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Soy sauce fermentation: Microorganisms, aroma formation, and process modification

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Abstract

Soy sauce is an increasingly popular oriental fermented condiment produced through a two-step fermentation process called koji (solid-state fermentation) and moromi (brine fermentation). Complex microbial interactions play an essential role in its flavor development during the fermentation. *Tetragenococcus halophilus* and *Zygosaccharomyces rouxii* are predominant among the microbes involved in the moromi stage. Despite their importance for producing a wide range of volatile compounds, antagonism can occur due to different growth condition requirements. Furthermore, microbial interactions in moromi fermentation are affected by current efforts to reduce salt in soy sauce, in order to tackle slow fermentation due to low metabolic activity of microbes and increased health risk related to high sodium intake. Attempts to enhance

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and accelerate flavor formation in the presence of high salt concentration include the inoculation with mixed starter cultures, genetic modification, cell, and enzyme immobilization. Although salt reduction can accelerate the microbial growth, the flavor quality of soy sauce is compromised. Several approaches have been applied to compensate such loss in quality, including the use of salt substitutes, combination of indigenous cultures, pretreatment of raw material and starter cultures encapsulation. This review discusses the role of microorganisms in soy sauce production in relation to flavor formation and changes in production practices.

Keywords: soy sauce; moromi; mixed cultures; antagonism; flavor; cell immobilization; cell encapsulation; low-salt soy sauce

1. Introduction

Soy sauce is a liquid condiment originating from China, which has light brown to black color with salty and intense umami taste (Steinkraus, 1983). Due to its distinctive taste and aroma, soy sauce is used as the main seasoning in Japan, China, Korea, and other Asian countries and it has been gaining popularity in the Western countries.

Soy sauce develops a complex microbial community of fungi, yeast, and bacteria during production. Although soy sauce varies greatly between countries, it shares a two-step fermentation process, namely koji (solid-state fermentation) and moromi (brine fermentation), in all cases (Feng, Cai, et al., 2014; Rolling, 1995; Song, Jeong, & Baik, 2015b; Tanaka, Watanabe, & Mogi, 2012; Yang et al., 2017). Koji is a solid-state fermentation of soybeans and wheat using mold spores, usually *Aspergillus oryzae* or *Aspergillus sojae*. Meanwhile, moromi is conducted by immersing the resulting koji in a brine solution and left to ferment for several months up to 4 years (Yang et al., 2017).

In traditional production, both koji and moromi stages are performed in a non-sterile environment which leads to spontaneous fermentation by indigenous microbes. A wide range of species have been isolated and identified during the soy sauce fermentation process and their activity strongly influences the flavor and aroma formation in the final product (Harada et al., 2016; Hayashida, Nishimura, & Slaughter, 1997; Song et al., 2015b; Sulaiman, Gan, Yin, & Chan, 2014; Q. Wei, Wang, Chen, et al., 2013; Yang et al., 2017). However, since the microbial community involved during soy sauce fermentation is very complex, controlling the process is difficult resulting in inconsistency in the quality of the final product.

Various approaches have been employed to study the soy sauce microflora and its role in flavor production, aiming to optimize and standardize the production process. The remarkable progress in metagenomics has allowed researchers to identify non-culturable microorganisms, and monitor the dynamic changes of microbial communities during koji and moromi fermentation. Some microbes have been characterized for their potential to produce essential aroma compounds in soy sauce. Despite the importance of some species in aroma formation, antagonistic interaction between microbes is possible due to differences in optimum growth conditions. Furthermore, metabolic products during moromi fermentation, such as organic acids could also suppress the growth of the other species. However, it is not always the case in soy sauce fermentation, and therefore, the exact cause and mechanism of the suppression remain poorly understood.

Since moromi fermentation can take several months, attempts have been made to enhance and accelerate the aroma formation during production, including the use of defined mixed starter cultures, genetic improvement of starter cultures, as well as

immobilization of cells and enzymes. Moreover, global efforts for reducing daily salt intake as recommended by the World Health Organization (WHO) have led to the production of low-salt soy sauce. Such salt reduction could also benefit the production process by accelerating the moromi fermentation stage. However, there are concerns related to microbiological safety and organoleptic properties when salt content is reduced.

With a deeper understanding of the microbiology of soy sauce, it becomes possible to steer its functionality and control the quality of the final product. This review discusses the microflora of soy sauce in relation to its fermentation process and aroma development as affected by traditional and emerging production practices.

2. Soy sauce production process

Soy sauce is made of four basic ingredients, soybeans, wheat, salt, and water. According to the amount of wheat used, soy sauce can be distinguished into two types: The Chinese-type produced using predominantly soybeans and less wheat, and the Japanese-type made using an equal amount of soybeans and wheat (Wanakhachornkrai & Lertsiri, 2003). The Chinese-type dominates Asian regions such as China, Indonesia, Malaysia, Philippines, Singapore, Thailand, while the Japanese-type is more popular in Japan and western countries (Zhu & Tramper, 2013). The difference in the amount of wheat used results in a different amount of sugar available during fermentation, and therefore affects the microbial composition in each type of soy sauce. Due to the lower amount of wheat used in Chinese soy sauce, yeast fermentation is not as significant as in the Japanese soy sauce (Roling & Van Verseveld, 1996).

Soy sauce production (Figure 1) varies from country to country in terms of process and duration of fermentation, ratios of water, salt, and soybeans used, as well as the addition of other ingredients (Feng, Cai, et al., 2014; Lioe, Wada, Aoki, & Yasuda, 2007; W. F M Röling, Apriyantono, & Van Verseveld, 1996; Song et al., 2015b; Valyasevi & Rolle, 2002; C. L. Wei et al., 2013; Zheng, Wu, Huang, Zhou, & Liao, 2013). For example, soy sauce can be classified into high-salt liquid-state fermentation soy sauce (HLFSS), low-salt solid-state fermentation soy sauce (LSFSS), and koikuchi soy sauce (KSS). HLFSS and LSFSS are mainly found in China, while KSS dominates both the Japanese and western market (Feng et al., 2015). In Japan, other types of soy sauce can be distinguished based on the composition of raw materials used, such as *usukuchi*, *tamari*, and *shiro shoyu* (Lioe et al., 2007). However, they all share a common two-step fermentation process, namely koji and moromi.

Koji fermentation begins by soaking soybeans in water which facilitates the removal of soybean hulls, increases soybeans moisture content, and removes inhibitors of fungi in the soybeans, which are necessary measures for the fungi to grow. Moreover, spontaneous fermentation occurs during the soaking process which decreases soybeans pH to 4.5-5.0. Such low pH supports the growth of fungi while restricts the growth of spoilage microorganisms during koji fermentation. According to Santhirasegaram et al. (2016), the number of lactic acid bacteria increased significantly during the soaking of soybeans. Mulyowidarso, Fleet, & Buckle (1989) identified several bacterial species (*Lactobacillus casei*, *Streptococcus faecium*, *Staphylococcus epidermidis*, *Streptococcus dysgalactiae*, *Klebsiella ozaenae*, *Enterobacter cloacae*, *Enterobacter agglomerans*, *Citrobacter diversus,* and *Bacillus breuis*) and yeast species (*Pichia burtonii*, *Candida*

diddensiae and *Rhodotorula rubra*) which dominated the fermentation during soybean soaking in tap water (Mulyowidarso et al., 1989).

After soaking, soybeans are cooked in batch using a pressure cooker with saturated steam at $0.8 - 1.0$ kg/cm^{-2} gauge pressure for $40 - 45$ min, or using continuous cooker at $6 - 7$ kg/cm^{-2} gauge pressure (about 170 °C) for $20 - 30$ s (Luh, 1995). Due to the high temperature and pressure used during cooking, the number of bacteria, yeasts, and molds in soybeans decrease at this stage (Santhirasegaram et al., 2016). Meanwhile, wheat is roasted in a hot air continuous cooker at 150 °C for $30 - 45$ s at atmospheric pressure. The roasted wheat is then ground to produce smaller particles of wheat flour. The soybeans and wheat flour are mixed with 0.05% - 0.3% w/w of fungal spores, with *A. oryzae* or *A. sojae* being the most commonly used species (Chancharoonpong, Hsieh, & Sheu, 2012; Feng, Chen, et al., 2014; Luh, 1995; Su, Wang, Kwok, & Lee, 2005; Yan, Qian, Ji, Chen, & Han, 2013). The mixture is loaded into trays with layers of 3-5 cm thick and incubated at 25 °C. During this stage, koji mold produces proteolytic enzymes which hydrolyze proteins into peptides and amino acids, as well as amylase to convert starch into simple sugars. The substrates are utilized as nutrients by bacteria and yeasts during the subsequent moromi stage. The enzymatic activity of fungi increases the pH of koji from around 6.5 to 7.3, accompanied by heat production. Regular stirring is applied in order to reduce the heat resulting from the fungal metabolic activity, thus maintaining the temperature between $25 - 28$ °C (Luh, 1995). Fungal mycelia grow on the surface of soybeans and sporulate, producing a greenish compact mass of soybeans after 3 days of incubation. In modern production, the koji making has shifted to an automatic process, including the use of continuous soybeans cooker and wheat roaster, mixer, cooler, automatic inoculator, mechanical mixer, temperature controllers, and

conveyors, and mechanical devices for stirring the mixture during incubation (Luh, 1995).

The second stage of soy sauce fermentation is moromi. Koji is immersed in a brine solution containing 18-22% NaCl, producing moromi mash (van der Sluis, Tramper, & Wijffels, 2001; Yong & Wood, 1977). The high salt concentration in brine suppresses the growth of spoilage microorganisms and pathogens, while favors the growth of halotolerant species that play an important role in the flavor formation. The growth and enzymatic activity of mold in koji are terminated due to high salt concentration. Thus, moromi fermentation is mainly driven by the indigenous halotolerant lactic acid bacteria (LAB) and yeast. LAB propagate rapidly at the beginning of the moromi fermentation and the pH gradually decreases due to lactic acid fermentation and other metabolic products. When the pH of moromi mash reaches 4.0 – 5.0, the bacterial population starts to decrease while the yeast population starts to increase (Yong & Wood, 1976). In modern production, mixed cultures of LAB *T. halophilus* and yeast *Z. rouxii* and *Candida* species, are utilized to achieve consistent product quality (van der Sluis, Tramper, et al., 2001).

After 3 – 6 months of fermentation, moromi is subjected to a refining process, including pressing, filtration, pasteurization, and packaging (Luh, 1995). The matured moromi is pressed through filters in order to separate solids from liquid raw soy sauce. The raw soy sauce is then pasteurized at 70 - 80°C for 30 min (Kaneko, Kumazawa, & Nishimura, 2013; Luh, 1995; Mar, Lynn, Aye, & Khaing, 2013) in order to prolong its shelf life by inactivating residual microorganisms and enzymes. Furthermore, a number of aroma compounds are generated during the heating process (X. L. Gao et al., 2010; Kaneko et al., 2013). Two of the most important aroma compounds in soy sauce, 4-hydroxy-2,5-

dimethyl-3(2H)-furanone (4-HDMF) and 4-hydroxy-2-ethyl-5-methyl-3(2H)-furanone (4-HEMF), are known to be produced from pentoses through Maillard reaction during heating (Sun, Jiang, & Zhao, 2010). Additional ingredients such as caramel can be added in the soy sauce to adjust the color. Soy sauce is then packaged in plastic or glass containers before being shipped to the market for consumption. Soy sauce packaged in plastic and glass containers typically has a shelf-life of 1.5 and 3 years, respectively.

3. Microbial community in soy sauce fermentation

Soy sauce production typically takes place in non-sterile conditions for a long period, ranging from 4 months (C. L. Wei et al., 2013) to 4 years (Yang et al., 2017). Such conditions allow the introduction and growth of a wide range of microorganisms which contribute to the aroma formation in soy sauce (Table 1). Microorganisms involved in soy sauce fermentation have been isolated and identified in several studies using culture-dependent methods (Wilfred F.M. Röling, Timotius, Prasetyo, Stouthamer, & Van Verseveld, 1994; Tanasupawat, Thongsanit, Okada, & Komagata, 2002). Such methods rely on the ability of microbes to grow on a nutrient medium and sometimes underestimate the true microbial diversity. Therefore, studies have included culture-independent methods, based on identification using total DNA and sequencing of phylogenetic markers (Song et al., 2015b; Sulaiman et al., 2014; Tanaka et al., 2012; C. L. Wei et al., 2013; Q. Wei, Wang, Chen, et al., 2013; Yan et al., 2013; Yang et al., 2017). Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) was used for the characterization of microbial community diversity and changes during soy sauce fermentation, and its combination with analysis by culture was able to reveal more microbial species than any method alone (Table 1) (Song et al., 2015b; Tanaka et al., 2012; C. L. Wei et al., 2013; Q. Wei, Wang, Chen, et al., 2013; Yang et al., 2017). For

example, by using PCR-DGGE analysis during 4 years long moromi fermentation, Yang et al. (2017) detected isolates belong to genera *Shimwellia*, *Weissella*, *Pantoea*, *Enterobacter*, *Scopulibacillus*, *Lactococcus*, and fungi genera *Absidia*, *Lichtheimia*, and *Sterigmatomyces*. On the other hand, analysis by culture was able to recover only some yeast species, such as *Meyerozyma guilliermondii*, *Candida parapsilosis*, and *Fusarium oxysporum*. However, more bacterial species could be detected during fermentation process of *inyu* (Taiwanese soy sauce), including *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus subtilis*, *Brachybacterium rhamnosum*, *Delftia tsuruhatensis*, *Enterobacter pulveris*, *Kurthia gibsonii*, *Pantoea dispersa*, *Staphylococcus cohnii*, *Staphylococcus condimenti*, *Staphylococcus gallinarum* and *Staphylococcus kloosii*, while fewer bacterial species, *Citrobacter farmeri*, *Pantoea agglomerans*, *Salmonella enterica*, *Serratia marcescens*, *Enterococcus faecium* and *Weissella confusa* were recovered by PCR-DGGE analysis (C. L. Wei et al., 2013).

Whole genome shotgun (WGS) analysis coupled with next-generation sequencing (Sulaiman et al., 2014) and clone libraries (Yan et al., 2013) provided information on the microbial dynamics as well as metabolic and functional diversity of the microbial population during fermentation. By using this method, the authors revealed the abundance of LAB in the first half of moromi fermentation, and a shift from homofermentative to heterofermentative fermentation. Clone libraries (Yan et al., 2013) revealed higher complexity in the koji bacterial community than PCR-DGGE (Tanaka et al., 2012).

The diversity and distribution of the microbial population in soy sauce tend to vary according to the country of origin, type of raw material, handling process, and fermentation conditions used (Table 1). For instance, moromi made with no or low

amount of wheat is usually associated with less complex yeast community (Wilfred F.M. Röling, Timotius, Stouthamer, & van Verseveld, 1994; Song et al., 2015b). Meanwhile, due to much higher salinity in the moromi, only two species of LAB (*E. faecium* and *W. confusa*) were found in inyu (C. L. Wei et al., 2013). Furthermore, different microbial dynamic changes were found between Chinese soy sauce made using high-salt diluted-state (HSDS, also reported as HLFSS) and low-salt solid-state (LSSS, also reported as LSFSS) fermentation process, due to different substrate composition and incubation conditions used (Zhang, Zhou, Cui, Huang, & Wu, 2016). In LSSS, *Weissella* and *Tetragenococcus* were found to be dominant throughout the fermentation process, while in HSDS *Weissella* population decreased as fermentation proceeds. The microbial diversity also differed between factories and within the same factory from batch to batch (Wilfred F.M. Röling, Timotius, Prasetyo, et al., 1994). However, the effect of differences in the koji incubation room, lipid content in soybean, and the type of *Aspergillus* species, on the bacterial composition during koji fermentation were negligible (Tanaka et al., 2012).

The microbial community of soy sauce is changing in every stage of the manufacturing process. Microbial diversity was found to be higher in koji than moromi due to the high salt concentration in moromi (Tanaka et al., 2012; C. L. Wei et al., 2013; Q. Wei, Wang, Chen, et al., 2013). Koji was dominated by bacteria, especially LAB, followed by yeasts and molds. Some groups of bacteria (*Staphylococcus*, *Bacillus*, and *Enterobacter*) remained present during the moromi stage, while *Klebsiella*, *Paenibacillus*, and *Corynebacterium* were only detected in koji. Fungal species including *A. sojae, Aspergillus parasiticus*, *Trichosporon ovoides*, and *Trichosporon asahii* disappeared during the moromi stage (Q. Wei, Wang, Chen, et al., 2013). Higher bacterial diversity

was detected during the koji fermentation of inyu by culture on media (C. L. Wei et al., 2013). However, several bacterial species including *Brachybacterium*, *Kurthia*, and *Staphylococcus* were no longer detectable in koji after the washing step (Figure 1). Similarly, members of the *Enterobacteriaceae* family were not detectable during the moromi fermentation stage. A wide range of bacterial species was detected in Japanese soy sauce during the koji step using the PCR-DGGE method (Tanaka et al., 2012). Among these, only *Weissella cibaria* (or *W. confusa, Weissella kimchii, Weissella salipiscis, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus iners*), *S. gallinarum* (or *Staphylococcus xylosus),* and *S. kloosii* were preserved during moromi stage.

The microbial diversity during moromi fermentation changes over time. As the fermentation progressed, the microbial diversity decreased especially in mid to late stage of moromi fermentation (Q. Wei, Wang, Chen, et al., 2013; Yang et al., 2017; Zhang et al., 2016). Such decrease is mainly due to high salinity in the moromi, which is unfavorable for the growth of non-halotolerant microbes.

According to Q. Wei, Wang, Chen, et al. (2013), moromi is dominated by bacteria, especially LAB, followed by yeasts and molds. *T. halophilus* has been reported as the most predominant LAB during moromi fermentation due to its halotolerance (Tanaka et al., 2012; Tanasupawat et al., 2002; Zhang et al., 2016). In moromi fermentation of inyu, *E. faecium* and *W. confusa* were the only LAB detected by PCR-DGGE, which could be due to much higher salt concentration than that typically found in soy sauce (30 – 42%) (C. L. Wei et al., 2013). Additionally, *Bacillus* and *Staphylococcus* have been repeatedly detected as dominant microbes in the whole moromi fermentation the by using PCR-DGGE (Song, Jeong, & Baik, 2015a; C. L. Wei et al., 2013; Q. Wei, Wang, Chen, et al., 2013). In Japanese soy sauce, in addition to *T. halophilus*, Tanaka et al. (2012) also found

W. cibaria (W. confusa, W. kimchii, W. salipiscis, L. fermentum, L. plantarum, L. iners or Streptococcus thermophilus), S. gallinarum (or S. xylosus), and S. kloosii in the moromi, even in the maturation stage. This indicates that the above bacteria had the ability to survive in high salt concentration.

Increase in acidity due to organic acid production by bacteria subsequently allows acid-tolerant yeasts to dominate the fermentation. The proportion of yeasts in the microbial population also changes during the moromi stage. *Z. rouxii* was reported to appear in the early stage of moromi fermentation, followed by *Candida etchellsii* and *Candida versatilis* in the middle stage, and *C. etchellsii* in the late stage (Tanaka et al., 2012). According to Q. Wei, Wang, Chen, et al. (2013), *Z. rouxii* was only detected in the mid to late stage of moromi fermentation and it was considered as the most dominant fungi in moromi stage (Q. Wei, Wang, Chen, et al., 2013). In contrast, yeasts from the *Candida* group were found to dominate the whole moromi stage of Korean soy sauce fermentation, accounted for 42.1% of the total yeasts (Song et al., 2015b). This group together with *Rhodotorula* and *Pichia* were consistently found throughout the whole process, while the population of other yeasts was fluctuating.

In modern production, soy sauce fermentation is performed under more controlled and sterile conditions. Unlike the traditional production which relies on the spontaneous growth of indigenous microorganisms, modern production of soy sauce utilizes a defined mixed starter culture (Luh, 1995). Therefore, the microbial diversity is less complex.

4. Antagonism between *T. halophilus* and *Z. rouxii* in moromi fermentation

T. halophilus and *Z. rouxii* have been reported as the most predominant microbes in moromi, regardless the soy sauce origin and production method (Wilfred F.M. Röling, Timotius, Stouthamer, et al., 1994; Tanaka et al., 2012; Tanasupawat et al., 2002; Zhang et al., 2016). During spontaneous fermentation, the growth of *T. halophilus* and *Z. rouxii* occurs sequentially. At the beginning of fermentation, moromi has relatively high pH ranging from 6.0 – 7.0 which supports the growth of *T. halophilus*. The propagation of *T. halophilus* results in organic acids production and moromi acidification. Once the pH drops below 5.0, *T. halophilus* is no longer able to grow. This pH is suitable for *Z. rouxii* to begin to grow and produce alcohol by utilizing glucose available in the moromi (Wilfred F.M. Röling, Timotius, Stouthamer, et al., 1994; van der Sluis, Tramper, et al., 2001; Yong & Wood, 1976).

When two or more microbial species co-exist in fermentation, interactions such as mutualism, competition, and antagonism are commonly observed. In moromi fermentation, although both *T. halophilus* and *Z. rouxii* contribute to the aroma formation, antagonism has been reported in several studies (Kobayashi & Hayashi, 1998; Kusumegi, Yoshida, & Tomiyama, 1998; Noda, Hayashi, & Mizunuma, 1980). Noda et al. (1980) demonstrated that acetic acid and lactic acid production by *T. halophilus* (previously named as *Pediococcus halophilus*) was able to inhibit the growth of *Z. rouxii* (previously named as *Saccharomyces rouxii*) and *Torulopsis versatilis* (Noda et al., 1980). Noda, Hayashi, & Mizunuma (1982) suggested that at comparable pH values, acetic acid exhibited higher inhibition activity compared to lactic acid and it increased significantly as the pH of the medium was reduced. Furthermore, a study by Kusumegi, Yoshida, & Tomiyama (1998) demonstrated that acetic acid could suppress the growth of *Z. rouxii*

R-1 by inhibiting its respiratory activity and cytochromes formation. Acetic acid was also found to inhibit the protons expulsion from the cells which is important for *Z. rouxii* salt tolerance. In a medium containing 18% NaCl, the growth was significantly reduced at an acetic acid concentration of 0.5%.

Moreover, the pH of moromi has been suspected to be the main factor affecting the antagonistic interaction between *T. halophilus* and *Z. rouxii* during fermentation, since these species require different acidity for optimum growth. *T. halophilus* grows at relatively higher pH values (5.5 – 9.0), while *Z. rouxii* needs slightly acidic pH values (4.0 – 5.0) in a substrate containing 18% salt (Wilfred F.M. Röling, Timotius, Stouthamer, et al., 1994). Antagonistic interaction due to changes in pH has been reported in reduced-salt moromi fermentation (10% w/v NaCl) (Devanthi, Linforth, Onyeaka, & Gkatzionis, 2018). Antagonism occurred when *T. halophilus* and *Z. rouxii* were co-cultured or added sequentially. The *T. halophilus* and *Z. rouxii* co-existence increased the pH to >6.0, which could be the underlying cause of antagonism. As the pH increased, *T. halophilus* growth was enhanced while *Z. rouxii* growth declined. The increase in pH could stimulate *T. halophilus* growth since the optimal pH for growth is around 7.0 (Justé et al., 2008; Wilred F. M. Röling & van Verseveld, 1997). On the other hand, the optimal pH range for *Z. rouxii* is 3.5-5.0, and therefore, the pH increase in moromi could inhibit *Z. rouxii* growth (Membré, Kubaczka, & Chéné, 1999). *Z. rouxii* cannot maintain its salt tolerance when the extracellular pH reaches above 5.5 due to the loss of the proton gradient across the plasma membrane (Watanabe & Tamai, 1992).

Oxygen supply was also suspected to be the cause for antagonism between *P. halophilus* and *S. rouxii* in a *shoyu* koji extract (Inamori, Miyauchi, Uchida, & Yoshino, 1984). At an initial pH 6.0, *P. halophilus* was inhibited by *S. rouxii* under aerobic conditions, while *S.*

rouxii was inhibited by *P. halophilus* under anaerobic conditions. Growth inhibition of *P. halophilus* and *S. rouxii* was also accompanied by poor lactic acid and alcohol fermentation, respectively.

5. Aroma compounds in soy sauce and the role of microorganisms in their production

Soy sauce has been consumed as the main seasoning in Asian countries to enhance the taste of food during cooking and it has been widely recognized all over the world because of its distinct flavor. The characteristic flavor of soy sauce is a combination of sweet, sour, salty and umami tastes generated by a variety of flavor compounds (Wanshoupeng, Wang, Hou, & Cao, 2011). Nearly 300 aroma compounds have been identified in soy sauce. These aroma compounds include alcohols, aldehydes, acids, acetals, hydrocarbons, esters, furans, furanones, ketones, lactones, nitrogen-containing compounds, phenols, pyrones, pyrazines, pyridines, sulfur-containing compounds, and thiazoles (Nunomura et al., 1980). Among them, alcohols, acids, esters, and aldehydes have been reported as the most abundant aroma compounds found in soy sauce (Feng, Cai, et al., 2014). According to Wei et al. (2013c), alcohols are mainly found during the initial stage of fermentation, esters during the middle stage, while alkanes and heterocyclic compounds at the end of fermentation.

Some important aroma compounds with regards to sensory characteristics are common in Japanese and Chinese-type soy sauce, including ethanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-octen-3-ol, 2-phenylethanol, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, benzeneacetaldehyde, acetic acid, ethyl acetate, guaiacol, 4-ethylguaiacol, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2,5-dimethylfuran, furfural, 2-furanmethanol, 5-methyl-2-

furancarboxaldehyde, 3-phenylfuran, dimethyl disulphide, dimethyl trisulphide, 3-(methylthio)propanal and maltol (Feng, Cai, et al., 2014). However, the aroma compounds may vary depending on raw materials, salt concentrations, microbial strains, and fermentation time and temperature used during the soy sauce manufacturing process. For instance, soy sauce made using whole soybeans has more fatty acid ester and milder flavor compared to that made using defatted soybeans, due to the high crude fat content in the whole soybeans (L. Gao et al., 2017). When whole soybeans are used, the lipophilic compounds including the aroma compounds prefer to reside in the oil fraction, which is further removed from the moromi mash before pasteurization. This leads to loss of flavor in the final product (Kinoshita, Sugimoto, Ozawa, & Aishima, 1998). Wheat bran is sometimes more preferred than wheat flour due to its ferulic acid content. During moromi fermentation, ferulic acid can be transformed by *Torulopsis* into 4-ethylguaiacol, which is a desirable aroma compound in soy sauce (Kinoshita et al., 1998). Furthermore, Feng et al. (2015) suggested that due to differences in manufacturing conditions, Japanese-type soy sauce contains higher amount of alcohols (ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol), esters (ethyl acetate, ethyl 2-methylpropanoate and acetic acid 2-phenylethyl ester), benzeneacetaldehyde, 4-ethylguaiacol, 4-vinylguaiacol and 4-hydroxy-2-ethyl-5-methyl-3-furanone (HEMF) compared to that of Chinese-type. Chinese soy sauce made using LSFSS has a higher concentration of 3-methylbutanal, 2-methylpropanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, 2-methylpyrazine and 2,5-dimethylpyrazine compared to that of Japanese or Chinese-type made using HLFSS.

Moromi fermentation is considered as important stage that determines the quality of flavor and aroma in soy sauce. During this stage, a vast range of microorganisms present in the moromi produce aroma compounds that contribute to the overall aroma profile of soy sauce. Among these microorganisms, *T. halophilus* and *Z. rouxii* are predominant and their contribution to the soy sauce aroma is the most studied. *T. halophilus* and *Z. rouxii* produce various secondary metabolites including important aroma compounds through lactic acid and alcoholic fermentation pathway, respectively (Harada et al., 2016; Lee, Lee, Choi, Hurh, & Kim, 2013). *T. halophilus* contributes to the production of acetic acid, formic acid, benzaldehyde, methyl acetate, ethyl 2-hydroxypropanoate, 2-hydroxy-3-methyl-2-cyclopenten-1-one, and 4-hydroxy-3-methoxybenzaldehyde. A metabolomic study by Harada et al. (2016) demonstrated a correlation between *T. halophilus* and an increase in furfural, furfury alcohol, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), and methional during moromi fermentation. These compounds are known to be produced through Maillard reaction and the production rate increases in an acidic environment which is caused by acetic acid and lactic acid production by *T. halophilus* (Harada et al., 2018).

Other aroma compounds important to the soy sauce flavor including ethanol, acetaldehyde, ethyl propanoate, 2,3-methylbutanol, 1-butanol, and ethyl 2-methylpropanoate are mainly produced by *Z. rouxii* (Lee et al., 2013). Ethanol is produced by *Z. rouxii* from sugar available in the moromi and when the pH is lower than 5.0 as at higher pH it loses its ability to maintain a proton gradient required for salt tolerance. However, due to the high salt concentration in moromi, only some sugars can be fermented into ethanol, e.g., glucose, while maltose can only be fermented in a salt-free medium (van der Sluis, Tramper, et al., 2001)

Alcohols including isobutyl alcohol, isoamyl alcohol, 2-phenyl ethanol, methionol, and polyol and two furan derivatives, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (HDMF) and HEMF, have been reported as important flavor compounds in Japanese-type soy sauce, and are known to be produced by *Z. rouxii* (Hecquet, Sancelme, Bolte, & Demuynck, 1996; Sasaki, 1996). Higher alcohols are produced through α-keto acids pathways, comprising the amino acid biosynthetic and amino catabolic pathway, which is known as Ehrlich pathway (Van Der Sluis et al., 2001; Webb & Ingraham, 1963). The Ehrlich pathway involves deamination or transamination of extracellular amino acids, producing α-keto acids which serve as key intermediates for the formation of higher alcohols. The biosynthetic pathway for the production of HDMF and HEMF remains poorly understood. However, it has been reported that both are produced by *Z. rouxii* using Maillard reaction intermediates as precursors (Hayashida, Kuriyama, Nishimura, & Slaughter, 1999).

Apparently, the production of aroma compounds by *T. halophilus* and *Z. rouxii* is highly affected by biochemical changes in the medium caused by the metabolic activity of microbes, such as the production of acids (e.g., acetic acid and lactic acid) and ethanol. The production of acid can alter the pH of the medium which affects not only the microbial growth but also the enzymatic activity and pathways involved in the aroma formation that takes place during fermentation (Harada et al., 2018). Recently, Devanthi, Linforth, Onyeaka, et al. (2018) have demonstrated that the inoculation sequence of *T. halophilus* and *Z. rouxii* altered moromi pH and their interaction and eventually resulted in changes in aroma profile.

6. Enhancement of soy sauce flavor formation

6.1 Inoculation of moromi with mixed starter cultures

Since the fermentation conditions in the traditional process are poorly controlled, the consistency of the final product flavor quality is difficult to be maintained. In order to address this problem in modern large-scale production, soy sauce is produced using a well-defined mixed starter culture (Table 3). The mixed starter culture is made of a combination of microbial species, which is essential for flavor enhancement. Mixed cultures of *T. halophilus* and *Z. rouxii* have been used in the production and their contribution to the flavor of soy sauce was studied using high-resolution food metabolomics (Harada et al., 2016). Furthermore, mixed cultures of *Z. rouxii* A22 and *Pichia guilliermondii* EM1Y52 isolated from Thai soy sauce, have been shown to enhance the volatile compounds formation during moromi fermentation (Wah, Walaisri, Assavanig, Niamsiri, & Lertsiri, 2013). *Z. rouxii* A22 enhanced the formation of HEMF and HDMF, while *P. guilliermondii* EM1Y52 contributed to the production of phenolic volatiles, such as 2-methoxyphenol, 4-ethyl-2-methoxyphenol, and 4-ethylphenol. The co-culture of these two species was able to produce more alcohols, furanones, esters, maltol, and benzoic acid than the pure culture of *Z. rouxii*. The microbes used in koji fermentation also determine the aroma formation during the moromi stage (Liu et al., 2015).

When using mixed starter cultures, the inoculation time plays an important role in flavor enhancement. Delaying the inoculation of *Z. rouxii* of up to 14 days in moromi fermentation can significantly increase the formation of HEMF (Hayashida et al., 1997). Furthermore, Devanthi et al. (2018) have shown that inoculating *Z. rouxii* after *T. halophilus* decreases the pH of moromi to <5.0, can result in more complex aroma

profiles. Delaying the inoculation of *Z. rouxii* might have allowed the accumulation of the aroma compound precursors.

6.2 Genetic engineering of starter cultures

High salt content in moromi decreases the metabolic activity of microbes and as a consequence, a long period of incubation is required for the flavor formation. Therefore, it is essential to develop microbial strains with high salt-tolerance by genetic engineering. Cao et al. (2010) have demonstrated the production of a *Z. rouxii* mutant with improved salt-tolerance through genome shuffling. This method was able to enhance the aroma quality of soy sauce through significant production of ethyl acetate, HEMF, 4-EG and amino acid nitrogen within a shorter period. A strain of *A. oryzae* HG76 presenting 82.19% higher acid protease activity has been obtained through genetic recombination between *A. oryzae* HN3042 and *A. niger* CICC2377 (Xu et al., 2013). The mutant strain exhibited better soybean degradation ability compared to the parental strains, indicated by a higher content of soluble solid, total acid, total nitrogen, amino nitrogen and low molecular weight peptides in the moromi. In addition, *A. oryzae* HG76 produced higher content of taste-enhancing free amino acids (FAA) and volatile compounds (e.g., alcohols, aldehydes, ketenes, and acids) which resulted in preferable sensory profiles of soy sauce.

Although genetic engineering has made significant progress in microbial strain improvement, no study has yet reported its impact on commercial application. In the future studies, the impact of gene modification on the whole metabolic pathway responsible for the cellular functions and the overall fermentation performance as well as food safety need to be considered.

6.3 Microbial cells and enzymes immobilization

Immobilization of microbial cells and enzymes has been reported to shorten the fermentation period and increase the production efficiency due to 10-100 fold higher concentration of yeast cells (van der Sluis, Stoffelen, et al., 2001). The use of immobilized glutaminase and cells of *P. halophilus*, *Z. rouxii*, and *C. versatilis* in a bioreactor system was found to shorten the soy sauce fermentation process from 24 to 2 weeks (Hamada et al., 1991). The cells were immobilized using a mixture of colloidal silica and sodium alginate, extruded through a nozzle into calcium chloride solution to form gel beads. Although alginate shows the ability to shorten the fermentation period and is considered to be safe, it appears to be sensitive to heat and therefore it cannot be completely sterilized by steam (Horitsu, Maseda, & Kawai, 1990). Also, the structure of alginate beads is weak and can be damaged by high salt concentration at a similar level to soy sauce (van der Sluis et al., 2000) .

A ceramic carrier was developed as an alternative to alginate for cell immobilization in soy sauce fermentation *Z. rouxii* and *C. versatilis* (Horitsu et al., 1990). The cultures were separated due to the negative effect of mixed cultures of *Z. rouxii* and *C. versatilis* on the production of 4-ethyl guaiacol by *C. versatilis*. Soy sauce of good quality was obtained within 8 days of fermentation, compared to 6 months using the conventional method.

Polyethylene-oxide gel is another cell immobilization technique proposed to enhance the gel beads durability under high salt concentration (van der Sluis et al., 2000). Compared to alginate, polyethylene-oxide gel was not sensitive to abrasion caused by high salt concentration. In order to maintain high cell viability after the immobilization process, contact between the cross linker and yeast cells was prevented during the preparation step. This immobilization technique allowed the production of aroma

compounds essential to the final product quality, including 4-ethylguaiacol, ethanol, and higher alcohols by *Z. rouxii* and *C. versatilis*. However, the use of polyethylene-oxide gel in continuous fermentation is problematic as the particles tend to aggregate (van der Sluis, Stoffelen, et al., 2001).

7. Low salt in soy sauce production and its effect on moromi fermentation

The demand for low-salt soy sauce has increased due to health concerns related to high sodium intakes, such as hypertension and renal dysfunction. The WHO recommends a limitation of daily sodium intake to 2 g, which is equivalent to 5 g of salt (World Health Organization, 2012). Meanwhile, the daily consumption of soy sauce per person in Japan is 30 mL (Kobayashi, 2013), while the per capita daily consumption in Indonesia is 10 – 15 mL (Sasaki & Nunomura, 2003). Due to high NaCl content in soy sauce, the consumption of 1 tablespoon (15 mL) of soy sauce can contribute to 38% of the Recommended Daily Intake (RDI) of sodium. Therefore, the production of soy sauce with low salt content without decreasing its quality has become a challenge for the industry. The high salt concentration is used in soy sauce production to create anaerobic conditions in moromi, prevent putrefaction of the autolysate, and inhibit the growth of spoilage microorganisms (Luo, Ding, Chen, & Wan, 2009; Muramatsu, Sano, Uzuka, & Company, 1993). It is also important to the organoleptic properties of soy sauce, including texture and aroma (Song et al., 2015a).

There have been efforts to reduce salt to the downstream processing by physical removal using nanofiltration (Luo et al., 2009), ion exchange (Motoki, Sasaki, Uchida, & Yoshino, 1977), reverse osmosis (Otomi, Furukawa, Kitakura, Someya, & Hashimoto, 1992), freezing (Watanabe, Tesaki, & Arai, 1996) and extraction (Matsuyoshi, Tadano, Shirai, & Hasui, 1998). Although some of them are claimed to have no adverse effect

towards the soy sauce flavor, they seem to be nonviable for commercial purposes due to high operating cost. Other studies focused on reducing the amount of salt used during moromi fermentation. This approach was shown to benefit the soy sauce production by accelerating the fermentation (Goh, Lai, Abas, & Tan, 2017; Hoang et al., 2016; Muramatsu et al., 1993; van der Sluis, Tramper, et al., 2001). High salt content in moromi can slow down the microbial metabolic activity, therefore a minimum period of 6 months is needed to complete fermentation. In order to accelerate the fermentation process while maintaining quality, low NaCl concentration can be combined with some physical treatments. Muramatsu et al. (1993) have successfully shortened the soy sauce production process by lowering NaCl content in moromi to 4.6% and autolyzing the koji prior to moromi fermentation with high temperature. This treatment was performed to obtain soy sauce with chemical composition and flavor similar to the traditional one. A recent study by Hoang et al. (2016) has demonstrated a significant reduction in reduced-salt (NaCl 10%) moromi fermentation time to 4.6 days by conducting the fermentation process at high temperature (40.7°C). The application of 10 min sonication at daily intervals during moromi fermentation has also been shown to accelerate moromi fermentation in the presence of 5% NaCl (Goh et al., 2017). Such conditions allowed higher production of glutamic acid and MSG-like amino acid, total and essential free amino acid contents within 4 days of fermentation, while the same levels were reached at day 7 in the case of untreated moromi. The authors suggested that the ultrasonication could bring the peptide and proteolytic enzymes in closer proximity to each other, therefore accelerating the peptide cleavage rate, which further resulted in efficient extraction of free amino acids (FAAs) in moromi. The high content of FAAs is desirable to obtain a final product with high sensory qualities.

In order to enhance the perception of saltiness in low-salt soy sauce, several chloride salts, such as KCl, MgCI$_2$, and MnCI$_2$, and amino acid based saltiness enhancers could be used to replace NaCl. However, Segawa et al. (1995) suggested that MgCI$_2$ and MnCI$_2$ addition did not add any saltiness in soy sauce, while KCl caused bitterness, which became apparent when added at a concentration above 10%. The effect of partial substitution of NaCl with KCl on moromi fermentation pattern and aroma formation was studied by Devanthi, Linforth, El Kadri, & Gkatzionis (2018). The substitution with KCl was shown to enhance the growth of *T. halophilus*, while the effect on *Z. rouxii* was negligible. This also resulted in faster sugar consumption and higher lactic acid production by *T. halophilus*, which caused an alteration in the final aroma profile of moromi. In addition to fermentation pattern and organoleptic quality, NaCl reduction also raises a microbiological safety concern. Chiou (1999) demonstrated that partial substitution of NaCl with ethanol could prevent the growth of pathogenic and spoilage microorganisms. However, the use of ethanol could limit the growth of desirable microorganisms.

Compared to chloride salts, amino acid based saltiness enhancers (e.g glycine ethyl ester hydrochloride and taurine) have shown the ability to produce low-salt soy sauce with better taste quality (Segawa et al., 1995). However, the balance of the saltiness and umami taste produced is inferior. Also, the impact on fermentation pattern and microbiological safety have not been studied.

Song et al. (2015a) have utilized indigenous yeasts, *Torulaspora delbrueckii* and *P. guilliermondii*, isolated from Korean traditional soy sauce fermentation to produce low-salt soy sauce. This method was proven to compensate for several adverse effects caused by salt reduction such as the growth of spoilage microorganisms and

undesirable flavor characteristics. The resulting moromi had a more complex and richer flavor similar to that of high-salt due to high production of fusel alcohols by *T. delbrueckii* and *P. guilliermondii*. Moreover, the application of mixed cultures of *T. halophilus*, *Z. rouxii* and *P. guilliermondii* (found in Thai soy sauce), was able to produce low-salt soy sauce desirable aroma (Singracha, Niamsiri, Visessanguan, Lertsiri, & Assavanig, 2017). Such combination of LAB and yeast was reported to reduce the amount of biogenic amine compared to the use of yeast starter cultures only (Singracha et al., 2017). However, the use of *T. halophilus* and *Z. rouxii* during low-salt moromi fermentation was not found sufficient to produce soy sauce with similar aroma profile to that of high-salt (Devanthi, Linforth, El Kadri, et al., 2018). Water-in-oil-in-water ($W_1/O/W_2$) double emulsions (DEs) were introduced as a system to promote a sustained release of *Z. rouxii* into the moromi. As previously mentioned, delaying the inoculation time of *Z. rouxii* could allow the accumulation of precursors for aroma compounds formation. Therefore, *Z. rouxii* was encapsulated in the inner W_1 phase, while *T. halophilus* was added in the continuous phase/moromi (W_2). Sequential fermentation occurred as *Z. rouxii* was gradually released into the moromi due to the inherent instability of DEs. Such method allowed moromi production with a similar aroma profile to that obtained with high salt concentration.

8. Conclusion

A well-defined mixed starter culture is important in the manufacturing of soy sauce for better control of the process and consistent product quality. The development of starter cultures should take into account the properties of individual species, as well as the interactions between them, observed in real soy sauce fermentation. Such knowledge can accelerate the fermentation and/or improve the performance of the cultures by

genetic engineering or cell encapsulation and immobilization. The reduction of salt in soy sauce demands the development of robust starter cultures that are stable and active towards the environmental perturbation. Therefore, the soy sauce production process can be modified, such as by lowering the salt concentration during the moromi stage, without compromising the flavor quality. However, microbial species involved during fermentation varies greatly among soy sauce, depending on the region and the production procedure. As a result, the degree of microbial composition and interactions contributing to the aroma profile of soy sauce remains unclear. For the purpose of microbial control and flavor quality improvement, future studies could focus on linking the microbiome, e.g., by using amplicon-based Next Generation Sequencing (NGS), metabolome, and sensory profile of soy sauce.

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Table 1. The diversity of microbial communities during soy sauce fermentation

Type of soy sauce	Ingredients	Salt concentration	Incubation condition	Fungi/yeast	Bacteria
Indonesian (*kecap*)	Black soybeans	12.3 - 21.3% NaCl	Koji: room temperature, 3 days Moromi: outdoors under the sun (21-42 ºC)	*Candida parapsilosis*, *C. guilliermondii* var. *membranaefaciens, C. famata, C. pulcherrima, Debaromyces hansenii* var. *fabryi*	*P. halophilus, Staphylococcus, Sterigmatomyces, coryneform*
Korean	*Meju* (soybeans only)	8% NaCl	Koji: 30ºC for 4 days	*Aspergillus oryzae*	*Bacillus* spp., *B. vallismortis*, *B. subtilis*, *B. amyloliquefacien*, *B. methylotrophicus*, *Staphylococcus* spp., *S. epidermidis*, *S. xylosus*, *Enterococcus* spp., *E. faecium*

		11% NaCl	Moromi: 30 ºC		*Bacillus* spp., *B. vallismortis, B. subtilis,B. amyloliquefaciens, B. methylotrophicus*
		14% NaCl			*Bacillus* spp., *B. vallismortis, B. subtilis, B. amyloliquefaciens, B. methylotrophicus*
Korean	*Meju* (soybeans and wheat fermented for 3 months)	20% NaCl	n.a	*Wickerhamomyces anomalus, Torulaspora delbrueckii, Tetrapisispora blattae, Rhodotorula* sp., *R. mucilaginosa, R. glutinis, Pichia sorbitophila, P. gulliermondii, P. triangularis, Microbotryum violaceum, Debaryomyces hansenii, Cryptococcus albidus, C.*	n.a

				parapsilosis, C. orthopsilosis, C. lusitaniae, Candida sp., *C. albicans, C. temnochilae, C. versatilis, Zygosaccharomyces* sp., *Penicillium islandicum, P. aurantiogriseum, Aspergillus* sp., *A. flavus*	
Chinese	n.a	16.9% NaCl	n.a	*Candida, Starmerella, Wickerhamiella, Aspergillus, Saturnispora*	*Weisella, Bacillus, Lactobacillus, Leuconostoc, Lactococcus, Padiococcus, Enterococcus, Micrococcus, Streptococcus, Xanthomonas, Streptococcus, Staphylococcus, Salmonella, Pseudomonas, Pantoea, Klebsiella, Dechloromonas, Cupriavidus,*

				Arsenophonus, Propionibacteriacea, Acidobacteriaceae
Japanese	Yellow soybeans : roasted wheat = 2.67 : 1	20% - 25% NaCl	Koji: 25 - 38 ºC , for 40h	*A. oryzae, Zygosaccharomyces rouxii, Z. pseudorouxii, Candida etchellsii, C. nodaensis, C. versatilis, C. catenulata, Wickerhamomyces anomalus, Pichia anomala,Geotrichum silvicola, Trichosporon jirovecii, T. asahii, T. japonicum, T. inkin, T. insectorum, T. faecale, T. ovoides,*	*Weisella cibaria, W. confusa, W. kimchii, W. salipiscis, Lactobacillus fermentum, L. plantarum, L. raffinolactis, L. mesenteroides, Staphylococcus gallinarum, S. xylosus, S. arlettae, S. saprophyticus, S. succinus, S. cohnii, S. caprae, S. kloosii, Pediococcus pentosaceus, P. acidilactici, Tetragenococcus halophilus*
Thai	n.a	n.a	n.a	n.a	*T. halophilus, L. acidipiscis, L. farciminis, L.*

					pentosus, L. plantarum
Taiwanese (*inyu*)	Black soybeans : rice bran = 100 : 1	30% - 42% NaCl	Koji: room temperature, 7 days Moromi: outdoors under the sun 21.6ºC - 41ºC, 4 months	n.a	*Staphylococcus sciuri, S. cohnii, S. condimenti, S. gallinarum, S. kloosii, S. sciuri, Citrobacter farmeri, Enterobacter cloacae, E. hormaechei, E. pulveris, Klebsiella pneumoniae, Pantoea agglomerans, P. dispersa, Salmonella enterica, Serratia marcescens, Weisella confusa, Enterococcus faecium, Brachybacterium rhamnosum, Kurthia gibsonii, Delftia tsuruhatensis, Bacillus amyloliquefaciens, B. licheniformis, B.*

					pumilus, B. subtilis
n.a	Defatted yellow soybean : roasted wheat = 5.5 : 4.5	20% NaCl	Koji: 35ºC for 42h Moromi: 30ºC, > 6 months	*Aspergillus sojae, A. parasiticus, Trichosporon ovoides, T. asahii, T. lactis, Zygosaccharomyces rouxii, Saccharomycopsis fibuliger, Millerozyma farinosa, Peronospora farinosa, Pichia farinosa, Candida* sp*., C. rugosa, C. orthopsilosis, C. tropicalis*	*Staphylococcus, Kurthia, Bacillus, Klebsiella, Enterobacter, Paenibacillus, Corynebacterium*
Chinese (LSSF)	Defatted soybean, wheat, and wheat bran	n.a	Koji: 35ºC for 26 – 28 h	*Aspergillus oryzae, Wickerhmomyces anomalus, Saccharomycopsis fibuligeraKluyveromyces marxianus, Candida rugosa, C. glabrata, C. tropicalis, Pichia fabianii, Trichosporon*	*Weisella paramesenteroides, W. confusa, W. cibaria, Bacillus subtilis, B. licheniformis, B. pumilus, Staphylococcus sciuri, S. gallinarum, S. succinus, S. aureus, S.*

asahii	*cohnii, Klebsiella pneumoniae, Escherichia coli, Corynebacterium variabilis, C. qlutamicum, C. variabile, Kurthia gibsonii, Enterobacter hormaechei, E. saccharolyticus, Enterococcus gallinarum, E. faecalis, Lactobacillus dextrinicus, L. similis, L. farciminis, L. salivarius, L. sakei, Rothia mucilaginosa, Arthrobacter crystallopoietes, Pediococcus pentosaceus*

| Chinese (*Xianshi*) | Soybeans : wheat flour = 4 : 1 | 18 – 20% NaCl | Koji: n.a

Moromi:

outdoors

under the sun

for 4 years | *Meyerozyma guilliermondii, Candida parapsilosis, Aspergillus niger, Cladosporium sphaerospermum, C. cladosporioide, Fusarium oxysporum, Sterigmatomyces halophilus, Lichtheimia ramose, L. hyalospora, Absidia blakesleeana* | *Bacillus amyloliquefaciens, B. subtilis, B. lincheniformis, B. methylotrophicus, B. aerius, B. halmapalus, B. flexus, B. thuringiensis, B. coagulans, Scopulibacillus darangshiensis, Shimwellia blattae, Weissella confusa, Lactococcus piscium, Klebsiella pneumonia, K. variicola, Pantoea dispersa, Enterobacter sacchari, Bacillus amyloliquefaciens, Erwinia toletana, Trichodesmium, Clostridium oceanicum, Streptomyces hebeiensis, Microlunatus phosphovorus* |

Table 2. The aroma compounds produced by *T. halophilus* and *Z. rouxii* during soy sauce fermentation

Soy Sauce Types	Main aroma compounds		References
	Tetragenococcus halophilus	*Zygosaccharomyces rouxii*	
n/a*	*Acids* Acetic acid, formic acid,	*Alcohols* Ethanol, 2,3-methylbutanol, 1-butanol, 2-phenylethanol	Lee, Lee, Choi, Hurh, & Kim (2013)
	Aldehydes Benzaldehyde, 4-hydroxy-3-methoxybenzaldehyde	*Aldehydes* Acetaldehyde	
	Esters Methyl acetate, ethyl 2-hydroxypropanoate	*Esters* Ethyl propanoate, ethyl 2-methylpropanoate	
	Ketones 2-Hydroxy-3-methyl-2-cyclopenten-1-one	*Furans* 4-Hydroxy-2-ethyl-5-methyl-3(2H)-furanone	

	(HEMF)		
Japanese type	*Acids*	*Acids*	Harada et al. (2016)
	Acetic acid, lactic acid, 4-oxopentanoic acid	Succinic acid, 2-butenoic acid, butanoic acid	
	Alcohols	*Alcohols*	
	Furfuryl alcohol	1,3-Butanediol, 2-(4-hydroxyphenyl)ethanol, 3-methyl-1-butanol, 1-propanol,	
		2,3-butanediol, 2-methyl-1-propanol, 2-phenylethyl alcohol, glycerol, methionol, 1-butanol	
	Aldehydes	*Aldehydes*	

Furfural, methional	Benzeneacetaldehyde
Amino acid	*Esters*
Ornithine	Hexadecanoic acid, ethyl ester
Ketones	
2-Hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), (R)-Dihydro-3-hydroxy-4,4- dimethyl-2(3H)-furanone	*Furans*
	HEMF, 5-ethyl-2(3H)-furanone
	Ketones
	1-Hydroxy-2-butanone, 2-methoxy-6-methyl-4-pyran-4- one, 5-ethyldihydro-2(3H)-furanone, 3,5-dimethyl-4-heptanone
	Lactam
	Caprolactam

Japanese type	n/a	*Alcohols*	Sasaki (1996)
		Isobutyl alcohol, n-butyl alcohol, isoamyl alcohol, 3-(methylthio)propanol, 2-phenylethanol	
		Furans	
		4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF), HEMF, 4-hydroxy-5-methyl-3(2H)-furanone (HMMF)	
Japanese type	*Alcohols* Ethanol	*Alcohols* Ethanol, 2-methyl-propanol, 3-methyl-1-butano, 3-octanol, 2-phenylethanol	Devanthi, Linforth, Onyeaka, & Gkatzionis (2018)
	Acids Acetic acid, 3-	*Acids* 2-Methylpropanoic acid, 3-	

	methylbutanoic acid	methylbutanoic acid
		Esters
		2-Phenylethylacetate

*n/a, not applicable

Table 3. Mixed starter cultures used in soy sauce fermentation and their inoculation sequence

Starter cultures		Inoculation sequence in brine fermentation	References
Solid-sate fermentation	Brine fermentation		
Aspergillus sojae	*Tetragenococcus halophilus* *Zygosaccharomyces rouxii*	Sequential inoculation	Harada et al. (2016)
n.a*	*Z. rouxii*	Co-inoculation	Wah, Walaisri, Assavanig, Niamsiri,

	Pichia guilliermondii		& Lertsiri (2013)
Aspergillus oryzae	*T. halophilus* *Z. rouxii*	Sequential inoculation	Devanthi et al. (2018)
A. oryzae	*T. halophilus* *Z. rouxii* *P. guilliermondii*	Sequential inoculation	Singracha, Niamsiri, Visessanguan, Lertsiri, & Assavanig (2017)
A. oryzae	*T. halophilus* *Z. rouxii* *Candida versatilis*	Sequential inoculation	Cui, Zheng, Wu, & Zhou (2014)
A. oryzae *Bacillus licheniformis*	*Z. rouxii & P. guilliermondii* *Torulaspora delbrueckii & P. guilliermondii*	Co-inoculation	Song, Jeong, & Baik (2015)

*n/a, not applicable
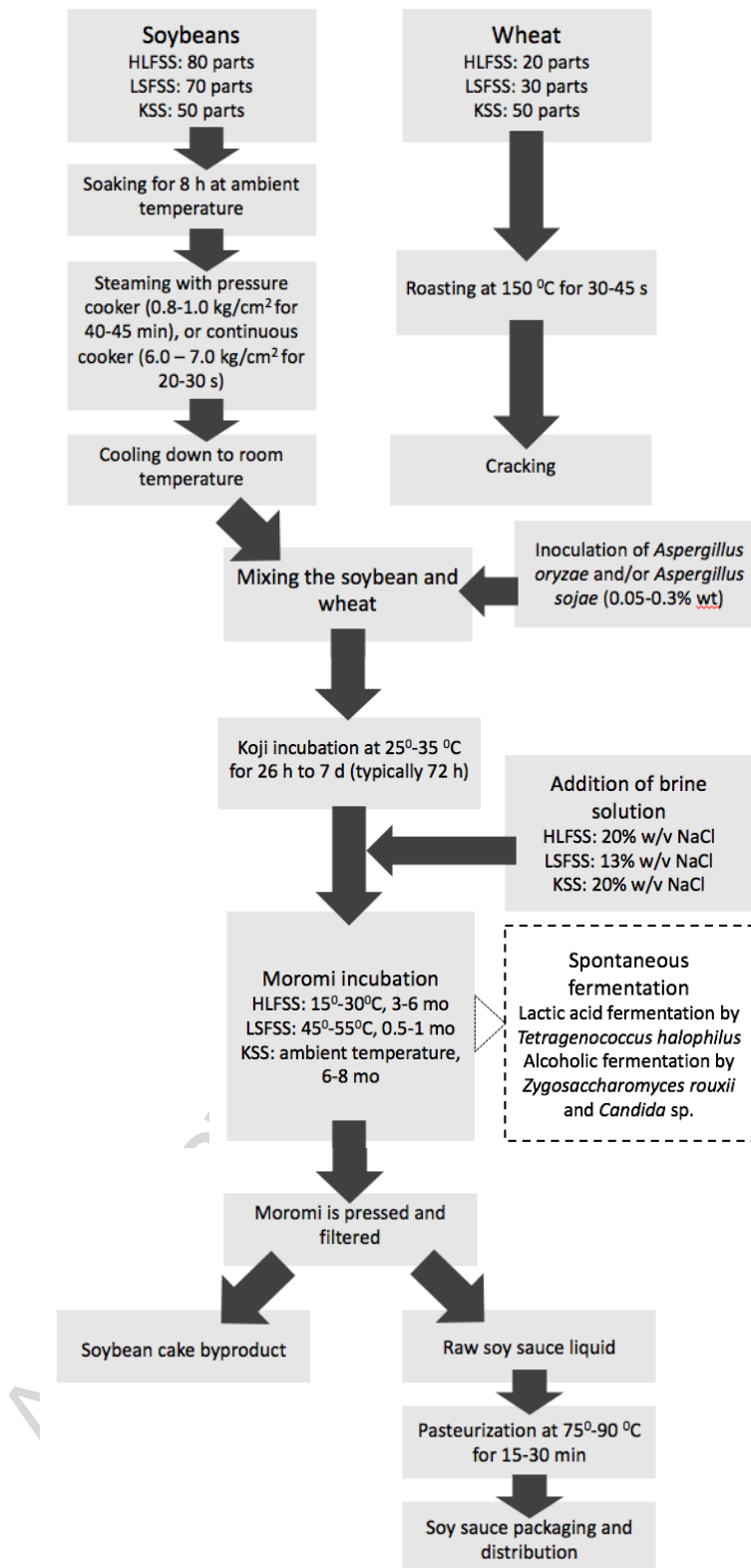
Figure 1 Flow chart summary of key stages and variations in soy sauce production.

Highlights

- Overview of microbial development in traditional and modern soy sauce production
- Review of the low-salt soy sauce production impact on microbes and aroma profile
- Certain fermentation conditions promote antagonism between microbes
- Variation in microbial diversity with impact on aroma profile of soy sauce
- Review of strategies for soy sauce flavor enhancement