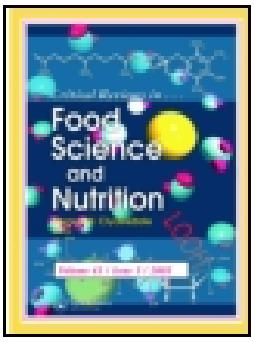

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Traditional fermented soybean products: processing, flavor formation, nutritional and biological activities

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ABSTRACT
Traditional fermented soybean food has emerged as an important part of people's dietary structure because of the unique flavors and improved health benefit. During fermentation, the nutrients in soybean undergo a series of biochemical reactions catalyzed naturally by microorganism secreted enzymes. Thereafter, many functional and bioactive substances such as bioactive peptides, unsaturated fatty acids, free soy isoflavones, vitamins and minerals are produced, making fermented soy products more advantageous in nutrition and health. This review comprehensively discusses the historical evolution, distribution, traditional fermentation processing, main sources and characteristics of fermented strains, flavor components, nutritional properties, and biological activities of four traditional fermented soybean foods including *douchi*, *sufu*, *dajiang*, and soy sauce. In the end, we introduce four major challenges encountered by traditional fermented soybean foods including high salt content, formation of biogenic amine, the presence of pathogenic microorganisms and mycotoxins, and quality inconsistency. We conclude that the establishment of scientific quality standard and innovated fermentation processing is the potential solutions to combat the issues and improve the safety of traditional fermented soybean products.

KEYWORDS
fermented soybean; traditional fermentation; functional ingredients; plant foods; nutrition and functionality; flavor

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important crops in the world and has been processed into a wide varieties of soybean products because of its excellent yield and rich nutrients. Among most of the industrial practices for soybean processing, fermentation is simple and inexpensive technique that can substantially improve the nutrition, texture, and flavor of soybean, thus becoming a popular process throughout the world (Caplice and Fitzgerald 1999; Granito et al. 2002).

Numerous fermented soybean products are available in the world. In particular, *douchi* (fermented black bean), *dajiang* (soybean paste), *sufu*, and soy sauce are the top fermented soybean products in terms of mass production especially in Asia. With the change of consumer's dietary concept to healthy eating, traditional fermented soybean products are still satisfying consumer's increasingly diversified dietary needs with their unique flavor and nutritional functions. This paper reviews the historical evolution, geographical distribution, traditional fermentation processing techniques, the application of strains for fermentation, flavor development during fermentation, nutritional and biological properties of the four most popular traditional fermented soybean products *douchi* (solid), *dajiang* (semi-solid), *sufu* (gel-like), and soy sauce (liquid). As fermentation has recently evoked much interests among consumers and the food industry, the aims of this paper are (i) to improve the modern processing methods of fermented soybean products, (ii) to enhance the safe production process of soybean fermentation, (iii) to point out a way of sustainable development for the future direction of traditional fermented soybean products, and (iv) to provide new insights into novel food ingredients innovation as well as ingredients flavor enhancements for other crops.

Historical development

Douchi

Douchi is a traditional fermented black soybean product originated in China, with a history of more than 3000 years. *Douchi* was clearly marked in Han Tomb No. 1 at the Mawangdui Han Tomb (165 BC) in South-Central China. Dating back to the Han Dynasty, the earliest record of *douchi* was discovered in *Shi Ming* (Explanations of Names, 200 AD) edited by Liu Xi. The ancients used *douchi* not only as a seasoning, but also as a medicine, and had attached great importance to it. In addition, the records are also found in *Han Shu* (Book of Han,105 AD), *Shi Ji* (Records of the

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Grand Historian, 104–91 BC), *Qimin Yaoshu* (Essential Techniques for the Peasantry, 533–544 AD), and *Bencao Gangmu* (Compendium of Materia Medica, 1578 AD), a magnum opus of Chinese historic significance compiled by Li Shizhen (published in 1596). The production history of *douchi* can be traced back to the Pre-Qin period (Paleolithic period–221 BC). The records of *douchi* making methods appeared in *Shi Jing* (The Classic of Poetry, 1100–700 BC). It is also recorded in the *Bencao Gangmu* (Compendium of Materia Medica, 1578 AD) of the Ming Dynasty that "*douchi* tastes delicious after adding salt, fresh ginger and pepper to the steamed soybean by a natural fermentation of three days in spring, two days in summer, and five days in winter".

In modern production, *douchi* is made from soybean using the main strain *Bacillus subtilis (natto)*. The process is divided into natural fermentation and modern inoculation fermentation (e.g. mold type and bacterial type) (Li et al. 2019). The protease and amylase produced from microbe during fermentation can decompose soy protein and starch to produce nutrients and flavorants. The bacteria type *douchi* is similar to Korean *chongkukjang/cheonggukjang* (Chang 2018), Japanese *natto* (Murooka and Yamshita 2008), *Kinema* in West Indian, Nepal and Libi ippa of Bhutan (Chettri, Bhutia, and Tamang 2016; Sugawara, Suzuki, and Yoshid 1998), *Thuo nao* in Thailand and Laos (Chukeatirote et al. 2018), *Tempeh* in Indonesia (Utami, Wijaya, and Lioe 2016), *Pepok* in northern Myanmar, and *Sieng* in Cambodia and Laos (Chettri and Tamang 2015).

Dajiang

Dajiang, a kind of soybean paste (also known as yellow bean paste), is a semi-solid fermented food with soybean as the main raw material. It is recorded that soy paste originated in China more than 2,500 years ago and was introduced to Japan in the 7th century (Fukushima 1979; Krezhova 2011). By Sui and Tang Dynasties (581–907 AD), the production of soybean paste had developed greatly, and it was an important condiment at that time (Sun 2010).

The ancient method of making soy paste in China is to mix soybeans with flour and then to add *koji* including *Aspergillus* to culture; but the production cycle of this fermented soy paste is long and seasonal. At present, the production technology of soybean paste has been drastically improved. Still, the mechanization and industrialization of soybean paste production are the future trend of soybean paste market. In fact, there are several soybean paste based food products in different countries, such as *dajiang* in China, *Miso* in Japan, *Doenjang* in Korea, *Tao-tjo* in Indonesia and Thailand, and *Tao-si* in Philippines. They have been widely used in preparing meat, seafood, poultry, vegetable dishes, and soups (Fukushima 1979).

Sufu

According to Chinese historical records, *sufu* has a history of more than 1800 years. It has become an ethnic food with traditional Chinese characteristics because of its rich nutrition, delicate texture, delicious taste and attractive appetite. The Three Kingdoms Period (220–265 AD) in China had a first historical record of "adding salt to dried *tofu* and then ripened into *sufu*" (Wang and Du 1998; Hong 1985). The descriptions of *sufu*, including "*sufu* is made by adding distillates or sauces to the pickled *tofu*", and "the product presents the nature with a sweet and salty and beneficial to the stomach" are found in *Bencao Gangmu* (Compendium of Materia Medica, 1578 AD). By the time of the Ming Dynasty (1368–1644 AD), *sufu* became popular, and its processing methods have recorded in many historical books (Zhang and Shi 1993).

In 1928, Wei Yanshou, for the first time, isolated a strain of *Mucor* from *sufu* which was named as *sufu Mucor* thereafter (Wei 1928). *Sufu Mucor* plays an important role in the fermentation of *sufu*. In 1942, Fang Xinfang isolated a strain from Wutongqiao *sufu* block in Sichuan China and named it *Wutongqiao Mucor*. These symbolize a new era of sterile fermentation to produce *sufu* in China. In the past 50 years, *sufu* was fermented by using wild microorganisms, *Mucor* and *Aspergillus*. Nowadays, more strains are utilized for *sufu* production and the products are mainly divided into four types: mold type, bacterial type, naturally ferment type, and enzymatically ferment type (Han, Rombouts, and Nout 2001). *Sufu* fermented by bacteria is found only in some regions of China such as Heilongjiang province and Wuhan city. The continuous improvement and innovation of the traditional *sufu* production process, together with its unique flavor, has made it a widely spread ethnic characteristic food in Asia. Now *sufu* has grown into a modern fermented food in many countries.

Soy sauce

China is the origin of soy sauce production with a history of more than 3,000 years. There are records of making soy sauce in the Zhou Dynasty. *Si Min Yue Ling* (Monthly ordinances for the four classes, 25–220 AD) edited by Cui Shi in the Eastern Han Dynasty and *Qi Min Yao Shu* (Important Arts for the People's Welfare, 533–544 AD) edited by Jia Sixie in the Northern Wei Dynasty have records of "sauce qing", a liquid juice seeping from the fermented soybean. There are clear records of "the beauty of cooking soup with soy sauce" in Shi Zanning's *Wu Lei Xiang Gang Zhi* (Compendium of Interaction Between Different Things, 919–1001 AD) and Zhao Xihu's *Tiao Xie Lei Bian* (Compilation of harmonious variation, 1190 AD) in the Northern Song Dynasty. The early soy sauce was actually made by marinating fresh meat. Later, soy sauce made from soybean was found to be similar in flavor and cheap to obtain, so it was widely used. After thousands of years of development, soy sauce now mainly adopts low-salt solid-state fermentation using defatted soybean as the raw material. After steaming and cooling, both *Aspergillus oryzae* and salt water are added into soybean to form a solid-state fermenting sauce, which is preserved and fermented at 42 °C. Because of its fast fermentation speed and short maturity, it has been used till now. Among many Oriental fermented products, soy sauce is one of the most widely used

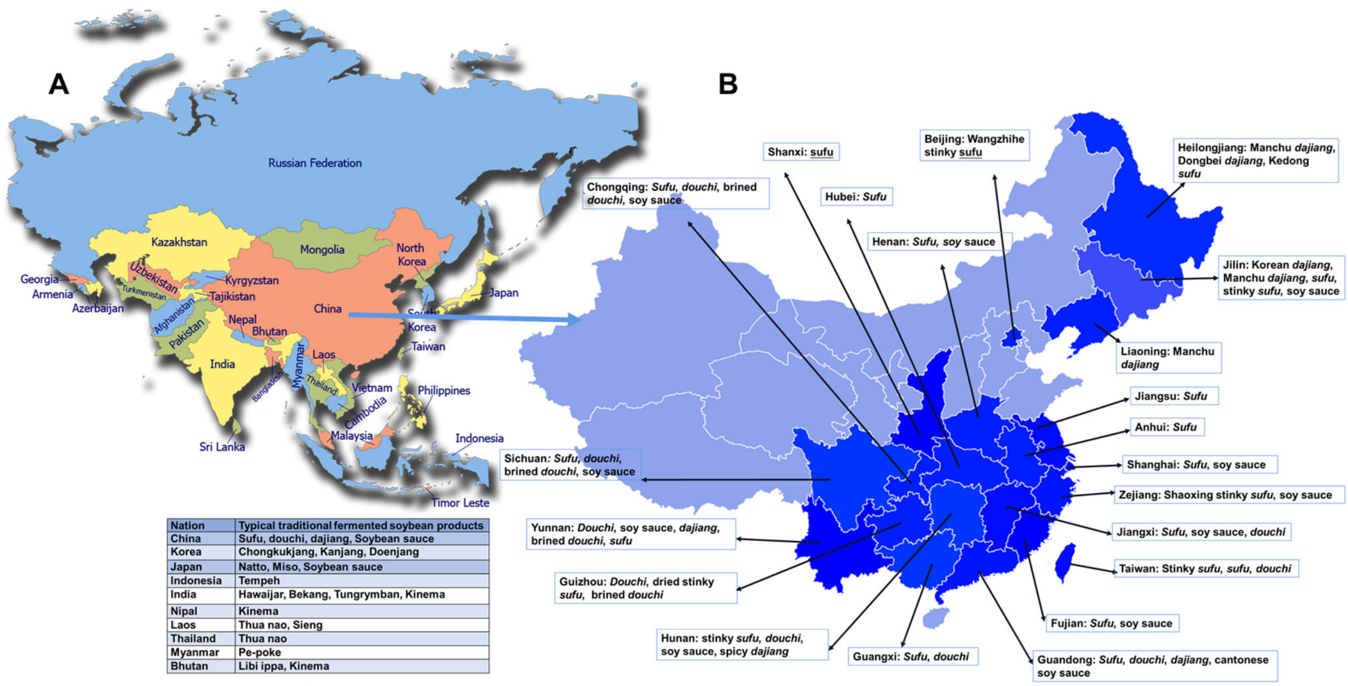

Figure 1. Geographical distribution and variety of traditional fermented soybean products (A) in Asia; and (B) in China.

condiments and colorants in in the world for food preparation and table use (Luh 1995).

Distribution of fermented soybean products in the world

The production of fermented soybean products has a long history of several thousand years and China is the birthplace of many fermented soybean products such as *douchi*, *dajiang*, *sufu*, soy sauce, etc. *Douchi* is one of the earliest foods brewed by microorganisms in China and was introduced overseas as early as the Tang Dynasty. Originating in China with thousands years of history, *dajiang* has played a very important role in the diet and health of Asian people. *Sufu* is one of the unique fermented soybean products in China with the earliest relevant records report in ancient books in the fifth century of the northern Wei Dynasty.

Figure 1 displays the distribution and a diverse variety of traditional fermented soybeans in some southeast Asian countries (A) and in China (B). There are many kinds and wide distribution of fermented soybean products in China, mainly in the north and south of China, and relatively few in the northwest.

According to records, the production of *douchi* was first spread from Taihe county in Jiangxi province, and then spread overseas through continuous development and improvement. It has become a local specialty in Guangdong, Guangxi, Hunan, Jiangxi, Shandong, Sichuan, Chongqing and the southern Shanxi and Gansu. *Dajiang*, as a traditional soybean paste in China, is made of fermented soybeans after stir-frying and grinding. *Dajiang* is a special paste in northeast China and is the traditional food of the Manzhu people, who have a long history of making soybean paste. In China, *sufu* is usually divided into three categories: white, green, and red. There are many places of origin, among which Suzhou, Shaoxing, Hangzhou, Ningbo, Guilin, Sichuan, and Heilongjiang are very famous in *sufu*. White type *sufu* is represented by Guilin *sufu* that has a long history and a long reputation. It was famous as far back as the Song Dynasty. Green *sufu* is a stinky soybean curd, represented by Wang Zhihe®, a century-old manufacturer in Beijing. Heilongjiang Kedong *sufu* is a bacterial type bean curd, a representative of red type *sufu*. Nowadays, most of the brand soy sauce in China are produced in the southern regions. Whereas the production in northeast China is small mainly because the production of soy sauce requires a dry and warm environment, while the temperature in this region is low and the heat is insufficient. However, the demand of soy sauce in northeast China is continuously growing.

These fermented soy products were introduced to Japan, Korea, Philippines, Indonesia, and other southeast Asian countries and regions in the early stage, and have integrated into traditional food with local characteristics, such as the famous Japanese *natto* and *shoyu*, Indonesian *tempeh*, and South Korean *cheonggukjang* (**Figure 1A**). Although traditional fermented soybean products are originated in Asia, these products are consumed, popularized, and produced worldwide as Asian food has prospered globally. In the United States, fermented soybean products are manufactured by numerous firms such as ConAgra, Wei-Chuan USA, Kikkoman USA, Yamasa Corp. USA, and San-J International, etc.

Traditional fermentation processing of soybean products

Douchi

The production principle of *douchi* making is to utilize protease and glucoamylase secreted by inoculated microorganisms to decompose large molecule protein and starch in soybean during early stage of fermentation. This process will

Figure 2. Flowchart of fermentation process for the production of (A) *Aspergillus* type *douchi*; (B) *Mucor* type *douchi*; (C) *bacteria* type *douchi*; and (D) *Rhizopus* type *douchi*.

produce polypeptides, amino acids, oligosaccharides, and monosaccharides, all of which serve as flavor precursors and undergo a series of biochemical reactions in the later fermentation process. Lastly, some spices are added to form characteristic color, flavor, and nutrient in *douchi*. In the production of *douchi,* soybean is generally processed through the steps of soaking, cooking, *koji*-making by natural or artificial inoculation, culture, *koji*-washing, mixing of adjuncts and *koji*, fermentation, and ripening. Depending on the types of microorganisms used, *douchi* products can be divided into *aspergillus* type (e.g., Liuyang *douchi* and Yangjiang *douchi*), *mucor* type (e.g., Yongchuan *douchi*), *rhizopus* type (e.g., Indian *Tempeh*), *bacterial* type (e.g., Qianxikou and Babao *douchi*), and *Rhizopus* type *douchi* (Chen et al. 2011).

Aspergillus type douchi

The production of *Aspergillus* type *douchi* is usually independent of season, yet its quality is moderate. *Aspergillus oryzae* or the mixture of *Aspergillus oryzae* and *Aspergillus niger* are usually used for fermentation of *Aspergillus* type *douchi*. The production steps of such *douchi* include soybean selection, soaking, cooking, *koji*-making, and fermentation (**Figure 2A**). After grain sorting, the mature soybeans are soaked in water until they are slightly wrinkled. This process usually takes 3–10 h depending on the weather. The soaked soybeans are then cooked for 1 h under the condition of 0.1 MPa, followed by a cooling to ~40 °C. After inoculating with *Aspergillus* species in the precooked soybeans, fermentation process is initiated at room temperature for 5–7 d. Salt, wine, and other seasonings are then introduced into sticky liquid soybeans. After fermentation for a period of 40 d, delicious *Aspergillus* type *douchi* is produced (He et al. 2016; Zhang et al. 2007).

Mucor type douchi

The *Mucor* type *douchi* is produced primarily in Sichuan province. Such *douchi* features with rich flavor and bright color. Tongchuan *douchi* and Yongchuan *douchi* produced in Sichuan province and Chongqing municipality,

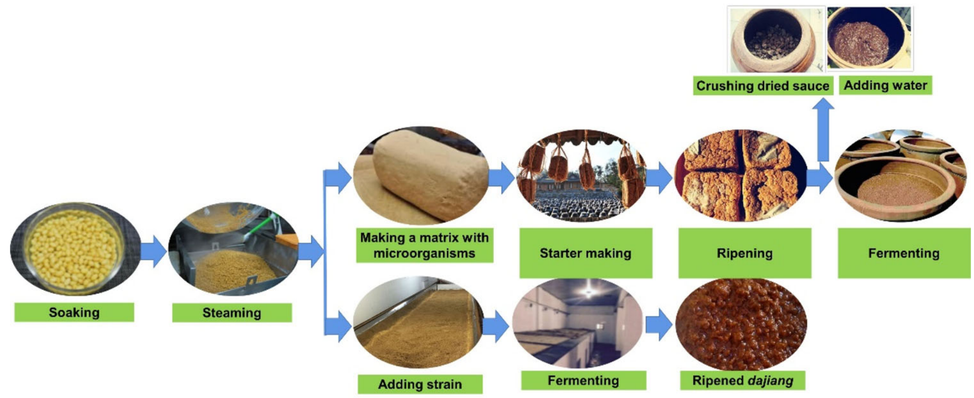

Figure 3. Flowchart of fermentation process for natural fermentation and artificial inoculation technology of *dajiang*.

respectively, are two of the most famous products symbolizing *Mucor* type *douchi*. The production process of *Mucor* products is similar to that of *Aspergillus* type *douchi*, including soybean selection, soaking, cooking, *koji* making, and fermentation (**Figure 2B**). After cooking, the soybeans are cooled to 35 °C, and then inoculated with *Mucor*, and the *koji* is prepared at 25–31 °C for 21 d. After the preparation, the fermentation is carried out at 20 °C for more than 6 months until reach to maturity (Wang et al. 2012).

Bacteria type douchi

Bacteria type *douchi* is mainly fermented by bacteria. Japanese *natto*, the product of steamed soybeans fermented by *Bacillus subtilis*, serves as a great example (Liu, Song, et al. 2018). The early bacterial fermentation of *douchi* was mainly performed by natural fermentation. In such way, the steamed soybeans are wrapped in straw where naturally exited *Bacillus subtilis* activates fermentation (**Figure 2C**). The modern technology is standardized by applying pure strains. The preparation process of *bacteria* type *douchi* includes soaking and cooking. The soaking time varies with the local temperature, and is generally 4–6 h in summer and 10–12 h in winter. The soaked soybeans are cooked at 130 °C and 0.15 MPa for 55 min. The bacteriological *douchi* is mostly prepared by *Bacillus subtilis*, and at the same time pre-fermentation should be completed at 40 °C. After pre-fermentation, the post-fermentation is also required which is carried out at 5 °C. At this stage, some ingredients can be added to increase the flavor. Generally, during the post-fermentation process, fermented soybeans appear to be dry (Kobayashi et al. 2017).

Rhizopus type douchi

Tempeh is the representative product of *Rhizopus* fermented soybean. Nowadays, the production of *Tempeh* is made by inoculating *Rhizopus* into pretreated soybean in a semi-sterile state which is then fermented in a short period of time. First, *Rhizopus* is cultured into liquid or solid to grow spore or hyphae, and then the soybeans are soaked in water for about one day, peeled and steamed (0.8 MPa, 20 min). Then the acidified matrix (0.8% lactic acid) is added to bacteria. Finally, soybeans are put into the container and fermented at a certain temperature (30–37 °C) for 1 to 3 d to obtain white, mature *Tempeh* (Setiawan et al. 2016) (**Figure 2D**).

Dajiang

Dajiang is made from soybean by microorganism fermentation, and is a kind of semi-solid condiment which is easy to digest and absorb by human body. There are two different production processes associated with *dajiang*, i.e., natural fermentation and artificial inoculation (**Figure 3**). Traditionally, *dajiang* is made by natural fermentation in the open air which facilitates the direct contact with microorganisms in the air. The process is generally divided into two stages. In the first stage of *koji-making*, soybeans are soaked overnight to a fully hydrated state, which are then minced into rectangular pieces before placing in the ventilated place for 1–2 months to dryness. The second stage begins by mixing 90% salt water (17% salt content) with ferment *koji* at room temperature. For the first month of this stage, stirring is required twice a day with a duration of 5–10 min. The purpose of agitation is to provide a sufficient amount of air for yeast and bacteria to breathe in *koji*/water mixture. This process is conducive to the growth of microorganisms which smooth the subsequent process of saccharification, alcohol fermentation, and protein decomposition. After a month stirring process, the materials are fermented with sun exposure for 1–2 months until the fermentation is mature to finish up the process. The high quality *dajiang* has a strong aroma with brown or reddish brown color (Zhao et al. 2009; Zhang, Wu, et al., 2018).

With the progress of modern food science and technology, *dajiang* has gradually evolved into artificial inoculation. Such process applies a single strain to produce *koji* which can markedly shorten the fermentation time; but the flavor is not rich enough. The main difference between natural fermentation and artificial inoculation lies in *koji*-making. Modern *koji* production mainly relies on the abundant enzyme system of mold, as well as the use of yeast and *Lactobacillus* to produce ester flavor substances such as alcohols and acids. *Aspergillus oryzae* is the most commonly used fermentation strain to produce *dajiang*. In addition to the accurate management of inoculated strain, the concentration of salt water is also strictly controlled within 14%,

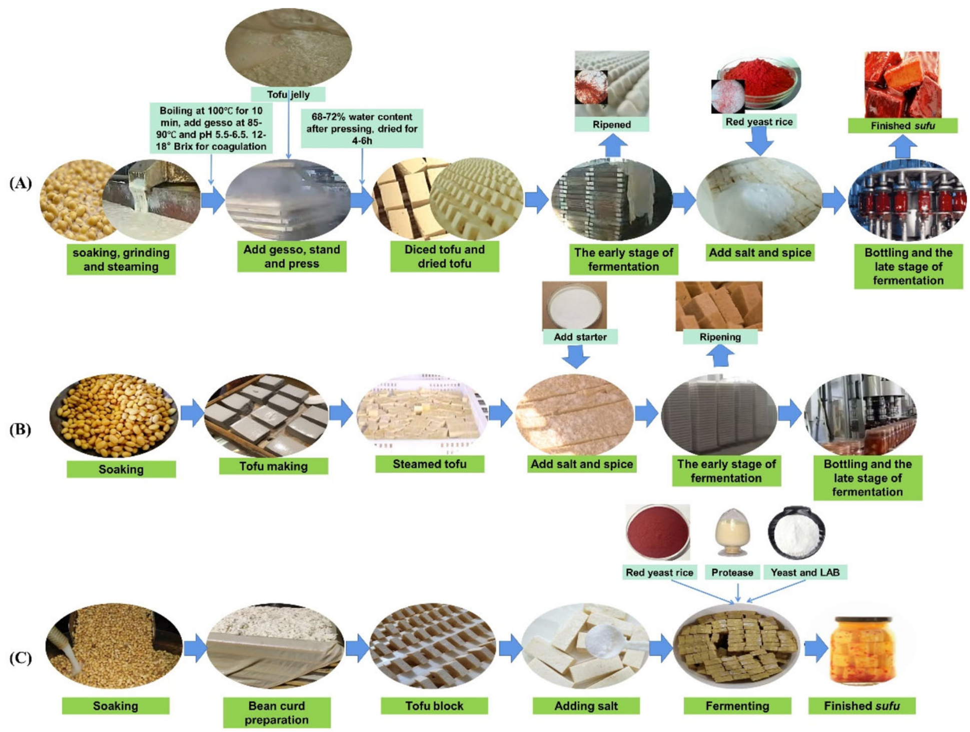

Figure 4. Flowchart of fermentation process for the production of (A) traditional *mold* type *sufu*; (B) traditional *Bacteria* type *sufu*; and (C) enzymatic *sufu*.

and the final salinity of *dajiang* is less than 10%. The fermentation is normally conducted at 45–47 °C for about 30 d (Li, Zhao, et al. 2018; Cui et al. 2012).

Sufu

Mold type sufu

Mold type *sufu* is produced by fermenting *tofu*; therefore, it mainly consists of *tofu* making and *tofu* fermentation processes (**Figure 4A**). After soaking, soybeans are ground to release oil body. Insoluble dregs/okara are filtered out and soy milk is cooked. Then, an appropriate amount of coagulant is added to the boiled soy milk, and coagulated bean curd is transferred into a box to solidify it into *tofu*. In the process of *sufu* making, mold growth is mainly controlled by adding salt and brine before fermentation. Enzymes produced by microorganisms degrade proteins, starch, and other macromolecular substances in *tofu*, the products of which are then esterified into distinct flavor substances in *sufu* (Han, Rombouts, and Nout 2001).

Bacteria type sufu

The process of *tofu* making is similar to the aforementioned step. After coagulating soy milk, soybean curd is poured into a press case with a *tofu* cloth placed on the bottom. In this way, prepared *tofu* is basically the same on each layer of plate (**Figure 4B**). The thickness is unified and the four corners are compacted. The pressed and shaped *tofu* blocks are steamed and then taken out of the pan to cool down. After cooling, the steamed *tofu* blocks are placed in a wooden tank and salted by sprinkling each layer of blocks with a layer of salt. After 24 h, *tofu* blocks are rinsed with clean water and loaded into a fermentation pan. Subsequently, the early fermentation culture is carried out, and the salted *tofu* blocks are inoculated and fermented to obtain the mature blocks. After drying the mature blocks in a drying chamber, the blocks are loaded into clay vat/jars with a layer of block accompanied with a layer of soup. The dried blocks need to be immersed in the soup and the mouth of the vat should be hermetically sealed with paper. After a period of airtight fermentation, the finished product is obtained (Xie et al. 2011).

Enzymatic sufu

The process of enzymatic fermentation to produce *sufu* can be divided into 5 steps (**Figure 4C**). To begin with, liquid culture is carried out in a liquid fermentation tank to promote protease metabolism. The fermentation broth bearing aromatic yeast and lactic acid bacteria is separated, purified and concentrated to obtain active protease. Meanwhile, cellulase is used in an enzyme reactor to hydrolyze the cellulose from soybean husk. Thermal processing is engaged to moderately denature soybean protein, which is cooled to an appropriate temperature. In the same enzyme reactor, the active protease is added to further hydrolyze protein. The fermentation broth of aromatic yeast and lactic acid bacteria, as well as a certain amount of salt and liquor or edible alcohol are added into partially denatured soybeans. After-ripening treatment is employed to produce enzymatic *sufu* (Wang and Li 2012).

Soy sauce

As concerns the production process of soy sauce in China, it has experienced transitions from traditional natural exposure

Figure 5. Flowchart of natural sun exposure fermentation of soy sauce.

fermentation to the current modern industrial production stage, from a single strain fermentation to multiple strains fermentation, from high-salt fermentation to low-salt fermentation.

Natural exposure fermentation

Fermentation with natural exposure is a traditional method to brew soy sauce (**Figure 5**). Soybean and wheat flour are used as raw materials with a mass ratio of 7:3. After washing, soybeans are soaked in water for 3 h in summer or 5 h in winter which allows kernel to imbibe enough water but without wrinkle. After the soaking, water is drained and soybeans are steamed at a high pressure of 0.15–0.2 MPa for 30 min. After cooled to 80 °C, soybeans are mixed with wheat flour, an adjunct to provide starch for microbial growth. The mixture is manually rubbed to loosely integrate soybeans with smooth surface, and ventilated during *koji*-making for 12 h. The maturity of *koji* is measured by the growth of chartreuse spores and a constant temperature. After formulating *koji* with 15% salt water at a mass ratio of 1:1.25, they are placed into large clay vats with bamboo cover to ease air flow. The brewing of soy sauce starts after *koji* is submerged into salt water and subsequently exposed to sunlight. In the beginning of fermentation, agitation is necessary to break up soybean into mashed thick semi-fluid state, which is generally known as sauce mash (also named moromi in *shoyu* brewing). In summer, the maximum temperature of sauce mash can reach to 45 °C. During fermentation when the color of sauce mash turns reddish-brown, stirring once until reddish-brown layer reaches several inches deep and redo it. When sauce mash is oily with a maroon color and a fresh smell, it indicates that sauce mash is fully matured for soy sauce extraction. The whole fermentation period for soy sauce brewing is more than half a year. Soy sauce is extracted using a bamboo ladle made of fine bamboo. In modern technology, soy sauce products made by high-salt dilute-state (HSDS) method, such as Japanese *shoyu* that are produced with the aid of traditional fermentation technology (Xu et al. 2014; Devanthi and Gkatzionis 2019).

Low-salt solid-state fermentation

Low-salt solid-state (LSSS) fermentation is the most commonly used technique in modern soy sauce production and has been adapted by >90% of Chinese firms. In contrast to the traditional method of brewing soy sauce with high salt and thin state, LSSS uses about 7% salt water to mix with *koji* and the water content in sauce mash is controlled to around 55%. In addition, LSSS only takes about 30 d to produce soy sauce. The raw materials for LSSS are treated and stirred in the same way as natural fermentation (**Figure 6**). The cooked soybeans are mixed evenly with wheat flour at a mass ratio of 7:3. The mixture is then transported to *koji* making room through a screw conveyor and the temperature is reduced to ∼38 °C. Practically, 0.3 wt% of strain is inoculated in *koji* and mixed thoroughly. The fermentation process to produce mashed sauce is divided into early stage of hydrolysis and late stage of fermentation. In the early stage of hydrolysis, the optimum temperature is controlled to 42–45 °C and maintained for about 10 d. Both heat of respiration and heat of decomposition are generated after the sauce mash enters into fermentation vat, which quickly rise the temperature of sauce mash up to 45–50 °C. Then the temperature of sauce mash will maintain steady. In the late stage of fermentation, the temperature of sauce mash rapidly decreases to ca. 35 °C after hydrolysis, which is generally completed in 15–20 d. Finished soy sauce is prepared by filtration and pasteurization of the fermented sauce mash (Cui, Zhao, et al. 2014; Sun, Jiang, and Zhao 2010).

Characteristics of main strains for soybean fermentation

Traditional fermented soybean products are produced in a natural environment. Therefore, the quality of fermented soybean products is reliant on the variety and quantity of microorganisms grown under specific environments. The inconsistent quality as well as the extremely long cycle has hampered the development of traditional fermented soybean products for industrial production. To overcome such problems in the industrial production of fermented soybean products, well characterized strains are utilized to maintain the quality, effectively improve product safety, and shorten fermentation time. In this section, we summarize the main strains that have been applied in soybean fermentation.

Douchi

As aforementioned, there are four types of *douchi* in terms of the dominant microorganisms. As a main strain for soybean fermentation, *Bacillus subtilis (natto)* with good protease activity was isolated from traditional food (e.g., *douchi*) in the mid-20th century. *B. subtilis (natto)* has the characteristics of acid resistance and heat resistance which enables to colonize the human intestinal tract and plays an important

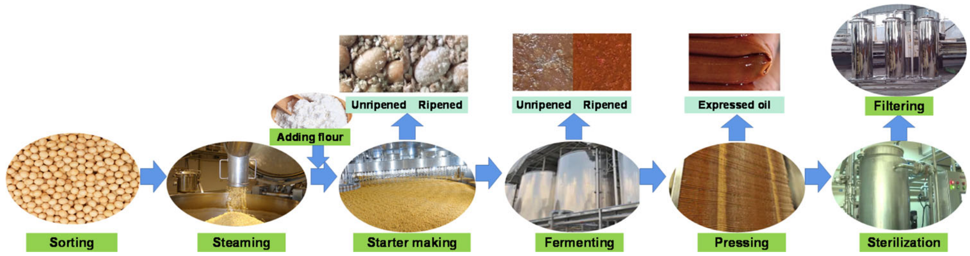

Figure 6. Flowchart of low salt solid-state fermentation of soy sauce.

role in the balance of microecology in human intestinal tract (Terada, Yamamoto, and Yoshimura 1999). It also shows secondary growth and its spores is resistant to high temperature, acid, alkali, salt, simulated gastrointestinal environment, and other harsh environment; but it is sensitive to antimicrobial agents (Hu, Wang, and Sun 2007). The physiological functions of *B. subtilis (natto)* such as dissolving platelet thrombus, antibacterial, lowering blood pressure, antioxidant activity, reducing blood viscosity, improving blood circulation, etc., have been previously reported (Abdel-Fattah et al. 2012; Cao et al. 2009). As an example, *B. subtilis (natto)* with high yield Nattokinase could synthesize anticoagulant components, which endowed *douchi* with anticoagulant activity (Wang et al. 2006).

In addition to *B. subtilis (natto)*, some other strains have also been isolated from *douchi* which are the potential strains for *douchi* production. These strains include *Bacillus subtilis* with high-yielding production of γ-polyglutamate (Inatsu, Kimura, and Itoh 2002), *Bacillus amyloliquefaciens* (Peng et al. 2003), *Bacillus subtilis* with broad-spectrum of antibacterial antibiotics (Wilson et al. 1987), *Lactic acid bacteria* (LAB) (Chen, Yanagida, and Hsu 2006), *Bacillus subtilis* with strong fibrinolytic activity (Wang et al. 2006), *Bacillus subtilis* with high protease production (Pant et al. 2015).

Dajiang

Traditional fermented *dajiang* is prominent among people because of its unique flavor, suitable taste and color, which cannot be replaced by industrial soybean paste. The fermentation of traditional soybean paste and the production of metabolites are completed under the joint action of a varieties of microorganisms (Gao et al. 2013). The microbial diversity in *dajiang* in northeast China was studied by using denaturation gradient gel electrophoresis (DGGE) technology which revealed that lactic acid bacteria were the predominant species. Wu et al. (2013) and Nam, Lee, & Lim (2012) analyzed the bacterial diversity in Korean *dajiang* (*doenjang*) by using 454 pyrosequencing technology, and pointed out that *dajiang* contained a total of 208 different bacterial species. In addition, *dajiang* reflected a region-specific bacterial community with *Bacillus* species and LAB being the most dominant members.

Although bacteria, yeast, and mold are the main strains identified, bacteria play a pivotal role in the fermentation process of *dajiang* (Wu et al. 2013; Zhang, Wu, et al., 2018; Zhang, Zhang, et al. 2018; Kim et al. 2010; Li, Wang, et al. 2018; Xie, Wu, et al. 2019). The crucial bacteria in the fermentation process of *dajiang* are *Bacillus* and *Lactobacillus* species, including *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus megaterium, Bacillus licheniformis*, *Enterobacter, Enterococcus, Lactococcus, Leuconostoc*, *Citrobacter*, and *Weissella*. In addition, *Tetragenococcus, Acinetobacter, Staphylococcus, Erwinia,* and *Pseudomonas* were also isolated in *dajiang*. The dominant fungi in the fermentation of *dajiang* in northeast China is related to the species of *Aspergillus oryzae*, *Eurotium,* and *Aspergillus chevalieri*. The prevalent yeast includes *Saccharomyces rouxii, Torulopsis, Candida humilis, Kluyveromyces lactis, Zygosaccharomyces rouxii,* and *Williopsis saturnus* (Zhao et al. 2009).

Sufu

As previously mentioned, there are four types of *sufu* depending on microorganisms. At present, the commonly used fermentation bacteria in industrial practice are *Mucor wutongqiao*, *Actinomucor elegans*, and *Mucor racemosus*. *Mucor* is particular importance with regarding to production of aromatic and umami flavor (Chen et al. 2015). The ability of *Mucor* to produce protease and lipase could effectively break down protein in *tofu* into soluble small molecule polypeptide and amino acid, and fat into glycerol and fatty acid, respectively. In addition, *Mucor* also has a strong saccharification force that can be used for the saccharification of alcohol, organic acids, and other industrial raw materials. Ma et al. (2015) used a space mutant strain of *Mucor* to ferment *sufu*, and found that both color and flavor, as well as the contents of amino acid nitrogen and water-soluble protein of resulting *sufu* was superior than that of commercial *sufu*. Other bacteria such as *Mucor flavus*, *Mucor racemosus*, *Mucor rouxianus*, *Lactobacillus*, and *Atopobium parvulus* have also been used in *sufu* production (Cheng et al. 2011; Han et al. 2004; Bao et al. 2020).

Rhizopus oryzae and *Rhizopus oligosporus* are the most commonly used fungus for mold type *sufu*. Recently, the production of *sufu* using mixed microorganisms has gained popularity. A comparative study on biochemical and textural changes during *sufu* fermentation using mixed *Mucor racemosus* and *Rhizopus oryzae* or a single *Mucor racemosus* found that the protease activity and the total amount of amino acids in *sufu* from mixed strains fermentation were higher than that of single strain (Li, Wang, et al. 2018).

Soy sauce

At present, the microorganisms utilized in brewing soy sauce are mainly *Aspergillus*, including *Aspergillus oryzae* and *Aspergillus niger* (Devanthi and Gkatzionis 2019). In industrial practice, the mixed fermentation by multiple strains are widely adopted for soy sauce brewing, which can overcome the weak enzyme production and poor flavor of soy sauce by a single strain fermentation. *Aspergillus oryzae* is suitable for the production of soy sauce with LSSS fermentation process and low temperature control. Attempts have been made to improve the brewing performance of *Aspergillus*. For instance, *Aspergillus oryzae* mutagenized by N^+ ion implantation yielded a remarkable improvement on the activities of protease, saccharifying enzyme, and cellulase (Zhao et al. 2012). The content of amino acid nitrogen in soy sauce was significantly elevated when brewed with an ultraviolet–diethyl sulfate induced *Aspergillus oryzae* mutation. (Ye et al. 2017; Ab Kadir et al. 2016) used a strain of *Aspergillus oryzae* with the capability of producing γ-aminobutyric acid (GABA) to develop GABA-rich soy sauce bearing exceptional health benefit.

Biological transformation of flavor components during soybean fermentation

Fermented soybean products have appeared to be an indispensable part of people's diet because of their irreplaceable flavor. As a matter of fact, volatile compounds presented in fermented soybean food are extremely complicated because different raw materials and strains can produce a wide varieties of flavor substances.

Douchi

Douchi has a unique flavor whose development is very complex. Mellow, ester, and umami are the three predominant descriptors associated with *douchi* flavor. As the bacteria used to make *koji* is different, the distinct type and amount of flavor have been observed in final *douchi*. During the post fermentation process of *douchi*, soy proteins are hydrolyzed into amino acids, organic acids, and small molecular peptides by the action of various proteases produce by *Mucor* and *Aspergillus*, which enrich the flavor substances in *douchi*. Umami components in *douchi* are mainly derived from free amino acids, especially free glutamic acid (Yamaguchi and Ninomiya 2000). Additionally, hydrocarbons, alcohols, aldehydes, ketones, acids, esters, heterocyclic compounds, sulfur compounds, phenols and other compounds are also identified from different kinds of *douchi*. These volatile compounds are mainly stemmed from lipid oxidation, amino acid degradation, carbohydrate fermentation, Maillard reaction, microbial metabolism, enzymatic catalysis, and Strecker degradation.

Chen et al. (2011) discovered a total of 28 volatile compounds in *Aspergillus oryzae* type Liuyang *douchi* including aldehydes, esters, pyrazines, furans, and alcohols, which may influence the overall flavor. He et al. (2016) found that Mucor type *douchi* had higher amounts of aldehydes, ketones, phenols, and pyrazines than that of natural fermented sample. Liu, Su, et al. (2018) and Liu, Song, et al. (2018) applied four flavor extraction methods to study the aroma in *bacterial* type *natto* and identified 14 key aroma compounds, among which pyrazine was a paramount compound with high flavor dilution value. Intriguingly, an earlier study from Tanaka et al. (1998) found major aromatic compounds in *natto* were acetone and methyl isobutyrate. Such discrepancy may again manifest the abstruse flavor formation in *douchi*. *Douchi* has a certain degree of undesirable ammonia odorants, which can be ameliorated by mixed fermentation of *Bacillus subtilis* with lactic acid bacteria (Lan et al. 2020), and mixed fermentation produces more abundant flavor substances than a single fermentation. The organic acids in *douchi* can not only enrich the flavor of the products, but also enhance the appetite.

Dajiang

Dajiang has bright color and pleasant flavor, whose development during the fermentation process involves lipid oxidation, Maillard reaction, protein degradation, amino acid conversion, starch saccharification, and alcohol fermentation. The key aromatic components of traditional fermented *dajiang* are esters, alcohols, aldehydes, and acids (Zhang et al. 2010). Proteins in soybean are hydrolyzed to polypeptide and small molecule by microorganisms and their enzymes, among which glutamic acid and aspartic acid are typical umami amino acids, phenylalanine and tyrosine are typical aromatic amino acids. In the late stage of *dajiang* fermentation, amino acids are catabolized to form amines, or undergo Maillard reactions to produce hexanal, nonanal, and heptanal. Some amino acids can also participate in Strecker degradation to form alcohols, aldehyde, and ketones. Carbohydrates in soybean are converted into dextrin and maltose by the action of amylase and cellulase, and finally into glucose and fructose. This change has a significant effect on the formation of color, odor, taste, and texture of *dajiang*. Maillard reaction contributes largely to the final sensory quality of *dajiang* which can be greatly accelerated upon sun exposure to form melanin and aromatic substances (Peng et al. 2014). Maillard reaction can form 40–50% of color of *dajiang*, as well as ketones, phenols, aldehydes and other aromatic components (Steinhart 2005). Using HS-SPME-GC/MS, Zhao et al. (2006) identified the highest contents of esters, furanones, and acids in traditional fermented *dajiang*.

The formation of flavor substances in *dajiang* mainly relies on several factors including raw materials and their pretreatment methods, *koji* type, fermentation time, and temperature. The quality of *dajiang* is highly dependent of the metabolites of products. The metabolites of microorganisms in *dajiang* are divided into primary metabolites such as sugar, amino acids, and organic acids, and secondary metabolites such as volatiles, isoflavones, tocopherol, and saponins. The former contributes to sweet, delicious, and umami taste, and improves the flavor quality of the products; while the latter are closely related to the function and antioxidant properties of the product. Kwon et al. (2019) found that primary metabolites were affected by the main ingredients, and that the fermentation time had the greatest influence on secondary metabolites. As a special fermented condiment, *dajiang* maintains exceptional color, aroma, taste and shape, and is rich in nutrients (Wang and Murphy 1994).

Sufu

The flavor formation of *sufu* is a complex biochemical process in which a large number of flavor substances arose through the synergistic action of various microorganisms. As a result, the number of mycelium inoculated in *tofu* block, along with raw material composition and heat treatment process, determine the final flavor of *sufu*. Hence, the flavor of *sufu* differs substantially with different fermentation methods and regions. Besides, aromatic substances of *sufu* change dynamically in different fermentation periods, and the variety increases as the fermentation proceeds. According to color and flavor, *sufu* is generally categorized into red, white, and green type flavor *sufu*. Liu et al. (2012) found that different kinds of *sufu* contained different flavor components, and that sulfur compounds were major flavor components in green *sufu*, while esters and alcohols were core flavor components in red *sufu* (Wang et al. 2019). A recent study stated that aldehydes and esters may be the pivotal flavor components in white *sufu* (He, Wan, et al. 2020).

The fermentation process of *sufu* is a complex biochemical process, and its special taste and nutritional value are closely related to the enzymes secreted by fermentation bacteria. During fermentation, a wide array of enzymes, such as protease, glutaminase, peptidase, lipase, cellulase, hemicellulase, α-amylase, β-glucosidase oligosaccharidase, etc., are released by salt solution in *tofu* block. These enzymes decompose proteins, amino acids, lipids, and carbohydrates in the raw materials into short peptides, free amino acids, fatty acids, and sugars to form the extraordinary flavor or flavor precursor compounds of *sufu* (Chung 2000). As salty and umami are the principal descriptors of *sufu*, protein hydrolysis is considered to be the crucial factor affecting the flavor and texture of *sufu*. The organic acids produced by LAB and yeast can be esterified with alcohols for flavor-active ester production. The characteristic sweetness and ester fragrance of rancid *sufu* are derived from the aromatic ester compounds produced by esterification of acetic acid, guanosinic acid, inosinic acid, and other organic acids with alcohols. The distinctive stinky odors are hydrogen sulfide and ammonia, which are mostly produced from the degradation of sulfur-containing amino acids, e.g., thiamin during fermentation.

As a traditional fermented food, the distinguishable and rich flavor is an important indicator of a high quality *sufu*. The aromatic components of *sufu* are mainly esters (e.g., ethyl 2-methylbutyrate, ethyl butyrate ethyl hexanoate, etc), aldehydes/ketones (e.g., 2-methyl-butanal, hexanal, octanal, heptanal, 2-pentanone, phenylacetaldehyde, etc.), and alcohols (Moy, Lu, and Chou 2012; Chung 2000; Liu, Kubota, and Kobayashi 1988; Kasankala, Xiong, and Chen 2011).

Soy sauce

The formation of soy sauce flavor is the synergistic effect of microorganisms in the fermentation process, which is fused by both raw material composition and microbial metabolism. The flavor compounds in soy sauce are extremely complex albeit it is always regarded as gustatory monotony. Indeed, researchers have identified a large number of aroma substances including alcohols, carboxylic acids, esters, carboxyl compounds, and furans in soy sauce (Zhang and Tao 2009; Yan et al. 2008). Nunomura, Sasaki, and Yokotsuka (1980, 1984) reported the flavor substances of soy sauce encompassing 93 acidic substances, 35 alkaline substances, and 142 neutral substances. Cui and coworkers identified aroma substances in soy sauce, which were divided into nine different groups of volatile compounds (Cui, Zheng, et al. 2014). Among all these identified aromatic compounds, 4-hydroxy-2-ethyl-5-methyl-3-furanone (HEMF), 4-ethyl guaiacol, 3-(methylthio)-1-propanol were the key aroma (Steinhaus and Schieberle 2007). Ethyl acetate and ethyl lactate are the representatives of esters in soy sauce flavor. Acid substances such as acetic acid can impart the flavor of soy sauce softer. Many alcohols in soy sauce not only contribute to flower fragrance, they can also interact with acids to produce esters and increase the smell and taste of soy sauce. Acetone mainly plays a coordinating role in soy sauce. Volatile phenols such as 4-ethyl guaiacol (4-EG) and 4-ethyl phenol (4-EP) bearing a fermented aroma and slightly sweet, are important factors determining the quality of soy sauce. Sulfur-containing compounds can produce off-flavor, and have a significant influence on the formation and content of flavor substances in soy sauce (Gao et al. 2010).

Soy sauce is generally characterized by mellow and *miso* odors. The type of brewing techniques, raw materials, formula, material pretreatment and microorganisms can affect the formation of aroma and taste of soy sauce. The preparation of soy sauce with soybean powder and flour mixture can add volatile compounds in the fermentation process, resulting in a better flavor (Zhang et al. 2017). Low-temperature fermented soy sauce has demonstrated the improvement on the types and total contents of flavor substances (Song, Jeong, and Baik 2015). The application of high quality strains can boost the formation of flavor compounds, giving rise to a stronger sweetness and a greater flavor property (Chen 2019). The taste of soy sauce is a combination of salty, umami, sweet, sour and bitter. Soy sauce produced in different regions has its own characteristics. For instance, Cantonese-style soy sauce has a strong aroma odor, while Japanese soy sauce has a more prominent sour and sweet taste.

Nutritional properties of fermented soybean products

Soybean is a good source of various nutrients comprising macronutrients (i.e. protein, lipid, and carbohydrate), nutritional minerals, dietary fiber, soybean isoflavone, saponins, and other functional substances. Protein, carbohydrate, lipid, and other components in soybean are degraded into small molecular substances by fermentation under the action of microorganisms. Anti-nutritional factors such as protease inhibitor, phytic acid, urease, and oxalic acid in soybean can be reduced or eliminated after fermentation (Samtiya, Aluko, and Dhewa 2020). Some nutraceuticals such as soy isoflavones are existed in the form of glycosides. During the fermentation process, the beta-glucosidic bond in soybean is cleaved and the isoflavone glycosides are transformed into free isoflavone aglycone, which has been shown to exert a higher biological activity (Yin, et al. 2005). With the improvements of human living standard and health consciousness worldwide, there is a growing demand on the nutritional function of food. In this section, the main nutrients and functions of fermented soybean products are analyzed, which is helpful for further understanding the functional properties of fermented soybean products.

Douchi

The processing of traditional fermentation can improve the bioaccessibility of minerals in *douchi*. At the same time, the glycoside soybean isoflavones are converted into free soybean isoflavones with a higher activity. The contents of water soluble nitrogen, soluble sugar, isoflavone, vitamin B1, B2 and B9, vitamin C, vitamin E, and other nutrients in *douchi* are also significantly increased (Xiang et al. 2019). *Natto*, a traditional fermented soy product in Japan, is high in vitamin K2, an important micronutrient to enhance the combination of calcium and vitamin D (Walther et al. 2013).

Trypsin inhibitors in soybean inhibit activity of trypsin and chymotrypsin in the small intestine, thus interfering with protein digestion, absorption and utilization. Additionally, cellulose in soybean (ca. 5%) can create a cell membrane to surround protein, making it difficult to contact with digestive enzymes and affecting digestion (He, Bai, and Wang 2014). *Douchi* also contains a variety of biological enzymes such as protease, amylase, cellulase (Chen et al. 2020). During the fermentation of *douchi*, protease can destroy trypsin inhibitors and hydrolyze some proteins in soybean to produce a series of intermediate products, such as peptides and amino acids. Cellulase can hydrolyze cellulose to produce monosaccharides, which makes it easier for protease to contact with proteins for hydrolysis. These hydrolyzed proteins with lower molecular weight can be absorbed directly by the intestinal mucosa to be easily digested by human body. When the fermentation is mature, the content of water-soluble nitrogen in the product is increased and the hardness of soybean is decreased. It also contains the most effective fibrinolytic enzyme for the treatment of thrombosis (Hu et al. 2019). The unique nutritional functions of *douchi* are closely related to the role of microorganisms.

Dajiang

Dajiang is rich in nutrients that are mainly composed of protein, polyunsaturated lipids (e.g., linoleic acid and linolenic acid), vitamins, nutritional minerals (e.g., calcium, phosphorus, and iron) (Jayachandran and Xu 2019). Compared with unfermented soybean, the protein and fat contents of *dajiang* are significantly reduced by the microbes to produce new active substances. The contents of retinol, riboflavin, and vitamin B12 in *dajiang* are significantly higher that these in raw materials. Retinol is an essential nutrient that promotes the formation of photosensitive pigments in vision cells and maintains normal visual response. Riboflavin, as the main component of intracellular dehydrogenase, is involved in a wealth of oxidative respiration processes. Riboflavin deficiency can affect the organism's biological oxidation, and make metabolic disorders. Vitamin B12 (cobalamin) can promote the development and maturation of red blood cells, and maintain hematopoietic and nervous system functions. Dietary vitamin B12 is found in high amounts in animal foods and is virtually absent in plant foods. Microorganisms produces vitamin B12 during *dajiang* fermentation; therefore, *dajiang* can be considered as an important source of vitamin B12 for vegetarians. In addition, a recent study found that vitamin K also exits in *dajiang* and *natto*, with a significant amount of menaquinone-6 and menaquinone-7, respectively (Tarvainen, Fabritius, and Yang 2019).

Sufu

A trivial name of *sufu* is Chinese cheese because of its high nutritional value. By microbial fermentation, the deficiency of bitter taste, gas production for indigestible oligosaccharides, and anti-nutritional factors of soybean can be drastically alleviated. Meanwhile, a large number of hydrolyzed proteins and free amino acids, along with a number of organic acids, alcohols, and esters are produced, which help promote the normal growth and development of human body (Xie, Zeng, et al. 2019).

Protein content of fermented *sufu* ranges from 12% to 17%. The amount of essential amino acids in fermented *sufu* is sufficient to meet the daily needs of an adult. *Sufu* contains a large number of hydrolyzed proteins, the digestibility of which is raised to 92–96%. During the fermentation process, a considerable amount of vitamin B12 is produced in *sufu* due to the action of microorganisms (Guan et al. 2013), presenting it a good source of vitamin B12 for plant protein food. Riboflavin is also found to be six to seven times higher than *tofu* (Li 1997). *Sufu* also contains nutritional minerals such as calcium, phosphorus, iron and zinc. Nowadays, *sufu*, as the most effective fermented soybean product, is attracting more attention from the consumers.

Soy sauce

High quality soy sauce contains rich protein, an assortment of amino acids, sugar, vitamins, and essential trace elements. The high contents of amino acid nitrogen and total nitrogen in soy sauce indicate that amino acid quality of soybean is greatly improved during the brewing of soy sauce. Soy sauce contains zinc, calcium, iron, manganese, and other nutritional minerals in the ionic state that can be directly absorbed by human body. Among them, divalent ion is easy for absorption. Soy sauce is rich in vitamin B1, B2, B12, and vitamin E (Chen 2019). In the process of brewing soy sauce, anti-nutritional factors such as phytic acid, trypsin inhibitor, lectin and antigen protein are eliminated from the raw materials, which improves the biological effectiveness of soy sauce. More uniquely, previously unavailable vitamin B12, nucleic acid, nucleotide, protein melanin, and aromatic compounds are produced in soy sauce, many of which have physiological activities (Nunomura, Sasaki, and Yokotsuka 1984).

Biological activities of fermented soybean products

Owning to the increased amount and type of nutrients/bioactive compounds in fermented soybean, multifarious biological activities of fermented soybean products have been reported in light of in vitro cell culture and animal studies. In this section, we provide an overview of the reported bioactivities of traditional fermented soybean products.

Antioxidant activity

Fermented soybean products are rich in antioxidant substances, such as antioxidant peptides and isoflavones, which play a decisive role in the total antioxidant capacity. During fermentation, the hydrolysis of protein produces more polypeptide substances. This results in the increased exposure of amino acid groups, some of which carry antioxidant activities, thus offering the products with stronger free radical scavenging capacity. Fan et al. (2009) found that prolonged fermentation time markedly improved the antioxidant activity of *douchi* which was ascribed to the increased amount of antioxidant peptides and isoflavones. Lee et al. (2014) investigated the primary metabolites of monosaccharides, amino acids, and fatty acids, as well as the dynamic changes of isoflavones and saponins in different fermentation stages for *doenjang* production. In particular, isoflavone glycosides decreased gradually after cooking, while isoflavone glycoside ligands such as daidzein, glycitein, and genistein increased significantly during salt immersion and post-ripening. This was positively correlated with the antioxidant capacity and total phenol content of *doenjang*. The reduction of soy saponins occurred after drying of *doenjang*. Polypeptide is also the main component responsible for antioxidant activity of *sufu* (Cai et al. 2016). A study found that *Bacillus subtilis* used by the typical bacterial type kedong *sufu* in northeast China had the capacity to boost antioxidant activity, fibrinolytic function, and sensory quality of *sufu* (Zhang et al. 2014). Esaki et al.(1999) identified a newly formed antioxidant 6-hydroxydaidzein in soybean *koji* fermented with *Aspergillus oryzae*. In addition to antioxidant peptides and isoflavones, Maillard reaction product melanoidin has been identified in some fermented soybean products, such as soy sauce, *dajiang*, *douchi*, and *tempeh*, accounting for the antioxidant activity of the fermented soybean products (Cao et al. 2019).

Antitumor effect

Fermentation of soy protein using microorganisms such as bacteria and fungi produces isoflavones, tocopherols, and other substances with specific antitumor activities (Sanjukta and Rai 2016). With the extension of maturity, the glycosylated isoflavones can be effectively converted to genistein and daidzein, which could significantly enhance the tumor inhibition effect (Jung, Park, and Park 2006). For instance, Jeong, Chang, and Park (2014) reported that the administration of *doenjang* produced by mixed fermentation of *Aspergillus oryzae*, *Bacillus subtilis*-SKM, and *Lactococcus lactis*-GAM ameliorated the symptoms of colon cancer of mouse model. Surfactin, a biosurfactant produced in *natto* and *doenjang*, has the potency to suppress a wide array of cancer cell growth, including human breast cancer cell and colon cancer cell (Wu et al. 2017; Lee et al. 2012). Antitumor proliferative effect of *sufu* against human colon cancer cells, Caco-2 and HT-29 was also noted (Chen et al. 2012).

Hypoglycemic effect

The consumption of soybean products is associated with reduced risk for metabolic syndrome; it however is not enough to relieve the symptoms of type 2 diabetes. After fermentation by microorganisms, some fermented soy products have shown better hypoglycemic and antidiabetic effects (Jayachandran and Xu 2019). A study from Kwon et al. (2007) found that dietary supplementation with *chungkookjang* enhanced insulinotropic action and exhibited hypoglycemic effect in diabetic rats. This research group also found that the elevated amounts of small peptides and isoflavones in *meju* (a dried fermented Korean soybean block to prepare *doenjang* and say sauce) raised the hypoglycemic effect in cell models (Kwon et al. 2011). They postulated that the improvement of insulin resistance and insulin secretion may serve as the mechanisms for fermented soybean to prevent or delay the development of type 2 diabetes (Kwon et al. 2010). Coincidentally, Yang et al. (2013) noted that the small molecular peptides in *chungkookjang* fermented with *Bacillus licheniformis* can significantly improve the insulin activity of type 2 diabetic rats, and as well improve the dynamic glucose balance in blood of type 2 diabetic rats.

Plasma cholesterol reduction

The phospholipid in soybean may reduce cholesterol, accelerate lipid metabolism, and promote the liver health (e.g., alleviating fatty liver and liver cirrhosis) for consumers. Phospholipid in soybean can also be used as a carrier for fat soluble vitamins to be smoothly absorbed by the body. B Vitamins (e.g., riboflavin) produced in the fermentation process have diverse effects, such as inhibiting cholesterol synthesis and participating in cell growth and metabolism (Xie, Zeng, and Qin 2018). A study found riboflavin in fermented soybean regulated liver lipid metabolism by reducing the level of 3-hydroxyl-3-methylglutaryl coenzyme A reductase. Sapbamrer, Visavarungroj, and Suttajit (2013) noticed that postmenopausal women with a supplementation of traditional fermented soybean (similar as *tempeh*) offering 60 mg of isoflavone daily for a period of 6-month had favorable effects on progesterone and cholesterol. Wu et al. (2015) fed high-fat mice with 10% *miso* in the diet for 5 weeks and found black soybean *miso* significantly decreased serum chelesterols.

Hypotensive effect

Angiotensin-converting enzyme (ACE) catalyzes the hydrolysis of angiotensin (decapeptide) I into angiotensin II (octapeptide), a powerful vasoconstrictor that causes elevated blood pressure. Some bioactive peptide produced by fermented soybean has shown antihypertensive activity to prevent or treat hypertension by inhibiting the activity of ACE. ACE inhibitory bioactive peptides has been found in several fermented soybean products such as *douchi*, soy sauce, and *tempeh*. Zhang et al. (2006) reported that ACE inhibitory activity of *douchi* produced by *Aspergillus aegyptiacus* was notable with the prolongation of fermentation time. After purification, high activity peptide including phenylalanine, isoleucine, and glycine with a ratio of 1:2:5 were obtained. Li et al. (2009) suggested ACE inhibitory activities were affected by the types of *douchi* (i.e., *Aspergillus*-type, *Mucor*-type, and *Bacteria*-type), and *Bacillus subtilis* would be a potential strain to produce *douchi* with high ACE inhibitory activity. Shimakage, Shinbo, and Yamada (2012) found that nicotianamine and peptide Ser-Trp in soy sauce, *miso*, and *natto* were responsible for the antihypertensive effects by inhibiting ACE activity. Kuba et al. (2003) isolated two kinds of bioactive peptides with the active peptide sequences of Ile-Phe-Leu and Trp-Leu in *sufu* which afforded ACE inhibitory activity. Zhu et al. (2008) demonstrated that bioactive peptides Ala-Phe and Ile-Phe from soy sauce exhibited ACE inhibitory activity with IC50 values of 165.3 μM and 65.8 μM, respectively.

Other activities

In addition to the aforementioned biological activities, the phytochemicals in fermented soybean products have demonstrated many other activities, such as improving the intestinal environment, preventing alzheimer's disease, increasing appetite, nourishing nerves, enhancing immune function, and reducing the risk of chronic diseases. Gamma-aminobutyric acid (GABA) is a natural non-protein amino acid commonly found in plants and animals in nature. It is the main inhibitory neurotransmitter in the central nervous system of mammals and has physiological functions such as regulating blood pressure and heart rate, regulating mood, antianxiety, antidepression, antitumor, protecting liver and kidney, and regulating hormone secretion (Aoki et al. 2003). Fermented soybean products are rich in glutamate, which provides a precursor for GABA synthesis. The production of glutamate decarboxylase by fermented microorganisms is conducive to its biological transformation to GABA. The ingestion of *doenjang* and *cheonggukjang* was also found to improve immune responses in mice through the consolidation of humoral and cellular immunity to Th1 response (Lee et al. 2017).

Challenges and potential improvement of traditional fermented soybean products

Traditional fermented soybean products, with the characteristics of distinct taste and flavor, richness in nutrients and easiness to digest and absorb, play an extremely indispensable role in human healthy diet. Nevertheless, some challenges are still remaining which hinder their broad acceptance worldwide. In this section, we summarize four challenges including high salt content, the formation of biogenic amines, contamination with pathogenic microorganisms and mycotoxins, and inconsistency in quality in the fermented soybean products, as well as the potential solutions.

High salt content

The majority of the traditional fermented soybean products requires a significant amount of salt (up to 20%). As an effective additive to control water activity, salt has a pronounced influence on the microbial growth and the rate of fermentation, and thereby on the safety and sensory quality of the products. In addition, salt could extend the shelf life of the final fermented products. In general, the salt content is above 18% in soy sauce, 10–15% in *douchi*, and over 10% in *dajiang*. As a consequence, the concerns about high salt intake associated with the increased consumption of fermented soybean products are on the rise since high salt intake is one of the major risk factors for hypertension. How to maintain the physicochemical properties and sensory quality of fermented soybean products with reduced salt addition has spring up as a bottleneck problem in the field.

In fact, salt reduction in fermentation has been the subject of ample researches. One common strategy is to identify the lowest salt concentration under which the growth of the fermenting microorganisms, as well as the flavor and texture of the fermented soybean products will not be adversely impacted. Some innovative fermentation processes have been developed to produce low salt or no salt fermented soybean products, such as *natto*, *Tempe* and *kinema* (Wang et al. 2007). Despite this, the increased levels of government oversight and control in response to salt reduction, such as set up maximum amount of salt per-serving target, is necessary to engage soybean fermentation manufactures to reduce salt use in fermented soybean products.

Formation of biogenic amine

Biogenic amines (BA) are low molecular weight nitrogenous organic bases which can cause toxic effects to consumers, such as headaches, nausea, cardiac palpitations, abnormal blood pressure, etc (Ladero et al. 2010). In fermented soybean products, BA are commonly produced through the decarboxylation of amino acids by amino acid decarboxylases (AADCs). During fermentation, proteins in soybean and adjuncts are hydrolyzed into small molecular weight peptides, amino acids and ammonia by proteases produced by fermenting microorganisms. Meanwhile, high amounts of free amino acid could be attacked by AADCs to form biogenic amines as such enzymes are widely present in microorganisms involving in fermentation (Alvarez and Moreno-Arribas 2014). In recent years, BA have been detected in *douchi*, *sufu*, soy sauce, *natto*, and *tempeh* produced all around the world (Moon et al. 2010; Guan et al. 2013). Although it has been proposed to control BA under 1000 mg/kg, the appropriate regulation on limiting the content of BA in fermented soybean products has yet to be implemented (Byun and Mah 2012). The employment of appropriate starter cultures or food additives are the two common practices to reduce BA in fermented soybean products (Park, Lee, and Mah 2019).

The occurrence of pathogenic microorganism and mycotoxin

As described in Section "Traditional fermentation processing of soybean products", the nature of traditional fermentation requires the exposure of minimally pretreated/unsterilized soybean and other adjuncts to the environment for a long period of time. Thus, contamination can take place in raw materials and during fermentation and storage by virtue of inadequate hygiene. How to improve product quality and safety on the premise of retaining original characteristics is another challenge. In fact, most countries have imposed harsh regulations and measures against high contagious food-borne pathogens, such as the FDA's "The Big Six". However, some pathogens that are off the list or official guidelines of ordinary foods can exist in fermented soybean products. A classic example is the discovery of *Bacillus cereus*, a pathogenic endospore forming and enterotoxins excreting bacterium that causes diarrhea and emesis, in *sufu*, *douchi*, and *doenjang* (Han, Rombouts, and Nout 2001; Zhou et al. 2014). In addition to the pathogenic microorganisms, fermented soybean products may also be contaminated with mycotoxins, especially aflatoxins (AFs) and ochratoxin A (OTA), which threaten human health. In our recent review, we have discussed the ubiquitous existence of mycotoxins in cereal based foods (Wan, Chen, and Rao 2020). *Aspergillus* species and *Penicillium* species, the main producers of AFs and OTA, can coexist with other fermenting strains. Both AFs and OTA were detected in *meju*, soybean paste, and *doenjang* produced in South Korea (Jeong, Chung, and Hong 2019; Lee et al. 2019; Ahn et al. 2016). Although many countries have enacted the rules on food pathogen in human food, *Bacillus cereus* is not included. Similarly, contamination of AFs in human food is strictly regulated worldwide, most countries have not set regulation on OTA content in food. Clearly, specific guidelines and regulations on pathogenic microorganisms and mycotoxins should be set up for fermented soybean products in order to guarantee food safety. The development of rapid detection methods to monitor pathogenic microorganisms and mycotoxins throughout the whole production process of fermented soybean products would be an effective control to avoid contamination.

Inconsistency in quality

The production of traditional fermented soybean products adopts natural fermentation technology. The fermentation process is difficult to be controlled, so does the quality of the fermented soybean products. In the real practice, the process of fermentation is extremely complex and the product quality is largely dependent on subjective factors and environmental conditions such as geographical location, environment, weather, season, etc. This has largely limited the spreading of traditional fermented soybean products globally.

The applications of pure strains, or multi-species under constant fermentation temperature have been shown to maintain the quality of fermented soybean products, thus worth promoting and improving (He and Chung 2020). In addition, a fundamental understanding on the dynamic changes of the quality and flavor of traditional fermented soybean products during the fermentation processes will underpin the role of the dominant flora and unravel the mechanisms (Chung 2020). Such knowledge could be applied to decipher the specific enzymes participating in the hydrolysis of constituents in raw material as well as in the enrichment of the taste and nutrients of the products (Hu et al. 2019). Lastly, the improvement or renovation of new fermentation equipment for steaming, cooling, mixing, and standardized production equipment can reduce labor intensity and ensure the stability and safety of process and product quality.

Conclusions and future remarks

In recent years, fermented soybean products have been the focus of scientific researchers and consumers due to their improved nutritional quality, broad biological activity, and exceptional flavor profiles and texture. The biological activities of fermented soybean products including antioxidant activity, antihypertensive, antitumor, lowering plasma cholesterol, liver protection, and anti-aging effects have been extensively studied. With the development of the society and the improvement of human living standard, consumers pay more attentions to not only the flavor and nutrition, but also the safety of fermented soybean products. Most of the traditional fermented soybean products are manufactured manually on a production line. The use of high concentration of salt during fermentation process, as well as the production of biogenic amines, acrylamide, pathogenic microorganisms and mycotoxins and other safety hazards are still the main bottlenecks in fermented soybean production.

In order to further develop and promote nutritional and safe fermented soybean products, the following three aspects are particularly critical and should be taken into consideration. First, efforts should be made to develop and diversify novel fermented soybean products, formulate various products with new flavors, and comprehensively utilize fermented soybean and by-products to fabricate nutritional and functional ingredients. Second, further research on the changes of functional bioactive components in the fermentation process and the development of functional fermented soybean products will be the new trend in the future. Lastly, in order to produce traditional fermented soybean products with high quality and safety, we should constantly improve the traditional fermented soybean process and parameters, incessantly search for excellent strains suitable for industrial fermentation, regulate fermentation process, clarify the fermentation mechanism, as well as actively discover the microbial structure, composition and metabolic characteristics in the soybean fermentation process.

Disclosure statement

The authors declare that there are no conflicts of interest.

Author contributions

Professor Libo Liu compiled data from literature review and drafted the manuscript. Xiaoqian Chen and Guofang Zhang collected data from literature review. Dr. Zhao Jin revised the manuscript. Professor Chun Li revised the manuscript and figures. Linlin Hao and Yuzhuo Yang provided industrial perspectives and collected data. Professor Jiajia Rao revised the manuscript and figures. Professor Bingcan Chen designed the table of contents of manuscript, supervised the work, and revised manuscript. All authors reviewed the manuscript before submission.

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