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**Review**

# Microbial consortia and up-to-date technologies in global soy sauce production: A review

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**Abstract**

Soy sauce is an Oriental fermented condiment, and key ingredient in many Asian cuisines. As consumers around the world are becoming more adventurous with their eating choices and preferences, the demand for and popularity of Asian cuisines are increasing globally. The underlying basis of soy sauce fermentation is intricate microbial interactions which play a vital role in defining the quality, flavour, and smell of the resulting soy sauce. Traditional soy sauce fermentation consists of a two-step process: *koji* and *moromi* fermentation. Despite the presence of beneficial microorganisms in soy sauce, various harmful microorganisms can also be found during the *koji* or *moromi* step, thus resulting in soy sauce contamination. Therefore, studying the biodiversity and interactions of microorganisms is critical in ensuring soy sauce quality. The present review thus discusses in depth the various bacterial and fungal species that are either beneficial or harmful to soy sauce fermentation. The present review also discusses the advances in soy sauce fermentation such as the enhancement of gamma-aminobutyric acid (GABA) in soy sauce by microorganisms, the enhancement of soy sauce flavour by mixed starter culture, and by genome shuffling starter culture.

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## Introduction

Soy sauce is a dark brown liquid commonly used as a dipping condiment and colouring agent during food preparation in China, Japan, Korea, and other Southeast Asia countries. China and Japan are the world's largest producers and consumers of soy sauce, which originated from the popular legume soybean (Cheng *et al.*, 2019; Mohd Zaini *et al.*, 2022). In China, soy sauce production is more than 5 million tons each year, accounting for more than 55% of the global output (Liujun *et al.*, 2008). In Japan, soy sauce production is 1.2 million tons each year, and the average annual consumption of soy sauce is about 10 L/capita (Kataoka, 2005). On average, the Japanese consume about 34.1 g of soy sauce per day, which provides 2.4 g protein, 0.2 g fat, 5.8 g salt, and 14 kcal (Nunomura and Sasaki, 1986). In the latest market research report published by Technavio (2020), the global soy sauce market is expected to grow by USD 6.39 billion in the forecast period of 2020 to 2024.

Due to its low water activity and high salt content, it is stable at room temperature and does not require refrigeration for storage. The high concentration of brine solution used to ferment soy sauce will subsequently help control bacterial propagation during soy sauce brewing, and acts as a preservative. Salt is crucial in soy sauce brewing; not only it helps in preserving the soy sauce, but it also

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provides minerals such as sodium and potassium to consumers. Due to its high potassium level, bamboo salt is a better choice as compared to table, coarse, French sea, or Himalayan pink salt (Tan *et al.*, 2016). Because bamboo salt is high in potassium and low in sodium, it is effective in lowering sodium intake, and as a result, lowering blood pressure or hypertension in the community (Henry and Appel, 2021). Soy sauce, in addition to contributing flavour to foods, contains phytochemicals such as saponins, isoflavones, phenolic acids, phytosterols, flavonoids, and sphingolipids which have both short- and long-term health advantages (Alghamdi *et al.*, 2018). Studies show that soy sauce helps boost gastric juice production, and has antihypertensive, antimicrobial, anticarcinogenic, and antioxidant effects (Kataoka, 2005; Dini and Laneri, 2021).

Food allergy is an uncommon immune response to protein-rich foods such as milk, eggs, wheat, tree nuts, soybeans, and peanuts (Muthukumar *et al.*, 2020). Despite the fact that soy sauce is mostly made up of soybeans and wheat, several studies have shown that fermented foods such as soy sauce do not contain allergens (hypoallergenic) (Kobayashi *et al.*, 2004). Biogenic amines (BAs; Kurtzman *et al.*, 2001) are a light-molecular-weight nitrogenous chemical molecule formed largely in foods by microorganisms capable of amino acid decarboxylation such as lactic acid bacteria (Shalaby, 1996), Enterobacteriaceae, and yeasts (Önal, 2007). The presence of BAs in fermented soy sauce and soybean paste has been investigated in Korea, China, and Malaysia. Despite the fact that most soy sauce and soybean paste had BAs, the levels were lower than those considered harmful to consumers' health (Stute *et al.,* 2002). When BAs are ingested in excess of the allowed limits, they cause headache, diarrhoea, nausea, rash, raised blood pressure, heart palpitation, and death (Feddern *et al.*, 2019).

There are over 300 flavour compounds that have been found in soy sauce which render the soy sauce having a well-balanced mix of the five primary flavours; umami, sweet, bitter, salty, and sour (Zhao *et al.*, 2020). The flavour-aroma creation in soy sauce is determined by its manufacturing processes, raw ingredients, and starter cultures. Therefore, soy sauce from diverse origins is dictated by their variation and complexity. Soy sauce can generally be divided into Chinese- and Japanese-type soy sauce based on their soybean and wheat compositions. Chinese-type soy sauce uses more soybeans and less wheat during production, and is primarily consumed in China, Thailand, the Philippines, Indonesia, Malaysia, and Singapore. For Japanese-type soy sauce, the ratio of soybean and wheat is equal, and it is primarily consumed in Japan and the western countries (Nunomura and Sasaki, 1986).

Based on the Japanese Agriculture Standards (JAS), Japanese-type soy sauce are classified into five categories: *koikuchi*, *usukuchi*, *tamari*, *shiro*, and *saishikomi*. *Koikuchi* accounts for around 84% of Japanese soy sauce production, and is prepared by using soybean and wheat at a ratio of 1:1. *Usukuchi* has similar soybean and wheat ratio as *koikuchi*; but it is lighter, thinner, and has more assertive and salty flavour than *koikuchi*, and has less production percentage in comparison to *koikuchi*, of only less than 13% of Japanese soy sauce production. *Tamari*, also known as Chinese-style soy sauce, is made of soybean with a small amount of wheat, originated from China, and accounts for only about 2% of Japanese soy sauce production. *Shiro* is the opposite of *tamari* as it is made of wheat with a small amount of soybean. For *saishikomi*, instead of the usual saltwater, it is made from enzymatically-degraded soybean and wheat. Both *shiro* and *saishikomi* account for less than 1% of Japanese soy sauce production (Reddy *et al.*, 2018).

Traditional soy sauce fermentation consists of two steps: *koji* and *moromi* fermentation, as described in Figure 1. In the first stage, the soybeans are soaked overnight to soften, and then steamed. The wheat is roasted and crushed lightly to open it up. Then, the cooked soybeans, roasted wheat grains, and starter mould (*Aspergillus oryzae*) are mixed in a wooden tray to make the *koji*. The *koji* is incubated at room temperature for one week. Following this, the matured *koji* is added into a brine solution to make a mash mixture which is known as *moromi*. The *moromi* mash is kept in a wooden barrel, and fermented for at least four months, after which, the *moromi* is poured onto pressed cheesecloths, and the raw soy sauce is squeezed out by pressing the layers of *moromi*. The raw soy sauce is then pasteurised, refined, inspected, and bottled up for consumption or shipment (Luh, 1995).

In traditional soy sauce making, fermentation can be divided into two states: solid-state fermentation (SSF) which refers to the *koji* fermentation, and submerged fermentation (SmF)


**Figure 1.** Traditional soy sauce brewing process.

which refers to the *moromi* fermentation. The substrate utilised during fermentation is the difference between these two processes. Solid substrates, such as soybean, wheat, and cereals are employed in SSF fermentation. These substrates are nutrient-dense, and can support extended fermentations because the bacteria progressively use the nutrients in them. Moulds, yeasts, and bacteria that require little water activity will thrive in SSF (Subramaniyam and Vimala, 2012). In soy sauce making, soybean and wheat are usually used as the solid substrate to produce the *koji*.

After making the *koji*, the second stage fermentation of soy sauce is the SmF. The substrates for SmF are free-flowing media like salt water, media broth, and molasses. In the SmF of soy sauce, the mature *koji* will be added into the salt water. The microorganisms present in the broth will consume the nutrient in the broth, and release a large number of bioactive chemicals which contributes to richer flavour (Subramaniyam and Vimala, 2012). The recent advances in soy sauce making will be discussed later in the subsection of advances in soy sauce microbial fermentation.

*The history of soy sauce and the microorganisms involved*

Soy sauce originated from '*jiang*', a fermented soybean paste that was used to preserve food, or as a condiment, and was initially found in the late Zhou dynasty (1046 - 256 BC) in China to preserve meat, fish, and grains. In the Han dynasty (206 BC - 220), soybeans became much more accessible, and could easily be cultivated even on poor land (Gao *et al.*, 2010; Shurtleff and Aoyagi, 2012b). Even though it was founded in China, the evolution of soybean pastes to soy sauce occurred in its neighbouring countries; Japan and Korea. Soybean paste was first introduced to Japan and Korea by Buddhist monks from China. After going through a series of trials and modifications, they had accidentally discovered soy sauce by collecting the by-product of soybean paste in the fermentation tanks, thus soy sauce was born (Fukushima, 2004; Gao *et al.*, 2010; Shurtleff and

Aoyagi, 2012b; Yue *et al.*, 2020). In China, soybean paste is known as '*doujiang*' or '*dajiang*'; in Korea, it is known as '*doenjang*' or '*cheonggukjang*'; and in Japan, it is known as '*miso*'. Soybean pastes from different countries and regions have distinctive tastes and flavours. The raw ingredients used, surrounding environments, and processing methods are the main factors that vary the types of the resulting soybean paste.

As earlier discussed, traditional soy sauce making consists of a two-step fermentation which includes *koji* and *moromi* fermentation. *Koji*-making technique has been a distinctive skill of the Orient for over 3,000 years (Abe *et al.*, 2006). During the Yayoi period (300 BC - 300), this *koji*-making technique was imported into Japan (Machida *et al.*, 2008), and since the 13th -15th centuries, filamentous fungal inoculation for fermentation has been available commercially as *koji*. During that period, people had cultivated *koji* without realising that it was made up of microorganisms (*koji* mould). Thus, the *koji* refers to the substance fermented by *Aspergillus oryzae*, and the *Aspergillus oryzae* mould itself (Machida *et al.*, 2008). *Koji* had different designations depending on the country, *kōji* in Japanese, *qu* in Chinese, and *nurukgyun* in Korean. It is a culture made up by inoculating different filamentous moulds (*Aspergillus* spp., *Rhizopus* spp., *Monascus* spp., *Mucor* spp., and *Absidia* spp.) on cooked grains or legumes, in a humid and warm place (Murooka and Yamshita, 2008; Chen *et al.*, 2009; Shurtleff and Aoyagi, 2012b). *Koji* can typically be divided into two types: the koji inoculated with *Aspergillus oryzae*, and red rice *koji* inoculated with *Monascus purpureus*. Red rice *koji* is also known as *beni koji* in Japanese, and *hong qu* in Mandarin (Samsudin and Abdullah, 2013). It is typically used as a natural food colouring and preservative (Shurtleff and Aoyagi, 2012b). Despite the various forms of *koji*, the *koji* mould appears to be the key to triggering the synthesis of hydrolytic enzymes which are responsible for the breakdown of macromolecules into micromolecules in traditional fermented foods (Machida *et al.*, 2008).

*Aspergillus oryzae* is the most common mould used to make *koji* (Shurtleff and Aoyagi, 2012b). In 1876, when H. Ahlburg was invited to the Japanese Medical College, he had isolated *Aspergillus oryzae* from *koji* for the first time. Later, F. Cohn renamed it as *Aspergillus oryzae* from its previous name, *Eurotium oryzae,* due to its inability to reproduce sexually (Machida *et al.*, 2008). In 2005, the genome of *Aspergillus oryzae* RIB40 (ATCC-42149) was entirely sequenced, and it was found out that the sequenced strain is a wild-type strain that most akin to those used in *sake* brewing, but still possessed one of the essential features in soy sauce brewing, which is producing proteases (Machida *et al.*, 2005). The *koji* mould is the critical ingredient in creating a spectacular umami flavour in soy sauce (Machida *et al.*, 2008). It plays a vital role in fermenting the elements, and producing many enzymes such as amylases, proteases, lipases, and speciality chemicals (Yu *et al.*, 2004). *Aspergillus oryzae* has been extensively used in fermented foods such as *sake* (rice wine), *miso* (soybean paste), and *shoyu* (soy sauce) (Abe *et al.*, 2006). Furthermore, *Aspergillus oryzae* has received the "Generally Recognized as Safe (GRAS)" status from the United States Food and Drug Administration (USFDA) for safe use in foods (Taylor and Richardson, 1979; Abe *et al.*, 2006; Machida *et al.*, 2008). The World Health Organization also supports that this microorganism is safe when used to ferment foods (WHO, 1989).

In the *moromi* stage, the main microorganisms present in the mash are LAB and yeasts. Studies since the early 1900s have revealed that *Tetragenococcus halophilus*, a halo-tolerant LAB, was present in the *moromi* mash (Tochikura *et al*., 2001). At present, there are four identified species in the *Tetragenococcus* genus which are *Tetragenococcus halophilus* (Collins *et al.*, 1990), *Tetragenococcus muriaticus* (Satomi *et al.*, 1997), *Tetragenococcus solitarius* (Ennahar and Cai, 2005), and *Tetragenococcus koreensis* (Lee *et al.,* 2005b). In the manufacturing of soy sauce, soy paste, fish sauce, shrimp paste, and Taiwanese fermented mustard '*suan-tsai*', both *Tetragenococcus halophilus* and *Tetragenococcus muriaticus* play a key role in halophilic fermentation (Juste *et al.,* 2008; Yee *et al.,* 2021).

Halo-tolerant yeasts were also found during the early (*Zygosaccharomyces rouxii*), middle (*Candida etchellsii* and *Candida versatilis*), and late (*Candida etchellsii*) fermentation stages (Tanaka *et al.*, 2012). *Zygosaccharomyces rouxii* is a yeast notable for its high osmotic stress tolerance (Pribylova *et al.*, 2007). Originally named *Saccharomyces rouxii*, Barnett *et al.* (1983) had reclassified it to the present name in 1983. In the latest edition of '*The Yeast, a Taxonomic Study*' by Kurtzman *et al.* (2011), they have accepted six species that belong to genus *Zygosaccharomyces* which are *Zygosaccharomyces bailii* (Linder)

(Guilliermond, 1912), *Zygosaccharomyces bisporus* (Naganishi, 1917), *Zygosaccharomyces kombuxhaensis* (Kurtzman *et al.*, 2001), *Zygosaccharomyces lentus* (Steels *et al.*, 1999), *Zygosaccharomyces mellis* (Fabian and Quinet, 1928), and *Zygosaccharomyces rouxii* (Boutroux) (von Arx *et al.*, 1977). There are numerous species in this genus that are substantially resistant to many standard food preservation techniques, and this yeast is known as a spoilage yeast in the food industry such as fruit juices, fruit concentrates, syrups, alcoholic beverages, honey, and jams (Stratford, 2006). However, in soy sauce making, the LAB *Tetragenococcus halophilus* and the yeast *Zygosaccharomyces rouxii* are the main microorganisms that drives the *moromi* fermentation (Singracha *et al.*, 2017). *Zygosaccharomyces rouxii* will undergo alcoholic fermentation and produce abundant secondary metabolites that give flavour and aroma to the soy sauce (Tanaka *et al.*, 2012).

Several reports investigated the interaction between these species. Kusumegi (2001) and Tochikura *et al*. (2001) had suggested that the *koji* moulds and yeasts may aid in the development of LAB by providing essential nutrients for them, while yeasts and LAB can survive using the glucose resulted from the enzyme activity of the *koji* moulds. Furthermore, when *Tetragenococcus halophilus* grows in high salt concentration brine, it will release lactic acid which subsequently lowers the pH, and provides conducive environment for the salt tolerant *Zygosaccharomyces rouxii* to grow. As a result, *Zygosaccharomyces rouxii* will take over, generating major soy sauce flavours such as alcohols, esters, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF), and (isobutyl alcohol, isoamyl alcohol, 2-phenylethanol) 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF).

*Microbial community involved in soy sauce production*

Soy sauce production varies among countries based on the fermentation methods, fermentation times, water ratios, salt concentrations, microbial communities, raw ingredients, and other ingredients. However, the production goes through the same two-step fermentation; *koji* and *moromi*. Soy sauce fermentation is often made in a non-disinfected environment for a lengthy period, and usually takes around four months to four years. During this period and under this condition, a wide range of microorganisms, mainly moulds, yeasts, and LAB such as *Aspergillus*, *Weissella*, *Tetragenococcus*, *Staphylococcus*, *Bacillus*, *Pichia*, *Zygosaccharomyces*, and *Candida* may be introduced and flourish (Tanaka *et al.*, 2012; Wei *et al.*, 2013a; Yang *et al.*, 2017). The microbial communities involved in soy sauce production are highlighted in Table 1 and Figure 2.

Each stage of soy sauce fermentation contains different amount of microbial community; microbial diversity present in the *koji* stage was found to be greater than that in the *moromi* stage. This is due to some of the bacterial groups in the *koji* stage (*Staphylococcus*, *Enterobacter*, and *Bacillus*) are also present in the *moromi* stage. Additionally, some bacteria (*Corynebacterium*, *Klebsiella*, *Kurthia*, and *Paenibacillus*) and fungi (*Aspergillus sojae*, *Aspergillus parasiticus*, *Trichosporon ovoides*, and *Trichosporon asahii*) present in the *koji* stage may not be preserved in the *moromi* stage due to the high salt concentration present in the *moromi* stage which becomes unfavourable for non-halotolerant microorganisms to develop and grow, notably in the middle to late stage of *moromi* fermentation. As a result, the microbial diversity present in the *moromi* stage decreases throughout the *moromi* fermentation period (Wei *et al.*, 2013a; 2013b; Zhang *et al.*, 2016).

According to Yang *et al.* (2017) who studied the microbial diversity and community dynamics for four long years in natural *moromi* of the Xianshi soy sauce by using the PCR-DGGE analysis, they discovered microbial isolates belonging to the bacterial genera of *Bacillus*, *Shimwellia*, *Weissella*, *Pantoea*, *Enterobacter*, *Scopulibacillus*, *Lactococcus*, and *Klebsiella*, and the fungal genera of *Aspergillus*, *Cladosporium*, *Absidia*, *Lichtheimia*, and *Sterigmatomyces*. Further, a previous study also revealed that bacterial species such as *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* were found during the *inyu* (Taiwanese soy sauce) fermentation, and by applying the PCR-DGGE method, they could discover more bacterial species such as *Citrobacter farmeri*, *Pantoea agglomerans*, *Salmonella enterica*, *Serratia marcescens*, *Enterococcus faecium*, and *Weissella confusa* (Wei *et al.*, 2013a).

**Table 1.** The main microorganisms involved in soy sauce fermentation.

| Class | Microorganism | Role / Remark | Reference |
|---|---|---|---|
| Mould | *Aspergillus oryzae*, *Aspergillus sojae* | - Do not produce the common *Aspergillus* spp. mycotoxins such as aflatoxins, ochratoxin A, or sterigmatocystin under any conditions.<br>- Possess high proteolytic, amylolytic, and macerating enzyme levels.<br>- Digest proteins and starches in the raw materials. | Sugiyama (1984) |
| | *Aspergillus tamarii* | Tamari brewing, a variety of soy sauce. | Lioe *et al.* (2010) |
| Lactic acid bacteria | *Tetragenococcus halophilus* KBC, *Weissella* spp. (*W. confusa*, *W. paramesenteroides*, and *W. cibaria*), *Enterococcus* spp., *Lactococcus* spp., *Leuconostoc* spp., *Lactobacillus fermentum* | - Produce lactic acid and other useful organic acids in a high concentration of salt brine.<br>- Reduce the pH and allow for yeast growth such as *Saccharomyces rouxii*.<br>- Do not possess tyrosine decarboxylase and histidine decarboxylase. | Sugiyama (1984), Wan-Mohtar *et al.*, (2020), Yee *et al.*, (2021) |
| | *Pediococcus soyae*, *Pediococcus soyae* nov. sp. | - Produce lactic acid and other useful organic acids in a high concentration of salt brine.<br>- Remove undesirable flavours.<br>- Create essential aromas and flavours for soy sauce. | Sakaguchi (1958, 1959) |
| | *Bacillus* spp., *Bacillus cereus* KBC | - Generate odours and ammonia.<br>- A specific drug-like odour of soy sauce brewed with *Aspergillus sojae* could be diminished by the addition of bran culture of *Bacillus subtilis*.<br>- The polypeptide-like turbidity of soy sauce could also be removed. | Sugiyama (1984), Wan-Mohtar *et al.* (2020) |
| | *Staphylococcus gallinarium*, *Staphylococcus kloosii*, *Staphylococcus arlette* | Create colour and flavour. | Wei *et al.* (2013a) |
| Osmophilic yeast | *Saccharomyces cerevisiae* | Convert some of the sugars to ethanol to create other flavour compounds. | Sugiyama (1984) |
| | *Zygosaccharomyces rouxii* | Produce alcohol and excellent flavour substances in a high salt concentration brine. | Sugiyama (1984) |
| | *Candida versatilis*, *Candida etchellsii* | - Produce excellent flavour substances in a high salt concentration brine.<br>- Over 270 flavour substances have been isolated. | Sugiyama (1984) |

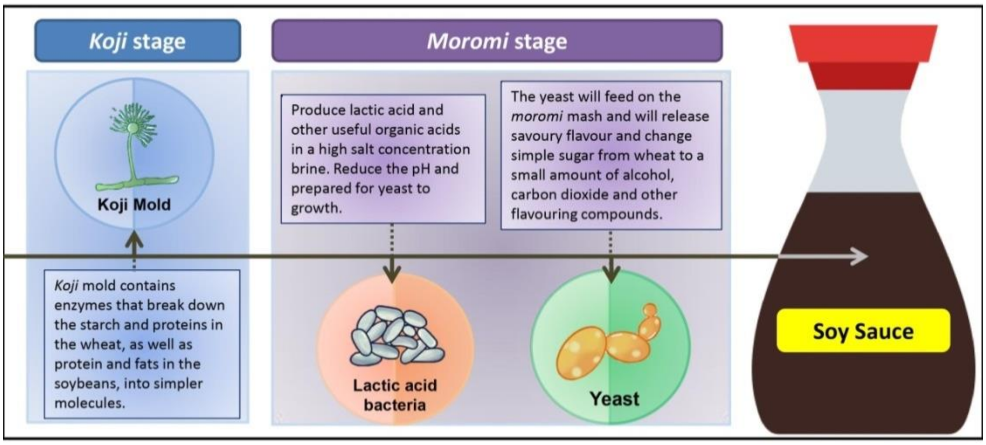


**Figure 2.** Three main microorganisms involved in soy sauce brewing.

The microbial community dynamics vary between the *koji* and *moromi* stages. *Koji* fermentation starts by soaking the soybeans in water overnight to soften them, and then beans' hulls are removed to eliminate fungal inhibitors in the soybeans; this is essential for the fungi to flourish during the *koji* fermentation. Additionally, during the soaking stage, the soybeans will start to ferment and indirectly lowers the pH of the soybeans. The low pH encourages fungal growth while inhibiting the growth of spoilage microorganisms (Santhirasegaram *et al.*, 2016). Several bacterial and yeast species have been found when the soybeans were soaked in the tap water; bacterial species included *Lactobacillus casei*, *Streptococcus faecium*, *Staphylococcus epidermidis*, *Streptococcus dysgalactiae*, *Klebsiella ozaenae*, *Enterobacter cloacae*, *Enterobacter agglomerans*, *Citrobacter diversus*, and *Bacillus breuis*, while yeast species included *Pichia burtonii*, *Candida diddensiae*, and *Rhodotorula rubra* (Devanthi and Gkatzionis, 2019). After soaking the soybeans overnight, they will be cooked at high temperatures, and this will eventually reduce the numbers of bacteria, yeasts, and moulds in the soybeans (Santhirasegaram *et al.*, 2016).

The next stage in soy sauce making is *koji* inoculation. According to Wei *et al*. (2013b), bacteria dominated the *koji*, notably LAB, followed by yeasts and moulds. *Lactobacillus* spp., *Staphylococcus* spp., and *Weissella* spp. have been found to be the most predominant bacteria in the *koji*, as compared to the other species. *Lactobacillus* spp. (*L. fermentum*, *L. iners*, and *L. plantarum*), *Staphylococcus* spp. (*S. arlettae*, *S. caprae*, *S. cohnii*, *S. gallinarum*, *S. kloosii*, *S. saprophyticus*, *S. succinus*, and *S. xylosus*), *Weissella* spp. (*W. cibaria*, *W. confusa*, *W. kimchii*, and *W. salipiscis*), *Streptococcus thermophilus*, *Lactococcus raffinolactis*, and *Leuconostoc mesenteroides* have also been found in the *koji* stage.

During the *moromi* fermentation, the microbial diversity varies over time. The microbial diversity reduces as the fermentation progresses, notably in the middle to late *moromi* fermentation (Wei *et al.*, 2013b; Zhang *et al.*, 2016; Yang *et al.*, 2017). This phenomenon occurs mainly due to high salt concentrations in the *moromi*, which eventually renders the *moromi* environment unfavourable to non-halotolerant bacteria, and inhibits their growth. Wei *et al.* (2013b) suggested that the *moromi* stage is dominated by bacteria, especially LAB, followed by yeasts and moulds. *Tetragenococcus halophilus* has been identified as the most common and predominant LAB during the *moromi* fermentation. This is mainly because *T. halophilus* is a salt-tolerant LAB that can survive in high salinity (Tanasupawat *et al.*, 2002; Tanaka *et al.*, 2012). Another study revealed that in high-salt dilute-state (HSDL) soy sauce, *Weissella* was found dominant. As the fermentation continued, the prevalent bacterial species evolved into *Weissella* and *Tetragenococcus* (Zhang *et al.*, 2016). Other bacterial species found in the *moromi* include *Lactobacillus* spp. (*L. fermentum*, *L. iners*, and *L. plantarum*), *Staphylococcus* spp. (*S. arlettae*, *S. caprae*, *S. cohnii*, *S. gallinarum*, *S. kloosii*, *S. saprophyticus*, *S. succinus*, and *S. xylosus*), *Weissella* spp. (*W. cibaria*, *W. confusa*, *W. kimchii*, and *W. salipiscis*), *Streptococcus thermophilus*, *Lactococcus raffinolactis*, *Leuconostoc mesenteroides*, *T. halophilus* strain KBC, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus* spp. (*B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, *B. methylotrophicus*, and *B. cereus* strain KBC), *Pediococcus pentosaceus*, and

*Pediococcus acidilactici* (Tanaka *et al.,* 2012; Yan *et al.*, 2013; Yang *et al.,* 2017; Wan-Mohtar *et al.,* 2020; Yee *et al.*, 2021). Among these bacterial species, *Bacillus* and *Staphylococcus* have been identified in both the *koji* and *moromi* stages (Wei *et al.,* 2013a; 2013b; Song *et al.,* 2015).

The second predominant microorganism present during soy sauce making, in both *koji* and *moromi* stages, is yeast (Wei *et al.,* 2013b). In the *koji* stage, *Candida catenulata*, *Candida glabrata*, *Candida rugosa*, *Candida tropicalis*, *Geotrichum silvicola*, *Kluyveromyces marxianus*, *Pichia anomala*, *Pichia fabianii*, *Trichosporon asahii*, *Trichosporon faecale*, *Trichosporon inkin*, *Trichosporon insectorum*, *Trichosporon japonicum*, *Trichosporon jirovecii*, and *Trichosporon ovoides* were detected (Tanaka *et al.,* 2012). However, these yeasts are only present in the *koji* stage, and are not found in the *moromi* stage. This implies that these yeasts have *koji*-specific roles in soy sauce making. For example, these yeasts may regulate the enzymatic activity of the *koji* moulds or control their growth. Therefore, they are absent in the *moromi* stage. Besides, according to Tanaka *et al.* (2012), *Kluyveromyces marxianus*, *Candida rugosa*, *Pichia fabianii*, *Candida glabrata*, and *Candida tropicalis* are the yeast species that are commonly found in plant fermentation, and have been related to flavour development when combined with LAB.

A dramatic fungal transition was observed in the *moromi* mash yeast analysis. The organic acid produced by LAB had increased the mash's acidity, thus allowing for acid-tolerant yeasts to flourish throughout the *moromi* fermentation. *Zygosaccharomyces rouxii*, a salt-tolerant and acid-tolerant yeast, was reported to appear in the early stage (Tanaka *et al.,* 2012), and proliferate in the middle to late stage of *moromi* fermentation when *Aspergillus sojae* started to diminish (Huang and Teng, 2004; Wei *et al.*, 2013b). Study shows that *Zygosaccharomyces rouxii* is the most dominant yeast present in the *moromi* mash. It is capable of alcoholic fermentation, hydrolysing different kinds of amino acids into their respective alcohols, and producing important aroma compound such as alcohol, ester, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF), and (isobutyl alcohol, isoamyl alcohol, 2-phenylethanol) 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) (Huang and Teng, 2004). After the alcohol fermentation is done by *Zygosaccharomyces rouxii*, *Candida etchellsii* and *Candida versatilis* were detected in the middle stage of *moromi* fermentation, while *Candida etchellsii* and *Torulopsis versatilis* were found in the late stage or maturation stage of the *moromi* fermentation (Tanaka *et al.,* 2012). Nunomura *et al.* (1980) have identified over 270 flavour compounds from soy sauce, and studies have showed that *Candida* spp. (or *Torulopsis* spp.) contribute to some of the excellent aroma and flavour compounds in high salt concentration brine (Wei *et al.,* 2013b). *Candida* spp. produce several aroma compounds such as 4-ethyl-guaiacyl phenol which gives the soy sauce a clove flavour, 4-ethylguaiacol (4-EG) and 4-ethyl phenol (4-EP) which gives a smoky flavour, and lastly 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF) which gives a caramel aroma (Tanaka *et al.,* 2012; Yang *et al.,* 2017). In short, among all the yeast species previously mentioned in both the *koji* and *moromi* stages, *Zygosaccharomyces rouxii* has been identified as the predominant yeast throughout the entire soy sauce fermentation (Wei *et al.*, 2013b).

According to Wei *et al.* (2013b), mould is the third predominant microorganism present during soy sauce making, in both *koji* and *moromi* stages, of which *Aspergillus oryzae* and *Aspergillus sojae* were found to be the most prevalent, producing extracellular enzymes such as proteolytic enzymes and saccharolytic enzymes. These enzymes will hydrolyse the soybean proteins into peptides and amino acids, and soybean carbohydrates into simple sugars, respectively (Liu, 2012).

*Microbial control during soy sauce fermentation*

Soy sauce, in general, has a high salt concentration, making it an unfavourable medium for microbial development (Yan *et al.,* 2013). Nevertheless, the *koji* fermentation is usually carried out in a non-sterile environment and under aerobic solid-state conditions which can often lead to microbial contamination. Therefore, to produce high-quality soy sauce, it is critical to prevent the *koji* stage from contamination. According to Jiang *et al.* (2019), *Lactobacillus pobuzihii*, *Aspergillus flavus*, and *Aspergillus parasiticus* will naturally contaminate the *koji* during the *koji* fermentation. Several *Bacillus* spp. have also been found to cause spoilage of the *koji*, and therefore, the resulting soy sauce. Sumague *et al*. (2008) reported that *Bacillus subtilis* contaminated the *koji* and competed with other *koji* moulds at high temperatures. Other researchers revealed that in both the *koji* and *moromi* stages, *Bacillus subtilis* and *Bacillus pumilus* were found in sour soy sauce (Devanthi and Gkatzionis, 2019). Researchers had isolated *Bacillus circulans, Bacillus maquariensis,* and *Bacillus pantothenticus* from sour soy sauce (Sumague *et al.*, 2008).

Considering the conditions of handling, incubation, and open-air storage, bacteria would simply infect the *koji* from air, dirt, hands, or even insects. Natural contamination is difficult to regulate and may even cause quality and safety risks. Traditional soy sauce brewing takes time, and due to this low efficiency in production, such contamination risks become much intense (Jiang *et al.,* 2019). Previous studies have highlighted the impact of microorganisms that were accidentally introduced into the *koji* stage during soy sauce production. When bacteria contaminate the *koji*, the *koji'*s pH will rise as a result of the ammonia released by the bacteria. This bacterial growth will inhibit the necessary *koji* mould growth, and subsequently suppress the *koji*'s enzyme activity (Takada, 2004).

After 20 - 24 h of *koji* fermentation, the number of bacteria reaches its peak, and after the *koji* moulds are spread, the bacterial number steadily declines. The drop in the number of bacteria has also been attributed to the reduction in the moisture content of the *koji* as suggested by Sano *et al.* (2007). Okuzawa (2003), on the other hand, suggested that the bacterial reduction could be due to the antibacterial compounds produced by the *koji* moulds. Therefore, the proper use of *koji* moulds that produce antibacterial compounds can achieve microbial control during *koji* production. The *koji* mould selection will enhance the quality of soy sauce brewing (Sano *et al.,* 2007).

During *moromi* fermentation, due to the microbial contamination by raw ingredients or from the surrounding environment, the *moromi* mash will easily get contaminated or spoiled (Wei *et al.,* 2013b). In order to mitigate this, the *moromi* mash will usually be submerged in 18 - 20% brine solution; the high salt content in the brine will create a high salinity environment which will then inhibit the growth of non-halotolerant microorganisms (Luh, 1995; Wei *et al.,* 2013b; Zhang *et al.,* 2016; Yang *et al.,* 2017). However, since the *moromi* surface is not submerged in the brine solution, and exposed to air, it could still be contaminated by ambient microorganisms (Wei *et al.,* 2013b). Another study reported that bacterial contamination primarily occurs in making low-salt soy sauce, as reducing the salt content in the soy sauce will allow more bacteria to grow and proliferate (Ferng *et al.*, 2020). The influence of salt content on microbial development is similar to other fermented products.

Yoon *et al.* (2008) and Nam *et al.* (2012) reported that *Enterococcus* spp. are opportunistic pathogens, but they also make up a substantial portion of the autochthonous microflora found in a variety of foods. *Enterococcus* spp. are most often found in traditional *moju* and other fermented soy products (Kim *et al.,* 2009; Nam *et al.,* 2012). Due to its strong glycolytic, lipolytic, and proteolytic activities, *Enterococcus* spp. add to the unique properties of soy products (Nam *et al.,* 2012). A strain isolated from soy products known as *Enterococcus faecium* did not contain virulence genes (Yoon *et al.,* 2008). Stute *et al.* (2002) and Yongmei *et al.* (2009) reported that the synthesis of BAs in soy sauce is assumed to be mainly attributable to the proteolytic *Enterococcus faecium.*

Staphylococci exist in various food items and environmental sources (Even *et al.,* 2010). *Staphylococcus* is the dominant microorganism in fermented seafood containing high salt content, thus demonstrating its high resistance to salt stress (Guan *et al.,* 2011). *Staphylococcus* has also emerged spontaneously during soy sauce manufacturing, especially in Japanese and Chinese soy sauce. However, in Korean soy products, it is rarely being detected (Kim *et al.,* 2009). *Staphylococcus* species such as *Staphylococcus epidermis* and *Staphylococcus xylosus* frequently possess enterotoxin-encoding genes, and could generate BAs (Even *et al.,* 2010). The emergence of enterococci and staphylococci by reducing salt might pose significant safety issues due to their potential contamination risk (Song *et al.,* 2015). To overcome this, Ferng *et al.* (2020) suggested that using ultra-violet treatment for fermenting low-salt soy sauce can effectively prevent microbial contamination. Meanwhile, Song *et al.* (2015) reported that adding *Torulaspora delbrueckii*, a strain that produces lots of ethanol, successfully suppressed the proliferation of putrefactive microorganisms present in the *moromi*, and by combining both *Torulaspora delbrueckii* with *Pichia guilliermondii*, a high-producing fuel alcohol strain, the *moromi* production resulted in a balance of more complex and richer taste with a flavour profile pattern similar to that of high-salt soy sauce.

*Asian traditional soy varieties and microbes involved*

Soybeans have been grown as a commercial crop for thousands of years. It was first cultivated in northern Asia, and then slowly expanded to North America (Hyten *et al.,* 2006). It is an essential plant-based protein source for millions of people and animal globally (Wan-Mohtar *et al.,* 2022). The bioactive compounds in soybeans provide various health benefits to humans such as antihypercholesterolaemic, chemopreventive, antihypertensive, antidiabetic, and immunomodulatory activities. As a result, soybean's market has been diversified to various soybean-based products worldwide (Naresh *et al.*, 2019). Generally, traditional soy-based foods are divided into fermented and non-fermented products (Kim *et al.*, 2011). This section will discuss the fermented soybean products in the Orient, and the overview of all the fermented soy products are highlighted in Table 2.

In China, there is a wide range of fermented soybean products such as *chee-fan*, *dou-chi*, *meitauza*, soybean milk, soy sauce, and *sufu*. *Chee-fan* is a soft cheese made from soybean and whey curd inoculated with *Mucor* spp. and *Aspergillus glaucus* for fermentation. It is traditionally consumed like cheese by the Chinese (Tamang *et al.,* 2015). *Dou-chi* is salted and fermented black soybean inoculated with *Aspergillus* sp. for about a month. It is also known as one of the popular seasoning agents among the Chinese worldwide (Chen *et al.*, 2005). *Meitauza* is a soybean cake inoculated with *Actinomucor elegans*, and people usually fry it in oil or cook it with vegetables. It is a popular dish or cooking ingredient in Taiwan and China (Nout *et al.*, 2007).

Soybean milk or soya milk or soy milk, is a beverage made from soybean extraction, and it resembles milk. It is first soaked and ground, then filtered through cheesecloth and boiled. Several varieties of soy milk are present in the market such as plain soy milk, dairy-like soy milk, soy milk soft drinks, cultured soy milk, soy milk infant formula, and soy milk blends. Cultured soy milk typically undergoes lactic acid fermentation by LAB (Shurtleff and Aoyagi, 2004).

Soy sauce is a dark brown liquid condiment made from soybean, wheat, salt, and water. As earlier discussed, soy sauce involves a two-step fermentation; *koji* and *moromi*. *Aspergillus oryzae*, *Aspergillus sojae*, *Lactobacillus* sp. and *Saccharomyces rouxii* are the microorganisms involved in soy sauce fermentation. Soy sauce is widely consumed and manufactured in Japan, China, the Philippines, and other oriental countries as a dipping sauce or cooking ingredient (Shurtleff and Aoyagi, 2012a).

*Sufu* or *furu* is a flavourful creamy bean paste made by fermenting the soybean curd with moulds followed by ageing or curing in saltwater or rice wine mixture. *Aspergillus elegans*, *Mucor hiemalis, Mucor silvaticus, Mucor subtilissimus,* and *Mucor sufu* are the moulds involved in *sufu* fermentation. *Sufu* can be classified into four types: red, white, grey, and sauce *sufu*, based on the different dressing ingredients used during *sufu* production. *Sufu* is usually being consumed as an appetiser and as well as a condiment (Han *et al.*, 2001, 2004).

In Japan, *natto*, *miso*, and *shoyu* (Japanese-style soy sauce) are the three most famous soybean fermented products. *Natto* is a traditional Japanese food consisting of fermented soybeans. There are three main types of *natto* available in the market which include *itohiki natto* (non-salted natto), *hama natto* (salted natto), and *yukiwari natto* (cracked natto). *Itohiki natto* is made from soybeans inoculated with *Bacillus natto*. *Hama natto* is made from soybeans with roasted wheat *koji* or barley *koji* inoculated with *Aspergillus oryzae, Streptococcus* sp. and *Pediococcus* sp. *Yukiwari natto* is made from fermented soybeans (*itohiki natto*) with rice *koji* inoculated with *Aspergillus oryzae* and *Bacillus natto.* All of them are usually eaten with rice or served as breakfast in Japan. Additionally, *natto* can also be used as topping for sushi, salad, or toast, especially *itohiki natto* and *yukiwari natto* (Shurtleff and Aoyagi, 2012b).

*Miso* is a traditional Japanese seasoning paste or soup base that is used in various Japanese cooking. It is made from soybeans, fermented rice, or barley *koji* and salt inoculated with *Aspergillus* sp., *Torulopsis etchellsii*, *Lactobacillus* sp. and *Saccharomyces rouxii.* The length of *miso* fermentation is similar to *shoyu*, which takes around six months to five years (Shurtleff and Aoyagi, 2012b).

In Indonesia, the examples of soybeans fermented products are *kecap*, *ketjap*, *taotjo*, *taoco*, and *tempeh*. Even though *kecap* and *ketjap* are both liquid condiments and flavouring agents, *kecap* is made from soybeans and wheat inoculated with

**Table 2.** Soy-fermented food history in the Orient.

| Product | Country / Region | Period | Substrate | End product form | Microorganism involved | Consumption / Role | Reference |
|---|---|---|---|---|---|---|---|
| *Chee-fan*  | China | Han dynasty (179 - 122 BC) | Soybean and whey curd | Solid | *Mucor* spp., *Aspergillus glaucus* | Cheese-like food, eaten fresh | Padmaja (1999) |
| *Dou-chi*  | China | 165 BC | Black soybean | Solid | *Aspergillus* sp. | Seasoning agent | Chen *et al.* (2005) |
| *Hama natto* (salted *natto*)  | Japan | Heian period (794 - 1185) | Soybean and roasted wheat *koji* or barley *koji* | Soft / raisin-like | *Aspergillus oryzae*, *Streptococcus* sp., *Pediococcus* sp. | Eaten with rice, as topping for sushi, salad, and toast, and also served as breakfast food | Kon and Ito (1974), Kiuchi *et al.* (1976), Blandino (2003), Shurtleff and Aoyagi (2012a) |
| *Itohiki natto* (non-salted *natto*)  | Japan | Heian period (794 - 1185) | Soybean | Soft / raisin-like | *Bacillus subtilis* var. *natto* | Eaten with rice, as topping for sushi, salad, and toast, and also served as breakfast food | Kon and Ito (1974), Kiuchi *et al.* (1976), Blandino (2003), Shurtleff and Aoyagi (2012a) |
| *Kecap*  | Indonesia | Mid-19th century (1830 - 1860) | Soybean and wheat | Liquid | *Aspergillus oryzae*, *Lactobacillus* sp. | Liquid condiment and flavouring agent | Lockwood and Smith (1950),Blandino (2003) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Ketjap*  | Indonesia | Mid-19th century (1830 - 1860) | Black soybean | Syrup | *Aspergillus oryzae* | Liquid condiment and flavouring agent | Lockwood and Smith (1950), Blandino (2003) |
| *Meitauza*  | China, Taiwan | 1937 | Soybean cake | Solid | *Actinomucor elegans* | Fried in oil or cooked with vegetables | Kronenberg and Hang (1984), Shurtleff and Aoyagi (1985), O'Toole (1999) |
| *Meju*  | Korea | 680 | Soybean | Paste | *Aspergillus oryzae*, *Rhizopus* sp. | Flavouring agent | Padmaja (1999), Shurtleff (2009) |
| *Miso*  | Japan | 800 - 900 | Soybean and rice | Paste | *Aspergillus* sp., *Torulopsis etchellsii*, *Lactobacillus* sp., *Saccharomyces rouxii* | Paste, soup base | Lockwood and Smith (1950), Shurtleff and Aoyagi (2012a) |
| Cultured soybean milk  | China, Japan | Han dynasty (179 - 122 BC) | Soybean | Liquid | Lactic acid bacteria | Beverage | Wang (1979), Blandino (2003), Shurtleff and Aoyagi (2004) |
| Soy sauce/ *Shoyu*  | Japan, China, the Philippines, and other oriental countries | Han dynasty (206 BC - 220 AD) | Soybean and wheat | Liquid | *Aspergillus oryzae*, *Aspergillus sojae*, *Lactobacillus* sp., *Saccharomyces rouxii* | Liquid condiment and flavouring agent | Shurtleff and Aoyagi (2012a) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Sufu / Furu*  | China, Taiwan | Wei dynasty (220 - 265) | Soybean whey curd | Solid | *Aspergillus elegans, Mucor hiemalis, Mucor silvaticus, Mucor subtilissimus, Mucor sufu* | Soybean cake and as a condiment | Han *et al.* (2001, 2004) |
| *Tao-si*  | The Philippines | 200 BC | Soybean and wheat | Solid | *Aspergillus oryzae* | Flavouring agent | Lockwood and Smith (1950), Shurtleff and Aoyagi (2011) |
| *Taotjo*  | East India, Indonesia | - | Soybean and roasted meal or glutinous rice | Solid | *Aspergillus oryzae* | Condiment and flavouring agent | Lockwood and Smith (1950), Shurtleff (2009) |
| *Taoco*  | West Java, Indonesia | - | Soybean and cereals | Solid | *Rhizopus oligosporus, Aspergillus oryzae* | Flavouring agent | Lockwood and Smith (1950), Shurtleff (2009) |
| *Tempeh*  | Indonesia and nearby regions | 400 | Soybean | Solid | *Rhizopus* sp. | Fried in oil or roasted; as a meat substitute | Djurtoft and Jensen (1977), Shurtleff and Aoyagi (1979) |
| *Yukiwari natto* (cracked *natto*)  | Japan | Middle of the Edo period (1603 - 1867) | Fermented soybean, *itohiki natto* with rice *koji* | Soft / raisin-like | *Aspergillus oryzae, Bacillus subtilis* var. *natto* | Served with rice | Kon and Ito (1974), Kiuchi *et al.* (1976), Blandino (2003), Shurtleff and Aoyagi (2012a) |

*Aspergillus oryzae* and *Lactobacillus* sp., while *ketjap* is made from black soybeans and inoculated with *Aspergillus oryzae*. Furthermore, *kecap* is in the form of liquid, while *ketjap* is in the form of syrup (Blandino, 2003). *Taotjo* and *taoco* are both flavouring agents and solid in form. *Taotjo* is made from soybeans and roasted meal or glutinous rice, while *taoco* is made from soybeans and cereals. The microorganisms involved during the fermentation of *taotjo* are *Aspergillus oryzae*, and for *taoco*, *Rhizopus oligosporus* and *Aspergillus oryzae*. *Tempeh* is a traditional Indonesian fermented soy-based food. It is in a cake form and was very popular on Javanese Island before slowly expanded worldwide. Unlike *tofu*, *tempeh* is made from cooked whole soybeans inoculated with tempeh starter *Rhizopus* sp., and fermented for 24 to 36 h to allow the spores to germinate and grow. The easiest way to cook *tempeh* is by deep-frying or stir-frying. *Tempeh* is also known as a meat substitution due to its high soy protein content (Joshi and Kumar, 2015).

There are several other soy fermented products from other countries such as *meju* from Korea and *tao-si* from the Philippines. *Meju* is a brick dried soybean that is made from soybeans inoculated with *Aspergillus oryzae* and *Rhizopus* sp. It is usually not consumed directly but used as the base for the Korean condiment or flavouring agents such as *doenjang* (soybean paste), *ganjiang* (soy sauce), and *gochujiang* (chilli paste) (Shurtleff, 2009). *Tao-si* is also known as salted black soybeans or soy nuggets. It is very similar to *dou-chi* from China, *hama natto* from Japan, and *taotjo* from Indonesia. The whole soybeans are first inoculated with *Aspergillus oryzae*, and then added into the brine solution to ferment and mature for about six months. *Tao-si* is not eaten alone, but typically used as a flavouring agent or condiment (Shurtleff and Aoyagi, 2011).

*Advances in soy sauce microbial fermentation*

Nowadays, soy sauce is commonly produced by three methods: traditional fermentation or brewing, chemical-hydrolysis or non-brewing, and a mixture of these. Traditional brewing involves microorganisms to carry out the fermentation, while chemical-hydrolysed soy sauce is produced by hydrolysing the soy protein with acid and heat. Chemical-hydrolysed soy sauce is also known as hydrolysed vegetable proteins (HVP) soy sauce, and created from soy protein that has been acid-hydrolysed by hydrochloric acid into amino acids and combined with sugars, colouring, and flavouring agents to create a sauce that resembles the naturally fermented soy sauce. This method is claimed to be cheaper and faster due to the lack of naturally fermentation steps. It can shorten the processing time from months to only one to three days. However, reports have proved that several unwanted compounds like 3-chloropropane-1,2-diol (3-MCPD) and 1,3-dichloropropanol (1,3-DCP) not produced during naturally fermentation are produced during the acid-hydrolysed reaction (Kataoka, 2005). According to a report from the International Agency for Research on Cancer (IARC, 2012), these compounds are categorised as carcinogenic, nephrotoxic, and reproductively toxic substances. Traditionally-brewed soy sauce provides the substances that benefit humans, and has more intense aroma and flavour as compared to HVP soy sauce.

Microorganisms are crucial in the fermentation of soy sauce. To isolate, identify, and classify bacteria, diagnostics and monitoring technologies have arisen in recent years. For decades, culture-dependent and culture-independent approaches have been regularly utilised to isolate bacteria found in the *moromi* stage (Tanaka *et al.*, 2012). However, in addition to being time-consuming and tedious, the culture-dependent method can only detect strains that can grow on nutritional media. As a result, the detection of additional bacteria that are difficult to develop on nutritional media is limited (Tanaka *et al*., 2012). In recent years, several advanced systematic microbial molecular analysis techniques involving rRNA base alignment and polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) have evolved significantly (Bano and Hollibaugh, 2002; Gray *et al.*, 2002; Norris *et al.*, 2002; Sekiguchi *et al.*, 2002; Crump *et al.*, 2003).

The PCR-DGGE approach has been widely employed in many sectors of microbial ecology including soil, sea, river, lake water, and wastewater treatment bioreactors to identify microbial community structure without cultivation, and to analyse community dynamics in response to environmental variables. Food microbiology has just begun to use it as well. Molecular approaches have been used by several research groups to discover and analyse the microorganisms found in food samples such as *shochu*, vinegar, soybean paste, wine, and *kimchi* (Cocolin *et al.*, 2000; Endo and Okada, 2005; Lee *et al.*, 2005a; Kim *et al.*, 2009; Okazaki *et al.*, 2010).

Clone libraries, 16 rRNA gene sequencing, and pyrosequencing are some of the other molecular approaches utilised to investigate microbial diversity in soy sauce production (Tanaka *et al.*, 2012; Yan *et al.*, 2013; Yang *et al.*, 2017; Sassi *et al.*, 2021; Yee *et al.*, 2021). These techniques aid researchers in gaining a better knowledge of the microorganisms involved in soy sauce production and identifying new microbial strains that can assist to improve soy sauce quality. Researchers can now identify the numerous microorganisms present during the *koji* and *moromi* stages using these technologies.

The following subsections discuss in depth the advances elicited by the various microorganisms involved in soy sauce fermentation, which are then summarised in Figure 3.

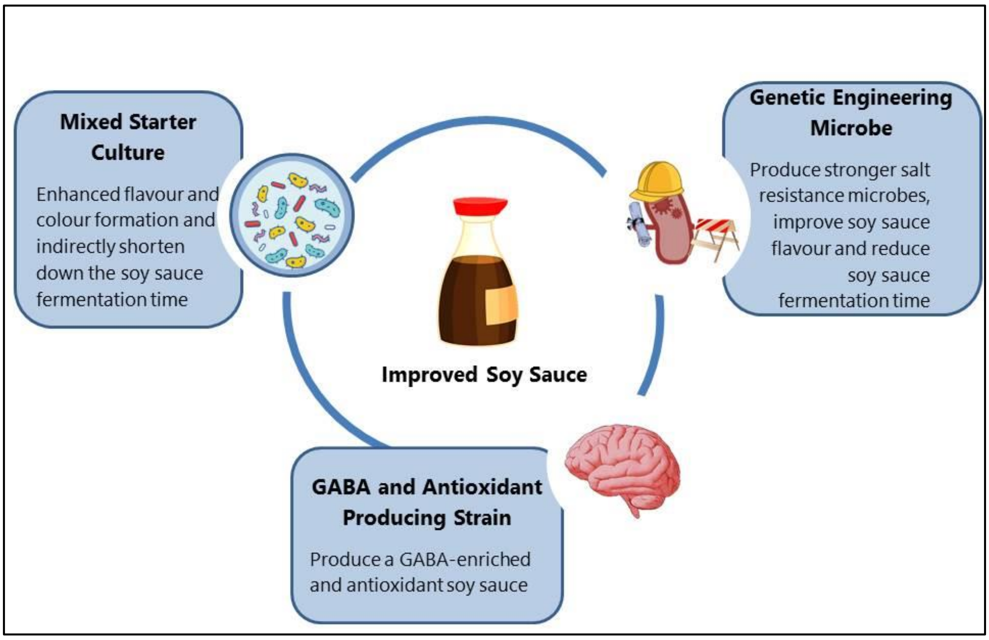


**Figure 3.** Improvement in soy sauce fermentation.

*Enhancement of GABA and other beneficial compound in soy sauce fermentation by microorganisms*

Fermentation has long been used to produce bioactive compounds that are beneficial to both consumers and industries. Over the years, researchers have found several ways to improve and enhance the functionality of soy sauce which includes chemicals that can help lower cholesterol levels, antihypertensive component, antioxidant, anti-carcinogenic properties, antimicrobial activities, anti-inflammation, gastric acid secretion, anti-allergy, and antiplatelet activities.

One such way to enhance the functionality of soy sauce is by enhancing the gamma-aminobutyric acid (GABA) production during soy sauce fermentation. The non-protein GABA is a prominent and influential inhibitory neurotransmitter in mammal's central nervous systems. Not only that, GABA is also claimed to be antihypertensive, anticancer, anti-inflammation, antidiabetic, anti-allergy, and have diuretic and tranquilliser effects, thus making it an excellent candidate for use in functional foods. GABA can be naturally produced during soy sauce fermentation either by breaking down the material proteins by proteases or peptidases, or by hydrolysing free L-glutamines by glutaminase.

According to Ab Kadir *et al.* (2016), four potential *koji* starter strains known as *Aspergillus oryzae* NSK, NSZ, NSJ, and NST were found to produce GABA during soy sauce fermentation, with *Aspergillus oryzae* NSK producing the highest (194 mg/L). In another study done by Hajar-Azhari *et al.* (2018), they found that when using 50 g/L of glucose cane molasses instead of native sugar syrup, sugarcane, and nipa as the fermentable substrate, *Aspergillus oryzae* NSK was able to produce the highest GABA concentration (354.08 mg/L). Wan-Mohtar *et al.* (2019) optimised seven key *Aspergillus oryzae* NSK culture parameters to produce the highest GABA yield (3,278.31 mg/L) in SmF; initial pH 5, temperature of 30°C, sucrose concentration of 100 g/L, a combination of yeast extract (YE) and glutamic acid (GA), and carbon to nitrogen ratio of C8:N3.

Apart from *koji* moulds, recent studies also found that certain bacterial strains originally found in the soy sauce *moromi* could produce GABA. Wan-Mohtar *et al.* (2020) had successfully isolated, identified, and optimised a novel high GABA-producing bacterial strain known as *Bacillus cereus* strain KBC in SmF. The isolated strain was capable of producing 523.74 mg/L of GABA under unoptimised conditions, while under optimised conditions (initial pH 7, 5 g/L of MSG concentration, at 40°C), the strain was able to produce 6.37-fold higher GABA (3,393.02 mg/L). Furthermore, another new bacterial strain known as *Tetragenococcus halophilus* strain KBC found by Yee *et al.* (2021) was claimed to be a new GABA-producing strain. In this study, *Tetragenococcus halophilus* strain KBC (TH)-*moromi* cultured in 20% molasses produced higher GABA (159 mg/L) when compared with *Bacillus cereus* strain KBC (BC)-*moromi* cultured in 20% molasses (118 mg/L). Interestingly, the GABA produced with the combination of BC:TH *moromi* in 5%:20% molasses was much lower (83 - 137 mg/L) when compared with singular 20% TH-*moromi*. Still, this combination of BC-TH *moromi* shortened the *moromi* fermentation period from 60 to 30 days.

Furthermore, previous research has shown that fermented soybean products are superior to raw soybeans in terms of antioxidant activity and phenolic content (Xu *et al.*, 2015). Other antioxidant-like compounds identified during soy sauce fermentation include melanoidin, phenolic acid, isoflavones, pyranones, and furanones. A mixed culture of fungi and bacteria not only helps to create a significant amount of bioactive phenolic compounds, but also helps to increase the antioxidant characteristics of soy sauce. Researchers discovered that combining fungal (*A. oryzae* J, *Mucor racemosus* 15, and *Mucor racemosus* 42) and bacterial (*B. subtilis* TKSP24) strains in a mixed starting culture improved antioxidant activity in *doenjang* samples. With 59.92% scavenging activity for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, and 92.83% inhibitory rate for nitric oxide radicals, this mixed culture demonstrated excellent antioxidant activity. Furthermore, the starting bacteria produced a significant amount of bioactive phenolic content, ranging from 25.06 to 29.49 mg/g gallic acid equivalents (Shukla *et al.*, 2016). Using a combination starting culture of *A. oryzae* HG-26 and *A. niger* HG-35 during *moromi* fermentation resulted in improved antioxidant activity, which was 12% greater on day 120 than using *A. niger* HG-35 alone, according to Peng *et al.* (2017).

*Enhancement of soy sauce flavour by mixed starter culture*

Mixed starter culture used in soy sauce fermentation is made up of two or more microorganisms; this is necessary for flavour development and enhancement as compared to single starter culture. The most commonly mixed starter culture used in *moromi* fermentation are the LAB *Tetragenococcus halophilus* and the yeast *Zygosaccharomyces rouxii* (Luh, 1995). Salt-tolerant yeasts such as *Zygosaccharomyces rouxii*, *Candida versatilis,* and *Candida etchellsii* have all been implicated in forming volatile flavour compounds (VFCs) during soy sauce fermentation (Cui *et al.*, 2014). Flavour compounds include ethanol, furanone, esters, aldehydes, acids, pyrone, and phenols. Differences have been noted in the total VFCs in the *moromi* when the starter cultures were single, mixed, or sequential (inoculated one after the other). For example, as previously discussed, the single use of *Torulaspora delbrueckii* JBCC-623 in low-salt soy sauce produced high ethanol and inhibited putrefactive microorganisms' growth. However, when *Torulaspora delbrueckii* JBCC-623 was combined with *Pichia guilliermondii*, more complex flavours were created, much similar to the flavour profile of high-salt soy sauce (Song *et al.*, 2015). In addition, Singracha *et al.* (2017) indicated that by adding *Tetragenococcus halophilus* TS71, *Zygosaccharomyces rouxii* A22, and *Meyerozyma* (*Pichia*) *guilliermondii* EM1Y52 into the *moromi*, higher amounts of key VFCs such as ethanol, 2-methyl-1-propanol, 4-hydroxyl-2,5-dimethyl-3(2H)-furanone (HDMF), and 3-hydroxy-2-methyl-4H-pyran-4-one (maltol) were produced, while lower amount of BAs was observed as compared to other treatments. Furthermore, the co-culture of *Zygosaccharomyces rouxii* and *Pichia guilliermondii* in the *moromi* produced more VPCs such as alcohols, furanone, esters, maltol, and benzoic acid as compared to single culture of only *Zygosaccharomyces rouxii* (Wah *et al.*, 2013).

*Enhancement of soy sauce flavour by genome shuffling starter culture*

The high salt content in the *moromi* makes it unfavourable for non-salt-tolerant microorganisms to grow. Therefore, lengthy fermentation is required to achieve sufficient flavour formation. It is thus crucial to employ genetic engineering techniques to produce high salt-tolerant microbial strains. Genome shuffling is a technique in which it shuffles multiple genes and recombines the whole genome to produce a novel and desired strain. Cao *et al.* (2012), Wei *et al.* (2013c), and Qi *et al.* (2016) have proved that the genome shuffling technique could increase the salt-tolerant properties of *Zygosaccharomyces rouxii* and subsequently increased the flavour formation in soy sauce. S3-2 is a *Zygosaccharomyces rouxii* mutant strain possessing better properties than the original strain. S3-2 could survive in the medium that contains high salt content, and against potassium chloride and lithium chloride, showing excellent resistance. Besides, S3-2 could also provide high level of amino acid nitrogen as well as a good flavour than the control. Flavour compounds produced included ethyl acetate, 4-hydroxy-2 (or 5)-ethyl-5 (or 2)- methyl-3(2*H*) –furanone and 4-ethylguaiacol. In short, S3-2 is able to enhance and accelerate the flavour development in soy sauce, and shorten the fermentation period (Cao *et al.*, 2012; Wei *et al.*, 2013c).

S3-5 is a mutant strain of *Candida versatilis*, and produced using the same genome shuffling technique. S3-5 had higher stress resistance against potassium chloride, lithium chloride, and sodium chloride. S3-5 also improved the soy sauce aroma by producing much higher ethanol and other main aroma compounds such as 4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3 (2H)-furanone (HEMF) as compared to the control (Cao *et al.*, 2010).

H3-8 is a mutant strain of *Hansenula anomala*, also produced using genome shuffling technique. Similarly, H3-8 showed better resistance against salts, and had a high survival rate in the YPD media that contained high sodium chloride content and within a wide range of pH. H3-8 produced high hydroxyethylmethylfuranone, ethyl acetate, and 4-ethylguaiacol amounts as compared to the control (Cao *et al.*, 2012).

Although this genetic engineering technique has improved the ability of the strains in terms of salt-resistance and flavour formation, no research has yet been published on its influence on industrial application. Concerns, however, arise on the genetically engineered strain’s safety such as human and environmental impacts, ethics, and food security. Therefore, to improve the present production process, to produce high-value and healthy food products, and to overcome the risks and problems, more studies and research are required.

## Conclusion

Due to their diversity, purported bioactive components, and health advantages, some fermented Asian foods of soy origin have been vital dietary for generations, and are now garnering attention and spreading to the Western hemisphere. Soy sauce, for example, is an important condiment with a large global market. Fermented soy meals are the result of complex and diverse microbial communities and interactions, and their quality and flavour are likely to be affected by this microbial component. Further study is therefore needed to determine and manage the effects of desirable bacteria, as well as to identify and avoid the unwanted ones.

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