

Chapter 6

Lactic Acid Bacteria and Fermented Cereals



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6.1 Introduction

6.1.1 Overview of Fermented Cereal Products

It is well-known that cereals are one of the traditional staple foods in many Asian countries. In China, the records of five cereals are reported as early as the Spring and Autumn and Warring States Period in the “Analects of Confucius,” including rice, wheat, soybeans, corn, and potatoes. Starch is one of the most important components in cereal; the content accounts for 60% of the total cereals weight and 90% of the total carbohydrate content. In addition, cereals also contain a variety of proteins, lipids, cellulose, minerals, and enzymes, which not only meet the nutritional and metabolic needs of microorganisms but also provide a good substrate for microbial growth (Cho et al. 2013; Waters et al. 2015). Fermentation technology has been known and mastered for thousands of years. As one of the traditional staple foods in northern China (Zhu 2014), steamed bun originated in the Three Kingdoms Period; soy sauce, as an ancient condiment, has a long history of more than 1800 years; fermented bean curd also had historical records in the ancient books of the Wei Dynasty as early as the fifth century AD. However, due to the lack of understanding in fermentation and microorganism at that time, the development of fermented food was limited. With the development of science and technology, we have come to realize that cereal is the natural medium for microbial growth and reproduction. Microorganisms utilize the carbohydrates and amino acids of cereal for fermentation (Oguntinyinbo and Narbad 2015), under a series of physiological and biochemical reactions, which improves the quality, flavor, and nutrition of products. Furthermore, the fermented cereal food also has the effects of regulating

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human intestinal health, alleviating constipation, and absorbing heavy metals in the body (Akanbi and Agarry 2014; Brandt 2014; Zhao et al. 2015).

At present, the common fermented cereal-based food on the market can be classified into the following three types:

- 1) Fermented wheat-based food, such as steamed stuffed bun, steamed bread, bread, rolls, etc.
- 2) Fermented soybean products, such as soy sauce, black bean, sufu, natto, sour soybean milk, etc.
- 3) Fermented cereal beverage based on coarse cereal, such as oat fermented milk, barley fermented milk, corn fermented milk, etc.

6.1.2 History and Current Situation of Fermented Cereal Products in China

China has a long history about the cereal fermentation; people have mastered the fermentation skills of fermented cereal products for thousands of years, such as steamed bread, soy sauce, tofu milk, vinegar, etc. The traditional cereal fermented foods are used to natural fermentation, which is one of the oldest methods to extend the shelf life of food due to the complex microbiota. With the development of modern fermentation technology, it is found that the flavor of products is improved after fermentation based on the culture-dependent, gene mutagenesis, and artificially controlled fermentation technology. Therefore, it gradually evolves into a unique food processing method.

6.1.2.1 Fermented Wheat-Based Food

Steamed bread as one of the traditional staple foods in China is a typical wheat-based fermented food. It is used to be made of flour, water, and starter through a series of processes, including mixing, leavening, and steaming (Su 2005). The original steamed bread is called “pastry,” which is a kind of unfermented wheat-based food. Until the Wei and Jin dynasties, people began to operate the fermentation skills and applied it to the processing of “pastry,” which steamed bread come from this (Su 2009).

In China, about 70% of the wheat flour is used in the processing of steamed bread each year, and the annual consumption of steamed bread is more than 12 million tons. The industrialization of staple food (steamed bread) is not only the trend of market development in the future but also the choice of market consumers. However, the industrialization of steamed bread in China has slowed development; the hand workshop still occupies the majority of the market share. The main reasons are the low profit, added value, and technology content of steamed bread. In addition, the hand workshop still follows the traditional fermentation technology. It takes a long time

but improves the flavor and texture of production, which is the important reason that's why the workshop-style processing products are deeply preferred by consumers.

6.1.2.2 Fermented Soybean Products

Soy sauce is a kind of traditional dressing with unique flavor, healthy nutrition, and delicious taste, which promote the appetite of consumers. It is made from cereals with high content of protein and starch and fermented with *Aspergillus* and other microorganisms for a long time (Feng et al. 2015). Soy sauce originated in China, and it evolved from the sauce in the early period. The process of sauce was recorded in the Zhou Dynasty, which has a history of more than 3000 years. The earliest soy sauce was made from fresh meat; the process is similar to the modern processing technology of fish sauce. For the higher demanding of consumer, the raw material of soy sauce evolved by using soybeans for fermentation, which greatly reduced the cost and extended rapidly.

In China, the industrial fermentation technology of soy sauce experienced three phases: natural brewing, traditional biotechnology, and modern bioengineering. A series of advanced technology are applied in the industrial production of soy sauce from strain screening, inoculated fermentation, genetic engineering, and enzyme engineering. Soy sauce industry is in the stage of rapid development and plays an important role in the Chinese traditional food industry. Based on the stable consumer group and high added value of products, soy sauce market has been in full bloom for a long time (Li 2013).

The statistics show that the annual growth rate of soy sauce is more than 10% in China. By the end of 2014, the national output of soy sauce had reached 9.39 million tons, with year-on-year growth of more than 10.63%. China is the birthplace of soy sauce, but it fails to carry forward the soy sauce process technology. Actually, the soy sauce process technology has been improved and innovated in Japan for a long time and forms a unique Japanese fermentation technology. By adding appropriate concentration of fresh yeast during fermentation, the products have the characteristic of intense delicious flavor. Nowadays, the high-end market of soy sauce in the world has been basically monopolized by Japan and Taiwan of China, which is closely related to the serious lack of traditional food culture in China for a long time. Therefore, paying attention on the introduction and application of advanced technology, as well as the continuous innovation and development of new products, has become a matter of urgency to accelerate the domestic soy sauce industry into the leading fermentation industry in the world.

6.1.2.3 Fermented Coarse Cereal Beverage

Fermented coarse cereal beverage is a new kind of beverage product on the market in recent years. However, fermented cereal drinks are not unprecedented product. For example, douzhir (fermented bean drink), the famous snack in old Beijing, has

the function of nourishing stomach and interpreting and relieving inflammation or fever, with a history of more than 200 years (Miao et al. 2013). In addition, Gwas, known as “liquid bread,” originated from East Slavs before the Principality of Kiev and has a history of 1000 years. At the end of nineteenth century, the declining royal family of Russia introduced the brewing technology of Gwas into China (Sui and Chu 2013). The traditional fermented cereal beverage has strong regional preference, which is the main reason why the popularization of fermented cereal beverage is limited in the market and the innovation has slowed development. With the pressing needs of consumers for the dietary supplement and healthy nutrition of food, fermented coarse cereal-based beverage comes into being. This kind of fermented beverage is mainly made of barley, oat, corn, etc. and fermented with probiotics such as yeast and LAB.

At present, the fermented coarse cereal beverage industry is still in the early stage of development, with fewer product categories and lower market share. However, the loss of national dietary structure balance results in the urgent desire to improve the level of dietary nutrition and health of food. It is an important developing direction of beverage market to develop coarse cereals-based fermented beverage in the future (Cheng et al. 2012; Zhang and Wang 2013).

6.1.3 Development Trend of Fermented Cereal Products in China

Cereal is one of the main sources of basic dietary composition, which plays an important role in the food industry and scientific research. As a large agricultural country, rice, wheat, and corn are the three major cereals in China, among which wheat and corn occupy the first and second place in the total output of the world, respectively. With the transformation of dietary structure of consumers and the understanding of the nutritional value of cereal food, fermented cereal products appear on the stage of global market. Rice as an important cereal is used to produce yellow wine, rice vinegar, and other products. It contains kinds of active ingredients such as threonine, which could effectively prevent memory loss, and a variety of amino acids and minerals also have the positive effects on reducing of blood pressure, blood sugar, and cholesterol (Chen and Xu 2013; Chen et al. 2014a, b). In addition, the total protein content of steamed bread is three to four times higher than that in the noodles and flat cakes, and fermentation also provides better aroma and taste (Rizzello et al. 2012; Katina and Poutanen 2013).

Chinese traditional cereal fermented food with a long history have a wide range of products, which occupy an important position in the dietary habits of consumers and have a wide market prospect. However, there are some disadvantages in the traditional fermented food, such as poor quality of product, imperfect technics and low techniques require, etc. There is still a gap on the process technology as compared with the food industry in the developed foreign countries. Therefore, the urgent affairs to carrying forward Chinese traditional fermented food is developing

the modern processing technology to simplify the traditional fermentation process and improving the production efficiency and batch stability of products effectively. With the development of molecular biotechnology method, the combination of culture-independent and culture-dependent analysis provides a more comprehensive and in-depth overview of the microbial communities and changes of fermented food, which plays an important role in the optimal regulation of the final product fermentation process (Minervini et al. 2014). As the interaction of strains during fermentation has been realized gradually, the isolation, identification, cultivation, and application of strain become the research hotspot in the field of fermented food. Modern biological techniques are applied to isolate and screen fermented strains with excellent traits, which are used to ferment food with appropriate ratio. Advances in research technology have greatly simplified the process of the product while increasing the batch stability of the product (Liao et al. 2015; Liu et al. 2015). The development of excellent strain is one of the core technologies in the application of fermented food. With the development of molecular biology, the genetic engineering techniques are used to modify the strains with superior fermentation characteristics and applied in the different fermented foods. However, in order to improve the flavor of product fermented with single strain, the mixed culture fermentation is also used to enhance the quality of fermented food by synergistic fermentation. In brief, the introduction of advanced fermentation equipment, the improvement of current fermentation technology, and the establishment of related technical indexes are the important means to promote the development of Chinese fermentation food industry.

6.2 Sourdough

6.2.1 *Microbial Ecosystem of Sourdough*

Sourdough is also known as Laomian in China. It is mainly made from wheat flour or miscellaneous cereal flour by mixing with water and spontaneous fermentation with back-slopping for a long time. It is the oldest natural dough starter and also a complex biochemical system, which contains a large number of lactic acid bacteria, yeast, and a small amount of other microorganisms. The total colony number of lactic acid bacteria and yeast is 10^7 – 10^9 cfu/g and 10^5 – 10^7 cfu/g, respectively, and the appropriate ratio of lactic acid bacteria to yeast in the “mature” sourdough is about 100:1 (Gobbetti and Gänzle 2012).

6.2.1.1 Yeast Diversity of Sourdough

Due to the acid and osmotic resistance of yeast, it has a good symbiotic relationship with lactic acid bacteria in the sourdough. At present, more than 25 species of yeast isolated from sourdough have been reported, mainly in the genus of *Saccharomyces* and *Candida*. In addition, it also contains a small amount of *Saccharomyces exiguus*,

Issatchenkia orientalis, *Pichia anomala*, *Hansenula subpelliculosa*, and *Candida holmii*. In recent years, the yeast diversity of sourdough collected from different regions has been investigated. Iacumin et al. (2009) focused on four sourdough bread samples collected from Northern Italy by culture-dependent and culture-independent method and found that *Saccharomyces cerevisiae* is the dominant yeast in sourdough; Vogelmann et al. (2009) collected from parts of Germany to explore the yeast diversity and found the similar results. In China, Wu Si Ri Gu Leng (2011) isolated and screened 85 strains of yeast from 28 sourdough samples in 7 leagues (cities) of Western Inner Mongolia. According to the 26S rDNA D1/D2 sequence analysis, 43 *Saccharomyces cerevisiae* strains, 22 *Candida mycoderma* strains, 6 *Torulaspora delbrueckii* strains, 3 *Pichia anomala* strains, 2 *Marine yeast* strains, 2 *Pichia kudriavzevii* strains, 2 *Candida glabrata* strains, 2 *Endosporium cerevisiae* strains, 1 *Starch Pichia pastoris* strain, 1 *Meyerozyma guilliermondii* strain and 1 *Rhodotorula mucilaginosa* strain. Liu Tongjie et al. (2014) screened and isolated 60 strains of yeast from 6 sourdough samples in northern China, of which 48 *Saccharomyces cerevisiae* strains were identified. Therefore, *Saccharomyces cerevisiae* is one of the most important yeast in the sourdough. In addition, the yeast diversity of sourdough was also affected by flour substrate, moisture content, fermentation temperature, and other process parameters. Brandt et al. (2004) found that the optimum growth temperature for *Candida* was 27–28 °C. Therefore, when the fermentation temperature is higher than 35 °C, the growth of *Candida* is inhibited, which reduced the competition with *Lactobacillus sanfranciscensis*.

6.2.1.2 Lactic Acid Bacteria Diversity of Sourdough

Sourdough is characterized by high relative abundance and diversity of lactic acid bacteria, which mainly consists of *Lactobacillus* and few amounts of *Weissella*, *Pediococcus*, *Streptococcus*, *Ascococcus*, and *Enterococcus* (Di Cagno et al. 2014). In recent years, various studies made systematic study to explore the LAB diversity of sourdough. More than 60 species of lactic acid bacteria isolated from sourdough have been reported, and heterofermentative LAB is the most representative species in the sourdough. The results showed that obligately heterofermentative LAB has good adaptability of glycometabolism, such as *Lactobacillus fermentum*, *Lactobacillus sanfranciscensis*, and *Lactobacillus reuteri*, which could metabolize and utilize maltose during sourdough fermentation. The metabolic pathway of arginine deiminase in *Lactobacillus fermentum* and *Lactobacillus reuteri* plays an active role in amino acid assimilation and acid stress regulation.

Scheirlinck et al. (2007) explored the effects of different regions on the LAB diversity of traditional Belgian sourdough. The results showed that 714 LAB strains from 21 different sourdough samples were mainly composed of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella*, and *Enterococcus*. Among them, *Lactobacillus sanfranciscensis*, *Lactobacillus plantarum*, and *Lactobacillus paralimentarius* are the dominant strains in the samples. Zhang et al. (2011a, b) analyzed and compared

the LAB diversity of 28 traditional sourdough bread samples from Western China by culture-dependent and culture-independent analysis. The results showed that *Lactobacillus plantarum* was the dominant strain in these sourdough samples. However, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus sanfranciscensis*, *Lactobacillus fermentum*, *Pediococcus pentosaceus*, and *Weissella cibaria* are predominant in the sourdough collected from the Italian region (Minervini et al. 2015). Therefore, regional environment is one of the key factors relating to the LAB diversity of sourdough. Similar to the yeast diversity, many studies confirmed that the substrate, cycle number of fermentation, fermentation temperature, pH, and other process parameters also influence the LAB diversity of sourdough. *Lactobacillus sanfranciscensis* is predominant in the sourdough by spontaneous fermentation due to the lower fermentation temperature and long-term fermentation time. Type II sourdough, which is generally used in industrial production, has a higher fermentation temperature (>30 °C) and dough yield (DY) value during fermentation. *Lactobacillus fermentum*, *Lactobacillus reuteri*, and *Lactobacillus amylovorus* are dominated in the type II sourdough (Gobbetti and Gänzle 2012). Interestingly, *Lactobacillus amylovorus* is predominant in the rye sourdough, but *Lactobacillus fermentum* are dominated in the rye sourdough by increasing the fermentation temperature (Ercolini et al. 2013; Minervini et al. 2014).

6.2.2 Biochemical Activity of LAB During Sourdough Fermentation

A series of biochemical reactions take place during sourdough fermentation, such as acidification, proteolysis, and the generation of exopolysaccharides (EPS) and flavor substrates (Sarfaraz et al. 2014; Wolter et al. 2014). It's a complex interaction, and the component changes greatly influence the sourdough properties and the quality of bread. The effects are associated with the metabolites and enzymes produced by LAB and yeast during fermentation (Corsetti 2013).

6.2.2.1 Acidification

Acidification is one of the important biochemical characteristics of sourdough fermentation (Corsetti 2013; Sarfaraz et al. 2015). Lactic acid bacteria metabolizes the production of organic acids during fermentation and leads to pH drop in the sourdough, which plays an important role in the rheological properties of the dough and the quality of the final product (Marti et al. 2014; Üçok and Hayta 2015).

Organic acids improve the solubility and intermolecular electrostatic repulsion of gluten proteins (Qiu et al. 2013; Robertson et al. 2014), because it is positively charged under acidic systems, which most commonly result in the spread of gluten proteins and expose more hydrophilic groups (Üçok and Hayta 2015).

The intermolecular electrostatic repulsion blocks the formation of new chemical bonds, which leads to dough softening and less time for stirring. The other components, such as starch particles and endogenous cereal protease, are also affected by acid environment. The endogenous protease activity is activated at the pH of around 4.0, which contributes to the proteolysis and the rheological properties of dough. The network structure of gluten greatly affects the physical properties of dough and final product, which is related to the acidification levels. The extension and gas retention capacity of dough are improved by LAB fermentation (Gobbetti et al. 2014). Clarke et al. (2004) focused on the effect of sourdough on the dough microstructure by confocal laser scanning microscope (CLSM). The results showed that the gluten fermented with sourdough was in an amorphous arrangement with aggregate structure, which increased the specific volume and delayed the staling of products. However, the excessive acidification of gluten is one of main barriers for the product-specific volume. Lopenon et al. (2007) found that acidification resulted in the hydrolysis of gliadin and glutenin in the dough through quantitative analysis. The degradation of macromolecule glutenin resulted in the decreasing of stability of gluten structure and gas retention capacity of dough. Therefore, the appropriate acidification is one of the important key processes for the high-quality products preparation. In the wheat flour, it mainly consisted of β -amylase but lack of α -amylase. β -amylase has little effect on the starch and has been completely inactivated during starch gelatinization. On the contrary, large amounts of α -amylase are present in rye flour, which leads to the structure of the dough and are generated by the water-binding pentosane (Corsetti et al. 2000). The previous study indicated that the decreasing of pH value of dough contributed to the activation of endogenous xylanase and significantly increased the solubility of arabinoxylan and water-insoluble arabinan, which resulted in the improvement of the dough elasticity and product quality characteristics (Gobbetti et al. 1999).

6.2.2.2 Proteolysis

Mckay and Baldwin (1974) firstly proved that the casein in the milk was hydrolyzed by the proteolytic system of LAB in order to meet the needs of growth and metabolism. The protease produced by LAB is distributed in the cytoplasm and cell wall, which degrades the protein into small molecular peptides and amino acids. In general, the protease activity of *Lactobacillus* is higher than that of *Lactococcus*. The amino acids produced by the *Lactobacillus* metabolism could also be utilized by *Lactococcus* and promote the growth of *Lactococcus*. The nitrogen source is presented in the forms of inorganic compounds in the cereals, which could not be degraded by LAB. Therefore, the protein degradation of LAB is limited in the process of cereal fermentation. As the previous study of Balestra et al. (2014) reported that the proteolysis is mainly dependent on the role of endogenous proteases in cereals during sourdough fermentation. During sourdough fermentation, the acid environment created by LAB activates the endogenous protease activity, which

contributes to the hydrolysis of macromolecular protein polymers (Yin et al. 2015). Two steps of proteolysis occur during sourdough fermentation: ① endogenous proteases hydrolyzed macromolecular polymeric proteins to form oligopeptides with low degree of polymerization in the appropriate pH value and the sulfhydryl groups accumulated accompanied by the hydrolysis of gluten and ② proteases produced by LAB transport low-polymerization oligopeptides (4–40 amino acids) into small molecular peptide and amino acids through a series of complex reactions. LAB converts amino acids into ketones, aldehydes, acids, and alcohols, which improve the flavor characteristics of products. In addition, glutathione (GSH) is a kind of important compound with strong reducibility, which reduced the molecular mass and polymerization degree of gluten polymers. The glutathione reductase existed in the heterofermentative LAB, which reduced extracellular oxidized glutathione (GSSG) to GSH and played an active role in dough rheology and product quality (Gänzle 2014).

6.2.2.3 Exopolysaccharides

In recent years, many studies have been reported that LAB produces exopolysaccharides during sourdough fermentation, which improve the tolerance ability of the strain and play a positive effect on the dough rheological property and the texture characteristic of final product (Wolter et al. 2014). Exopolysaccharides are categorized into homopolysaccharides and heteropolysaccharides according to their composition and biosynthesis pathway. Homopolysaccharides are synthesized from one kind of monosaccharide by extracellular glucanase and fructinase, while heteropolysaccharides are composed of one or more monosaccharides by intracellular glycosyltransferase during fermentation. Previous studies have indicated that exopolysaccharides with hydrophilic characteristics are metabolized by LAB, and it plays an important role in the quality of products: ① improving the water-binding capacity and delaying the staling of products and ② the interaction with other components in the dough, such as protein and starch, which improves the stability of network structure and the product quality (Galle and Arendt 2014). The dextran produced by *Leuconostoc* has been proven to extend the shelf life of bread. The optimization of fermentation parameter, such as substrate, dough yield, and fermentation time, which increase the yields of exopolysaccharides, have a great influence on the product quality. Exopolysaccharides are generally used to produce rye bread due to its poor taste and texture property. High molecular weight dextran has a significant effect on the improvement of rye bread quality (Galle and Arendt 2014; Wolter et al. 2014; Di Monaco et al. 2015). However, the screening and isolation of LAB with excellent exopolysaccharide-produced property is still far to work in the further study based on the requirement of fermented food process technology and product quality.

6.2.3 Sourdough Process Technology

6.2.3.1 Traditional Process Technology

In China, the traditional process technology of sourdough is produced by long-term fermentation with daily refreshments. Laomian and jiaozi are the two most representative of Chinese traditional dough starters. Laomian is one kind of type I sourdough, without adding baker's yeast and long-term fermentation with daily refreshment (Hu 2010). However, jiaozi is generally fermented with jiuqu for the sourdough propagation, and the preparation and propagation steps are different from laomian, which the propagation of jiaozi relies on more than three refreshments within 24 h and lasts for several days. Jiaozi is used to dry after fermentation in order to reduce the moisture content for long-term storage. Laomian is used to ferment with wheat, rye, and corn flour, but jiaozi is usually made from corn and rice flour or steamed bread crumbs due to its better dispersibility in the water during steamed bread processing. People in Shandong province used to produce steamed bread fermented with laomian, and jiaozi is usually applied in Henan province. The process technology of sourdough has the region specificity. In Henan province, Shangqiu area is mainly used to produce jiaozi with corn flour as raw material and mixed with Daqu, but rice flour and xiaoqu are used to prepare jiaozi in Nanyang area (Yang and Liu 2007). Han Chanjuan et al. (2010) developed the evaluation method of jiaozi by determining the fermentation and saccharification power. The results indicated that the fermentation and saccharification power of jiaozi are more than 800 mL and 15–300 U/g, respectively, which produced the steamed bread with better texture and flavor properties. Otherwise, there is a negative effect on the quality of steamed bread. In addition, Yang Jingyu et al. (2006) also focused on the changes of the physical and chemical properties of jiaozi. The results showed that the moisture content, protease, and amylase activities of jiaozi gradually increased during fermentation.

6.2.3.2 Modern Process Technology

Type II and type III sourdough are mainly applied in the process of industrialization. The former one is liquid sourdough and dried after preparation and is named type III sourdough. The dough yield of type II sourdough is about 200 and fermented at a controlled temperature that exceeds 30 °C in order to shorten the fermentation time. Long-term fermentation and high dough yield of sourdough resulted in low pH, which the strains with better resistant to low pH are dominated in type II sourdough. Due to the low moisture content of type III sourdough, it's convenient to storage and application. However, according to the preparation of type III sourdough, high temperature is used for sourdough drying, and the stress-resistant strains are dominated in type III sourdough (Brandt 2014). In addition, *Lactobacillus sanfranciscensis*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, and

Lactobacillus brevis are used for type II and type III sourdough fermentation. Unlike type I sourdough, type II and type III sourdough are not suitable for dough leavening. In order to ensure that the dough has better fermentation power, it is necessary to add a proper amount of baker's yeast for dough leavening (Corsetti 2013).

In China, the application of industrial sourdough develops slowly and still has many problems, such as poor fermentation stability of stains, extensive process technology, and simple processing equipment. Therefore, the market and consumer requirements desire us to develop the modern process technology of sourdough rapidly and enhance the added value of products as soon as possible.

6.2.4 Effect of Sourdough on the Quality of Product

Sourdough has been widely used for producing the wheat-based fermented food for a long time, such as steamed bread, bread, and cookie, which have a positive effect on the sensory quality, delay the staling and prolong the shelf life of products (Plessas et al. 2015).

6.2.4.1 Effect of Sourdough on the Sensory Quality of Product

Sourdough fermentation improves the sensory quality of product, such as texture and specific volume, which is related to the enzyme activity and metabolites formation by LAB during fermentation. Proteolysis plays an important role in the texture of bread or steamed bread and is mainly based on the pH-mediated activation of endogenous flour proteases. Katina et al. (2006) found that proteolysis by LAB resulted in better texture of bread compared to the chemically acidified products, which may have been associated with the slow-release acidification during sourdough fermented with LAB. It's indicated that biological acidification plays an important role in improving product sensory quality. In addition, the significant role of proteolysis by LAB and exopolysaccharides produced by LAB in the staling of product also has been confirmed. In addition to the endogenous cereal protease and protease metabolized by LAB, heterofermentative LAB-liberated glutathione reductase also plays a positive effect on the depolymerization of gluten protein. Glutathione reductase reduces GSSG to -GSH, resulting in an increase in the -SH group in gluten. GSH is mainly involved in the thiol-exchange reaction between gluten proteins and reduces cross-linking of disulfide bonds, which results in a decrease in the molecular weight of glutenin macropolymer (Jänsch et al. 2007).

6.2.4.2 Effect of Sourdough on the Flavor of Product

The flavor of steamed bread is one of the most important attributes, which is directly related to the consumers' acceptability. Although the ease of using baker's yeast, sourdough still preferred to apply in the fermented food process due to the improvement of product flavor. Food fermented with sourdough is valued for their odor and taste characteristics. The aroma compounds and taste compounds are generated by LAB and yeast during sourdough fermentation. The volatile compounds are mainly composed of alcohols, esters, aldehydes, and ketones, and taste-active amino acids, amino acid derivatives, and peptides play an important role in the food taste.

6.2.4.2.1 Taste Compounds

The interaction between lactic acid bacteria and exogenous enzymes affects microbial acidification kinetics, the content of acetic acid, and product texture. The generation of flavor compounds has been proven to be related to the activity of enzymes, which are mainly glucose oxidase, lipase, xylanase, amylase, and protease secreted by microorganisms (Pétel et al. 2016). Studies have shown that the addition of enzymes can increase the lactic acid content produced by the metabolism of *Leuconostoc*, *Lactococcus lactis*, and *Lactobacillus hilgardii*. During dough fermentation, moderate acetic acid content has obviously influence on flavor improvement. The synergistic fermentation of *Lactobacillus sanfranciscensis* and yeast can promote the formation of acetic acid, and the symbiosis of *Lactobacillus sanfranciscensis* and *Lactobacillus plantarum* can enhance the acid production capacity. Moreover, the fermentation temperature has a positive effect on the metabolism of the bacteria to produce aroma compounds. Because the optimum fermentation temperature of *Lactobacillus sanfranciscensis* is 32 °C, when it was cultured at 35 °C, the acetic acid content of *Lactobacillus sanfranciscensis* decreased significantly, but the metabolism of lactic acid and ethanol was not affected. In general, the product flavor is preferred with the lactic acid content of 3.11–5.14 g/kg.

6.2.4.2.2 Aroma Compounds

Microbial fermentation has been proved that different strain starters have a significant effect on the variation of product flavor. The aroma compounds produced by yeast fermentation are mainly alcohols, such as 2-methyl-1-propanol and 3-methyl-1-butanol. However, heterofermentative lactic acid bacteria fermentation mainly produced esters, specific alcohols, and aldehyde (Hansen and Schieberle 2005). Lactic acid bacteria play an important role in the improvement of the flavor of the product, and homofermentative lactic acid bacteria and heterofermentative lactic acid bacteria have a synergistic effect with yeast in the generation of aroma compounds. Due to the complex microbial community of sourdough, the flavor of the product is more abundant (Thiele et al. 2002). Free amino acids are important

substrates for the formation of volatile aroma compounds. The total amount of free amino acids in environmental system is mainly related to the hydrolysis degree of protein. Furthermore, the acid environment created by the lactic acid bacteria fermentation can increase the production of free amino acids and thus contribute to the formation of aroma compounds such as thiazole, thiophene sulfide, thiophene ketone, pyrrole, and pyrazine.

6.2.4.3 Effect of Sourdough on the Shelf Life of Products

Fermented noodle food is often accompanied by product staling phenomenon during storage, mainly manifested as internal tissue hardening, easy to drop slag, serious water loss, flavor fission, etc. The main causes of this phenomenon are as follows:

- ① The staling of amylose. Since the amylose is heated and cooled by the product, it is easy to complete its staling process. Therefore, the staling of the product during storage is mainly due to the staling of amylose.
- ② The migration of water. During the storage process, the water will gradually transfer from the core to the epidermis, resulting in water loss and tissue hardening of the product.

With the development of fermentation technology, it is found that lactic acid bacteria fermentation plays an important role in the anti-staling characteristics of products (Meng 2007). Min Weihong's (Min et al. 2004) research shows that after long-term fermentation of starchy raw materials, the lactic acid produced by the metabolism of lactic acid bacteria can affect the proportion of starch crystallization zone, which leads to the chain scission and debranching of macromolecular amylopectin and the increase of amylose content. In addition, Tiekling et al. (2003) also found that some lactic acid bacteria derived from sourdough can be metabolized to produce extracellular polysaccharides, and some exopolysaccharides have good hydrophilic properties, which can replace the use of hydrophilic colloids in fermented foods, in order to reduce the moisture transfer rate of the product in the storage process, effectively improving the anti-staling characteristics of the product.

6.3 Cereal Condiments Fermented with Lactic Acid Bacteria

6.3.1 *Microbial Community of Fermented Condiments*

6.3.1.1 Vinegar

Vinegar, with complex microbial community and diversity, is one of the traditional fermented condiments in China. The brewing process of vinegar consists of three parts: ethanol fermentation, acetic acid fermentation, and post-fermentation, which

is similar to soy sauce fermentation process. The main process of alcohol fermentation is attributed to the fermentation of molds and yeasts. The enzyme produced by microbial metabolism decomposes the macromolecular nutrients to amino acids and sugars, which are utilized by acetic acid bacteria and LAB. Moreover, these metabolites are metabolized and utilized in acetic acid fermentation, and some free amino acids are also the precursor substances of flavor compounds. In the acetic acid fermentation of vinegar brewing, numerous kinds of organic acids such as oxalic acid, tartaric acid, pyruvate, and malic acid are produced by the multi-strain fermentation, which provides amounts of substrates for the improvement of product flavor. In the post-fermentation stage, the color and aroma characteristics of vinegar are improved by microbial fermentation metabolism (Li et al. 2015).

6.3.1.1.1 Fungal Diversity of Vinegar

Different microorganisms play their respective roles in different stages of vinegar brewing. In the process of **solid fermentation substrate of vinegar** from vinegar, the inoculation of *Aspergillus* causes a large amount of mold to be introduced into the vinegar. Mold mainly acts on the alcohol fermentation stage of vinegar. Because of its good saccharification ability and protein hydrolysis ability, it provides a material basis for the growth and metabolism of yeast, lactic acid bacteria, and acetic acid bacteria in the post-fermentation process of the vinegar. Han Qinghui (2013) analyzed the dynamic structure changes of microbial flora in Liangzhou traditional fumigated vinegar brewing process. It was found that in the early stage of brewing, all kinds of microorganisms showed a tendency of rapid reproduction. However, on the second day of fermentation, the number of mold and yeast showed a rapid decline. On the fourth day, the total number of bacteria peaked and then gradually decreased, eventually gradual. The cultivation of vinegar usually uses *Aspergillus* as the key strain for fermentation. The *Aspergillus* commonly used in vinegar brewing includes *Aspergillus oryzae*, *Aspergillus niger*, and *Monascus*. Haruta et al. (2006) analyzed the molds in the traditional Japanese rice vinegar fermentation process, and they found that *Aspergillus oryzae* was the main fermentation strain in the alcohol fermentation stage. In addition, *Aspergillus oryzae* is the key fermentation strain for the production of traditional fermented condiments in Japan. The study confirms that *Aspergillus oryzae* has a high rate of enzyme secretion and plays a key role in vinegar brewing.

The yeast mainly acts on the ethanol fermentation stage and the acetic acid fermentation stage of the vinegar. In the alcohol fermentation stage, yeast metabolism utilizes monosaccharides to produce ethanol and carbon dioxide. In the acetic acid fermentation stage, the yeast is partially autolyzed, and its intracellular soluble matter can be used by other microorganisms as the **nutrient substance**. With the deepening of the modern molecular biology technology research, the composition of yeast in the structure of vinegar bacteria has been further understood. Wu et al. (2012) found that the yeast in **Shanxi super-mature vinegar** is mainly *Saccharomyces cerevisiae*. This is mainly due to the ability of *Saccharomyces cerevisiae* to better

adapt to the external environment in the later stage of alcohol fermentation. In addition, Xu Wei et al. (2007) used the molecular biology method to analyze the structure of fungal flora in the fermentation stage of acetic acid in [Zhenjiang fragrance vinegar](#). They found that vinegar was mainly composed of yeasts such as *Saccharomyces cerevisiae*, *Saccharomyces paradoxus*, and *Saccharomyces bayanus*. In the process of brewing super-mature vinegar in China, the yeast is mainly composed of *Dekkera*, *Brettanomyces*, *Oosporium*, *Kluyveromyces*, and *Pichia*. In traditional Italian [aromatic vinegar](#), it is mainly composed of *Zygosaccharomyces* and *Saccharomyces cerevisiae*. Among them, the abundance of *Zygosaccharomyces bailii* accounted for 41%. This is mainly due to the high sugar content in the traditional Italian aromatic vinegar brewing process, and the *Zygosaccharomyces* can still have good growth characteristics under the condition of higher sugar concentration (Solieri and Giudici 2008).

6.3.1.1.2 Bacterial Diversity of Vinegar

The key bacteria for vinegar brewing are acetic acid bacteria, lactic acid bacteria, and *Bacillus*. Acetic acid bacteria are one of the important strains, which oxidize ethanol to acetic acid during vinegar brewing. Acetic acid bacteria are mainly divided into two groups based on their physiological and biochemical characteristics: *Acetobacter* and *Gluconobacter*. However, *Gluconobacter* has a strong ability to oxidize glucose and produce sorbic acid, while *Acetobacter* has higher ethanol oxidation capacity than *Gluconobacter*. Due to the physiological metabolic activity of *Acetobacter pasteurianus*, it is dominated at the end of acetic acid fermentation stage (Li et al. 2015). Lactic acid bacteria produce a large amount of lactic acid during vinegar brewing, which alleviate acetic acid irritation and improve the taste of vinegar. It also produces the fatty acids and reacts with alcohol to generate aromatic esters, resulting in a better mellow and thick aroma of vinegar. Zou et al. (2011) found that *Lactobacillus sanfranciscensis* and *Lactobacillus plantarum* increased the content of lactic acid in the products and play an important role in the flavor compounds formation. *Bacillus* is a class of aerobic bacteria; the organic acids are mainly generated by tricarboxylic acid cycle and improve the sour taste of vinegar. In addition, the highly active protease produced by *Bacillus* hydrolyzes proteins into amino acids, which also play an important role in flavor formation and color improvement.

6.3.1.2 Soy Sauce

6.3.1.2.1 Fungal Diversity of Soy Sauce

Koji-making is an indispensable process in soy sauce production. Mold can fully grow and metabolize in this stage, producing rich enzymes, which contribute to enhance the flavor and quality of soy sauce. The molds commonly used in

koji-making of soy sauce are mainly *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, and *Botryosporium*, among which *Aspergillus oryzae* is the dominant species. It is found that mold usually commence to grow at the early stage of soy sauce fermentation. However, the growth of mold was inhibited, and the number decreased gradually with the consumption of oxygen in fermentation process. In addition, molds can generate a large number of enzyme systems, on the one hand, proteins are decomposed to produce small molecular peptide and amino acids by secreted protease, and the amylase can decompose the starch to dextrin and glucose, providing essential nutrients for the growth of lactic acid bacteria and yeasts in the subsequent fermentation; on the other hand, the free glutamine from the raw material was hydrolyzed by glutamate to generate glutamate, which could enhance the umami taste of soy sauce.

Yeast is considered to be another important fermentation strain in brewing of soy sauce. *Zygosaccharomyces rouxii* and *Torulopsis bombicola* are the main yeasts species in the brewing process. *Zygosaccharomyces rouxii* usually contributes to the early stage of fermentation and converts sugars such as glucose and maltose to alcohol. In addition to the formation of aromatic substances, the contents of succinic acid and furfuryl alcohol in soy sauce are increased; these compounds also greatly improved the aroma of soy sauce. Contrarily, *Torulopsis bombicola* has the main advantage in the later fermentation stage, which can reduce the production of sugars and amino acids, and mitigate occurrence of the Maillard reaction. Therefore, the ideal color can be well maintained in the final soy sauce products. In addition, guaiacol, phenylethanol, and other aroma components produced by *Torulopsis bombicola* makes the aroma of soy sauce more mellow. The amount of yeast in the initial stage of fermentation is very low, only 7.6×10^2 cfu/g. However, the growth rate is significantly accelerated when the fermentation is initiating; the highest content can be reached up to 3.2×10^6 cfu/g, then gradually slowed down, and became more balanced, and the number of yeast flora could be finally stabilized at 10^3 cfu/g (Yang et al. 2016). In the modern soy sauce brewing technology, the flavor of soy sauce products is usually improved by adding aroma producing yeast, and the interaction between yeast and *Aspergillus oryzae* can reduce the contents of reducing sugars and increase the content of amino nitrogen.

6.3.1.2.2 Bacterial Diversity of Soy Sauce

The amount of NaCl in soy sauce is as high as 18%, which prevents most bacteria from growing. However, the salt-tolerant lactic acid bacteria mainly include *Pediococcus halophilus* and *Tetracoccus Sojiae*, which survive and reproduce in the sauce mash with 18~20% salt content. In addition, it has positive effect on the color and flavor of sauce mash, which makes the product bright, ruddy, and rich in flavor. Lactic acid bacteria with good salt-resistant grow in the early stages of soy sauce brewing. With the decreased of pH during fermentation, yeasts are promoted to grow, and the content of lactic acid bacteria decreased rapidly in the late fermentation stage (Chen et al. 2014a, b).

6.3.1.3 Yellow Wine

6.3.1.3.1 Fungal Diversity of Yellow Wine

Mold existed in wheat koji plays an important saccharification in the brewing process of yellow wine, mainly in the genus *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium*, and *Absidia*. *Rhizopus* fungi produces glucoamylase, liquefaction enzyme, and some flavor substances. Moreover, *Aspergillus* fungi can secrete amylase, protease, peptidase, and other enzymes into the environment, which plays an important role in the hydrolysis of residual starch in post-fermentation. *Mucor* has a rich complex enzyme system, which can secrete glucoamylase, α -amylase, and protease. Fang Hua (2006) analyzed the fungi in wheat koji of yellow wine using traditional separation technology. It was found that the main fungi in wheat koji were *Aspergillus oryzae*, *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor pusillus*, and *Rhizopus microspores*. However, due to the water evaporation, the fungi were inhibited during process of koji. In addition, Cao et al. (2008) also found that straw and wheat raw materials had a great impact on the dynamic changes of fungal flora during wheat koji fermentation.

The fermented mash of yellow wine is rich in yeasts including *Saccharomyces cerevisiae*, *Hansenula anomala*, *Saccharomyces cinerea*, *Saccharomyces diastaticus*, and *Trichosporon pullulans*. In the early stage of mash fermentation, starch is converted to glucose, and yeast with saccharification ability was dominant in this process (Lv et al. 2013). With the development of fermentation, the alcohol concentration in the wine mash increased, and the *Saccharomyces cerevisiae* with higher alcohol tolerance gradually occupied the dominant position. When the fermentation time exceeds 90 days or more, the alcohol content reaches 19.0% (V/V), and the other yeasts will disappear in the late fermentation period due to the high alcohol content.

6.3.1.3.2 Bacterial Diversity of Yellow Wine

Lactobacillus is one of the main lactic acid bacteria in yellow wine brewing, including *Lactobacillus pasteurii*, *Lactobacillus amylophilus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus thermophilus*. *Lactobacillus pasteurii* decomposes starch, dextrin, and produces exopolysaccharide. The amount of *Lactobacillus* in Lin-fan yeast starter generally is in the range of $0.3 \times 10^9 \sim 1.0 \times 10^9$ cfu/mL. *Lactobacillus pasteurii*, *Lactobacillus amylophilus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus delbrueckii*, and *Lactobacillus thermophilus* are the most abundant in Lin-fan yeast starter (Fang et al. 2015). In addition, *Lactococcus* is another kind of main lactic acid bacteria in brewing of yellow wine, which stopped growing when pH was lower than 4.5 (Lv et al. 2016).

6.3.2 Biochemical Activity of Lactic Acid Bacteria During Fermentation

Vinegar and soy sauce are made of cereals through microbial fermentation. The fermentation process is accompanied with a series of biochemical metabolism and reaction, such as proteolysis, starch saccharification, alcohol fermentation, and the formation of organic acids.

6.3.2.1 Proteolysis

The secondary structure of cereal protein can be changed after high-temperature treatments, and protease in the fermentation system hydrolyze proteins into low molecular peptides and amino acids. Numerous studies have demonstrated that most of free amino acids have taste activity and can be used as precursor substances for the formation of flavor substances, which play an important role in the flavor characteristics of the product. During the soy sauce brewing, *Aspergillus oryzae* produce many kinds of proteases and alkaline protease. Therefore, it is necessary to prevent protease activity reduced that caused by low pH in the fermentation process, thus affecting the hydrolysis of protein in the fermentation process and going against the formation of flavor compounds in soy sauce.

6.3.2.2 Starch Saccharification

Cereals are rich in carbohydrates and high temperature of process releases starch granule. Macromolecular dextrin, maltose, and glucose are generated by amylase during fermentation. Glucose is an important carbon source for microbial metabolism in condiment fermentation, and it can also participate in Maillard reaction during high-temperature process. In addition, glucose is also a basic metabolic substrate for alcohol fermentation and organic acid generation. Therefore, the starch saccharification should be strictly controlled in the fermentation process to avoid excessive acidification.

6.3.2.3 Alcohol fermentation

Yeast is an indispensable fermentation strain for alcohol fermentation in the condiment process. The optimum growth temperature of yeast is 28~34 °C. Yeast has a good acid-resistant capability, but its growth can be inhibited on the condition of pH < 3.5. The saccharification of starch in vinegar, soy sauce, and other condiments resulted in the accumulation of a large amount of glucose in the fermentation system, which provides plenty of carbon sources for saccharide metabolism in yeast. In addition to producing alcohols, cereal fermentation also produce aldehydes, acids

and esters, and other important aroma components, which plays a crucial role in flavor characteristics of vinegar and soy sauce.

6.3.2.4 Organic Acid Formation

Condiments, such as vinegar and soy sauce, are rich in many organic acids, including lactic acid, acetic acid, succinic acid, malic acid, and citric acid. It was found that different organic acids in condiments had synergistic effects on the flavor characteristics of products. Moreover, the organic acid can also be esterified with alcohols in the later stage of the fermentation, thereby improving the content of ester compounds in the fermented products, which play an active role in the improvement of products flavor.

6.3.3 Processing Technology of Cereal-Based Condiments Fermented by Lactic Acid Bacteria

6.3.3.1 Vinegar

Vinegar is mainly fermented from starchy cereals; the procedure of microbial metabolism and enzymatic conversion converts starch to ethanol and acetic acid. There are significant differences in vinegar brewed in different regions, which is related to the brewing methods and raw materials characteristics. According to fermentation process, it can be divided into three types: solid-state fermentation, liquid state fermentation, and solid-liquid state fermentation.

6.3.3.1.1 Solid-State Fermentation

The traditional vinegar brewing technology in China mostly adopts solid-state fermentation, namely, the fermentation materials is solid-state in acetic acid fermentation stage. Due to acetic acid bacteria which need a certain amount of oxygen to oxidize ethanol to produce acetic acid, it is necessary to add more fluffy food materials, such as millet shell, sorghum shell, and bran. The fermentation environment of traditional solid-state fermentation is open-ended, and the microbial flora structure is complex. So the vinegar produced by this method is rich in flavor compounds and good quality. For example, both of the famous Shanxi aged vinegar and Zhenjiang vinegar are produced by solid-state fermentation. However, this approach has its drawbacks, such as wide coverage, low yield, and low equipments utilization. In recent years, with the improvement of new brewing technology, modern solid-state fermentation technology has been widely used in vinegar processing. Compared with the traditional brewing technology, the production rate has been significantly increased, but the product quality has significantly decreased. How to keep the

quality and flavor of traditional vinegar brewing on the basis of improving technology has become a research problem in the field of vinegar industry (Su 2015).

6.3.3.1.2 Liquid State Fermentation

In China, the common liquid state fermentation technology of vinegar brewing are mainly composed of surface liquid fermentation, quick-brewed vinegar fermentation, and submerged fermentation. Some famous vinegar is also produced by liquid state fermentation technology, such as Jiangsu and Zhejiang rose vinegar, Fujian red koji vinegar, etc.

Surface Liquid Fermentation Process

According to the different operations of the former process, the vinegar produced by surface liquid fermentation can be divided into white vinegar, sweet vinegar, and rice vinegar, and the specific fermentation process is as follows:

1) White Vinegar Process

White vinegar is produced by placing the vinegar source in a special container, adding ethanol solution and a small amount of nutrients to cover the cylinder head, and fermenting about 20 days at natural temperature or in a greenhouse at 30 °C. The vinegar source is generally the mature vinegar of the previous batch, and the ethanol solution can be prepared by diluting the liquor with water and diluting to 3% ethanol contents. The specific fermentation period depends on the environment temperature. The fermentation period should be extended if the temperature is low. The mature vinegar is clear and colorless with the content of acetic acid of 2.5~39.0 g/100 mL.

2) Sweet Vinegar Process

Sweet vinegar is made from maltose, which mainly exists in Beijing. After inoculation of acetic acid, the fermentation is carried out in a paper-sealed cylinder at 30 °C. The matured period of fermentation is about 30 days, which is a slightly longer than white vinegar. The acetic acid content of final sweet vinegar is 3~4.59 g/100 mL.

3) Rice Vinegar Process

Rice vinegar is made from rice by liquid surface fermentation. However, the processing methods have regional divergence. For instance, rice koji can be made by adding *Aspergillus oryzae* to rice, then saccharifying rice by adding water, or saccharifying rice by adding koji. Certainly, wheat flour was inoculated with *Aspergillus oryzae* to make dough and then added with water to saccharify the rice. After the saccharification solution was prepared, yeast was added for ethanol fermentation, and acetic acid bacteria were eventually added to system for surface liquid fermentation. The acetic acid content of rice vinegar is generally 3~5 g/100 mL.

Quick-Brewed Vinegar Process

Quick-brewed vinegar is brewed in a quick brewing tower; raw materials such as liquor are oxidized to acetic acid by acetic acid bacteria and then through aging process. Quick-brewed vinegar is clear, colorless, or yellowish.

Submerged Fermentation Process

Vinegar made by submerged fermentation technology is a new technology developed in modern technology. The period of vinegar brewing is very short with comparatively higher productivity. In addition, most of the production equipments are standard fermentation tank; hence the floor space is small, and no rice husk fillers are used. This new process is a great progress from traditional fermentation to modern mechanized vinegar production. However, the flavor of vinegar by modern process is slightly inferior to that of traditional vinegar.

6.3.3.1.3 Solid-Liquid State Fermentation

The primary difference between solid-liquid state fermentation and other fermentation approaches is that the process of acetic acid fermentation is solid-state, while the saccharification of raw materials and the fermentation of ethanol are carried out in liquid state. Natural ventilation and vinegar reflux combined with enzymatic method are used in solid-liquid state fermentation, which not only reduced the labor force but also improved the utilization ratio of raw materials. In general, 8 kg vinegar can be obtained from 1 kg broken rice material.

6.3.3.2 Soy Sauce

As a traditional fermented condiment in China, soy sauce has a delicious taste and a wide range of soy sauce. Generally speaking, soy sauce is mainly divided into two types: dark soy sauce and light soy sauce. The tastes of dark soy sauce is salty, heavy color, and often used for color improvement, while the light soy sauce has a strong umami taste and is often used for flavor enhancement. At present, the main technology for soy sauce brewing are natural fermentation, high saline diluting fermentation, low saline solid-state fermentation, part-brewing solid-diluted state fermentation, etc. (Zhao 2009).

6.3.3.2.1 Natural Fermentation

Natural fermentation method is the traditional processing technology of soy sauce brewing in China. This method naturally exposed the fermented ingredients to the sun in an open environment. On the one hand, a large number of fungus, yeast, and lactic

acid bacteria can be introduced into the fermented system, which is a natural way of koji-making; on the other hand, the moisture in the fermented grains can be evaporated effectively by long-time sunshine. Therefore, the maturation time of fermented soy sauce is longer, and the salt content is higher. However, the higher salt content and lower fermentation temperature can inhibit the enzyme activity in soy sauce cereal.

6.3.3.2.2 High Saline Diluting Fermentation

High saline diluting fermentation is a combinatorial brewing method that combines natural fermentation and modern soy sauce brewing technology. Generally, the fermentation temperature is 10–30 °C; the fermentation time is 6–12 months, and the salt content is 15% or more (Lu and Wei 2006). Compared with the traditional natural fermentation method, modern processing and temperature control technology can effectively eliminate the potential safety problems caused by long-time natural exposure. However, the high saline diluting fermentation cannot avoid the disadvantage of long fermentation period and high investment cost.

6.3.3.2.3 Low-Saline Solid-State Fermentation

Low saline solid-state fermentation is evolved from salt-free solid-state fermentation combined with Chinese brewing technology. It is the most widely used fermentation technology for soy sauce production in China. On account of the salt content in sauce is less than 10%; the inhibitory effect on the enzyme activity is not significant; the ripening period is very short for soy sauce fermentation. Therefore, this brewing approach is suitable for industrial scale production of soy sauce with good quality. However, the flavor taste of this kind of soy sauce is obviously inadequate than the traditional method of natural fermentation and high saline diluting fermentation products (Deng et al. 2015). Moreover, the traditional salt-free solid-state fermentation process is maintained in some rural areas of China, in which the amount of salt added in the brewing process is very little or lacking. In order to ensure that the product is not contaminated by miscellaneous bacteria, the fermentation temperature range is usually 55–60 °C; the increase of temperature can significantly promote the activity of the enzyme, and the fermentation ripening cycle is only 60–72 h, but the products have defects such as serious lack of flavor and insufficient quality.

6.3.3.2.4 Part-Brewing Solid-Diluted State Fermentation

In order to shorten the fermentation period of soy sauce and ensure the flavor of the products, the part-brewing solid-diluted state fermentation was developed as a rapid brewing technology, which is combined with the characteristics of solid and dilute fermentation technology. This method mainly separately prepared sauce mash from protein and starch and optimized the temperature, salt content, and fermentation

conditions of solid-liquid mash. Solid-state fermentation is first conducted under a lower salt condition, and then brine is added for diluting fermentation, which effectively reduces the inhibitory effect of salt on enzyme activity. Furthermore, this process can also promote metabolism and growth of fungi and yeast in the early fermentation stage. However, this process is more cumbersome, and the control of segmental fermentation is strict, so that it is not widely used in the manufacturing of soy sauce (Deng et al. 2015).

6.3.3.3 Yellow Wine

Yellow wine is one of the oldest liquors in the world. It is produced by the interaction of different kinds of fungus, yeasts, and bacteria using rice and millet as the main raw materials, and after a series of manufacturing procedure of cooking, saccharifying, fermenting, and squeezing, the low-alcohol brewing yellow wine is obtained, and the alcohol content is usually 14~20%. In addition, yellow wine contains eight kinds of essential amino acids and other nutrients, so it is always called “liquid cake.” Due to the differences in raw materials, starter, process technology, and natural conditions yellow wine production, the quality of yellow wines are distinguishable. According to the content of sugar, yellow wines can be divided into dry yellow wine, semidry yellow wine, semisweet yellow wine, and sweet yellow wine. The famous yellow wines include Shaoxing rice wine, Fujian old wine, Jiangxi Jiujiang Sealed Liquor, and Wuxi Huiquan wine. The manufacturing technology of these wines belongs to the traditional rice wine fermentation process, which can be divided into Lin-fan wine, Tan-fan rice wine, and Wei-fan wine.

6.3.3.3.1 Lin-Fan Wine

The name of Lin-fan wine comes from the operation of sprinkling cold water to steaming rice. The process is as follows: the glutinous rice is immersed in water for 48 h, then is steamed and matured into rice, after cooling with cold water to the optimum temperature for saccharification and fermentation of rice, and then mixed with wine, special koji, clear water, etc., and Lin-fan wine produced in fermented condition for 45 days. Lin-fan wine is mainly used in the production of sweet yellow wine. Although the mature Lin-fan wine holds a poor flavor characteristics and monotonous taste, the high yield, cheap price, and fast available market of yellow wine make it popular in China.

6.3.3.3.2 Tan-Fan Wine

Tan-fan wine is made by immersing white glutinous rice with water for 16–20 days, separating the rice water and steaming the raw rice into rice, then spread the rice and cool down to 35 °C, and then mixing it with rice syrup, wheat koji, yeast, and

appropriate amount of water for saccharification and fermentation. After 60–80 days, Tan-fan wine can be formed, such as Yuanhong wine. Using the traditional technology of winter brewing, on account of long brewing period, the starch hydrolysis, protein, and fat decomposition of material in the fermentation process are more intensive. Moreover, the degree of formation of various organic acids and flavoring substances are more adequate.

6.3.3.3.3 Wei-Fan Wine

When Wei-fan wine is brewed, the raw rice for brewing is divided into multiple portions; the first one is used to produce yeast starter using Lin-fan method; the first Wei-fan operation is carried out after 27~28 h. Then the materials need to be stir immediately so that the yeast starter of the first fermentation is evenly distributed. Subsequently, add water every 24 h to make the saccharification and fermentation uniform, and control the temperature of the fermentation product. After repeating four times, the Wei-fan wine is produced. Compared with Lin-fan wine and Tan-fan wine, the degree of fermentation is deeper, and the utilization ratio of raw material is higher. There are still many Chinese areas that adopt this traditional technology for yellow wine brewing; a typical representation of this kind of wine is Shaoxing yellow wine.

6.3.4 *Effect of Lactic Acid Bacteria Fermentation on the Condiment Quality*

6.3.4.1 Effect of Microbial Fermentation on the Vinegar Quality

Vinegar is metabolized and fermented by many microbes and is rich in many organic acids that are mainly classified into volatile acids and nonvolatile acids. Volatile acids are mainly acetic acids produced by the metabolism of acetic acid bacteria, and propionic acid, butyric acid, and valeric acid are present in trace amounts. The smaller the molecular mass of volatile acids makes the stronger flavor irritation. Therefore, the irritative taste of vinegar mainly comes from volatile acetic acid. The nonvolatile acids in vinegar were mainly lactic acid, malic acid, citric acid, and succinic acid, which were also produced by the metabolism of lactic acid bacteria. Such substances can regulate the pH in environmental system, inhibit the growth of spoilage bacteria, and effectively improve the quality of products (Wang et al. 2013). Moreover, nonvolatile acids have a buffer effect on the irritating odor of volatile acetic acid; the products with higher content of nonvolatile acids will weaken flavor irritation of volatile acids. In general, the content of nonvolatile acid in solid-state fermentation vinegar is higher than that in liquid state fermentation vinegar. In addition, esterification reaction occurred in the fermentation process of lactic acid, and bacteria can facilitate the odor of vinegar (Zhao et al. 2014).

The aroma components of vinegar mainly come from acid, aldehyde, alcohol, ester, phenol and furan, and other volatile flavor substances, which are lower in vinegar, but these compounds will give the product a strong acidic flavor in the appropriate proportion. Moreover, the excessive content of aldehydes will lead to vinegar possessing a spicy flavor and strong irritation, while moderate aldehydes concentration can give vinegar rich desirable flavor. Esters are important flavor components that form the unique aroma of vinegar. They are mainly produced by the esterification reaction of organic acids and alcohols. Generally, the high-quality vinegar are rich in ester compounds, such as butyl acetate, ethyl acetate, ethyl lactate, etc. (Zhu et al. 2016). Li Danya's (2008) found that acetic acid and 3-methyl butyric acid were the characteristic aroma components of Zhenjiang fragrant vinegar. In the flavor study of Shanxi aged vinegar, there were significant differences between high-quality vinegar and middling vinegar in the contents of acetaldehyde, ethyl acetate, ethanol, furfural, and propionic acid (Miao et al. 2010). High-quality vinegar needs to go through the aging process, which result in the evaporation of water, so that the content of characteristic flavor substances was improved significantly. Compared with Japanese vinegar, the amino acid level in Chinese vinegar is significantly higher. Taking Japanese rice vinegar as an example, the average amino acid content is 137 mg/100 mL, which is only about one-tenth of Chinese vinegar.

6.3.4.2 Effect of Microbial Fermentation on the Soy Sauce Quality

The flavor formation of soy sauce mainly generate in the later fermentation period. Currently, more than 200 flavor compounds have been reported from soy sauce. Thereinto, there are 20~30 kinds of representative flavoring substances in soy sauce including alcohols, aldehydes, organic acids, phenols, esters etc. The flavor formation mechanism of soy sauce is relatively complex; there are four main generation pathways: ① from the ingredients, different ingredients have a significant impact on the flavor characteristics; ② in the stage of koji-making, metabolism of *Aspergillus* can produce many biochemical enzymes and decompose macromolecular to many flavor compounds, which is beneficial for enhancing the flavor of soy sauce; ③ in the later fermentation stage, acetic acid bacteria, lactic acid bacteria, and yeast produced various flavor compounds by metabolizing the small molecule produced by the decomposition of mold and yeast in the koji-making stage, thus achieving the synergistic effect of enhancing flavor; and ④ the change of nonenzymatic chemical reaction involved in the whole brewing process of soy sauce, which can promote the enhancement of flavor (Singracha et al. 2016).

Lee et al. (2006) compared the volatile flavor compounds of fermented soy sauce and acid hydrolyzed soy sauce. It was demonstrated that alcohols and esters were the major flavor compounds in fermented soy sauce, while heterocyclic compounds were the main components in acid hydrolyzed soy sauce. Alcohol is mainly produced by yeast fermentation metabolism, and ethanol is one of the main substances produced by yeast fermentation with hexacarose; amyl alcohol and isopentanol are

important products of yeast decomposing leucine and isoleucine. It was also found that ethanol, pentanol, isoamyl alcohol, phenylethanol, furfuryl alcohol, and other hydroxyl flavor compounds were the main flavor components of low salt solid soy sauce. Esters are the main components of aroma in soy sauce. They are mainly produced by esterification of alcohols and organic acids produced by microbial metabolism (Gao et al. 2010). At present, more than 40 esters were identified from soy sauce, which endows the soy sauce with strong flavor. Aldehydes and acids are also important components of the flavor components of soy sauce. The aldehydes are mainly converted from the organic acid produced by yeast and *Aspergillus* during the koji-making process. Up to now, 18 aldehydes in soy sauce have been reported. The brewing process of soy sauce is based on the growth metabolism of microorganisms. In addition, there is an interaction between different substances, which has the characteristics of synergistic fermentation and flavor enhancement (Cui et al. 2014). Mixed strain fermentation is mainly used in the stage of koji-making and post-fermentation. Multi-strain koji is composed of *Aspergillus oryzae* and mixed with other strains. The main purpose of this method is to improve the utilization rate of raw materials and the productivity of amino acids through the enzymes secreted by strains. The multi-strain fermentation is mainly in the post-fermentation stage, and the flavor characteristics of product are improved by additionally adding yeast and lactic acid bacteria. It was found that mixed fermentation could promote the growth rate of starters; the difference of metabolic pathways between starters led to different metabolites formation, which resulted in the reciprocal growth of microorganisms. It is noteworthy that the later fermentation process is easy to be contaminated by miscellaneous bacteria, and the addition of lactic acid bacteria in mixed fermentation can effectively inhibit the breeding of miscellaneous bacteria (Zhao 2005). In addition, the study also found that *Lactobacillus plantarum* has good salt tolerance, and it has a significant synergistic effect with salt-tolerant yeast under suitable ratio conditions. Therefore, it plays an active role in improving the flavor of soy sauce prepared by low salt solid-state quick-brewing process.

6.4 Other Cereal Products Fermented with Lactic Acid Bacteria

6.4.1 Fermented Rice Flour

6.4.1.1 Microbial Diversity of Fermented Rice Flour

Natural fermentation is usually used in the process of fermented rice flour. Tong Litao et al. (2013) have found that the microbial diversity of rice flour is changing during the process of fermentation, in which lactic acid bacteria are the main bacteria with dominant microflora in the process of fermentation. The growth rate

of yeast was faster during the first 24 h of fermentation and then tended to be stable. Due to the large amount of oxygen needed for the growth of mold, most of them are grown on the surface of fermentation broth in the process of rice flour fermentation, which has a limited effect on the fermentation process of rice flour. In recent years, many scholars have studied on the diversity of microbial flora in fermented rice flour. Lu Zhanhui et al. (2006) found that rice flour in Changde (Hunan Province) were mainly composed of *Lactobacillus*, *Streptococcus*, and *Saccharomyces cerevisiae*. The analysis of microbial community showed that *Lactobacillus plantarum* and *Saccharomyces cerevisiae* were the dominant fermentation strains for rice flour according to the 14 samples from 3 regions of China and South Asia.

6.4.1.2 Process Technology of Fermented Rice Flour

Rice flour, which is similar to the noodles in northern China with long strips form, is an important dietary component in Southern China and Southeast Asia. The processing technology of common rice flour consists three types: cutting powder, pressing powder, and the other kinds. Among them, the cutting-powder type of rice flour is prepared by gelatinizing part of the rice milk in advance, then mixing it with raw rice milk, and coating on the conveyor belt, which is heated by the steam tunnel furnace, air-cooled, and sliced into shape. The pressing-powder type of rice flour is mainly processed by extrusion molding, that is, the rice is soaked, wet-milled, filtered, and dehydrated until the moisture content is 38%~42%, and then extruding inside the machine after boiling or steaming. In addition, rice flour is divided into fermented and non-fermented. Compared with the non-fermented type, the fermented rice flour has special texture characteristics and chewiness. Ma Xia et al. (2015) found that soaking time and temperature are two important factors to determine the quality of fermented rice flour.

6.4.1.3 Effect of Lactic Acid Bacteria on the Rice Flour Quality

Lactic acid bacteria play an indispensable role in the natural fermentation of rice flour, which has a positive effect on the texture, nutritional, and functional characteristics of rice flour. Starch, the main component of rice, which makes rice flour can be regarded as a kind of starch gel. The previous studies found that the rice flour produced by *Lactobacillus* fermentation can produce organic acids such as extracellular enzyme and lactic acid during the fermentation process, and the content and average degree of polymerization of amylose are significantly increased after fermentation. The elasticity of starch gel was improved by increasing the average polymerization degree of amylose. Therefore, compared with the non-fermented rice flour, the product has better edible quality, such as flexibility, chewiness, and tensile properties. Li Lite et al. (2001) further confirmed that the main reason for the increase of amylose content in rice flour was that lactic acid bacteria metabolized

during the fermentation process could affect the proportion of starch crystalline region; thus, the amylopectin of macromolecular was broken and debranched, and the content of amylose was increased. In addition, the fermentation of lactic acid bacteria can metabolize many bacteriocins with the inhibition of spoilage bacteria and effectively prolong the shelf life of products.

6.4.2 Fermented Cereal Beverage

6.4.2.1 Microbial Diversity of Fermented Cereal Beverage

The bacteria commonly used in traditional cereals fermented drinks are *Leuconostoc mesenteroides*, *Lactobacillus*, *Streptococcus*, and *Pediococcus*, the fungus include *Aspergillus*, *Paecilomyces*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Trichothecium*. Boza in Turkey is a mixture of wheat, rye, corn, and other cereals with sucrose and fermented for a long time at 30 °C. Based on the culture-dependent and culture-independent analysis. Boza is mainly composed of *Lactobacillus sanfranciscensis*, *Leuconostoc mesenteroides*, *Lactobacillus coryniformis*, *Leuconostoc dextranicum*, *Lactobacillus fermenti*, *Leuconostoc oenos*, *Saccharomyces uvarum*, and *Saccharomyces cerevisiae*. Mahewu is a traditional fermented coarse grain beverage in Zimbabwe, which is mainly made from corn and sorghum by 16 h fermentation at 45 °C. It has been proved that the main fermentation microorganism is *Lactococcus*. Due to *Lactococcus lactis* which can metabolize *nisin*, the products could be resistance to *Salmonella*, *Campylobacter jejunii*, and *Escherichia coli*.

6.4.2.2 Process Technology of Fermented Cereal Beverage

There are many kinds of fermented coarse grain beverages reported in China, which are mainly processed from oats, corn, barley, etc. Yan Haiyan et al. (2008) mixed corn with water according to 1:10 (w/V), inoculated with lactic acid bacteria by 12 h fermentation at 37 °C, and then added 0.1% xanthan gum and 0.15% sodium carboxymethyl cellulose after fermentation to produce a lactic acid corn fermented beverage. Jia Jianbo (2002) focused on a mixture of skim milk powders and oats, which was fermented by *Lactobacillus rhamnosus*. Firstly, the oats were hydrolyzed by two enzymes, then add skim milk powders, sterilized and cooled, inoculated and fermented to make the biological milk with active ingredients and healthcare function. Its viable count reached 10¹⁰ cfu/ml; acidity was 108 °T, and β-glucan content was 236 mg/L. It can be seen that fermented coarse grain beverage has a good market prospect not only due to the effectively enhancement of nutritional value of grain itself but also has the same healthcare function as fermented products such as lactic acid bacteria.

6.4.2.3 Effect of Lactic Acid Bacteria on the Cereal Beverage Quality

After fermentation with lactic acid bacteria, the contents and types of trace nutrients such as vitamins, amino acids, and minerals in cereals were significantly increased, and organic acids, alcohols, aldehydes, and ketones were produced by metabolism, which endow the products with more intense aroma. Onyango et al. (2004b) used corn and millet milk as raw materials to produce Uji, a traditional food in East Africa. By comparing the amino acid content in the mixture before and after fermentation, it was found that the contents of Lys, Tyr, Met, Cys, Gly, and Asp were significantly increased after fermentation of Uji. Wang Fengling and Liu Aiguo (2003) also found that based on the combined action of microbes and enzymes, the protein, starch, fat, and other macromolecular nutrients decompose into small molecular substances such as amino acids, polysaccharides, monosaccharides, and fatty acids, which improve the absorption of body. Onyango et al. (2004a) processed the fermented mixture of corn and millet into a kind of food for nourishing and weaning, which is more suitable for children because of lower content of fiber and tannin after fermentation. Nanson and Fields (1984) reported that the content of lysine and tryptophan increased greatly after natural fermentation of corn flour.

6.4.3 Fermented Soya Beans

6.4.3.1 Microbial Diversity of Fermented Soya Beans

As one of the traditional fermented products in China, the fermentation process of fermented soya beans is coordinated by a variety of complex microorganisms. Traditional fermented soya beans fermentation is mainly divided into koji-making stage and brine stage. The main fermentation bacteria were filamentous fungi, such as *Aspergillus*, *Mucor*, and *Rhizopus*. The types of filamentous fungi used in koji-making stage are mainly affected by local environment and climate due to the natural fermentation processing. When the fermentation temperature is about 15 °C, the main fermentation bacteria are low-temperature type of *Mucor*, and when the temperature is 20~30 °C, the main fermentation bacteria is medium-temperature type of *Rhizopus*. Chen et al. (2011) used modern molecular biological techniques to analyze the diversity of microbial communities in the koji-making stage of “Daoxiangyuan fermented soya beans.” It was found that *Aspergillus oryzae* and *Bacillus subtilis* were the main fermentation bacteria in the stage. In addition, they found a variety of microbes known as *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Aspergillus Niger*, *Staphylococcus saprophyticus*, and *Saccharomyces cerevisiae*. But the brine stage is mainly the anaerobic fermentation process with high osmotic pressure, which needs long time and plays an important role in the formation of flavor and quality improvement of fermented soybeans. The mold is useless in the process because of the anaerobic environment. However, due to the metabolism of

many enzymes in the stage of koji-making, sufficient material basis was provided by the mold for the brine stage of fermentation process. The selection of salt concentration in brine stage plays an important role in microbial growth. High concentration of salt can inhibit the growth of bacteria and prolong the ripening process. In contrast, low salt concentration lead to the rapid growth of lactic acid bacteria and excessive acidification of the product, which is not conducive to the sensory quality of the product. Therefore, suitable selection of salt concentration determines the growth and metabolism of microorganisms and affects the quality characteristics of the product. Chen et al. (2011) used traditional separation techniques combined with PCR-DGGE to investigate the microbial community composition of the fermented soya beans meal brine phase. The study found *Lactococcus lactis* and *Staphylococcus aureus* were the main bacteria in the post-fermentation process, and a variety of microbes, such as *Bacillus subtilis* and *Enterobacter*, has been found in the process.

6.4.3.2 Process Technology of Fermented Soya Beans

Fermented soya beans are mainly made of beans, processed by raw material handling, stewing, koji-making, fermentation, and drying. As one of the four traditional fermented soybean products in China, these not only have many types but also use the natural fermentation method, which is made by using the microorganism in the environment and raw materials combined with the local climate fermentation. However, the open fermentation methods are often accompanied by serious safety risks and poor batch stability, which is not conducive to industrial standardized production. Fermented soya beans have only a limited presence in marketplace due to the long fermentation cycle, high storage cost, and low market value. In addition, in order to prevent the spoilage of the products, high concentration of salt was used to extend the shelf life, which further discourages the consumers. In recent years, with the development of modern fermentation technology, directional fermentation with specific dominant strains has become the direction of industrial processing of modern fermented foods. Therefore, the strains can be divided into *Aspergillus*, *Mucor*, *Rhizopus*, *Bacteria*, and *Neurospora* according to the different strains used in the stage of koji-making. Among them, *Aspergillus*-type of fermented soya beans are mostly used in China, and tempeh of Indonesia and natto of Japan are representatives of *Rhizopus*-type and Bacterial-type, respectively. Therefore, there were significant differences in sensory and texture properties of fermented soya beans by different strains.

6.4.3.3 Effect of Lactic Acid Bacteria on Fermented Soya Bean Quality

Lactic acid bacteria are not the key fermentation bacteria in the process of fermented soya beans production, but they play an important role in the formation of flavor, enhancements to the functionality, and nutritional characteristics of

fermented soybeans during the post-fermentation stage. Similar to other fermented soybean products, the lactic acid bacteria with good salt-tolerant characteristics, such as *Tetragenococcus halophilus*, can use small molecular sugar to produce a variety of organic acids, which can form esters with unique aroma with the alcohols produced by yeast fermentation. At the same time, a large number of small molecules of saccharides in the system are metabolized and utilized by microorganisms in the post-fermentation process so as to reduce the Maillard reaction in the post-ripening process and effectively maintain the colors of the products. In addition, it was found that *Lactobacillus plantarum* and *Lactococcus lactis* contained in fermented soya beans can metabolically produce bacteriocins, which can inhibit the growth of spoilage bacteria, to effectively extend shelf life to meet current consumer demand for green and chemical-free food market.

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