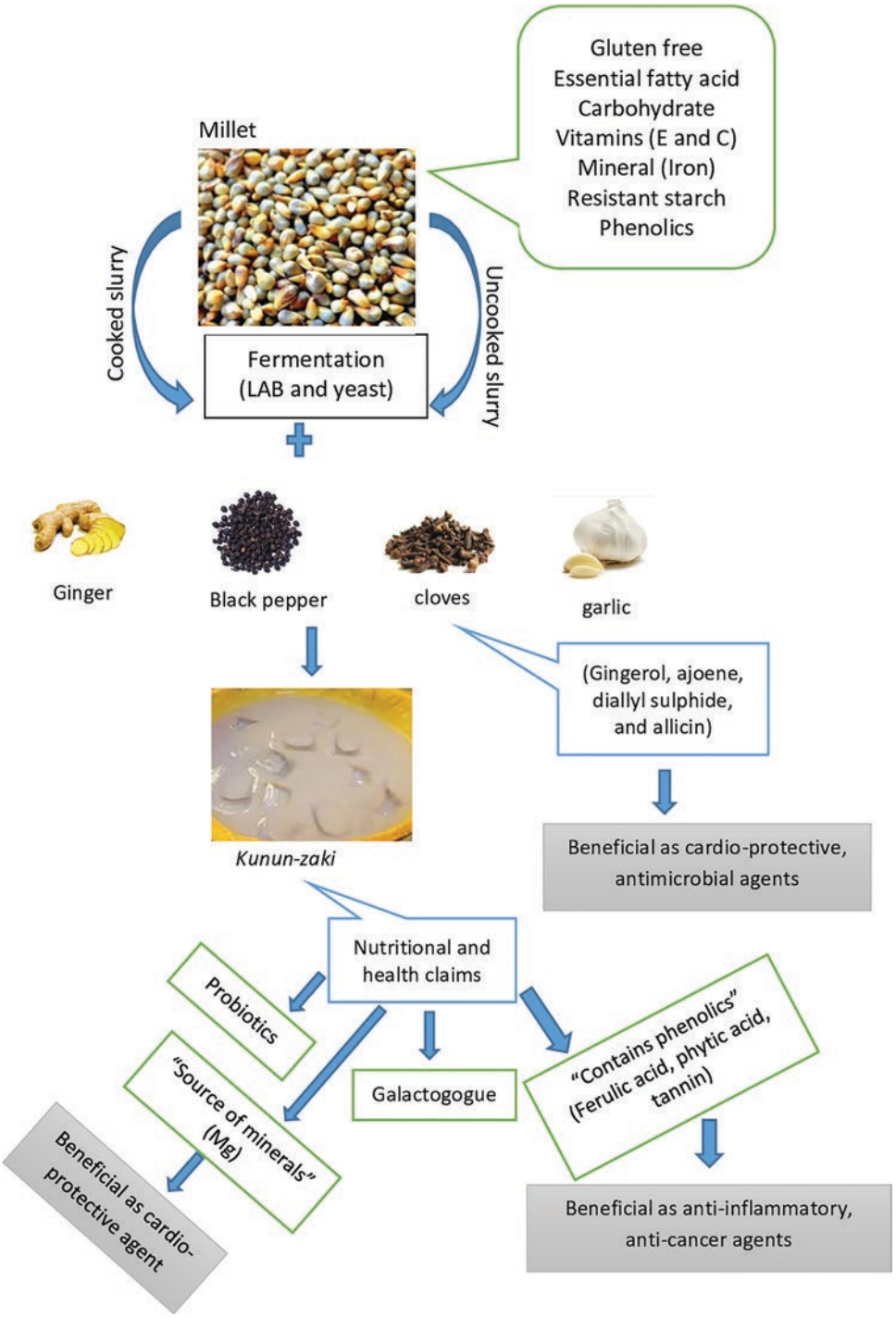

Fig. 36.2 Nutritional and health claim of *Kunun-zaki*

might be due to the higher fibre content of millet when compared with other cereals such as rice and wheat. Phenolics in millet can inhibit enzymes (alpha-glucosidase, pancreatic amylase) responsible for the hydrolysis of complex carbohydrates thus reducing postprandial hyperglycaemia. Also, the presence of phenolic compounds such as gallic, protocatechuic, ρ-hydroxybenzoic, ρ-coumaric, and the flavonoid quercetin in millet inhibits the enzyme aldose reductase (Saleh et al. 2013) that causes accumulation of sorbitol, thereby reducing the risk of diabetes-induced cataract diseases (Kim et al. 2011).

Consumption of millet helps to control blood glucose level, improves antioxidant status and nerve growth factor (Sarita and Singh 2016). Diabetic rats showed decreased levels of oxidative stress markers, and increased antioxidants levels thus hastening the dermal wound healing process after consuming millet (Kim et al. 2011). Consumption of millet is also able to increase the levels of antioxidants such as glutathione and vitamins E and C, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in diabetic animals (Saleh et al. 2013). Furthermore, the antidiabetic effect of *kunun* is not solely due to millet, spices (ginger, garlic and black pepper) added during production have also been implicated in its anti-diabetic activity. These spices increase insulin secretion and inhibit the endogenous production of glucose. Phytochemicals (polyphenols, alkaloids) contained in the spices help to modulate various metabolic reactions which directly or indirectly reduce blood glucose (Otunola and Afolayan 2015).

***Kunun* as a Cardio-Protective Food** Millets from which *kunun* is produced, are good sources of magnesium, a mineral capable of reducing migraine and heart attack (Sarita and Singh 2016), and are also rich in phytic acid, a substance known to lower cholesterol (Coulibaly et al. 2010). The consumption of millet prevented cardiovascular disease in hyperlipidemic rats by reducing plasma triglycerides (Sarita and Singh 2016), and its protein improved plasma levels of adiponectin and high-density lipoprotein (HDL) cholesterol in genetically obese type-2 diabetic mice (Choi et al. 2005). Millet has shown evidence in preventing cardiovascular diseases in hyperlipidemic rats than sorghum (Lee et al. 2010). Millet based food is also associated with a decrease in the prevalence of hypertension, as it causes a significant decline in both systolic and diastolic blood pressure after 12 weeks of administration (Singh et al. 2020). This effect is due to the inhibition of the angiotensin-converting enzyme and increased production of nitrate. Genetic and epigenetic damage of cells, associated with cardiovascular diseases are also preventable by consuming millet (Singh et al. 2020).

Garlic, ginger, black pepper and other spices mixed with *kunun* during production possess cardioprotective properties. Allicin, an alkaloid in garlic inhibit cholesterol synthesis while also protecting against atherosclerosis by reducing lipid content on the arterial wall (Mikaili et al. 2013). Ginger prevents platelet aggregation and thromboxane formation thus reducing the risk of ischemia, while black pepper decreases cholesterol, triglycerides and free fatty acids in rats fed with high-fat diets (Banewal et al. 2013).

Kunun **as Anti-Cancer and Anti-Celiac Disease Food** Phytochemicals such as phenolic acids, tannins, and phytate contained in millet are well known to reduce the risk for colon and breast cancer in animals (Saleh et al. 2013). Millet-based foods lowered oesophageal cancer, indicating that consuming millet may be effective in the prevention of initiation and progression of cancer (Chandrasekara and Shahidi 2011). Millet is also able to inhibit the proliferation of human HepG2 liver cancer cells *in vitro* due to the presence of phenolic acids such as ferulic, chlorogenic, caffeic and syringic acids (Zhang et al. 2014). The spices added to *kunun* contributes to its anti-cancer effect. Cloves are effective anti-cancer and anti-mutagenic agents, in modifying the cellular detoxification process in mice (Kaefer and Milner 2011) and inhibiting carcinogen-induced genotoxicity. Garlic, on the other hand, is acclaimed for its anti-cancer effect. It can lower the incidence of breast, skin, colon, oesophagus, lung and uterine cancers due to the presence of compounds such as ajoene, diallyl sulphide, and so on. Ginger addition to *kunun* will cause gingerol to inhibit the formation of reactive oxygen species in human keratinocyte cells, and 6-paradol to induce apoptosis in human promyelocyte leukaemia cells (Kaefer and Milner 2011).

Celiac disease, an immune-mediated enteropathy prompted by the ingestion of gluten in genetically susceptible individuals is common to cereals such as wheat and rye (Sarita and Singh 2016). Millets are gluten-free grains, *kunun* is a suitable beverage for persons suffering from celiac disease or adhering to a gluten-free diet.

Kunun **as Anti-Ageing and Anti-Inflammatory Food** The ability of these polyphenols in millet to scavenge reactive oxygen species and free radicals helps slow down complications associated with the ageing process. Ferulic acid, a bioactive component of millet have strong anti-inflammatory activity that prevents tissue damage and stimulates the wound healing process (Saleh et al. 2013). Spices in *kunun* beverage possess anti-inflammatory activity. The bioactive components in these spices have modulates several inflammatory pathways, upregulate the production of several anti-inflammatory cytokines or downregulate the production of pro-inflammatory cytokines. Gingerol in ginger downregulates the production of pro-inflammatory cytokines interleukin 6 (IL-6), diallyl sulphide (DAS) in garlic inhibits inflammatory factor nuclear factor kappa B (NF-KB) and expression of interleukin 1B (IL-1B) while protecting against osteoarthritis (Kunnumakkara et al. 2018).

Kunun **as Anti-Microbial Food** Lactic acid bacteria isolated from *kunun-zaki* inhibits the growth of pathogenic bacteria, thus reducing microbial spoilage and maintaining the nutritive quality of the beverage. The lactic acid bacteria present in the beverage during consumption help to enhance gastric mucosa integrity by protecting against mucosa injury. Lactic acid bacteria can inhibit the growth of the toxigenic fungi *Aspergillus flavus* which causes some food-borne illnesses, via the biosynthesis of antimicrobial compounds such as organic acids, and bacteriocins during lactic acid fermentation (Olonisakin et al. 2017). Spices also contribute to the anti-microbial effect of *kunun*. Cloves inhibit the production of amylase and

protease in *Bacillus cereus*, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas fluorescens*; garlic can cause the death of the fungi *Candida albicans* while ginger possesses antimicrobial activities against *Pseudomonas aeruginosa, Bacillus subtilis, Salmonella enteritidis, Fusarium spp* (Liu et al. 2017).

36.4 *IRU* (African Locust Beans)

Parkia biglobosa is a multipurpose native tree in tropical regions of West Africa, commonly called African locust beans. It is of the family Mimosaceae (Leguminosae - Mimosoideae), serving both nutritional and medicinal purposes due to its richness in phytochemicals such as alkaloid, flavonoids, tannins, saponins, cardiac glycosides, terpenes, and other nutrients like free amino acids, fatty acids (arachidic acid, linoleic acid) and minerals thiamine, riboflavin, magnesium, sodium, potassium, zinc, calcium, copper and iron (Daramola 2015). In different countries, *Parkia biglobosa* has been used in the therapy of a variety of diseases ranging from hypertension, inflammation, wound healing, diabetes and ulcer (Alinde et al. 2014). The seeds are usually not consumed in their natural state, but fermented and used as a seasoning, to impact the flavour *or enhance taste in soups, stews and meals in West African cuisine* (Sackle 2013*).* Proteolytic *Bacillus species, such as B. subtilis, B. megaterium, B. circulan* are the fermenting microorganisms in most condiments.

In Nigeria, the fermented African locust bean seeds are referred to as 'iru', 'ogiri', or 'dadawa kalwa' in Yoruba, Igbo, and Hausa languages respectively. *Iru* is a good source of protein and vitamins, it is normally used as a condiment in soup and sauces, however, poor families' especially rural dwellers add a generous quantity of *iru* to their food to serve as a low-cost meat substitute. The traditional methods of processing i*ru* are time-consuming, and the end products are of low quality, which is why the optimised process conditions of the seeds before fermentation is suggested (Koledoye and Akanbi 2013). To extend the shelf life, and increase the acceptability of the condiment (especially to those who dislike the sight of the seed in foods), fresh *iru* is sometimes pounded into a paste, dried, milled or even processed into bouillon cubes.

Traditionally in various countries and cultures of West Africa, *Parkia biglobosa* has been used in the treatment of malaria, urinary tract infection, inflammation, diarrhoea, infertility, leprosy, stroke and hypertension (Alinde et al. 2014). Other therapeutic and nutritional uses (Fig. 36.3) of *iru* are in:

Protein-Energy Malnutrition This is a major health challenge in Sub-Saharan Africa, where animal proteins such as fish and meat are quite expensive and barely affordable for the poor populace. As a result, there is a need to look for an alternative in plants, especially the under-utilized, available, accessible and cheap ones. With a crude protein content of about 31% and a vast array of vitamins and minerals, *Parkia biglobosa* is a worthy alternative for animal protein. Diet supplemented with *Parkia biglobosa* ameliorate protein-energy malnutrition, this may be as a

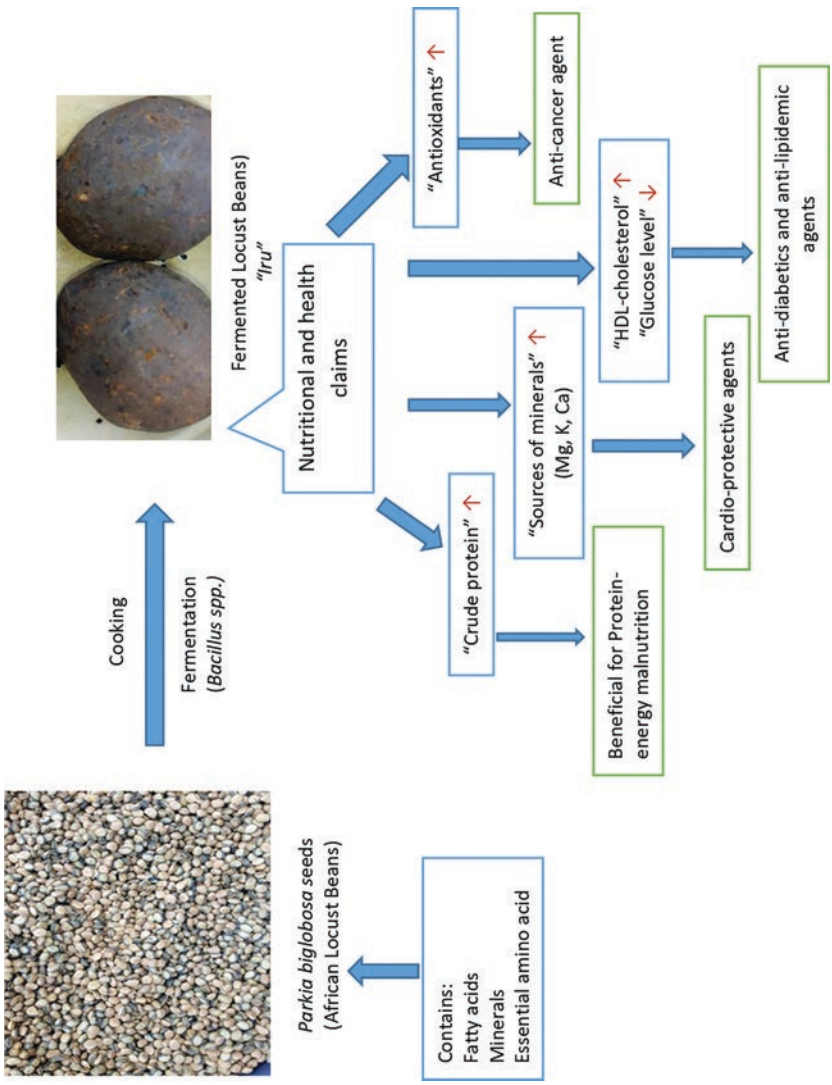

Fig. 36.3 Nutritional and health claim of *Iru*

result of the essential amino acids (phenylalanine, threonine, serine, glycine and tyrosine) present In the seeds (Biobaku et al. 2017) as considerable weight gain were observed in the malnourished rats. The presence of arachidic acid in *Parkia biglobosa* also alleviates neuronal degenerative changes that occur due to protein-energy malnutrition. (Biobaku et al. 2017).

Cardiovascular Diseases Studies have indicated the hypotensive potential of *Parkia biglobosa* seed extracts, reducing serum cholesterol and triglycerides in diabetic rats (Alinde et al. 2014). *Iru*, a fermented seed of *Parkia biglobosa* can inhibit isoproterenol-induced myocardial infarction. It improves lipid profile by inhibiting lipid peroxidation, and improve antioxidant capacity in rats, providing protection against the cytotoxic effect of isoproterenol (Kodjo et al. 2013). The consumption of *Parkia biglobosa* condiment had an antihypertensive and cardioprotective effect on the people of Bogou in Togo who consume a lot of the condiment in their diet. Plasma levels of cardioprotective minerals such as magnesium, potassium and calcium increased with the consumption of the condiment when compared with those who do not consume the condiment in their diet (Ognatan et al. 2011).

Diabetes and Hypolipidemia *Parkia biglobosa* possess antidiabetic and hypolipidemic activities. The fermented seeds have significantly reduced the glucose level in alloxan-induced diabetic rats, and ameliorate weight loss due to diabetes in the test animals. The condiment also improved the lipid profile by increasing serum HDL-cholesterol and reducing LDL-cholesterol (Odetola et al. 2006).

Cancer The *in vitro* cytotoxic effect of methanolic extract of fermented *Parkia biglobosa* seeds was evident in MCF-7 breast cancer cells and leukaemia cell lines. The cytotoxic activity of the seed is due to the fermentation process that enhances its antioxidant, nutritional and chemical components, making the condiment more biologically and therapeutically active (Ayo-Lawal et al. 2020).

36.5 Conclusion

The nutritive and therapeutics effects of fermented food in Nigeria cannot be overemphasized. The health benefits of all the fermented foods all over Nigeria has helped low-income families combat malnutrition and some diet-related non-communicable diseases. Lack of information about the health benefits of these foods always relegates them when compared to other types of foods. The food experts need to educate the citizen about these benefits.

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Chapter 37
Omics in Traditional Fermented Foods and Beverages

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37.1 Introduction

Fermentation is the most ancient and cost-effective technique of food production and preservation. It's a natural technique to boost the nutritional content and digestion of meals. Fermentation removes undesirable flavors, decreases cooking energy, and destroys anti-nutritional components such phytic acid, tannins, and polyphenols (Kabak and Dobson 2011). Traditional microbiological techniques have been used to study food microbiota for a long time. A strong grasp of microbiota dynamics has been established with the arrival of molecular or culture-independent approaches. In the research of fermented foods, various methodologies have been used. Microbiology has traditionally relied on culture-dependent procedures, in which the separation of microorganisms is required for subsequent phenotypic and biochemical analyses (Rizo et al. 2020).

Analyzing the functional role of microbial communities, on the other hand, is not straightforward. These approaches can only detect culturable bacteria, despite the fact that culturable bacteria make up a very small portion of wild microbial diversity, according to the recognized paradigm. (Stackebrandt and Embley 2000). As a result, we only have a limited understanding of microbial populations. Omics is a broad term that includes (meta)genomics, (meta)transcriptomics, metabolomics,

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and (meta)proteomics, as well as other HTS methods (among others). The use of omics science in the study of fermented foods would provide a metabolic and functional understanding of microbial populations and their impact on the fermented product, including the chemicals that characterize its scent and flavor, as well as nutritional qualities. The term foodomics (a fusion of two terms, food and 'omics') was used in 2009 to describe the study of food and nutrition using omics technology, particularly in food science research (Adebo et al. 2021). Proteomics, transcriptomics, and metabolomics are some of the omics techniques utilized to unravel basic molecular food mechanisms in relation to health in this area (Li et al. 2021). Food contamination, food safety, food microbiology, food processing, food traceability and food authenticity, food fraud, and food functioning are all examples of areas where progress is being made in this area.

The use of omics technology to examine food fermentation processes and functional starter cultures has grown in popularity during the last decade (Bigot et al. 2015; Kergourlay et al. 2015). Several research have dealt with metagenetics, metagenomics, metatranscriptomics, or metaproteomics in the context of sourdough fermentations (De Filippis et al. 2017; Gobbetti et al. 2016). Additionally, genomics was used to decipher the whole-genome sequences of many microbial strains obtained from spontaneous sourdough fermentation processes (De Vuyst et al. 2017; Martino et al. 2016; Zheng et al. 2015). Fermentation properties such as flavor, texture, and microorganism metabolites can be explored using genomic analysis of individual fermented microorganisms, which cannot be identified by microbial community analysis (Chun et al. 2017; Jung et al. 2019). Metagenomics and metatranscriptomic techniques can also be utilized to investigate the fermentation features of the complete microbiome in the fermented food environment (Jeong et al. 2018; Kim et al. 2020). The generation of fermentative metabolites generated by lactic acid bacteria (LAB) genes in fermented meals is verified using metabolomic methods (Jung et al. 2018; Park et al. 2016). The development of these meta-omics methods has resulted in the discovery of hitherto unknown fermented microbiomes. As a result, the food fermentation mechanism of these bacteria can be clearly identified. As a result, the goal of this chapter is to provide light on the widespread use of omics technologies in fermentation in relation to African fermented foods (Fig. 37.1).

37.2 Metaproteomics

Metaproteomics can be used to explore the nature of microbial function in distinct habitats and states, as well as to understand complicated substrate-microbiome interactions (Wilmes and Bond 2004). Since 2004, when the ideas of the metaproteome and metaproteomics were established, research aimed at characterizing the overall protein profiles of microbial communities at precise time points in samples has increased each year (Rodríguez-Valera 2004). Metaproteomics has revealed a wealth of information about microbial ecosystem function in a variety of

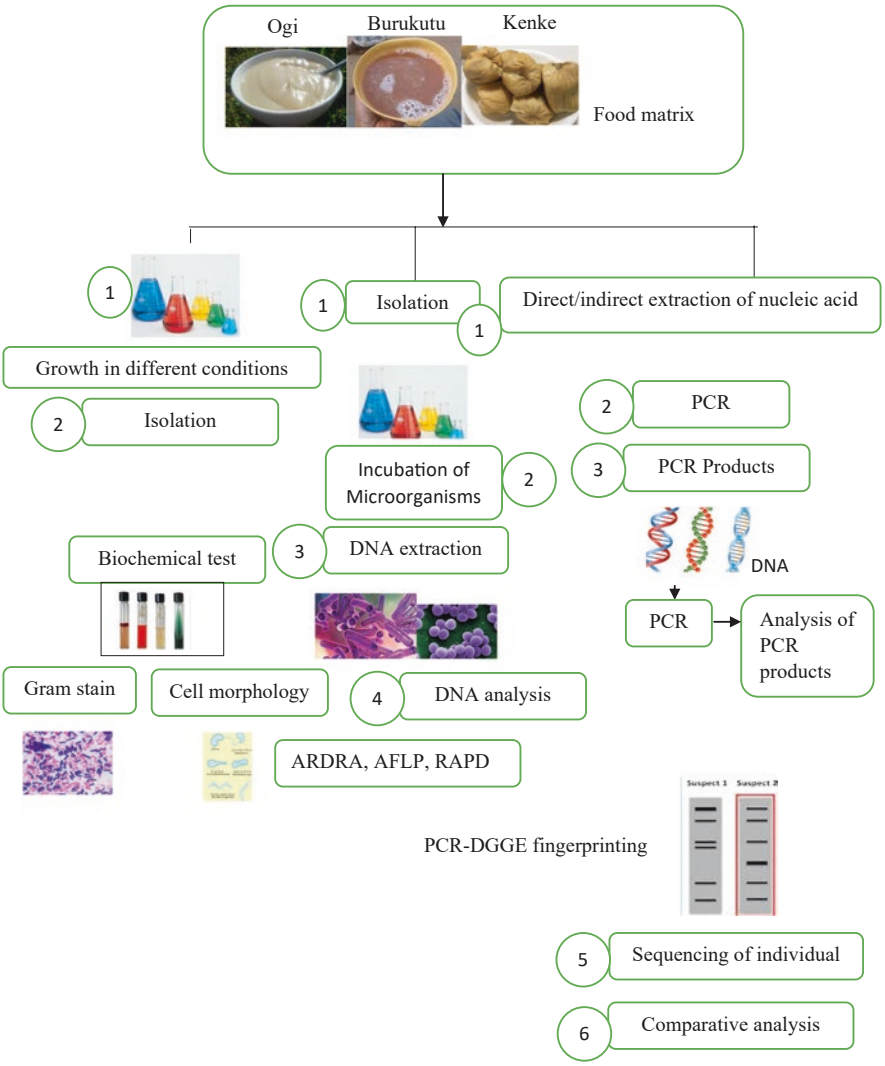

Fig. 37.1 General schematic diagram of methodologies for the microbial study of fermented foods. (Rizo et al. 2020)

environments, ranging from activated sludge to soil to the microbiota of the human gut (Wilmes et al. 2015). In terms of protein isolation and identification, metaproteomics has advanced significantly, but it is still underutilized, owing to several challenges. These challenges stem from the high complexity of the microbial community, the low coverage of the complete metaproteome, and the high sequence similarity between many proteins (Haange and Jehmlich 2016), the inadequacy of publicly accessible databases (Rechenberger et al. 2019), as well as the significant costs (Jansson and Baker 2016; Schiebenhoefer et al. 2019; Xu et al. 2017).

Metaproteomics research has primarily focused on areas such as intestine micro-ecology (Zhang et al. 2017), biology of the sea (Williams and Cavicchioli 2014), as well as soil biology (Starke et al. 2019). Although there has been a study of the use of proteomics in food quality, safety, microbes, and allergens, which has increased our understanding of food composition, (Piras et al. 2016) metaproteomics isn't discussed at all. Metaproteomics of sourdough fermentation processes entails the separation of proteins present in the ecosystem, for example using two-dimensional acrylamide gel electrophoresis, and then their identification using sophisticated mass spectrometry[For example, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS), liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), or quadrupole time-of-flight mass spectrometry (Q-TOF-MS) A direct, gel-free approach can also be used, in which the proteins are separated and identified instantly using advanced mass spectrometric techniques such as MALDI-TOF-MS, LC-MS/MS, or Q-TOF-MS. As a result, (meta)proteomics can be considered a preferred methodology for revealing the real functional features of microbial strains (proteomics) or an entire microbial ecosystem (meta)proteomics) (metaproteomics). If the organism genome sequence or draft sequence is known, interpreting the proteomic data can be very simple; however, if the organism is distantly related to well-characterized organisms or in the analysis of mixtures of organisms, it can be difficult (Armengaud 2016), This is frequently the case in fermented food metaproteomics. Despite these challenges, a few proteome and metaproteomic studies in fermented foods have been published. Metaproteomics is separated into two types of analysis: descriptive and comparative. The descriptive analysis sheds light on the metabolic processes that microbial communities engage in under various circumstances (Matallana-Surget et al. 2018). Comparative study isn't limited to a descriptive technique; it can also reveal the functional responses of microbial communities to various fermentation processes and micro-ecology types (Morris et al. 2010).

37.3 Metagenomics

Metagenomics is the use of high-throughput techniques to sequence the complete DNA (or RNA) content of a sample, regardless of where it came from. Without any preliminary marker gene amplification, template DNA included in a sample of interest is sequenced immediately. Metagenomic data can not only offer a detailed taxonomic identification of the microbiome, but it can also compare the relative abundance of all organisms in the microbiome at the same time. After that, large amounts of sequencing data generated by a metagenomic technique is queried against databases like k-mer. (Compeau et al. 2011) as well as SILVA (Quast et al. 2013) to figure out the taxonomic makeup of the microorganisms in the sample. The capacity to characterize bacteria present in the microbiome to species/strain level is the main advantage of metagenomic techniques over 16S rDNA sequencing. Metagenomics also gives detailed information on the full gene repertoire, genome

structure and organization, microbial community structure, and evolutionary relationships found in the sample. As a result, this method has a number of advantages over the 16S rDNA marker gene method. 16S rRNA gene sequencing (hereinafter referred to as 16S) and internal transcribed spacer (ITS) gene sequencing are the two most extensively used amplicon sequencing technologies for profiling bacterial and fungal communities, respectively (Caporaso et al. 2011; Schoch et al. 2012).

Typically, amplicon sequencing is confined to genus-level identification, albeit thanks to dedicated species classifiers and the use of longer read technology, some studies have been able to obtain species-level assignments (Allard et al. 2015; Mosher et al. 2014). Genes involved in amino acid catabolism were discovered to be involved in flavor synthesis using metagenomics. The process of surface ripened cheese was better understood because to a collaborative effort involving metagenomics, metatranscriptomics, and biochemical analyses (Dugat-Bony et al. 2015). All of the information presented above demonstrated that metagenomic analysis provided an in-depth examination of how microbial communities interacted during fermentation. Metagenomics opens a doorway into a world of hitherto undiscovered microbial variety of incredible magnitude, allowing researchers to tap into the huge genetic potential of microorganisms to create biotechnologically useful products and processes. In the industrial sector, the application of metagenomics for accessing the entire microbiome of a given environmental sample has yielded a number of significant discoveries. The search for novel biocatalysts using metagenomics has generated promising findings, with new enzymes being discovered in genetically untouched resources that have potential in a variety of industries. This current trend of directly cloning metagenomic DNA to acquire novel and natural sequences is assisting in the screening and discovery of previously undiscovered microbial consortia for important bioactive chemicals, biocatalysts, and other important products and processes.

37.4 Transcriptomics

Metatranscriptomics can be used to figure out how much gene expression and activity there is. However, because cell activity is regulated at the protein level, a microbial community study cannot identify microorganisms that are directly linked to metabolites in traditional fermented foods and beverages (TFFB) raw materials. Metatranscriptomics also played a pivotal role in understanding the process of fermentation, especially the ripening of cheese. Camembert-type cheese ripening is driven by fungal microflora including Geotrichum candidum and Penicillium camemberti. Metatranscriptomics-based functional gene expression studies revealed that genes related with metabolic processes, cell development, and stress responses were differentially expressed during cheese ripening, with changes in expression patterns occurring over the first two weeks of ripening (Lessard et al. 2014). Through metatranscriptomics, Jung et al. looked at the active gene expression in Kimchi fermentation (Jung et al. 2013). Six typical lactic acid bacteria have had their

genomic sequences completed (LAB), *The metatranscriptomic profiles of Leuconostoc (Lc.) mesenteroides, Lactobacillus (Lb.) sakei, Weissella (W.) koreensis, Leuconostoc (Lc.) gelidum, Lc. carnosum, and Leuconostoc (Lc.) gasicomitatum were investigated at five time points during a 29-day fermentation using reference genomes from Leu.* The mRNA sequencing data were mapped onto the genomes of the six LAB strains, revealing that Lc. mesenteroides was the most active during early-stage fermentation, whereas gene expression by Lb. sakei and W. koreensis was high at later stages. Metabolomics.

Chromatography combined with mass spectrometry is a noteworthy technological achievement in the field of biological sciences. This analytical platform has been developed over the previous decade to improve dependability and sensitivity (Gröger et al. 2020; Lesur et al. 2016). Medicine, biology and life sciences, nutrition, agriculture, and, more recently, food science and technology research are all areas where metabolomics is being used (Adamski 2020; Adebo et al. 2017).

Understanding such a complicated and multidimensional metabolic space with a wide range of concentrations, chemical structures, affinities, and polarity can be difficult using traditional methods. As a result, metabolomics – which involves the global qualitative and quantitative profiling of metabolites in a biological matrix – can be used to characterize the end products of food processing. This establishes metabolomics as a promising method for gaining a deeper knowledge of the multifunctionality and complexity of cereal and legume fermented diets (CLFFs). The most commonly utilized analytical systems in metabolomics investigations are capillary electrophoresis-mass spectrometry (CE-MS), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) (Adamski 2020; Ten-Doménech et al. 2020). Fourier Transform Infrared (FTIR) and Raman spectroscopy are two more analytical methods employed in metabolomics. Food fermentation can be better understood with the help of metabolomics. Recent research has proved the resilience of this 'omics' approach for cereal and legume fermented foods by looking at a variety of biological concerns in order to provide a complete understanding of the fermentation process. The untargeted pathway, in particular, was long used to characterize the fermentation of cereal-based foods (alcoholic and non-alcoholic beverages, bread, dough, gruel, among others) Seo et al. (2016), GC-MS based untargeted metabolomics was used to assess the fermentative behavior of yeast strains in Makgeolli (a traditional fermented rice wine) during alcoholic fermentation and aging. The study found that fermentation advanced quickly during the early stages, with lower quantities of glucose and phosphoric acid and higher quantities of other identified metabolites. The varied fermentation behaviors induced by the cultivated yeast strains were blamed for the observed metabolite alterations. On the other hand, metabolites in the product rarely altered through the age period (up to 70 days).

Mu et al. (2019) used a similar metabolomics methodology to study the association between metabolites and fermentation duration of black glutinous rice wine. Liquid extraction followed by derivatization [methoxy amination and addition of N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA)], The scientists discovered 28 substantially distinct metabolites (SDMs) using a gas chromatography time of flight

mass spectrometry (GC-TOF-MS) instrument and multivariate data analysis (MVDA). Half of these metabolites (phenolic acid, organic acid, and sugar) were enriched at 60 h. The authors found that alanine, aspartate, and glutamate metabolism, starch and sucrose metabolism, and the pentose phosphate pathway were the most relevant to pre-fermentation, with 60 h being identified as the potentially optimal time for pre-fermentation of black glutinous rice wine using pathway analysis. The majority of investigations have focused on elucidating metabolite alterations in fermented soybean products using GC-MS-based metabolomics (Park et al. 2019; Seo et al. 2018a; b). Starter ingredient (*Koji,* for *koji*-derived fermented products), fast-fermented bean paste (*cheonggukjang* and *soksungjang*), fermented paste (doenjang), and others (*douchi, meju, moromi,* soy sauce, and *tempe* or *tempeh*) are among them (Seo et al. 2018b) *Koji* made from soybeans and a mix of cereals was studied. Using a combination of SPME-GC-MS and GC-TOFMS-based metabolomics, the researchers discovered that (Seo et al. 2018a) In koji samples fermented separately with *Bacillus amyloliquefaciens* and *Aspergillus oryzae*, the volatile organic components and primary metabolites were compared. The authors found that the volatile profile of koji is mostly governed by the inocula choice, which alters the primary metabolites in koji substrates, hence changing its fragrance characteristics, using these integrated methodologies. The same authors used a GC-TOF-MS investigation to investigate the effects of different substrates (soybean, wheat, and rice) and the same inocula (*A. oryzae* and *B. amyloliquefaciens*) on koji metabolite compositions (Seo et al. 2018a). Soybean had a stronger impact on main metabolite compositions in koji varieties than wheat and rice. *A. oryzae* was found to stimulate higher levels of carbohydrates, lipid derivatives, and organic acids in the koji types studied, while *B. amyloliquefaciens* produced higher levels of amino acids, implying that the metabolomic approach has potential applications in koji product production, bioprocess, and quality control. During the fermentation of fast-fermented soybean pastes (*cheonggukjang* and *soksungjang*), certain research has revealed metabolite profiling with the goal of answering various biological issues. *Cheonggukjang* was infected with various Bacillus strains, and metabolite variations were observed in relation to fermentation times (Baek et al. 2010; Kim et al. 2012). On the one hand, different patterns of amino acids, organic acids, sugars, and sugar alcohols were seen depending on the fermentation length (0–72 h), but significant changes in pre-determined metabolite contents were dependent on the inocula strains (Baek et al. 2010). A metabolomics investigation follows a general multi-step procedure in the lab, regardless of the analytical platform used Fig. 37.2.

37.5 Genomics of Foodborne Pathogens

Foodborne pathogen detection, prevention, and treatment are quickly changing thanks to genomics, transcriptomics, and proteomics. Microbial genome sequencing, in particular, has progressed from a research tool to a method for characterizing foodborne pathogen isolates in routine surveillance systems. Genome sequencing

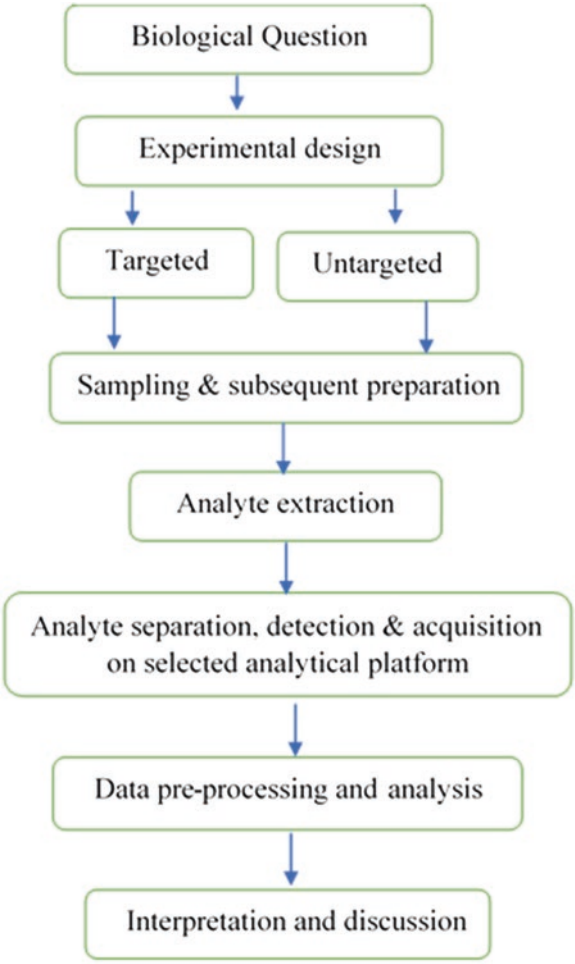

Fig. 37.2 General flow chart of metabolomics

efforts will not only improve outbreak detection and source tracking, but will also generate large amounts of foodborne pathogen genome sequence data, which will be available for datamining efforts that could help with source attribution and provide new insights into the biology and transmission of foodborne pathogens. Although the practical applications and uses of metagenomics, transcriptomics, and proteomics data and techniques are less common, these methods are beginning to yield real food safety answers. Bacteria, viruses, and parasites that cause foodborne illness are responsible for a significant portion of the world's illness burden. Foodborne disease loads are difficult to collect in many parts of the world, including many underdeveloped countries (Bergholz et al. 2014). The use of metagenomics methods to discover and identify the causative agent of foodborne disease cases from clinical specimens has been well reported (Bergholz et al. 2014). Despite the numerous opportunities to use metagenomics tools to support the detection of

foodborne pathogens from foods and food-associated environments, the majority of metagenomics studies on the detection of microbes in foods have focused on characterizing the microbial ecology and microbial successions during fermentations (van Hijum et al. 2013), for instance, *kimchi* (Jung et al. 2011; Park et al. 2012). A study that used metagenomics approaches to characterize the species composition associated with the tomato phyllosphere, both on the native plant and in the pre-enrichment and enrichment media used to isolate salmonella, demonstrates the potential for metagenomics approaches to improve foodborne pathogen detection (Ottesen et al. 2013). Despite the fact that tomatoes have been implicated as the source of multiple huma salmonellosis epidemics, isolation of salmonella from the tomato phyllosphere has historically proven hard. Transcriptomics can be used to analyze how bacteria respond to physical, chemical, or biological food preservation interventions, in addition to knowing the physiological status of pathogens on foods. In many cases, a single compound's antimicrobial activity is recognized, but there is little knowledge on the antimicrobial's actions at the molecular level. A study of *E. coli* O157:transcriptional H7's response to the antimicrobial cinnamaldehyde revealed that the pathogen's initial response was to activate the oxidative stress response, which the pathogen was able to overcome after a relatively short period of time by converting cinnamaldehyde to cinnamic alcohol (Visvalingam et al. 2013).

37.6 Conclusion

The use of microbial starter cultures distinguishes most African traditional fermented dishes from other fermented foods such as cheese and yogurt. The majority of African traditional fermented food manufacturing methods rely on spontaneous fermentation by microbes derived from the products' source materials. Bacteria, eukaryotic microorganisms, archaea, and viruses make up the microbiome of African traditional fermented foods. The microbial profile, taste, and quality of African traditional fermented foods are all determined by these microbial species. Microbial community investigations of African traditional fermented foods yielded minimal results. The quick investigation of the full genome of the African traditional fermented foods microbiome and accurate identification of the associated fermentative properties has been made possible thanks to recent advances in omics technology and gene analysis tools. Comparative genomic investigations of microbial strains can reveal intrinsic differences and similarities between them. Metagenomic and metatranscriptomic investigations reveal each microorganism's preferred carbon sources, as well as the genes and intermediate products engaged in fermentative metabolic activities that use these carbon sources. Through metabolomic research, it is now possible to discover many compounds produced by gene expression in the fermented food microbiome at the same time. In this context, a comprehensive database spanning all microbial genomes found in fermented foods would help us better comprehend the microbial communities' complicated relationships.

Although omics techniques have the potential to have a significant influence in several areas of microbiological food safety, there are some areas where their usage is still in its infancy. Adoption of next-generation sequencing as a standard technique for epidemic detection and research in food microbiology will necessitate not only financial investments, but also staff training. To make these approaches more widely used, scientists that can bridge food and public health microbiology, omics techniques, and bioinformatics are needed. The development of quicker, less computationally intensive, and easier-to-use bioinformatics tools will be critical in allowing the continued use of omics techniques, and may be more significant than the nearly certain advancements in sequencing technology. Furthermore, developing proper legal frameworks for the use of omics data and outcomes will be critical in facilitating corporate and government adoption of these tools.

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