# 秋田県産発酵調味料（味噌・醤油）の品質特性の解明および その品質評価法の開発に関する研究

# Studies on the Quality Characteristics of Fermented Seasonings (Miso and Soy sauce) from Akita Prefecture and Development of Quality Evaluation Methods

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# Studies on the Quality Characteristics of Fermented Seasonings (Miso and Soy sauce) from Akita Prefecture and Development of Quality Evaluation Methods

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# ABSTRACT

Soy sauce and miso are two traditional Japanese fermented staple condiments and are consumed for the preparation of Japanese cuisine for hundreds of years. The traditional Japanese soy sauce and miso are made from soybeans, grains, salt, and water. According to a long period of fermentation, microbial fermentation and enzymatic activity occur, resulting in color, amino acids, sugars, organic acids, and flavor compounds. Although soy sauce is a liquid whereas miso is a soybean paste, they have similar aroma and flavor due to their fermentations are almost the same. Both soy sauce and miso are typically used to improve the color, flavor, and taste of the cooked cuisines. These two fermented foods increasingly consumed around the world. However, the quality of Japanese fermented soy sauce and miso products were mainly determined by traditional sensory evaluation using a panel of human assessors so the quality of products is described in long hours and subjective terms, and thus cannot satisfy the production management needs of food manufacturers. There is a lack of understanding of the final quality for Japanese fermented soy sauce and miso products as well as effective methods for rapidly assessing their final qualities.

The research described in this thesis sought to: I) analyze the chemical composition that related to the color and taste to find the relationship between the chemical composition and sensory evaluation then clarified the essential chemical characteristics for soy sauce and miso products produced in the Akita area; II) investigate the correlation between the volatile compounds and sensory evaluation to clarify the effects of flavor compounds on the final qualities of soy sauce and miso, respectively; III) investigate the feasibility of the predictive models developed by using the measured chemical composition and volatile compounds in order to assess the final qualities of soy sauce and miso products, respectively; IV) develop a practical technique for rapid assessment of the final qualities for soy sauce and miso, respectively, with the aim to meet the accuracy and real-time capability for industrial spots.

**In Chapter 1**, the research background and a general literature review that stated the knowledge of soy sauce and miso, their fermentation processes for the formation of chemical composition and flavor compounds, the effects of constituents on the final qualities, and the current research on quality assessment methods for soy sauce and miso.

**In Chapter 2**, a wide range of soy sauce and miso samples were collected from the Akita Prefectural Miso and Soy Sauce Products Competition held in 2016, 2017, and 2018 by the Akita Prefectural Miso and Soy Sauce Manufacturer Cooperative (Akita, Japan). The quality of each collected sample was assessed by sensory evaluation and then these samples were ranked annually according to their sensory scores. The chemical compositions of soy sauce and miso collected were analyzed for color, salt, soluble salt-free solids, acids, carbohydrates, amino acids, pH, and moisture. The relationship between the chemical compositions and the results of sensory evaluation was then determined. Finally, the measured chemical compositions for all of soy sauce and miso samples were further summarized and subjected to principal component analysis (PCA) to determine the chemical compositions that most contributed to the final quality of the tested soy sauce and miso samples, respectively.

Results showed that $a^*$ values and soluble salt-free solids content, as well as the content of glucose, ethanol, phosphoric acid, acetic acid, and the total amino acids, contributed to the quality improvement of soy sauce products; while the salt content showed a negative effect on the final quality of soy sauce products. Particularly, the level of proline in soy sauce were found contribute significantly to the improvement of final quality. PCA results obtained from soy sauce samples is in accordance with the previous findings, and also highlighted some acids and amino acids which were not very obvious before. With regard to the tested miso samples, there is an obvious difference in color between different sensory qualities of miso samples, with higher $a^*$ values found to improve the quality, while the other two parameters showed the opposite effect. The level of glucose was found to negatively correlate to the final quality of miso samples might be due to the consumption of glucose to produce other beneficial substances during fermentation. Additionally, there was an optimized range from 35% to 40% for the soluble salt-free solids content of miso, and the optimal salt content was about 12%. PCA results of miso samples from 2016 to 2018 confirmed the previous results.

Based on the results obtained from this study, the different roles and reasons for the chemical characteristics of the final qualities of both soy sauce and miso products produced in the Akita area were identified. This provided the novel classification information for both soy sauce and miso products and allow optimization of their final qualities. This is a part of a wider project for developing an objective tool complementary to the sensory evaluation, which can be applied to routine use.

**In Chapter 3**, the effects of different volatile compounds on the flavor characteristics of Japanese fermented soy sauce and miso produced in the Akita area were investigated. Sensory evaluation ranked the overall sensory scores of soy sauce and miso samples manufactured in 4 consecutive years (2015-2018). To reduce the steps in sample preparation and to minimize any interference, the volatile compounds in the tested soy sauce and miso samples were analyzed by extracting them directly from the headspace. The different roles and relationships of the determined volatile compounds on the aromatic composition of the tested samples were identified. Finally, PCA was performed to gain a comprehensive understanding of the differences in volatile compounds between the tested samples.

Results showed a total of 62 peaks were detected by GC-MS analysis from the tested soy sauce samples, including ten aldehydes, ten ketones, nine furan(one)s, seven alcohols, seven esters, six sulfur-containing compounds, five pyrazines, three phenols, and other two compounds that divided into a group named "others". Among them, 35 volatile compounds were detected in all samples thus considered to be common in soy sauce. The effect of these 62 volatile compounds on the final qualities of the tested soy sauces were then investigated, respectively. Particularly, 19 detected volatile compounds were found to play a positive role in the quality improvement of soy sauce, whereas 23 detected volatile compounds deteriorated the final quality of soy sauce samples produced in the Akita area. The statistical analysis of the data using PCA confirmed the effect of the volatile compounds on soy sauce in the Akita area suggested in this study.

With regard to the miso samples, results showed forty-eight volatile compounds were detected from the tested miso samples evaluated in the annual competition from 2015 to 2018, and all were positively identified, including nineteen esters, nine aldehydes (including furan-2-carbaldehyde), eight alcohols, four ketones, three acids, two sulfur-containing compounds, and three others. Of these forty-eight volatile compounds, twenty-one were present in all the miso samples tested. Most volatile compounds in miso showed a positive correlation between their concentrations and the results of sensory evaluation. There are almost no compounds showed a significantly negative correlation with the quality improvement, indicating the high-quality miso products were correlated to an intense flavor. During PCA analysis, most volatile compounds found in miso samples contributed positively to the PC1, except for pentanal, ethyl 2-hydroxypropanoate, and 2-phenylethyl acetate, thus in good agreement with the previous findings.

Based on the results obtained from this study, new information for classifying soy sauce and miso products and to allow the optimization of their sensory quality was provided. The results of this study will not only provide valuable information for investigating the flavor characteristic of both soy sauce and miso products produced in the Akita area but also provide guidance for the soy sauce industry to improve the final product quality. It was promising to combine the studies of chemical compositions and volatile compounds for developing an objective tool for routine use that can complement sensory evaluation for soy sauce and miso products.

**In Chapter 4**, on the basis of understanding the chemical and flavor characteristics for soy sauce and miso products produced in the Akita area, an investigation into the potential of the development of predictive models by using the relevant sensory variables as a simple technique for the rapid determination of the final qualities of soy sauce and miso products was conducted using partial least-squares (PLS) regression. The relevant variables were selected by calculating the contribution of each variable to the sensory scores through the Compressed Sensing (CS) theory.

A total of 74 parameters that related to the overall acceptance of soy sauce including physical, chemical, and volatile compounds, were measured. Sensory evaluation ranked the overall sensory scores of samples manufactured in 3 consecutive years (2016-2018). The contribution of these 74 parameters to sensory scores was measured using CS-based method. Subsequently, 30 predictive variables shown to affect the quality of soy sauce were successfully selected when the tolerated error $\epsilon$ was set as 0.05. Using these 30 predictive variables to establish the predictive model for the final quality of soy sauce, the evaluation results showed the values of determination coefficient ($R^2$) and the root-mean-square error of prediction (RMSEP) obtained for the validation samples were 0.80 and 11.47, respectively. This indicated the obtained model provided a reasonable accuracy for determining the final quality of soy sauce products.

On the other hand, a total of 87 variables that related to the sensory quality of miso products were measured. The contribution of each variable to the sensory score was also calculated using CS-based method, thus, 32 variables were selected and used to develop the predictive model using PLS regression. The miso products produced in 2016 and 2017 were selected into calibration sample set, and miso products produced in 2017 were selected into validation sample set and used for model evaluation. The obtained results showed that the values of $R^2$ and RMSEP for the obtained model with 2018 miso products were 0.64 and 21.14, respectively.

Based on the results obtained from this study, the developed predictive models provided an objective tool for routine use, during the production of soy sauce and miso products in the Akita area, which can complement sensory evaluation. Moreover, this is the first time used the CS-based method as a variable selection approach for developing a predictive model in foods, the superiority of the CS-based method on selecting variables was also confirmed.

**In Chapter 5**, with the aim to furtherly develop a rapid and simple method for the quality assessment of soy sauce and miso products manufactured in the Akita area, the near-infrared (NIR) spectroscopy technology was applied. The advantage of NIR models is to estimate the final quality of soy sauce or miso products without any sample preparations. The models were constructed on the calibration data set (soy sauce samples from 2016 and 2017, sample numbers = 76; miso samples from 2016 and 2017, sample numbers = 77), the validation set (soy sauce samples from 2018, sample numbers = 34; soy sauce samples from 2018, sample numbers = 38) was used to evaluate the performance of the constructed models.

Results showed results showed that the models constructed using the full spectra region performed a certain accuracy for the prediction of the sensory quality of soy sauce and miso samples. Comparing the influence of different regions in the acquired spectra enabled the accuracy of the models to be improved. The optimal model for the quality prediction of soy sauce samples was the model that involves standard normal variate (SNV) and first derivative in the wavelength range of 2050-2400 nm of transflectance spectra, with the $R_P^2$ of 0.78 and the RMSEP of 11.13 for validation. Similarly, the most accurate model for assessing the final quality of miso was constructed in the region of reflection spectra from 400 to 1100 nm, with the value of $R_P^2$ and RMSEP were 0.56 and 24.80, respectively. Based on the results of this study, it is believed that the use of NIR spectroscopy directly on monitoring the final product quality in the field of fermented foods in the Akita area was of great interest to the food manufacturers.

**In Chapter 6**, a general conclusion, limitations, and a suggestion for future research to this study are included. On the whole, the results presented in chapters 2, 3, 4, and 5 comprising this work can serve as a guide to current literature for those who wish to understand the chemical and flavor characteristics of fermented soybean foods in Japanese traditional way, and also developed two types of model for monitoring the final qualities of soy sauce and miso products that produced in Akita area.

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Shuo Wang

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# LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| > | Greater than |
| < | Less than |
| = | Equals |
| ≥ | Greater than or equal to |
| ± | Plus or minus |
| + | Addition |
| / | Per |
| – | Minus |
| × | Times |
| & | and |
| ℃ | Degree Centigrade |
| µL | Microliter |
| µm | Micrometer |
| µmol | Micromole |
| DHS | Dynamic headspace |
| DSE | Direct solvent extraction |
| e.g. | Exempli gratia (for example) |
| et al. | Et allii (and others) |
| etc | Et Cetera |
| FD | Flavor dilution |
| g | Gram |
| GC | Gas chromatography |
| GC-MS | Gas chromatography-mass spectrometry |
| h | Hour |
| $H_2O$ | Water |
| HPLC | High performance liquid chromatography |
| i.d. | Internal diameter |
| i.e | id est |
| kg | Kilogram |
| L | Liter or lightness |
| Ltd | Limited |
| m | Meters |
| mg | Milligram |
| min | Minutes |
| mL | Milliliter |
| mm | Millimeters |

| | |
|---|---|
| mM | Millimolar |
| mmol/L | Millimole per liter |
| MRL | Multiple linear regression |
| N | Normality |
| nd | Not detected |
| NIR | Near infrared spectroscopy |
| No. | Number |
| nm | Nano meter |
| O | Oxygen |
| OVA | Odor Activity Values |
| PC | Principle component |
| PCA | Principle component analysis |
| PLS | Partial least squares |
| PRC | Principle component regression |
| $r$ | Correlation coefficient |
| $R^2$ | Coefficient of determination |
| RMSEC | Root mean square error of calibration |
| RMSECV | Root mean standard error of cross validation |
| RMSEP | Root mean standard error of prediction |
| rpm | Revolution per minute |
| SD | Standard deviation |
| SEC | Standard error of the calibration |
| SECV | Standard errors of cross-validation |
| SNV | Standard normal variate |
| SPME | Solid-phase micro-extraction |
| SPSS | Statistical Package for the Social Sciences |
| SSFSC | Soluble salt-free solids content |
| USA | United States of America |
| v/v | Volume to volume |
| V-SDE | Vacuum simultaneous steam distillation-solvent extraction |
| w/w | Weight to weight |

# CHAPTER 1 LITERATURE REVIEW

## 1.1 Soy Sauce and Miso

Nations throughout the world have their own fermented foods and beverages. The people in the Orient mastered the techniques of cultivating the molds on the raw materials to produce appealing and nourishing fermented foods from ancient times. For daily cuisine, eating a proper amount of fermented products every day is considered safe for people. The various fermented products have shown to support several health benefits, such as improving digestion and absorption, antioxidant, antidiabetic, anticancer, and boosting the immune system (Byun & Mah, 2012; Giri et al., 2012; Kung, Tsai, & Wei, 2007; Shimizu et al., 2015; Yamabe et al., 2007; Ye, Dudley, & Shaw, 2017; Yu et al., 2014; Zaid & El-Shenawy, 2010). It is also showed that the people in the Orient had a lower incidence of diabetes compared with those in western countries due to the consumption of fermented soybean products in the Orient (Kwon et al., 2010). The fermented foods, especially for the product derived from soybean, had received increasing attention for now. Moreover, various countries in Orient used fermented foods as condiments. For example, *doenjang* (a fermented soybean paste) is popular in South Korea; China utilized *douchi* (a traditional soybean-fermented food), *doubanjiang* (chili bean paste), and *tianmianjiang* (sweet bean paste) in daily dishes; and other Southeast Asian countries also prepared different fish soy as condiments.

In Japan, there is a very long history of utilizing *koij* (*Aspergillus oryzae*, a filamentous fungus) to produce various kinds of fermented foods, such as soy sauce, mirin (sweet rice wine), su (rice vinegar), and miso (fermented soybean paste) (Rai & Kusumoto, 2017). Among these fermented foods, soy sauce and miso are two typical traditional fermented condiments with soybean cultivation. Both soy sauce and miso had a close relationship with *Washoku* (the traditional dietary cultures of the Japanese), which was inscribed on the List of Intangible Cultural Heritage established by United Nations Educational Scientific and Cultural Organization (Kumazawa et al., 2018). *Washoku* encompasses the regional and traditional foods of Japan, which consists of rice with miso soup and other dishes, such as *sashimi* and *sushi* (seafood), *tempura* (seafood and vegetables deep-fried in a light batter), *soba* and *udon* (noodles), and so on. The key element for the preparation of *Washoku* to achieve a palatable umami taste was contributed by the condiments used, mostly soy sauce and miso. Against this background, both soy sauce and miso have been essential Japanese seasonings according to their characteristic flavor, aroma, and taste, and have also been developed through cultural fusion.

It is said that the fundamental principles of the production of soy sauce and miso was introduced from China and Korea to Japan over 1000 years ago, then the Japanese made technological innovation in the manufacturing of the fermented soybean condiments in 400 years ago and it is still in use today (Yokotsuka & Sasaki, 1997). Japanese fermented soy sauce is the most well-known Japanese condiment and in recent times has become widely consumed not only in Asian countries, but also around the world (Chavasit & Photi, 2018). Soy sauce (*shoyu* in Japanese) was invented in China and the first documentation related to soy sauce has more than 3000 years of history (Yokotsuka & Sasaki, 1997). Firstly, *chu*, the same as *koji* in Japanese was found in *Shu-Ching* about 3000 years ago, then the more detail information was written in *Shih-Chi*, *Chi-Min-Yao-Shu*, *Bei-Shau-Chiu-Ching*, and more, the first written record about the soy sauce production was found in *Ben-Chao-Gong-Mu* (AD 1590) (Yokotsuka, 1986). From China, the production of soy sauce was introduced to Japan by Buddhism, and to other Southeast Asian countries over 1000 years ago (Fukushima, 2004; O'toole, 2019). *Hishio*, which is derived from fish and salt, is considered the antecedent of the *shoyu* and miso. It is speculate that the change of *hishio* raw materials form fish or meat to beans or grains was due to the principle of Buddhism of abstaining from meat foods. The production method and production scale of manufacturing soy sauce have made great progress in the Edo era in Japan. For now, soy sauce was widely used as an all-purpose seasoning to deliver intense umami, salty sensation and characteristic aroma to the Japanese diet, including for sushi, sashimi and grilled foods.

According to the Japanese Agricultural Standard (JAS), the genuine fermented soy sauces produced in Japan were divided into five types, that is, *koikuchi shoyu*, *usukuchi shoyu*, *tamari shoyu*, *saishikomi shoyu*, and *shiro shoyu*. *Koikuchi shoyu*, i.e. dark soy sauce, accounted for approximately 85% of all Japnese soy sauces. This type of soy sauce was originated in Japan's Kanto region, then has come to be used all over the country, and further become popular around the world, to the present day. The soy sauce produced in Akita area mainly belonged to *koikuchi shoyu*. The production of *koikuchi shoyu* uses soybeans and wheat in equal amounts and the products after aging needs to take pasteurization at a rather high temperature (about 80°C) resulting in the dark reddish brown color and strong heat flavor for the final products (Kaneko, Kumazawa, & Nishimura, 2013; Kataoka, 2005). A mixture containing more amount of soybeans and less amount of wheat are used as starting materials for *usukuchi shoyu* (light-colored soy sauce), and nearly accounts for 13% of all Japanese soy sauce consumed. *Usukuchi shoyu* were originally from Japan's Kansai region. A higher level of salt was added for the manufacturing process of *usukuchi shoyu* compared with *koikuchi shoyu*, causing the final product both saltier and less strongly fermented than most other soy sauces. Sometimes, the saccharified rice *koji* with water is added to *usukuchi* mash to mitigate the salty. *Tamari shoyu* (aged soy sauce) is a darker type than *koikuchi shoyu*, and mostly consumed in Japan's Chubu region. The raw materials for the production of this type of soy sauce were mainly soybeans, only a little or without wheat was used. Thus, the final products were imparted with an intense umami flavor that nicely suitable for sushi, sashimi, and grilled foods. In contrast, *shiro shoyu* (white soy sauce) is produced mostly from wheat and only a little soybean. This kind of soy sauce are imparted with a mild and uniquely sweet flavor. The special part of *shiro shoyu* was to take advantage of the flavor of soy sauce without the color change during cooking, such as soups. The consumption of *shiro shoyu* and *saishikomi shoyu* only accounts for 1% of total soy sauces produced in Japan, respectively. Unlike other soy sauces, *saishikomi shoyu* is made by fermenting the sauce twice. Therefore, the price of this soy sauce was higher and used particularly for sushi and sashimi.

Miso is a paste-like half-solid product that is a fermented and aged mixture of cooked soybeans and mold rice, barley or wheat (Hitomi, 1994; Inoue et al., 2016; Ogasawara, Yamada, & Egi, 2006), and salt. It is used commonly as a seasoning in cooking soup and in side dishes with various kinds of vegetables, even as a broth for certain types of noodles for over 1000 years (Hideo, 2004; Nikkuni, Okada, & Itoh, 1988). According to historical records, miso appears in Japan earlier than soy sauce. The predecessor of the miso named *koma* (meaning Korea) –*sho* is introduced from Korea. In the later period, the production technology of miso was also influenced by Chinese fermented foods, which gradually formed the current miso products (Yokotsuka & Sasaki, 1997). From the difference of raw materials of *koji* used, miso products can be roughly classified into three types: (i) rice miso, made by adding rice *koji* to soybeans; (ii) barley miso, made by adding barley *koji* to soybeans; and (iii) soy miso, made using soybeans only (Abiose, Allan, & Wood, 1982; Hesseltine, 1983). The most common type of miso is rice miso, accounting for 80% of total miso produced in Japan and having the shortest fermentation period (Kitamura et al., 2016). Interestingly, because the people live in different environments and locations of Japan, there is a different tradition of dietary culture for the production of varieties of miso. The rice miso can be subdivided into six types according to taste and color, namely red salty, yellowish salty, white sweet, red sweet, brown, and white. Red salty rice miso is the most popular type in northern Japan (Inoue et al., 2016). Akita Prefecture in northeast Japan is famous for rice production owing to favorable weather and clean water. Miso manufactured in the Akita are is mostly red salty rice miso, which has a harmonious taste with salty and umami features. For the other two types of miso: soybean miso is popular in Japan's central region including Aichi Prefecture, Gifu Prefecture, and Mie prefecture; barley miso is mainly consumed in Japan's Kyushu region and has become the most widely accepted by Westerners.

Nowadays, Japanese traditional fermented soy sauce and miso products have gained a global reputation in recent years. According to the record, the transport of soy sauce from Japan to foreign countries was started since 1668. And then the industrial level of soy sauce and miso was greatly improved by Western civilization so it increased rapidly. The data showed that the amount of export of Japanese fermented soy sauce products from 2013 to 2017 were 42.7 tons, 51.8 tons, 61.9 tons, 66.1 tons and 71.5 tons (Reported by the Ministry of Agriculture, Forestry and Fisheries based on the Ministry of Finance's Trade Statistics, Japan), respectively. With regard to miso, approximately 2,800 tons were exported in 1990. In 2017, the export level increased to approximately 17,010 tons. The increases in their export volume indicated the popularity of Japanese fermented soy sauce and miso around the world. With more and more studies proving the health benefits of eating fermented soybean products, such as heart-healthy, lowered risks for several cancers, and more (Cao et al., 2019; Jayachandran & Xu, 2019; Li et al., 2019; Mah et al., 2019; Yang et al., 2011), soy sauce and miso will attract more interest in the further.

## 1.2 Fermentation Process

Soy sauce and miso are important fermented seasonings that are widely used in the cuisine of East and Southeast Asian countries, especially in Japan. Generally, soy sauce and miso products are manufactured using a natural fermentation process for between six and twelve months under controlled temperature conditions (Noda, Hayashi, & Mizunuma, 1980). The long period of fermentation provides products with a better aroma and umami taste as well as more functional substances than products manufactured chemically or by limited fermentation, which are sold globally except in Japan (Kataoka, 2005). Because of their high quality and functional properties, soy sauce and miso products are becoming increasingly popular around the world (Moon & Rhee, 2016; Song et al., 2008). Consequently, the fermentation process is largely responsible for making soy sauce and miso so accepting and beneficial.

Fermentation is an ancient method for processing and preserving foods known today (Abiose et al., 1982; Fukushima, 1979). The fermented foods are generally produced by multi-species microbial communities during fermentation that related to the formation of flavor, taste, texture, and aroma of fermented foods (Cao et al., 2019; Wolfe & Dutton, 2015). In soy sauce fermentation, raw materials used mainly included soybean, wheat, salt, and water. Soybean, which is rich in protein and oil content, is used as protein source in soy sauce fermentation (Kinoshita et al., 1998). Due to the oil component in soybean did not contribute to the production of soy sauce, defatted bean was widely used. The ratios of defatted soybean to wheat determined the desired type of fermented soy sauce (O'toole, 2019). Soy sauce in Japan is commonly prepared by a traditional method, in which the steamed and presoaked-soybean mixed with roasted wheat flour at a proper ratio, followed by cultivation using *A. oryzae* or *A. sojae* to make koji, then koji is mixed with brine to make moromi (Xu et al., 2013). The moromi is fermented with lactobacilli and yeasts mainly including enzymatic degradation of raw materials, lactic fermentation, and yeast fermentation occurred(Rhee, Lee, & Lee, 2011; Zhu & Tramper, 2013). During the early stage of moromi fermentation, the high brine concentration can suppress the growth of salt-intolerant microbes and only halotolerant lactic acid bacteria (LAB) and yeasts grow that accelerated the lactic acid production and the degradation of raw materials into amino acids and lower peptides, which results in the decrease of the initial pH value of moromi, about 6.5 – 7.0. Yeast fermentation would take the place of lactic acid fermentation when the pH value of moromi had fallen to 5.5. The predominant yeast in soy sauce fermentation is *S. rouxii* and was commonly added into moromi at the pH value of 5.5 – 5.0 in Japan (Hoang et al., 2016; Ishihara et al., 1996). The yeast fermentation produces alcohol and volatile compounds that help to produce the desirable flavors and improve the final quality of soy sauce products (Singracha et al., 2017; Song et al., 2015). The lactic acid and yeast fermentation generally finished after 3 – 4 months, but an additional period is necessary for the aging of moromi at room temperature, usually 3 – 4 months (Luh, 1995). About Fifty percentages of the color of soy sauce is formed during aging due to the Maillard reaction between amino acids and sugars. At the same time, some flavor compounds, such as furan-2-carbaldehyde, 3-methylsulfanylpropanal, and so on, were also derived from the so-called browning reactions, such as

Maillard reaction, Strecker degradation, and other reactions (Feng et al., 2012; Harada et al., 2017; Smit, Engels, & Smit, 2009). After fermentation, the aged moromi is taken to filter and pasteurize to finally yield soy sauce.

During the fermentation of soy sauce, koji produces enzymes that decompose the raw materials into small molecules, then used to produce constituents to improve the quality of soy sauce. The proteins in soybeans are hydrolyzed to amino acids, peptides, and other water-soluble nitrogen content by hydrolytic enzymes in koji. The α-amylases and glucoamylases are responsible for the production of glucose or other reducing sugars by breaking down the starches of wheat. The generated reducing sugars could further work with amino acids to produce the dark brown color and various volatile compounds, known as Maillard reaction. Also, sugars are used as basic substrates for LAB and yeast fermentations that related to the production of organic acids, alcohol, glutamic acid, and so on (Devanthi & Gkatzionis, 2019; Liu, 2017; Singracha et al., 2017). As a result, these formed constituents during fermentation was considered as important factors that determines the quality of soy sauce.

The fermentation process between soy sauce and miso is very similar in Japan, including koji production, brine fermentation, and aging. Raw materials used for miso fermentation include soybean, salt, water, and cereal grain (i.e. rice, barley or wheat). The type of miso depends on the brine concentration and the type of koji being made. As mentioned above, there are three kinds of koji made of rice, barley, and wheat, respectively. The rice koji accounted for 80% of the total miso production is made most frequently. To produce the koji, soybean which preferring the large grain type and the selected cereal grain was firstly soaked in water to absorb enough water then steamed. After cooking, the substrate (rice, barley, or only soybean) were cultured with koji starter, usually *A. oryzae*, and then mixed with brine to stop the growth of koji mold, and finally mixed with the cooked soybeans and a proper amount of water for fermentation (Rai & Kusumoto, 2017). Sometimes yeast and/ LAB was also added for miso fermentation. Similar to the fermentation of soy sauce, in the early stage of miso fermentation, components in raw materials, i.e. rice and soybean, are broken down into smaller fragments by microbes produced from koji then further digested to generate the essential constituents for miso product. The amylase generated from koji could digest the starch of rice into zymohexose, including glucose and maltose. Glucose was used as basic substrates to take part in the lactic acid fermentation for the production of lactic acid and acetic acid, which could provide acidity to the moromi mash then inhibited contamination. The proteases generated from koji could catalyze the hydrolysis of soybean protein for the production of amino acids and peptides. The alcoholic fermentation was also taken place by the added yeast, usually *Zygosaccharomyces rouxii*, to utilize glucose for the production of ethyl alcohol, higher alcohols, and organic acids. These generated constituents could react with each other chemically to produce esters. Due to the production of soy sauce used the whole soybean, there is also the presence of enzymatic activity from lipase to convert the soybean oil into free fatty acids. Moreover, the Maillard reaction between the reducing sugars and amino acids was also simultaneously occurred that influenced the formation of pigments. The continuous production of various constituents of miso through microbial fermentation and enzymatic activity was continued during aging. Also, the obtained organic acids reacted with fatty acids through esterization lead to the formation of fatty acid ethyl esters during aging (Yamabe et al., 2004). After fermentation, miso directly becomes a product without heat and pasteurization. The final product was a soybean paste that still contained weakly microbial fermentation.

As mentioned above, the spontaneous fermentation by indigenous microbes played a key role in producing essential constituents for soy sauce and miso. Particularly, well-fermented products of soy sauce and miso produced the optimal amount of chemical compositions and flavor compounds, which had a significant influence on the determination of a final product with high sensory quality.

## 1.3 Effect of Constituents on the Qualities of Soy Sauce and Miso

The final product of soy sauce and miso contained salt, carbohydrates, organic acids, amino acids, and volatile compounds that played a major role in the sensory qualities of the product. Salt, mainly sodium chloride, is directly added before the fermentation. The proper concentration of salt could inhibit contamination during fermentation and provide salty taste to the final product. Carbohydrates were mainly produced by amylase and give the sweet taste to the final product. Also, carbohydrates had a major influence on the formation of organic acids and some volatile compounds, which had an influence on the sensory qualities of the final product. The generated organic acids in soy sauce and miso could improve the acidity of moromi then inhibits contamination during fermentation and give the sour taste to the final product. The amino acids, together with their salts, enhance the flavor of the final products, especially for glutamic acid and aspartic acid, which could significantly improve the umami taste to make the final products of soy sauce and miso more appealing. Volatile compounds that have various smells and odors were related to the sense of taste and smell for soy sauce and miso. Apparently, the investigation of these compounds would help to move toward an understanding of quality improvement for soy sauce and miso.

### 1.3.1 Soy sauce

#### 1.3.1.1 Chemical Composition

The original research and development work on the chemical compositions soy sauce was mainly made by Japanese researchers at the beginning of the study. Nishimura (1897) published the first scientific article written by a Japanese in English about Japanese fermented soy sauce. The production of soy sauce, including the preparation of raw materials, koji production, and chemical changes during fermentation, was described in this article with the aim to provide a deep insight into the industrial of soy sauce. The content of chemical compositions for the final product of soy sauce including salt, alcohol, carbohydrates, organic acids, and some amino acids, and the formation of flavor during manufacturing were also reported in detail. Later, Saito (1905) carried out the microbiological research on Japanese fermented soy sauce and reported the chemical composition and changes during brewing. He proposed the saccharification of starch, decomposition of albumen, and the formation of organic acids and alcohol during moromi fermentation. On the basis of these works, more and more research was conducted on the chemical changes of soy sauce (Iwamura, 1936; Oka & Nagata, 1974; Oshima & Church, 1923; Sakaguchi, 1958; Watanabe & Kiyoshi, 1962).

Recently, with people's living standard has improved, people pay attention to living quality and have need more high sensory quality food of daily life. Therefore, the research on soy sauce, as well as the other fermented foods, were turned to correlate with the sensory results. The extensively studies have conducted by Japanese researchers on the umami taste of soy sauce to clarify the key chemical compositions involved in the sugars, organic acids, and amino acids. (Kaneko, Kumazawa, & Nishimura, 2011; Lioe, et al., 2005; Lioe, Selamat, & Yasuda, 2010; Shiga et al., 2014; Yamaguchi & Ninomiya, 2000). Miyagi (2012) carried out research on the color preference of soy sauce that related to the acceptance of soy sauce. They found that lighter-colored soy sauce was preferred by consumers who lived in the Chiba region. They then investigated the decolorizing technologies to improve the color preference of soy sauce without the change of chemical compositions (Miyagi, Nabetani, & Nakajima, 2013; Miyagi et al., 2013). Some research

(Segawa et al., 1995; Tamura et al., 1989) were carried out to find the saltiness enhancers among organic acids, amino acids and their esters in order to decrease the salty intensity of soy sauce so that help to decrease the side effects for a high intake of salt. These works on the chemical compositions of soy sauce significantly improved the palatability and function of the final products produced in Japan.

Moreover, the study of the chemical composition of soy sauce was also contributed by China, Korea, and the other Southeast Asian countries, even American and European countries (Chen et al., 2015; Heo & Lee, 2017; Kim et al., 2017; Klinke, Thomsen, & Ahring, 2004; Li et al., 2009; Qin et al., 2013; Song et al., 2015; Wan et al., 2013; D. Xu et al., 2013; Zhou et al., 2019). Kim & Lee (2008) investigated the changes of chemical composition of Korean mixed soy sauce in comparison with traditional fermented soy sauce during fermentation. The results showed that the traditional fermented soy sauce mixed fishes or meat was imparted with a strong and complicated umami taste which had the potential to in placement of the traditional fermented soy sauce in Korea for soup and more prepared foods. Choi et al. (2011) germinated soybeans under both dark conditions and light conditions, and used them to prepare soy sauce, which was then compared with soy sauce prepared from non-germinated soybeans. According to the evaluation panel, the germinated soybeans increased the organoleptic preference. Zhang (2010) carried out research on chemical characteristic changes, including the content of amino acids, sugars, pH value, and color, of soy sauce fermented by using the short fermentation process named low-salt solid-state fermentation in China. These studies highlighted the changes in chemical compositions of soy sauce produced involved in various raw materials, fermentation conditions, and manufacturing processes that help to understand the chemical characteristics influencing the final quality of the product.

#### 1.3.1.2 Flavor Compounds

During fermentation of soy sauce, yeasts produced several hundreds of volatile compouds through alcohol fermentation. Also, the heating process for pasteurization played an important role in the formation of volatile compounds (Cui et al., 2014; Harada et al., 2017; Landaud, Helinck, & Bonnarme, 2008; Sasaki, Nunomura, & Matsudo, 1991; Sluis, Tramper, & Wijffels, 2001). This is due to the browning reactions, including the Maillard reaction and the Strecker degradation, which took place during heating. It was reported that the contents of aldehydes, pyrazines, phenols, sulfur compounds, and other volatile compounds, increased during this process (Kaneko, 2015; Kato, 1960; Nunomura et al., 1978; Yokotsuka, 1958). The identification of volatile compounds in soy sauce was started in 1887, and then more than 20 researchers in Japan identified about 130 flavor compounds from the fermented soy sauce (Obata & Yamanishi, 1950; Yamanishi, Obata, & Sano, 1952; Yokotsuka & Sasaki, 1997). With the development of gas chromatography–mass spectrometry (GC-MS), more aroma components have been successfully extracted from the Japanese fermented soy sauce (Aishima & Nobuhara, 1976; Nunomura et al., 1976). To date, approximately 300 volatile compounds have been identified in soy sauce, which can be classified into different chemical classes, such as alcohols, esters, ketones, aldehydes, acids, furanones, phenols, pyrazines, and sulfur-containing compounds (Feng et al., 2014; Sun, Jiang, & Zhao, 2010; Yamada, 1927). The important volatile compounds on the quality of Japanese fermented soy sauce were identified gradually by using different methods (Meng, Hatakeyama, & Sugawara, 2014; Meng et al., 2017; Meng, Isikawa, et al., 2012; Meng, Kakuta, & Sugawara, 2012; Nunomura, Sasaki, & Yokotsuka, 1980; Sasaki, 1996; Sasaki et al., 1991). The important flavor compounds to the koikuchi type soy sauce, which is the most common type in Japan, were identified by the aroma extract dilution analysis (Steinhaus & Schieberle, 2007). Thirty compounds, especially for sotolon, 4-HEMF, 3-methylbutanal, 2-methylbutanal, and methional, detected with different odor notes showed a high flavor dilution factor then assigned as odor-active compounds. Kaneko et al. (2012) further applied the aroma extract dilution analysis technique of the aroma concentrate on the five typical types of Japanese fermented soy sauce, that is, koikuchi, usukuchi, tmari, shiro, and saishikomi soy sauce. In comparison of flavor dilution factors of each volatile compounds detected from these five types of soy sauce, a total of 23 volatile compounds were considered to be important for the flavor of Japanese soy sauces and also the key volatile compounds that highly correlated to the aroma characteristic of each type of Japanese soy sauce were identified, respectively. They also performed quantitative analysis on both the raw and heated Japanese fermented soy sauce in order to clarify the difference in the flavor characteristic between the raw and heated Japanese fermented soy sauce (Kaneko, 2015; Kaneko et al., 2013).

Several investigations were also carried out for the flavor compounds of soy sauce produced in other places except Japan (Gao et al., 2017; Gao et al., 2010; Lee, Chiu, & Dou, 2007; Sun et al., 2010; Wah et al., 2013; Wang, Fan, & Xu, 2014; Yan et al., 2008). Feng et al. (2014, 2015) investigated the aroma-active components in two types of

Chinese soy sauce, including high-salt liquid-state fermentation soy sauce and low-salt solid-state fermentation soy sauce. They also performed the experiments on clarifying the various effects on the changes of volatile compounds, for example, koji fermentation process (Feng et al., 2013), fatty acid composition and lipid profile of koji samples (Feng, Chen, et al., 2014), and Optimization of extraction techniques (Feng et al., 2017). Moreover, the identification of volatile compounds to soy sauces was extensively studied by various preparation methods (Kaneko et al., 2012; Lee, Seo, & Kim, 2006; Wanakhachornkrai & Lertsiri, 2003). the mechanism of formation of various volatile compounds during the production of soy sauce has also been investigated recently (Huang & Barringer, 2016; Lee et al., 2013; Zhao et al., 2018, 2015; Zhou et al., 2019).

### 1.3.2 Miso

#### 1.3.2.1 Chemical Composition

After fermentation there are a wide range of biochemical changes that play a major role in determination of the final quality of Japanese fermented miso. The taste of miso is mainly governed by salt, amino acids, carbohydrates, and organic acids. Shibasaki & Hesseltine (1962) described the production process of miso in detail. The chemical changes occurring during fermentation including the formation of zymohexose, amino acids, organic acids, and flavor compounds through the koji fermentation, lactic acid fermentation, alcoholic fermentation, and esterization between compounds, were summarized in this study systematically. Abiose et al. (1982) followed to investigate the technology updates of miso fermentation to increase the understanding of miso to the Occidental. The microbial and biochemical events that took place during miso fermentation were newly summarized and introduced the contents of moisture, sugars, amino acids, lipids, free fatty acids, vitamins, organic acids, and volatile compounds. Chiou et al. (Chiou, Ferng, & Beuchat, 1999; Chiou, 1999) conducted experiments on the development of novel low-salt miso with the aim of satisfying the comsumer demand. Ogasawara et al. (Ogasawara et al., 2006) investigated the taste profile of various ripened miso samples from 10 days up to 20 months then linked sensory results with the chemical changes during ripening to clarify the key taste enhancers for the mouthfulness and continuity of miso. These studies greatly contributed to the quality and applicability of miso. Moreover, the chemical components related to the nutritional and health benefits have also been received increasing attention (Chin & Koehler, 1986; Coward et al., 1993; Esaki et al., 1997; Kung et al., 2007; Murphy et al., 1999; Sookwong et al., 2010; Win et al., 2018; Yang et al., 2018; Zarkadas et al., 1997).

#### 1.3.2.2 Flavor Compounds

Comprehensive studies of Japanese fermented miso have been published regarding the flavor compounds, which are not only derived from yeast fermentation, but also involved in several reactions during ripening (Honma, 1987; Hosaka & Sugawara, 2002; Kaku, Sugawara, & Takahashi, 2000; Kojo et al., 2019). Sugawara et al. carried out a series of research on the changes in flavor of miso, such as the sample preparation methods (Sugawara et al. 1990), varied types (Sugawara & Yonekura, 1998), fermentation periods (Sugawara, 1991a), sensory evaluation (Sugawara, Saiga, & Kobayashi, 1992, 1994). To date, more than 200 volatile compounds had been identified from miso products (Mori, Kiuchi, & Tabei, 1983; Ohata et al., 2007). Particularly, several important volatile compounds had been reported in varieties of miso, for example, HEMF (Sugawara, 1991b; Sugawara et al., 1994; Sugawara & Sakurai, 1999), HDMF (Hayashida, Nishimura, & Slaughter, 1998), 2-furanmethanethiol (Ohata et al., 2009), 4-ethylguaiacol & methionol (Sugawara & Yonekura, 1998), and methional (Kumazawa, Kaneko, & Nishimura, 2013). These studies elucidated the significance of the volatile compounds in the miso quality.

## 1.4 Assessment of Soy Sauce and Miso Qualites

Soy sauce and miso are two traditional Japanese fermented condiments, which representing the food culture in Japan and are indispensable for use in the Japanese cuisine for a long time. In general, soy sauce and miso are manufactured by the same fermentation process through various microbial and chemical reactions and now widely enjoyed for not only their characteristic flavors and tastes but also their nutrients and health benefits. Serval investigations have carried out for both soy sauce and miso to describe the differences among them or explain the common points for fermented soybean foods (Hamano & Sugimoto, 1978; Kobayashi & Sugawara, 1999; Nakamura et al., 2003; Suezawa et al., 2006; Suezawa & Suzuki, 2007). Due to the demand of both soy sauce and miso has been increasing all over the world in recent years, the objective of increasing their qualities to fulfill consumer acceptability in today’s highly innovative and global food environment has brought new challenges to evaluation methods of foreign and domestic food manufacturers: how to assess the final qualities of their products.

The qualities of both soy sauce and miso are governed by their sensory properties. Measuring the quality of fermented soy sauce requires a comprehensive understanding of chemical compositions, flavors, and sensory evaluation. Several previous studies have investigated the correlation between the chemical compositions or flavors present and the sensory evaluation. In particular, Aishima & Nobuhara (1977) and Sugawara et al. (Sugawara, Saiga, et al., 1994) separately performed the multiple regression analysis on the sensory evaluation of soy sauce/miso products and their volatile compounds to elucidate the contribution of each compound detected to the qualities of the product. The obtained results for soy sauce and miso then enabled to help the quality improvements of flavor and their standardizations. Recently, many emerging methods have been used to descript the relationship between sensory evaluation and the sensory attributes of product. Feng et al. (2013) performed principal component analysis on ten aroma-active components to find the relationship with the sensory evaluation of six soy sauce koji samples. Sun et al. (2010) also used principal component analysis to establish the relationship between the twelve types of soy sauce products and their volatile compounds. In order to facilitate sensory evaluation of soy sauce qualities, Imamura (2016) built a flavor wheel by using descriptive analysis to define the attributes of soy sauce for sensory evaluation. These studies suggested that linking the key product attributes with sensory evaluation to find the conformance could elucidate the quality of product.

Generally, color, taste, and flavor played a major role, accounting for 96.8% of the evaluation results, during the sensory evaluation. Specifically, nine types of taste characteristics account for 97.6% for the taste preference of soy sauce, and also the cumulative contribution ratio of seventeen types of odorous characteristics was 96.5%. It is reported that the flavor and taste have more impact than color during sensory evaluation (Yokotsuka & Sasaki, 1997). The quality of Japanese soy sauce and miso were generally assessed by sensory evaluation (Hitomi, 1994; Imamura & Sato, 2008). For example, the Akita Prefectural Miso and Soy Sauce Competition has used sensory evaluation to assess the quality of soy sauce and miso products annually since 1953 (Kaoru, 2006). However, although the competition has contributed to quality improvements of soy sauce and miso products and still influences the development of the products in Akita area, the traditional sensory evaluation performed by human assessors is relatively subjective, costly and time-consuming that cannot meet the production management needs for soy sauce and miso products from the food manufacturers.

Therefore, additional scientific data are needed for the standardization and quality management of these products. To date, it is believed that the development of related predictive model would evaluate the final quality of food products rapidly and objectively, which more efficient than the traditional sensory evaluation (Civille, 1991; Nozaki & Nakamoto, 2018; Xu et al., 2019).

## 1.5 References


Abiose, S. H., Allan, M. C., & Wood, B. J. (1982). Microbiology and Biochemistry of Miso (Soy Paste) Fermentation. *Advances in Applied Microbiology*, *28*, 239–265.

Aishima, T., & Nobuhara, A. (1976). Evaluation of Soy Sauce Flavor by Stepwise Multiple Regression Analysis of Gas Chromatographic Profiles. *Agricultural and Biological Chemistry*, *40*, 2159–2167.

Aishima, T., & Nobuhara, A. (1977). Flavour evaluation by multiple regression analysis of gas chromatographic patterns in soy sauce. *Food Chemistry*, *2*, 161–175.

Byun, B. Y., & Mah, J. H. (2012). Occurrence of Biogenic Amines in Miso, Japanese Traditional Fermented Soybean Paste. *Journal of Food Science*, *77*, 216–223.

Cao, Z. H., Green-Johnson, J. M., Buckley, N. D., & Lin, Q.-Y. (2019). Bioactivity of soy-based fermented foods: A review. *Biotechnology Advances*, *37*, 223–238.

Chavasit, V., & Photi, J. (2018). Chapter 15—Condiments and Sauces. In M. G. V. Mannar & R. F. Hurrell (Eds.), *Food Fortification in a Globalized World* (pp. 153–158). Academic Press.

Chen, Z. Y., Feng, Y. Z., Cui, C., Zhao, H. F., & Zhao, M. M. (2015). Effects of *koji* -making with mixed strains on physicochemical and sensory properties of Chinese-type soy sauce: Effects of *koji* -making with mixed strains on soy sauce. *Journal of the Science of Food and Agriculture*, *95*, 2145–2154.

Chin, K. D. H., & Koehler, P. E. (1986). Effect of Salt Concentration and Incubation Temperature on Formation of Histamine, Phenethylamine, Tryptamine and Tyramine During Miso Fermentation. *Journal of Food Protection*, *49*, 423–427.

Chiou, R. Y. Y., Ferng, S., & Beuchat, L. R. (1999). Fermentation of low-salt miso as affected by supplementation with ethanol. *International Journal of Food Microbiology*, *48*, 11–20.

Chiou, R. Y. Y. (1999). Salt-free Miso Fermentation Using Ethanol, Sugars, and Polyols. *Journal of Food Science*, *64*, 918–920.

Choi, U. K., Jeong, Y.-S., Kwon, O. J., Park, J. D., Kim, Y. C., Choi, U. K., & Kim, Y. C. (2011). Comparative Study of Quality Characteristics of Korean Soy Sauce Made with Soybeans Germinated Under Dark and Light Conditions. *International Journal of Molecular Sciences*, *12*, 8105–8118.

Civille, G. V. (1991). FOOD QUALITY: CONSUMER ACCEPTANCE AND SENSORY ATTRIBUTES. *Journal of Food Quality*, *14*, 1–8.

Coward, L., Barnes, N. C., Setchell, K. D., & Barnes, S. (1993). Genistein, daidzein, and their. Beta.-glycoside conjugates: Antitumor isoflavones in soybean foods from American and Asian diets. *Journal of Agricultural and Food Chemistry*, *41*, 1961–1967.

Cui, R. Y., Zheng, J., Wu, C. D., & Zhou, R. Q. (2014). Effect of different halophilic microbial fermentation patterns on the volatile compound profiles and sensory properties of soy sauce moromi. *European Food Research and Technology*, *239*, 321–331.

Devanthi, P. V. P., & Gkatzionis, K. (2019). Soy sauce fermentation: Microorganisms, aroma formation, and process modification. *Food Research International*, *120*, 364–374.

Esaki, H., Onozaki, H., Kawakishi, S., & Osawa, T. (1997). Antioxidant Activity and Isolation from Soybeans Fermented with *Aspergillus* spp. *Journal of Agricultural and Food Chemistry*, *45*, 2020–2024.

Feng, J., Zhan, X. B., Zheng, Z. Y., Wang, D., Zhang, L. M., & Lin, C. C. (2012). A two-step inoculation of Candida etchellsii to enhance soy sauce flavour and quality: 2-step yeast inoculum enhances soy sauce. *International Journal of Food Science & Technology*, *47*, 2072–2078.

Feng, Y., Cai, Y., Su, G., Zhao, H., Wang, C., & Zhao, M. (2014). Evaluation of aroma differences between high-salt liquid-state fermentation and low-salt solid-state fermentation soy sauces from China. *Food Chemistry*, *145*, 126–134.

Feng, Y., Chen, Z., Liu, N., Zhao, H., Cui, C., & Zhao, M. (2014). Changes in fatty acid composition and lipid profile during koji fermentation and their relationships with soy sauce flavour. *Food Chemistry*, *158*, 438–444.

Feng, Y., Cui, C., Zhao, H., Gao, X., Zhao, M., & Sun, W. (2013). Effect of koji fermentation on generation of volatile compounds in soy sauce production. *International Journal of Food Science & Technology*, *48*, 609–619.

Feng, Y., Su, G., Sun-Waterhouse, D., Cai, Y., Zhao, H., Cui, C., & Zhao, M. (2017). Optimization of Headspace Solid-Phase Micro-extraction (HS-SPME) for Analyzing Soy Sauce Aroma Compounds via Coupling with Direct GC-Olfactometry (D-GC-O) and Gas Chromatography-Mass Spectrometry (GC-MS). *Food Analytical Methods*, *10*, 713–726.

Feng, Y., Su, G., Zhao, H., Cai, Y., Cui, C., Sun-Waterhouse, D., & Zhao, M. (2015). Characterisation of aroma profiles of commercial soy sauce by odour activity value and omission test. *Food Chemistry*, *167*, 220–228.

Fukushima, D. (1979). Fermented vegetable (soybean) protein and related foods of Japan and China. *Journal of the American Oil Chemists' Society*, *56*, 357–362.

Fukushima, Danji. (2004). Industrialization of fermented soy sauce production centering around Japanese shoyu. In *Industrialization of Indigenous Fermented Foods, Revised and Expanded* (K.H. Steinkraus, pp. 1–98). New York: Marcel Dekker, Inc.

Gao, L., Liu, T., An, X., Zhang, J., Ma, X., & Cui, J. (2017). Analysis of volatile flavor compounds influencing Chinese-type soy sauces using GC–MS combined with HS-SPME and discrimination with electronic nose. *Journal of Food Science and Technology, 54*, 130–143.

Gao, X. L., Cui, C., Zhao, H. F., Zhao, M. M., Yang, L., & Ren, J. Y. (2010). Changes in volatile aroma compounds of traditional Chinese-type soy sauce during moromi fermentation and heat treatment. *Food Science and Biotechnology, 19*, 889–898.

Giri, A., Osako, K., Okamoto, A., Okazaki, E., & Ohshima, T. (2012). Effects of Koji Fermented Phenolic Compounds on the Oxidative Stability of Fish Miso. *Journal of Food Science*, *77*, C228–C235.

Hamano, M., & Sugimoto, H. (1978). Water Sorption, Reduction of Caking and Improvement of Free Flowingness of Powdered Soy Sauce and Miso. *Journal of Food Processing and Preservation, 2*, 185–196.

Harada, R., Yuzuki, M., Ito, K., Shiga, K., Bamba, T., & Fukusaki, E. (2017). Influence of yeast and lactic acid bacterium on the constituent profile of soy sauce during fermentation. *Journal of Bioscience and Bioengineering*, *123*, 203–208.

Hayashida, Y., Nishimura, K., & Slaughter, J. C. (1998). The importance of the furanones HDMF and HEMF in the flavour profile of Japanese barley miso and their production during fermentation. *Journal of the Science of Food and Agriculture, 78*, 88–94.

Heo, J., & Lee, J. (2017). US consumers' acceptability of soy sauce and bulgogi. *Food Science and Biotechnology*, *26*, 1271–1279.

Hesseltine, C. W. (1983). Microbiology of Oriental Fermented Foods. *Annual Review of Microbiology, 37*, 575–601.

Hideo, E. (2004). Industrialization of Japanese Miso Fermentation. In K. Steinkraus (Ed.), *Industrialization of Indigenous Fermented Foods, Revised and Expanded* (pp. 99–147). New York: Marcel Dekker, Inc.

Hitomi Y. (1994). Quality Evaluation of Rice Miso. *Brewing Society of Japan*, *89*, 200–206.

Hoang, N. X., Ferng, S., Ting, C. H., Huang, W. H., Chiou, R. Y. Y., & Hsu, C. K. (2016). Optimizing the initial moromi fermentation conditions to improve the quality of soy sauce. *LWT*, *74*, 242–250.

Honma, N. (1987). Aroma and aroma components of miso (2). *Journal of the Society of Brewing,Japan*, *82*, 547–553.

Hosaka, Y., & Sugawara, E. (2002). Studies on aroma change of the miso soup by heating. *Abstracts of the Annual Meeting of the Japan Society of Cookery Science*, *14*, 9–9.

Huang, X., & Barringer, S. A. (2016). Kinetics of furan formation during pasteurization of soy sauce. *LWT - Food Science and Technology*, *67*, 200–205.

Imamura, M. (2016). Descriptive terminology for the sensory evaluation of soy sauce: I MAMURA. *Journal of Sensory Studies*, *31*, 393–407.

Imamura, M., & Sato, H. (2008). Development of Sensory Tests in Selecting Descriptive Panelists for Evaluating Soy Sauce and Its Related Products. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *55*, 468–480.

Inoue, Y., Kato, S., Saikusa, M., Suzuki, C., Otsubo, Y., Tanaka, Y., & Hayase, F. (2016). Analysis of the cooked aroma and odorants that contribute to umami aftertaste of soy miso (Japanese soybean paste). *Food Chemistry*, *213*, 521–528.

Ishihara, K., Honma, N., Matsumoto, I., Imai, S., Nakazawa, S., & Iwafuchi, H. (1996). Comparison of Volatile Components in Soy Sauce (*Koikuchi Shoyu*) Produced Using *Aspergillus sojae* and *Aspergillus oryzae*. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *43*, 1063–1074.

Iwamura, I. (1936). Determination of Cystine in“Shoyu.” *Bulletin of the Agricultural Chemical Society of Japan*, *12*, 89–89.

Jayachandran, M., & Xu, B. (2019). An insight into the health benefits of fermented soy products. *Food Chemistry*, *271*, 362–371.

Kaku, Y., Sugawara, E., & Takahashi, K. (2000). Effect of the Manufacturing Process (the Hardness of Steamed-cooked Soybeans and the Temperature During the Aging Process) on the Formation of Aroma Components of Red Salty Rice *Miso* with Aging. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *47*, 919–925.

Kaneko, S. (2015). Studies on the Key Aroma Compounds in Raw (Unheated) Japanese Soy Sauce and those Changes during Heating. *Journal of the Brewing Society of Japan*, *110*, 20–25.

Kaneko, S., Kumazawa, K., & Nishimura, O. (2011). Isolation and Identification of the Umami Enhancing Compounds in Japanese Soy Sauce. *Bioscience, Biotechnology, and Biochemistry*, *advpub*, 1105282507–1105282507.

Kaneko, S., Kumazawa, K., & Nishimura, O. (2012). Comparison of Key Aroma Compounds in Five Different Types of Japanese Soy Sauces by Aroma Extract Dilution Analysis (AEDA). *Journal of Agricultural and Food Chemistry*, *60*, 3831–3836.

Kaneko, S., Kumazawa, K., & Nishimura, O. (2013). Studies on the Key Aroma Compounds in Raw (Unheated) and Heated Japanese Soy Sauce. *Journal of Agricultural and Food Chemistry*, *61*, 3396–3402.

Kaoru O. (2006). A Comparison of the Analytical Data on Misos and Soysauces Submitted to Akita Prefectural Competitions during the Last 50 Years. *Akita Research of Food & Brewing*, *101*, 81–87.

Kataoka, S. (2005). Functional effects of Japanese style fermented soy sauce (shoyu) and its components. *Journal of Bioscience and Bioengineering*, *100*, 227–234.

Kato, H. (1960). Studies on Browning Reactions between Sugars and Amino Compounds. *Bulletin of the Agricultural Chemical Society of Japan*, *24*, 1–12.

Kim, J. S., & Lee, Y. S. (2008). A study of chemical characteristics of soy sauce and mixed soy sauce: Chemical characteristics of soy sauce. *European Food Research and Technology*, *227*, 933–944.

Kim, Y., Kim, E. Y., Son, H. J., Lee, J., Choi, Y., & Rhyu, M. R. (2017). Identification of a key umami -active fraction in modernized Korean soy sauce and the impact thereof on bitter-masking. *Food Chemistry*, *233*, 256–262.

Kinoshita, E., Sugimoto, T., Ozawa, Y., & Aishima, T. (1998). Differentiation of Soy Sauce Produced from Whole Soybeans and Defatted Soybeans by Pattern Recognition Analysis of HPLC Profiles. *Journal of Agricultural and Food Chemistry*, *46*, 877–883.

Kitamura, Y., Kusumoto, K., Oguma, T., Nagai, T., Furukawa, S., Suzuki, C., & Tamaki, H. (2016). Ethnic Fermented Foods and Alcoholic Beverages of Japan. In P. T. Jyoti (Ed.), *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (pp. 193–236). New Delhi: Springer.

Klinke, H. B., Thomsen, A. B., & Ahring, B. K. (2004). Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology*, *66*, 10–26.

Kobayashi, A., & Sugawara, E. (1999). Flavor Components of Shoyu and Miso Japanese Fermented Soybean Seasonings. In F. Shahidi & C. T. Ho (Eds.), *Flavor Chemistry of Ethnic Foods* (pp. 5–14). Boston, MA: Springer US.

Kojo, T., Kawai, M., Shiraishi, Y., Kurazono, S., Kadooka, C., Okutsu, K., & Tamaki, H. (2019). Effect of Maturation Time on Koji-like Smell and Volatile Compounds of Barley Miso (Japanese Soybean Paste) during Fermentation. *Food Science and Technology Research*, *25*, 313–319.

Kumazawa, K., Kaneko, S., & Nishimura, O. (2013). Identification and Characterization of Volatile Components Causing the Characteristic Flavor in Miso (Japanese Fermented Soybean Paste) and Heat-Processed Miso Products. *Journal of Agricultural and Food Chemistry*, *61*, 11968–11973.

Kumazawa, T., Nishimura, A., Asai, N., & Adachi, T. (2018). Isolation of immune-regulatory Tetragenococcus halophilus from miso. *PLOS ONE*, *13*, e0208821.

Kung, H. F., Tsai, Y. H., & Wei, C. I. (2007). Histamine and other biogenic amines and histamine-forming bacteria in miso products. *Food Chemistry*, *101*, 351–356.

Kwon, D. Y., Daily, J. W., Kim, H. J., & Park, S. (2010). Antidiabetic effects of fermented soybean products on type 2 diabetes. *Nutrition Research*, *30*, 1–13.

Landaud, S., Helinck, S., & Bonnarme, P. (2008). Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food. *Applied Microbiology and Biotechnology*, *77*, 1191–1205.

Lee, K. E., Lee, S. M., Choi, Y. H., Hurh, B. S., & Kim, Y. S. (2013). Comparative Volatile Profiles in Soy Sauce According to Inoculated Microorganisms. *Bioscience, Biotechnology, and Biochemistry*, *77*, 2192–2200.

Lee, M. R., Chiu, T. C., & Dou, J. (2007). Determination of 1, 3-dichloro-2-propanol and 3-chloro-1, 2-propandiol in soy sauce by headspace derivatization solid-phase microextraction combined with gas chromatography–mass spectrometry. *Analytica Chimica Acta*, *591*, 167–172.

Lee, S. M., Seo, B. C., & Kim, Y. S. (2006). Volatile Compounds in Fermented and Acid-hydrolyzed Soy Sauces. *Journal of Food Science*, *71*, C146–C156.

Li, C., Zhang, B., Zhou, H., Wang, X., Pi, X., Wang, X., & He, G. (2019). Beneficial influences of dietary Aspergillus awamori fermented soybean meal on oxidative homoeostasis and inflammatory response in turbot (Scophthalmus maximus L.). *Fish & Shellfish Immunology*, *93*, 8–16.

Li, Y., Zhao, H., Zhao, M., & Cui, C. (2009). Relationships between antioxidant activity and quality indices of soy sauce: An application of multivariate analysis: Antioxidant activity and quality of soy sauce. *International Journal of Food Science & Technology*, *45*, 133–139.

Lioe, Hanifah N., Apriyantono, A., Takara, K., Wada, K., & Yasuda, M. (2005). Umami Taste Enhancement of MSG/NaCl Mixtures by Subthreshold L-α-Aromatic Amino Acids. *Journal of Food Science*, *70*, s401–s405.

Lioe, Hanifah Nuryani, Selamat, J., & Yasuda, M. (2010). Soy Sauce and Its Umami Taste: A Link from the Past to Current Situation. *Journal of Food Science*, *75*, R71–R76.

Liu, S. (2017). Chapter 7—Soy Sauce Fermentation. In *Fermented Foods, Part II* (1st Edition, pp. 135–149). Boca Raton: CRC Press.

Luh, B. (1995). Industrial production of soy sauce. *Journal of Industrial Microbiology*, *14*, 467–471.

Mah, J. H., Park, Y. K., Jin, Y. H., Lee, J. H., & Hwang, H. J. (2019). Bacterial Production and Control of Biogenic Amines in Asian Fermented Soybean Foods. *Foods*, *8*, 85.

Meng, Q., Hatakeyama, M., & Sugawara, E. (2014). Formation by yeast of 2-furanmethanethiol and ethyl 2-mercaptopropionate aroma compounds in Japanese soy sauce. *Bioscience, Biotechnology, and Biochemistry*, *78*, 109–114.

Meng, Q., Isikawa, N., Kumamaru, H., Imamura, M., Yamasita, Y., Obata, A., & Sugawara, E. (2012). Characterization of the aroma compounds in raw soy sauce by aroma extract dilution analysis. *Abstracts of the Annual Meeting of the Japan Society of Cookery Science*, *24*, 23.

Meng, Q., Kakuta, T., & Sugawara, E. (2012). Quantification and Odor Contribution of Volatile Thiols in Japanese Soy Sauce. *Food Science and Technology Research*, *18*, 429–436.

Meng, Q., Kitagawa, R., Imamura, M., Katayama, H., Obata, A., & Sugawara, E. (2017). Contribution of 2-methyl-3-furanthiol to the cooked meat-like aroma of fermented soy sauce. *Bioscience, Biotechnology, and Biochemistry*, *81*, 168–172.

Miyagi, A. (2012). Research on purchase consciousness and color preference of Japanese soy sauce among general consumers in Chiba prefecture. *Journal for the Integrated Study of Dietary Habits*, *22*, 320–324.

Miyagi, A., Nabetani, H., & Nakajima, M. (2013). Decolorization of Japanese soy sauce (shoyu) using adsorption. *Journal of Food Engineering*, *116*, 749–757.

Miyagi, A., Suzuki, T., Nabetani, H., & Nakajima, M. (2013). Color control of Japanese soy sauce (shoyu) using membrane technology. *Food and Bioproducts Processing*, *91*, 507–514.

Moon, H., & Rhee, M. S. (2016). Synergism between carvacrol or thymol increases the antimicrobial efficacy of soy sauce with no sensory impact. *International Journal of Food Microbiology*, *217*, 35–41.

Mori, Y., Kiuchi, K., & Tabei, H. (1983). Flavor Componentsof Miso: Basic Fraction. *Agricultural and Biological Chemistry*, *47*, 1487–1492.

Murphy, P. A., Song, T., Buseman, G., Barua, K., Beecher, G. R., Trainer, D., & Holden, J. (1999). Isoflavones in Retail and Institutional Soy Foods. *Journal of Agricultural and Food Chemistry*, *47*, 2697–2704.

Nakamura, M., Aoki, N., Yamada, T., & Kubo, N. (2003). Feasibility and effect on blood pressure of 6-week trial of low sodium soy sauce and miso (fermented soybean paste). *Circulation Journal: Official Journal of the Japanese Circulation Society*, *67*, 530–534.

Nikkuni, S. A., Okada, N. O., & Itoh, H. I. (1988). Effect of Soybean Cooking Temperature on the Texture and Protein Digestibility of Miso. *Journal of Food Science*, *53*, 445–449.

Nishimura, Y. (1897). The chemistry of soja sauce manufacture. *Bulletin of the College of Agriculture, Tokyo Imperial University*, *3*, 191–206.

Noda, F., Hayashi, K., & Mizunuma, T. (1980). Antagonism between osmophilic lactic acid bacteria and yeasts in brine fermentation of soy sauce. *Appl. Environ. Microbiol.*, *40*, 452–457.

Nozaki, Y., & Nakamoto, T. (2018). Predictive modeling for odor character of a chemical using machine learning combined with natural language processing. *PloS One*, *13*, e0198475.

Nunomura, N., Sasaki, M., Asao, Y., & Yokotsuka, T. (1976). Identification of Volatile Components in *Shoyu* (Soy Sauce) by Gas Chromatography-Mass Spectrometry. *Agricultural and Biological Chemistry*, *40*, 485–490.

Nunomura, N., Sasaki, M., Asao, Y., & Yokotsuka, T. (1978). *Shoyu* (Soy Sauce) Volatile Flavor Components: Basic Fraction. *Agricultural and Biological Chemistry*, *42*, 2123–2128.

Nunomura, N., Sasaki, M., & Yokotsuka, T. (1980). Shoyu (Soy Sauce) Flavor Components: Acidic Fractions and the Characteristic Flavor Component. *Agricultural and Biological Chemistry*, *44*, 339–351.

Obata, Y., & Yamanishi, T. (1950). Studies on the Flavors of Soy. Part 1. On the Flavors which developed from Hydrolyzed Protein and Amino Acids. *Nippon Nōgeikagaku Kaishi*, *24*, 226–229.

Ogasawara, M., Yamada, Y., & Egi, M. (2006). Taste enhancer from the long-term ripening of miso (soybean paste). *Food Chemistry*, *99*, 736–741.

Ohata, M., Kohama, K., Morimitsu, Y., Kubota, K., & Sugawara, E. (2007). The Formation Mechanism by Yeast of 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone in Miso. *Bioscience, Biotechnology, and Biochemistry*, *71*, 407–413.

Ohata, M., Tominaga, T., Dubourdieu, D., Kubota, K., & Sugawara, E. (2009). Quantification and Odor Contribution of 2-Furanmethanethiol in Different Types of Fermented Soybean Paste Miso. *Journal of Agricultural and Food Chemistry*, *57*, 2481–2485.

Oka, S., & Nagata, K. (1974). Isolation and Characterization of Acidic Peptides in Soy Sauce. *Agricultural and Biological Chemistry*, *38*, 1195–1202.

Oshima, Kokichi., & Church, M. B. (1923). Industrial Mold Enzymes. *Industrial & Engineering Chemistry*, *15*, 67–70.

O'toole, D. K. (2019). Chapter Two—The role of microorganisms in soy sauce production. In G. M. Gadd & S. Sariaslani (Eds.), *Advances in Applied Microbiology* (Vol. 108, pp. 45–113). Academic Press.

Qin, O., Chen, Q., Zhao, J., & Lin, H. (2013). Determination of Amino Acid Nitrogen in Soy Sauce Using Near Infrared Spectroscopy Combined with Characteristic Variables Selection and Extreme Learning Machine. *Food and Bioprocess Technology*, *6*, 2486–2493.

Rai, A. K., & Kusumoto, K. I. (2017). Miso, the Traditional Fermented Soybean Paste of Japan. In *Fermented Foods, Part II: Technological Interventions* (pp. 122–134). Florida: CRC Press, Taylor and Francis Group.

Rhee, S., Lee, J. E., & Lee, C. H. (2011). Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories*, *10*, S5.

Saito, K. (1905). Microbiological studies on the brewing of japanese Soja-Sauce. *Shokubutsugaku Zasshi*, *19*, 75–77.

Sakaguchi, K. (1958). Studies on the Activities of Bacteria in Soy Sauce Brewing. *Bulletin of the Agricultural Chemical Society of Japan*, *22*, 345–352.

Sasaki, M. (1996). Influence of Sodium Chloride on the Levels of Flavor Compounds Produced by Shoyu Yeast. *Journal of Agricultural and Food Chemistry*, *44*, 3273–3275.

Sasaki, M., Nunomura, N., & Matsudo, T. (1991). Biosynthesis of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone by yeasts. *Journal of Agricultural and Food Chemistry*, *39*, 934–938.

Segawa, D., Nakamura, K., Kuramitsu, R., Muramatsu, S., Sano, Y., Uzuka, Y., & Okai, H. (1995). Preparation of Low Sodium Chloride Containing Soy Sauce Using Amino Acid Based Saltiness Enhancers. *Bioscience, Biotechnology, and Biochemistry*, *59*, 35–39.

Shibasaki, K., & Hessbltine, C. W. (1962). Miso fermentation. *Economic Botany*, *16*, 180–195.

Shiga, K., Yamamoto, S., Nakajima, A., Kodama, Y., Imamura, M., Sato, T., & Fukusaki, E. (2014). Metabolic profiling approach to explore compounds related to the umami intensity of soy sauce. *Journal of Agricultural and Food Chemistry*, *62*, 7317–7322.

Shimizu, N., Du, D. D., Sakuyama, H., Ito, Y., Sonoda, M., Kawakubo, K., & Uehara, Y. (2015). Continuous subcutaneous administration of Miso extracts attenuates salt-induced hypertension in Dahl salt-sensitive rats. *Food and Nutrition Sciences*, *6*, 693–702.

Singracha, P., Niamsiri, N., Visessanguan, W., Lertsiri, S., & Assavanig, A. (2017). Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (moromi) fermentation. *LWT - Food Science and Technology*, *78*, 181–188.

Smit, B. A., Engels, W. J. M., & Smit, G. (2009). Branched chain aldehydes: Production and breakdown pathways and relevance for flavour in foods. *Applied Microbiology and Biotechnology*, *81*, 987–999.

Song, Y. S., Frias, J., Martinez-Villaluenga, C., Vidal-Valdeverde, C., & de Mejia, E. G. (2008). Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. *Food Chemistry*, *108*, 571–581.

Song, Y. R., Jeong, D. Y., & Baik, S. H. (2015). Effects of indigenous yeasts on physicochemical and microbial properties of Korean soy sauce prepared by low-salt fermentation. *Food Microbiology, 51*, 171–178.

Sookwong, P., Nakagawa, K., Yamaguchi, Y., Miyazawa, T., Kato, S., Kimura, F., & Miyazawa, T. (2010). Tocotrienol Distribution in Foods: Estimation of Daily Tocotrienol Intake of Japanese Population. *Journal of Agricultural and Food Chemistry*, *58*, 3350–3355.

Steinhaus, P., & Schieberle, P. (2007). Characterization of the Key Aroma Compounds in Soy Sauce Using Approaches of Molecular Sensory Science. *Journal of Agricultural and Food Chemistry, 55*, 6262–6269.

Suezawa, Y., Kimura, I., Inoue, M., Gohda, N., & Suzuki, M. (2006). Identification and Typing of Miso and Soy Sauce Fermentation Yeasts, *Candida etchellsii* and *C. versatilis*, Based on Sequence Analyses of the D1D2 Domain of the 26S Ribosomal RNA Gene, and the Region of Internal Transcribed Spacer 1, 5.8S Ribosomal RNA Gene and Internal Transcribed Spacer 2. *Bioscience, Biotechnology, and Biochemistry, 70*, 348–354.

Suezawa, Y., & Suzuki, M. (2007). Bioconversion of Ferulic Acid to 4-Vinylguaiacol and 4-Ethylguaiacol and of 4-Vinylguaiacol to 4-Ethylguaiacol by Halotolerant Yeasts Belonging to the Genus *Candida*. *Bioscience, Biotechnology, and Biochemistry, 71*, 1058–1062.

Sugawara E. (1991a). Change in Aroma Components of Miso with Aging. *NIPPON SHOKUHIN KOGYO GAKKAISHI*, *38*, 1093–1097.

Sugawara, E. (1991b). Identification of 4-Hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3 (2 H)-furanone as a Flavor Component in Miso. *Nippon Shokuhin Kogyo Gakkaishi*, *38*, 491–493.

Sugawara, E., Hashimoto, S., Sakurai, Y., & Kobayashi, A. (1994). Formation by Yeast of the HEMF (4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone) Aroma Component in Miso with Aging. *Bioscience, Biotechnology, and Biochemistry*, *58*, 1134–1135.

Sugawara, E., Ito, T., Odagiri, S., Kubota, K., & Kobayashi, A. (1990). Comparison of Miso Aroma Components in Preparations by Different Methods. *Nippon Nōgeikagaku Kaishi*, *64*, 171–176.

Sugawara, E., Saiga, S., & Kobayashi, A. (1992). Relationships between Aroma Components and Sensory Evaluation of Miso. *Nippon Shokuhin Kogyo Gakkaishi*, *39*, 1098–1104.

Sugawara, E., Saiga, S., & Kobayashi, A. (1994). Multiple Regression Analysis of Aroma Components and Sensory Evaluation of Miso. *Nippon Shokuhin Kogyo Gakkaishi*, *41*, 844–846.

Sugawara, E., & Sakurai, Y. (1999). Effect of Media Constituents on the Formation by Halophilic Yeast of the 2 (or 5)-Ethyl-5 (or 2)-methyl-4-hydroxy-3 (2*H*)-furanone Aroma Component Specific to *Miso*. *Bioscience, Biotechnology, and Biochemistry*, *63*, 749–752.

Sugawara, E., & Yonekura, Y. (1998). Comparison of Aroma Components in Five Types of Miso. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *45*, 323–329.

Sun, S. Y., Jiang, W. G., & Zhao, Y. P. (2010). Profile of Volatile Compounds in 12 Chinese Soy Sauces Produced by a High-Salt-Diluted State Fermentation. *Journal of the Institute of Brewing*, *116*, 316–328.

Tamura, M., Seki, T., Kawasaki, Y., Tada, M., Kikuchi, E., & Okai, H. (1989). An Enhancing Effect on the Saltiness of Sodium Chloride of Added Amino Acids and Their Esters. *Agricultural and Biological Chemistry*, *53*, 1625–1633.

Van der Sluis, C., Tramper, J., & Wijffels, R. H. (2001). Enhancing and accelerating flavour formation by salt-tolerant yeasts in Japanese soy-sauce processes. *Trends in Food Science & Technology*, *12*, 322–327.

Wah, T. T., Walaisri, S., Assavanig, A., Niamsiri, N., & Lertsiri, S. (2013). Co-culturing of Pichia guilliermondii enhanced volatile flavor compound formation by Zygosaccharomyces rouxii in the model system of Thai soy sauce fermentation. *International Journal of Food Microbiology*, *160*, 282–289.

Wan, S., Wu, Y., Wang, C., Wang, C., & Hou, L. (2013). The development of soy sauce from organic soy bean. *Agricultural Sciences*, *04*, 116–121.

Wanakhachornkrai, P., & Lertsiri, S. (2003). Comparison of determination method for volatile compounds in Thai soy sauce. *Food Chemistry*, *83*, 619–629.

Wang, X., Fan, W., & Xu, Y. (2014). Comparison on aroma compounds in Chinese soy sauce and strong aroma type liquors by gas chromatography–olfactometry, chemical quantitative and odor activity values analysis. *European Food Research and Technology*, *239*, 813–825.

Watanabe, T., & Kiyoshi, A. S. O. (1962). ON THE SUGAR COMPOSITION OF SHIRO SHOYU (WHITE SOY SAUCE). *Tohoku Journal of Agricultural Research*, *13*, 265–271.

Win, N. N., Kanda, T., Nakamoto, S., Moriyama, M., Jiang, X., Suganami, A., & Shirasawa, H. (2018). Inhibitory effect of Japanese rice-koji miso extracts on hepatitis A virus replication in association with the elevation of glucose-regulated protein 78 expression. *International Journal of Medical Sciences*, *15*, 1153–1159.

Wolfe, B. E., & Dutton, R. J. (2015). Fermented Foods as Experimentally Tractable Microbial Ecosystems. *Cell*, *161*, 49–55.

Xu, D., Li, C., Zhao, M., Fengc, Y., Sun, L., & Wang, Y. (2013). Assessment on the improvement of soy sauce fermentation by Aspergillus oryzae HG76. *Biocatalysis and Agricultural Biotechnology*, *2*, 344–351.

Xu, L., Wang, X., Huang, Y., Wang, Y., Zhu, L., & Wu, R. (2019). A predictive model for the evaluation of flavor attributes of raw and cooked beef based on sensor array analyses. *Food Research International*, *122*, 16–24.

Yamabe, S., Kaneko, K., Inoue, H., & Takita, T. (2004). Maturation of Fermented Rice-koji Miso Can Be Monitored by an Increase in Fatty Acid Ethyl Ester. *Bioscience, Biotechnology, and Biochemistry*, *68*, 250–252.

Yamabe, S., Kobayashi-Hattori, K., Kaneko, K., Endo, H., & Takita, T. (2007). Effect of soybean varieties on the content and composition of isoflavone in rice-koji miso. *Food Chemistry*, *100*, 369–374.

Yamada, M. (1927). On the Origin of Aldehydes in Fermentation Products Ii. *Bulletin of the Agricultural Chemical Society of Japan*, *3*, 80–83.

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. *The Journal of Nutrition*, *130*, 921S-926S.

Yamanishi, T., Obata, Y., & Sano, M. (1952). Studies on the Flavors of Soy. Part 5. Synthesis of Sulfur-containing Compounds. *Nippon Nōgeikagaku Kaishi*, *26*, 125–128.

Yan, L., Zhang, Y., Tao, W., Wang, L., & Wu, S. (2008). Rapid determination of volatile flavor components in soy sauce using head space solid-phase microextraction and gas chromatography-mass spectrometry. *Chinese Journal of Chromatography*, *26*, 285–291.

Yang, H. J., Park, S., Pak, V., Chung, K. R., & Kwon, D. Y. (2011). Fermented Soybean Products and Their Bioactive Compounds. *Soybean and Health*. https://doi.org/10.5772/10670

Yang, X., Nakamoto, M., Shuto, E., Hata, A., Aki, N., Shikama, Y., & Kuwamura, Y. (2018). Associations between intake of dietary fermented soy food and concentrations of inflammatory markers: A cross-sectional study in Japanese workers. *The Journal of Medical Investigation*, *65*, 74–80.

Ye, H., Dudley, S. Z., & Shaw, I. C. (2017). Escherichia coli biotransformation of daidzein fermentation products from soy-based foods—Relevance to food oestrogenicity-based functionality. *International Journal of Food Science & Technology*, *52*, 1082–1091.

Yokotsuka, T., & Sasaki, M. (1997). Fermented protein foods in the Orient: Shoyu and miso in Japan. In B. J. B. Wood (Ed.), *Microbiology of Fermented Foods* (pp. 351–415). Boston, MA: Springer US.

Yokotsuka, T. (1958). Studies on Flavorous Substances in Shoyu. Part XV. Flavorous Substances in Heated Shoyu (2). *Nippon Nōgeikagaku Kaishi*, *32*, 23–26.

Yokotsuka, T. (1986). Soy Sauce Biochemistry. In *Advances in Food Research* (Vol. 30, pp. 195–329). Elsevier.

Yu, C. P., Hsieh, Y. W., Lin, S. P., Chi, Y. C., Hariharan, P., Chao, P. D. L., & Hou, Y. C. (2014). Potential modulation on P-glycoprotein and CYP3A by soymilk and miso: In vivo and ex-vivo studies. *Food Chemistry*, *149*, 25–30.

Zaid, A. A., & El-Shenawy, N. (2010). Effect of miso (A soybean fermented food) on some human cell lines; HEPG2, MCF7 and HCT116.pdf. *Journal of Journal of American Science*, *6*, 1274–1282.

Zarkadas, C. G., Voldeng, H. D., Yu, Z. R., & Choi, V. K. (1997). Determination of the Protein Quality of Three New Northern Adapted Cultivars of Common and Miso Type Soybeans by Amino Acid Analysis. *Journal of Agricultural and Food Chemistry*, *45*, 1161–1168.

Zhang, Y. (2010). Biochemical changes in low-salt solid-state fermented soy sauce. *African Journal of Biotechnology*, *9*, 8215–8221.

Zhao, G., Ding, L. L., Yao, Y., Cao, Y., Pan, Z. H., & Kong, D. H. (2018). Extracellular Proteome Analysis and Flavor Formation During Soy Sauce Fermentation. *Frontiers in Microbiology*, *9*, 1872.

Zhao, G., Yao, Y., Hao, G., Fang, D., Yin, B., Cao, X., & Chen, W. (2015). Gene regulation in Aspergillus oryzae promotes hyphal growth and flavor formation in soy sauce koji. *RSC Advances*, *5*, 24224–24230.

Zhou, K., Siroli, L., Patrignani, F., Sun, Y., Lanciotti, R., & Xu, Z. (2019). Formation of Ethyl Carbamate during the Production Process of Cantonese Soy Sauce. *Molecules*, *24*, 1474.

Zhou, W., Sun-Waterhouse, D., Xiong, J., Cui, C., Wang, W., & Dong, K. (2019). Desired soy sauce characteristics and autolysis of Aspergillus oryzae induced by low temperature conditions during initial moromi fermentation. *Journal of Food Science and Technology, 56*, 2888–2898.

Zhu, Y., & Tramper, J. (2013). Koji – where East meets West in fermentation. *Biotechnology Advances*, *31*, 1448–1457.

# CHAPTER 2 EFFECT OF THE CHEMICAL COMPOSITION OF SOY SAUCE AND MISO UPON THE SENSORY EVALUATION

## 2.1 Abstract

Understanding the effect of chemical characteristics on sensory evaluation elucidates the classification of fermented condiment quality. The chemical composition of soy sauce and miso products from the Akita Prefectural Miso and Soy Sauce Competition over three consecutive years from 2016 to 2018 were measured to determine the contribution of each composition to the sensory evaluation. The obtained results showed: I) the good quality soy sauce should was positively related to the *a** values, soluble salt-free solids content, glucose, phosphoric acid, acetic acid, and the total amino acid content; while the salt content, moisture, formic acid were found negatively affected soy sauce quality; furtherly, a comprehensive understanding of the differences in the contribution of each measured compositions for the soy sauce samples was obtained by using principal component analysis. 2) For miso products, the external appearance contributed significantly to the quality classification; Carbohydrate analysis showed that the derived sugars were digested during fermentation to contribute to improved sensory properties of miso; The increase in acid (except succinic acid and formic acid) and amino acid contents was also responsible for increased sensory quality. Consequently, the obtained results from this study suggest that the analytical data presented herein contributes to providing the detail information for the quality characteristics of the industrial soy sauce and miso products in Akita area.

## 2.2 Introduction

The Akita Prefectural Miso and Soy Sauce Products Competition was founded in 1953 with the aim to improve the sensory qualities of soy sauce and miso, in order to provide consumers with high quality products, and contribute to the healthy development of Akita Prefectural companies. In the course of nearly 65 years of development, the competition has formed a mature sensory evaluation system and the sensory results for various kinds of soy sauce and miso are generally considered reliable. Kaoru (2006) summarized data from the first 50 years of this competition and provided valuable insight into the development of the soy sauce and miso industries in Akita Prefecture.

The main type of soy sauce submitted to the competition, *koikuchi* soy sauce, has an intense flavor that is prepared using a complex fermentation process (Kobayashi & Sugawara, 1999; O'toole, 2019). *Koikuchi* soy sauce is the most common soy sauce in Japan, accounting for approximately 85% of domestic production (Fukushima, 2004; Sasaki, 1996). In addition to its saltiness, *koikuchi* soy sauce is also characterized by an intense umami, a mellow sweetness, and a refreshing acidity (Kaneko, Kumazawa, & Nishimura, 2011). It is an all-purpose seasoning characterized by a strong aroma, myriad flavors, and deep reddish brown color that can be used widely in cooking. Many types of miso have been developed in Japan, with different local traditions according to the ingredients available or taste desired. The most common type is rice miso, accounting for 80% of total miso output (Kitamura et al., 2016). Akita Prefecture in northeast Japan is famous for rice production owing to favorable weather and clean water. Miso manufactured in the Akita area is red salty rice miso, which has a harmonious taste with salty and umami features.

Comprehensive studies have been published regarding the fermentation process, composition, and flavor components for soy sauce (Bonkohara et al., 2016; Luh, 1995; Nunomura, Sasaki, & Yokotsuka, 1980; Steinhaus & Schieberle, 2007; Yokotsuka, 1961) and miso (Giri et al., 2010; Suezawa & Suzuki, 2007; Yoshida et al., 2009). Presently, research is being conducted on components in soy sauce which contribute to the intense umami taste of fermented soy sauce (Lioe, Selamat, & Yasuda, 2010; Zhuang et al., 2016). Miyagi et al. (2012) conducted research on color preference for *koikuchi* soy sauce and found that lighter-colored soy sauce was preferred by consumers. For the research of miso, the differences in the preferences of miso due to the change of chemical composition have also been reported (Chiou, Ferng, & Beuchat, 1999; Ratnaningrum et al., 2018). Kumazawa et al. (2013) compared raw miso and heat-processed miso to explore flavor changes in industrial miso production, and the effect of amino acids in miso on the odor during heat processing. The changes in protein and sugar during long-term miso ripening were also investigated confirming the significant influence of protein and sugar on generation of the characteristic flavor of long-ripened miso (Ogasawara, Yamada, & Egi, 2006). To sum up, previous studies conducted research on various compositions of soy sauce and miso to identify their effect on the products. However, these results either lack the connections of sensory quality or just one aspect of sensory quality. Therefore, the results obtained cannot be used to draw conclusions about the standardization of the final soy sauce and miso products.

Moreover, although the Akita Prefectural Miso and Soy Sauce Products Competition has contributed to improvements in the quality of miso and still influences the development of miso produced in the Akita area, additional scientific data are needed for the standardization and quality improvement of miso products. The present study was therefore conducted in order to indicate the relationship between the chemical compositions and sensory characteristic of Japanese fermented soy sauce and miso products. The soy sauce and miso products subjected to the Akita Prefectural Miso and Soy Sauce Products Competition from 2016 to 2018 were collected and their chemical profiles were measured. The chemical characteristics influencing the sensory quality, mainly external appearance and taste of products, were then systematically elucidated.

## 2.3 Materials and Methods

### 2.3.1 Materials and Reagents

#### 2.3.1.1 Soy Sauce Sample

Forty-five soy sauce samples were collected from the Akita Prefectural Miso and Soy Sauce Products Competition held in 2016, 2017, and 2018 by the Akita Prefectural Miso and Soy Sauce Manufacturer Cooperative (Akita, Japan). The total consisted of 16, 15, and 14 samples from the competitions held in 2016, 2017, and 2018, respectively. The samples subjected to the annual competition were produced by different companies and artisan workshops located in the Akita Prefecture in traditionally fermented process. In general, the manufacturing processes consisted of three major steps: koji production, brine fermentation, and refining. All samples were immediately stored in a refrigerator at –25 °C before analysis.

### 2.3.1.2 Miso sample

Twenty-six Miso samples entered into the competition each year (from 2016 to 2018), a total of 78 samples, were produced by different companies located in Akita Prefecture. These tested miso samples belong to rice miso, which is manufactured in the traditional method by grinding a mixture of cooked soybeans, rice koji, and salt. The manufacturing process generally consists of koji preparation, soybean preparation, mixing, inoculation, fermentation, and refining. All raw miso products were immediately stored in a refrigerator at –25 °C prior to use.

As miso is a semisolid fermented food with ingredients that continue fermenting during storage, the collected miso samples were pretreated to determine the chemical composition of miso by preparing water-soluble fractions (Ogasawara, Yamada and Egi 2006). The pretreatment process was as follows: Distilled water (45 g) was added to miso (5 g) and then homogenized using a Polytron homogenizer (Kinematica AG, Littau, Switzerland) at 8,000 rpm for 1 min. This 10-fold (w/w) diluted mixture of miso was then heated over a hot-water bath at 100 °C for 10 min and cooled in an ice-water bath. The cooled solution was centrifuged at 3,000 rpm for 30 min and the supernatant (soluble fraction of miso) was collected and used to determine the salt, sugars, acids, and amino acids in miso samples.

### 2.3.1.3 Reagents

A water distillation apparatus (Advantec, Tokyo, Japan) was responsible for the distilled water used throughout this research. For carbohydrate analysis, Milli-Q water used as the eluent was obtained from a Milli-Q Integral system (Merck, Darmstadt, Germany). Standard substances and the ninhydrin coloring solution for the amino acids analysis were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Other chemicals were of the best grade available supplied from Wako Pure Chemical Industries Ltd. (Osaka, Japan), Kanto Chemical Co. Inc., (Tokyo, Japan), and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

### 2.3.2 Analytical Methods

#### 2.3.2.1 Color Analysis

Color determination was performed on both the raw miso samples and raw soy sauce samples in triplicate. The color of each sample was determined using a Minolta CM-700d/600d spectrophotometer (Tokyo, Japan) and expressed in CIELab and CIELCH color spaces (Manta et al., 2013). The measurements were conducted with a measuring aperture diameter of 8 mm in specular component included mode using illuminant D65 at an observer angle of 10°. CIELab parameters $a^*$, $b^*$, and $L^*$ were measured. The parameter $L^*$ represents the lightness that ranged from 100 (white) to 0 (black); The parameters $a^*$ and $b^*$ represent for the green (negative) – red (positive) and blue (negative) – yellow (positive) color components, respectively.

#### 2.3.2.2 Determination of Soluble Salt-free solids Content and Salt Content

To measure the soluble salt-free solids content (SSFSC) and salt content, the raw soy sauce samples were subjected to pretreatment by adding the sample (1 g) to distilled water (9 g) and centrifuging at 3,000 rpm. The total soluble solids content and salt content were conducted using a digital refractometer (model PR-101, Atago, Japan) and a salt meter (model ES-421, Atago) on the 10-fold (w/w) diluted soy sauce samples and the soluble fraction of miso samples, respectively. Each sample was measured three times in parallel and the final result was averaged. The value of SSFSC was equal to the difference between the total soluble solids content and the salt content.

#### 2.3.2.3 Determination of Acids

The acids found in soy sauce and miso were analyzed by high performance liquid chromatography (HPLC) using a Nexera UHPLC/HPLC System chromatograph (Shimadzu, Japan) consisting of two liquid delivery pumps (model LC-30AD), a conductivity detector (model CDD-10Avp), an autosampler (model SIL-30AC), a column oven (model CTO-20AC), and a system controller (model CBM-20Alite).

To decrease the concentration of sodium salt in soy sauce and miso, the collected soy sauce samples and the soluble fraction of miso samples were prepared by diluting 30-fold (w/w) with distilled water and filtering through a

0.45-μm membrane filter, respectively. The mobile phase solution (5.0 mmol/L *p*-toluene sulfonic acid aquerous solution, Shimadzu) and pH buffering reagent solution (Aqueous solution mixture (5.0 mmol/L *p*-toluene sulfonic acid, 20 mM Bis(2-hydroxyethyl)-amino-tris(hydroxymethyl)-methane and 0.1 mM ethylenediaminetetra acetic acid), Shimadzu) were used exclusively for acid analysis. Each solution was deaerated using the degasser and a flow rate of 0.8 mL/min was used in each flow line. The test solution (10 μL) was injected into the RSpak KC-811 column (i.d. 8 mm × 300 mm, Shodex, Japan) maintained at 40 ℃ using the column oven and the chromatogram was recorded for 20 min. The presented values were obtained on at least three replicates.

### 2.3.2.4 Determination of Carbohydrates

Carbohydrates in soy sauce and miso was performed using an HPLC system equipped with a Sugar KS-801 column (Shodex, Japan), in conjunction with a pump (LC-10ADvp, Shimadzu) coupled to a refractive index detector (RID-10A, Shimadzu) and an auto injector (SIL-10ADvp). Fructose, mannose, and galactose could not be separated on this column and were, therefore, analyzed as a single fructose peak. The quantitative analysis of sugars in the collected samples was mainly focused on isomaltose, glucose, and fructose. The presented values were obtained on at least three replicates. As for acid analysis, both the soy sauce samples and the soluble fraction of miso samples were first diluted 30-fold (w/w) with distilled water and filtered through a 0.45-μm membrane filter. The samples were analyzed under the following conditions: Column temperature, 80 °C; eluent, Milli-Q water; flow rate, 0.7 mL/min; acquisition time, 20 min.

### 2.3.2.5 Determination of Amino Acids

For the determination of amino acid compositions in soy sauce and miso products, the collected soy sauce samples and the soluble fraction of miso samples were first diluted 30-fold (w/w) with distilled water and further diluted with 0.02 N hydrochloric acid to give a final concentration 900-fold (w/w) and 600-fold (w/w) lower than the original soy sauce and miso, respectively. The analysis of the amino acid compositions for the prepared samples were conducted by using an L-8900 high-speed amino acid analyzer (Hitachi, Japan).

2.3.2.6 Determination of pH

The pH of the collected soy sauce and miso samples were determined from the raw samples by using a pH-meter, model HI99161N (HANNA, Italy).

2.3.2.7 Determination of Moisture Content

Freeze-drying method was used determine the moisture content of soy sauce and miso samples. Raw samples (1 g) were weighed, placed onto a plate, and transferred to a vacuum freeze dryer (EYELA, Tokyo, Japan) to freeze-dry under vacuum at -80 °C for 48 h (Koyama, Nakamura, & Nakamura, 2013). The moisture of each sample were measured by gravimetry (loss on drying). Each chemical analysis was performed in triplicate.

### 2.3.3 Sensory Evaluation

During the annual Akita Prefectural Miso and Soy Sauce Products Competition, to evaluate and classify the sensory quality of the tested soy sauce miso samples, nine specially trained panelists (including 7 males and 2 females) working in the field of fermented foods and with expertise in food sensory evaluation were selected for sensory evaluation, which was conducted in two stages. The first stage used the 5-point preference test, in which scores of 1, 2, 3, 4, and 5 indicate much better, better, moderate, poorer, and much poorer, respectively (Hitomi, 1994). After evaluation, the sum of the scores evaluated by these nine panelists was the score of product in the first stage, and therefore the evaluated samples were ranked according to the ascendant order, with a best possible score of 9 points.

After the first stage of sensory evaluation, miso products with scores lower than 20 were able to enter the second stage. Therefore, the remaining miso products that did not enter the second stage had final sensory scores only in double digits and ranked according to the ascendant order. In the second stage, the nine panel members performed a 100-point classification test of the advanced samples with regard to external appearance, texture, aroma, taste, and overall acceptance. The final scores of the evaluation were computed by summing the scores obtained from panelists, and the samples were ranked according the descending order, with a better quality indicated by a higher score. The total possible score for each sample was 900 points.

### 2.3.4 Statistical Analysis

Means and standard deviations were calculated to analyze the chemical parameters of miso samples tested. All results are reported as the mean ± standard deviation of at least three measurements. The Student's *t*-test was applied to the data to determine significant differences. Principal component analysis (PCA) were conducted on the correlation matrices between chemical and the results of sensory quality using the mean values. All statistical analyses were performed using the SPSS software package (Version 22.0 for Windows; SPSS Inc., Chicago, IL, U.S.A.).

## 2.4 Results and Discussion

### 2.4.1 Soy Sauce

To understand the relationships between sensory and chemical parameters, the soy sauce products evaluated were divided into two groups based on the results of sensory evaluation obtained from 2016 and 2018 (Table 2-1), respectively. The detail group information was as follows: the products which enter the second stage during evaluation from 2016 to 2018 were classified into group S1, and the remaining products were classified into group S2, respectively. Generally, the overall preference of the S1 samples was better than that of the S2 samples. The average chemical parameters of the tested soy sauce products from different groups were used to determine different sensory preferences for soy sauce products.

Table 2-1 Sensory scores of soy sauce products submitted to the Akita Prefectural Miso and Soy Sauce Products Competition from 2016 to 2018. The products from each year are divided into groups according to the ranking results.

| Rank | Sensory Score | | | Group | Information |
|---|---|---|---|---|---|
| | 2016 | 2017 | 2018 | | |
| 1 | 856 | 844 | 852 | S1 | Samples evaluated by both the first and the second stage of sensory evaluation. Ascendant ranking order based on sensory scores.. |
| 2 | 853 | 838 | 845 | | |
| 3 | 852 | 836 | 843 | | |
| 4 | 834 | 831 | 840 | | |
| 5 | 824 | 829 | 827 | | |
| 6 | 822 | 817 | 817 | | |
| 7 | 808 | 801 | 817 | | |
| 8 | 805 | 794 | 803 | | |
| 9 | 796 | 793 | 802 | | |
| 10 | 781 | 787 | 785 | | |
| 11 | 780 | 784 | 781 | | |
| 12 | 779 | 778 | 768 | | |
| 13 | 771 | 21 | 22 | S2 | Samples only evaluated by the first stage of sensory evaluation. Descending ranking order based on sensory scores. |
| 14 | 22 | 22 | 22 | | |
| 15 | 25 | 24 | | | |
| 16 | 27 | | | | |

2.4.1.1 Color

The average values of *a**, *b**, and *L** of the tested soy sauce samples in two different groups are shown in Figure 2-1. For 2016 soy sauce samples, the average value of *a** ranged from 0.26 (S2) to 0.39 (S1), *b** ranged from 0.03 (S2) to 0.06 (S1), and *L** ranged from 24.00 (S1) to 24.09 (S2), respectively. For 2017 soy sauce samples, the average values of *a** ranged from 0.24 (S2) to 0.37 (S1), *b** ranged from 0.41 (S2) to 0.53 (S1), and *L** ranged from 24.09 (S2) to 24.20 (S1), respectively. Finally, the average value of *a** for 2018 soy sauce samples ranged from -0.05 (S2) to 0.17 (S1), *b** ranged from 0.32 (S1) to 0.42 (S2), and *L** ranged from 20.73 (S1) to 21.43 (S2), respectively.

It was observed that *a** values of the high-ranking soy sauces were significantly higher than those of low-ranking soy sauces (Figure 2-1(a)). In general, *koikuchi* soy sauce naturally has a deep reddish-brown color mainly resulting from nonoxidative and nonenzymatic browning reactions (Fukushima, 2004). During evaluation, the color of *koikuchi* soy sauce was an important indicator for good appearance. This observation was suggested that $a^*$ values was a key contributors to the desired appearance of soy sauce.

Though the $b^*$ and $L^*$ values also had effect on the deep reddish-brown color, the difference in $b^*$ and $L^*$ values between these two different groups from 2016 to 2018 were not very significant. Therefore, these two parameters might not be directly correlated to the final quality of soy sauce.

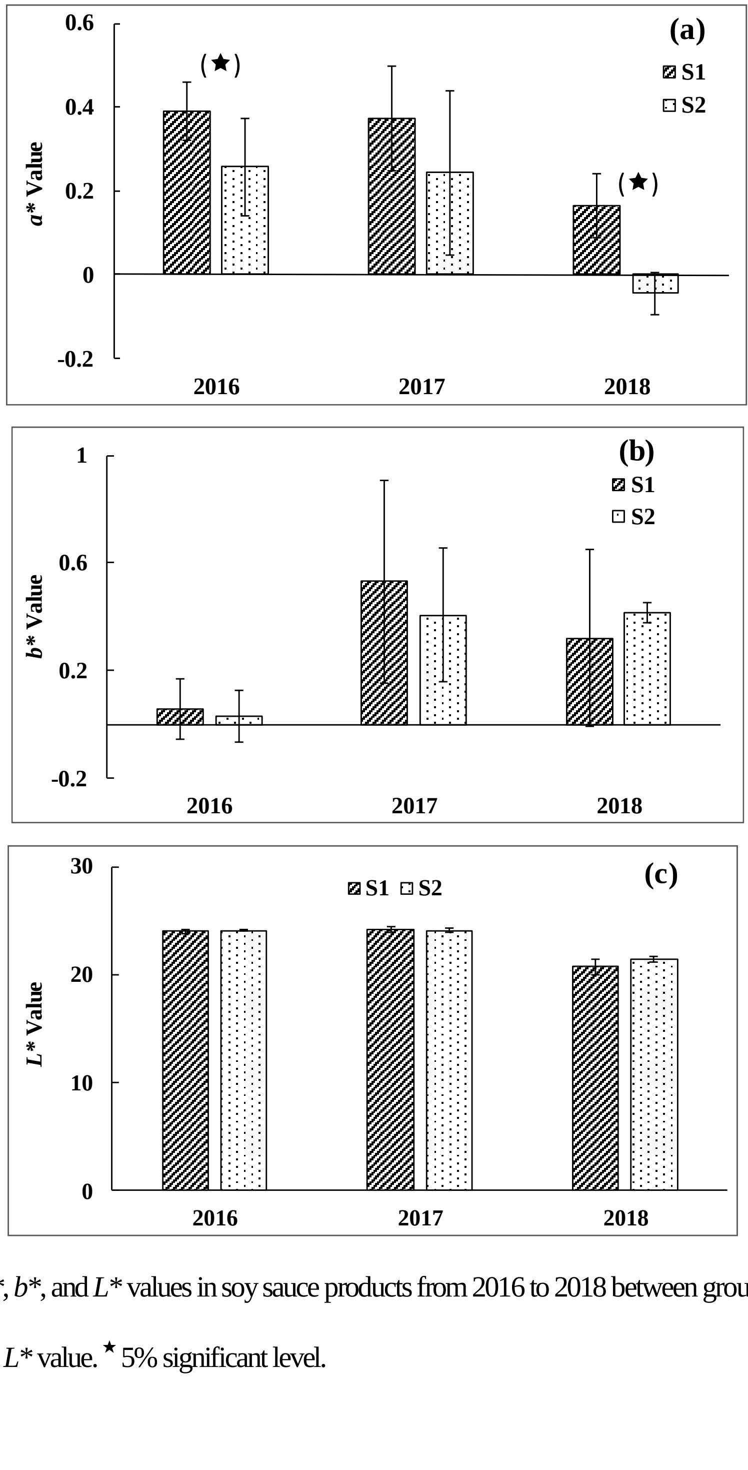


Figure 2-1 Average $a^*$, $b^*$, and $L^*$ values in soy sauce products from 2016 to 2018 between groups S1 and S2: (a) $a^*$ value, (b) $b^*$ value, (c) $L^*$ value. ★ 5% significant level.

2.4.1.2 Salt Content and SSFSC

The effect of the relationship between salt content and SSFSC (as shown in Figure 2-2) on the sensory quality of soy sauce was studied. Except for the sample that ranked at 14th in 2017, the salt content of all tested soy sauces was in the range 10-15%. However, there were obvious differences in the SSFSC, which ranged from 15% to 45%. Interestingly, the sensory quality of the tested soy sauces was markedly affected by the salt content and SSFSC, with the top three soy sauces showing low salt contents (about 10% – 12%) and SSFSC greater than 40%. Intermediate ranking soy sauces showed higher salt contents (12 – 14%) and lower SSFSC (28 – 36%). The lower ranking soy sauces comprised those with the highest salt contents and the lowest SSFSC. From the clear group information collected for the tested soy sauces, it could be suggested that the SSFSC and salt content were important indicators for the sensory quality of soy sauce.

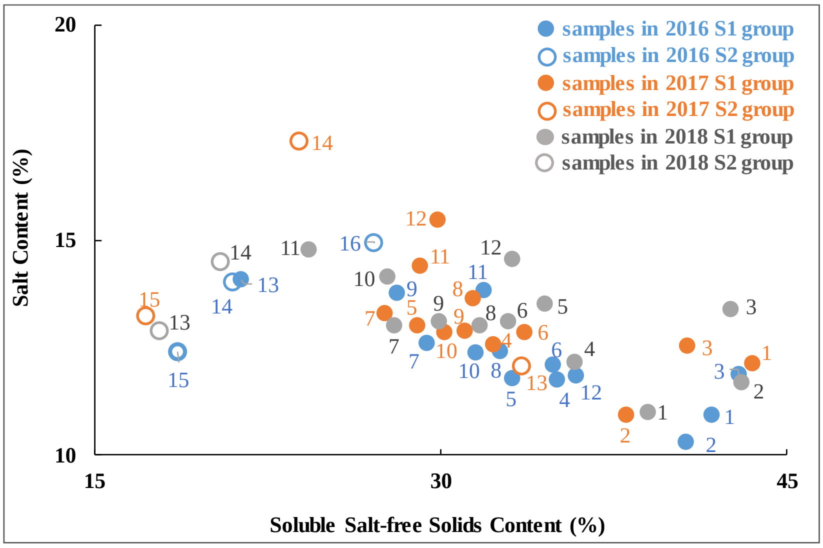


Figure 2-2 Plot of salt content versus SSFSC for soy sauce products from 2016 to 2018. The numbers in the figure represent the rankings of each sample during the annual competition.

The SSFSC comprises substances other than water, salt, and insoluble substances, and mainly includes proteins, amino acids, peptides, sugars, and organic acids. SSFSC is also an important indicator of flavor for commercial soy sauce. The good relationship found between SSFSC and soy sauce quality indicating the good quality of soy sauce closely related to a higher SSFSC (more than 40 %).

The salt in soy sauce was mainly sodium chloride, which influenced not only the salty taste, but also the texture, and played an important role in preserving soy sauce against microbes (Hoang et al., 2016; Kremer, Mojet, & Shimojo, 2009). salt is associated with the aroma of soy sauce (Lioe et al., 2004) and reportedly able to interact with some free amino acids, especially glutamic acid and phenylalanine, synergistically to enhance the umami of soy sauce (Lioe et al., 2005). However, excessive salt can cause the flavor to diminish and generate an astringent taste. It is found that the high salty contents should be one of the key reasons that lead to the samples ranked at the lower positions, for example, the samples that ranked at 14th in 2017.

#### 2.4.1.3 Carbohydrates and ethanol

The carbohydrates in soy sauce not only had an important influence on the sweet taste, but also helped subdue its saltiness. The main carbohydrates found in the tested soy sauces were isomaltose, glucose and fructose (as shown in Figure 2-3**)**. During the determination of the carbohydrates, there was also a peak assigned to ethanol was detected, therefore, the ethanol content in soy sauce products was also measured. The total sugar content was found to be associated with the glucose content due to the glucose content being the highest. As shown in Figure 2-3, the carbohydrates and ethanol content was closely related to the results of sensory evaluation, with the carbohydrates and ethanol content in high-ranking soy sauces was clearly higher than those in low-ranking soy sauces. As a result, these compositions in soy sauce products was considered to be a key contributor to improve the quality of soy sauce.

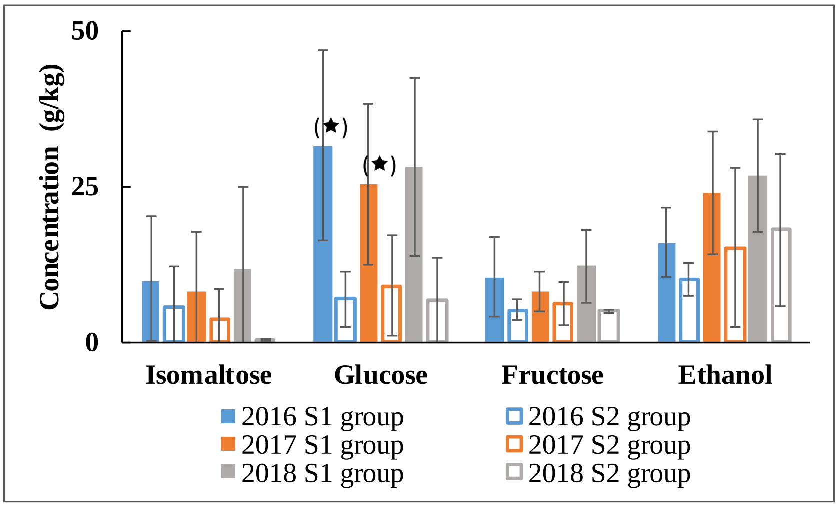


Figure 2-3 Average carbohydrates and ethanol content in soy sauce products between groups S1 and S2 from 2016 to 2018. ★ 5% significant level.

#### 2.4.1.4 Acids

Next, the acid content of the tested soy sauces was further studied with the goal of understanding the influence of acid on sensory quality. The major acid substances detected in the tested soy sauces were phosphoric acid, citric acid, malic acid, succinic acid, lactic acid, formic acid, acetic acid, and pyroglutamic acid. The average concentration of each group for the soy sauces tested from 2016 to 2018 are shown in Figure 2-4. The phosphoric acid, lactic acid, citric acid, and pyroglutamic acid were the main components of *koikuchi* soy sauce produced in Akita area. Prior work has documented that acids were important to produce a good flavor of Japanese fermented soy sauce in brine fermentation (Noda, Hayashi, & Mizunuma, 1980; Osaki et al., 1985). It can be observed from Figure 2-4 that the sensory quality increased with increasing acid concentration for all acids except formic acid, and especially phosphoric acid and acetic acid. The concentration of formic acid showed an opposite trend to the quality improved. These results demonstrate that acids in soy sauce had an important influence on the determination of quality.

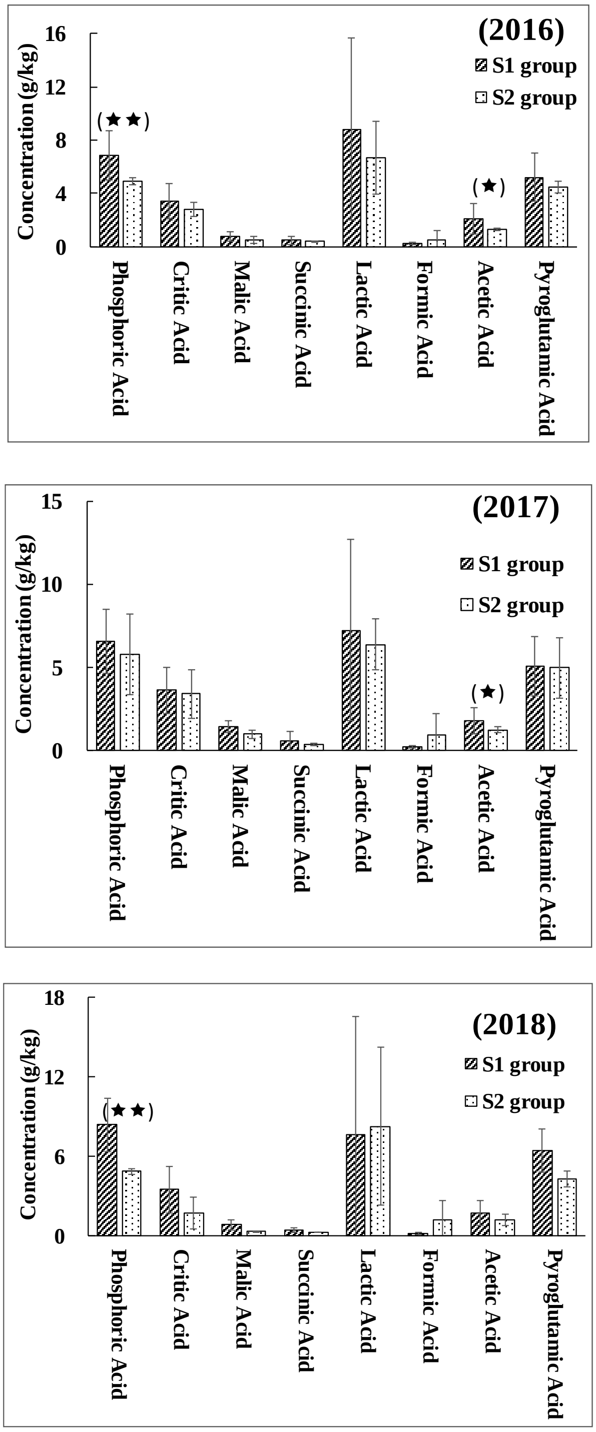


Figure 2-4 Average acids content in soy sauce products from 2016 to 2018 between groups S1 and S2. ★ 5% significant level. ★★ 1% significant level.

2.4.1.5 Amino Acids

A total of 20 amino acids was detected, including taurine, aspartic acid, threonine, serine, glutamic acid, α-aminoadipic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, β-alanine, γ-aminobutyric acid, lysine, histidine, and arginine, and the total amino acid concentrations of all tested soy sauces were calculated as shown in Figure 2-5. The average value of the total amino acid concentration for the tested soy sauce samples in S1 group was obviously higher than those of S2 group. Moreover, the concentration of glutamic acid in soy sauce products ranged at 5.91 g/kg – 17.01 g/kg in 2016, 8.77 g/kg – 16.26 g/kg in 2017, and 8.37 g/kg – 14.38 g/kg in 2018, had the highest content among the overall amino acids in soy sauce. It had been previously reported that glutamic acid is a key compound responsible for umami in soy sauce (Yamaguchi & Ninomiya, 2000). However, in combination with the results of sensory evaluation, the correlation coefficient between the concentration of glutamic acid and the rankings of products were 0.55 of 2016, -0.33 of 2017, and -0.21 of 2018, which was not as high as expected. Lioe et al. (2007) had also reported that *koikuchi* soy sauce containing a much lower glutamic acid content would have an intense umami taste when salt and phenylalanine were present. Consequently, the contribution of glutamic acid in soy sauce to the classification of quality was not very obvious as expected.

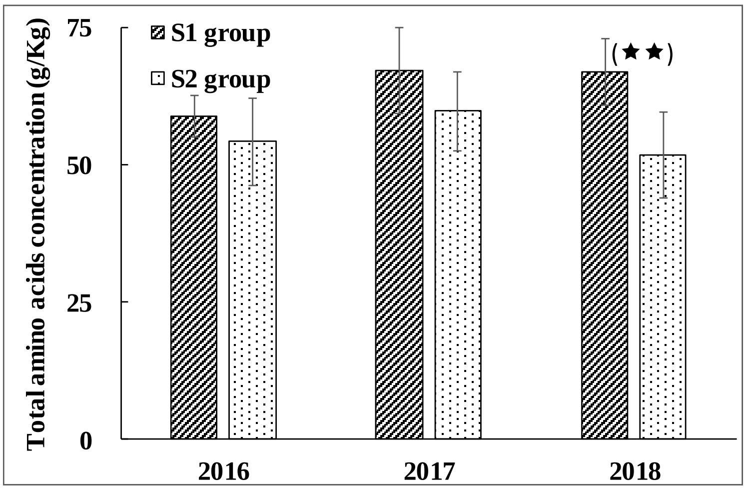


Figure 2-5 Average of the total amino acids concentration between groups S1 and S2 from 2016 to 2018. ★★ 1% significant level.

Furthermore, proline, which imparts sweetness, was shown to improve the soy sauce quality. The correlation coefficient between the concentration of proline in soy sauce sample from 2016 to 2018 and the ranking of these soy sauce samples were -0.75 of 2016, -0.73 of 2017, and -0.91 of 2018, indicating the sample at a higher ranking position contained a higher proline concentration. Nevertheless, the total contents of sweet taste-eliciting amino acids (including proline, glycine, alanine, threonine, and serine) had no related influence on the classification of sensory quality. As a result, this observation indicated that proline might contribute to the classification of the tested soy sauces. The previous study found that the presence of proline and glucose in gluten-free bread produced a much improved and acceptable aroma (Pacyński, Wojtasiak, & Mildner-Szkudlarz, 2015). This might suggest that the interaction between proline and glucose could exhibit a synergistic effect resulting in quality improvement of soy sauce.

#### 2.4.1.6 pH and Moisture

The sour taste is related to the acidity of the fermented soy sauces, therefore, the pH value of the tested soy sauces was determined, and also the average pH value for the groups S1 and S2 were calculated and recorded in Figure 2-6. The little difference of the available acidity in the classification of tested soy sauces was observed by pH values with a negligible difference between groups S1 and S2 in each year (as shown in Figure 2-6). This observation explained that the acidity had no direct relationship with the classification of soy sauce.

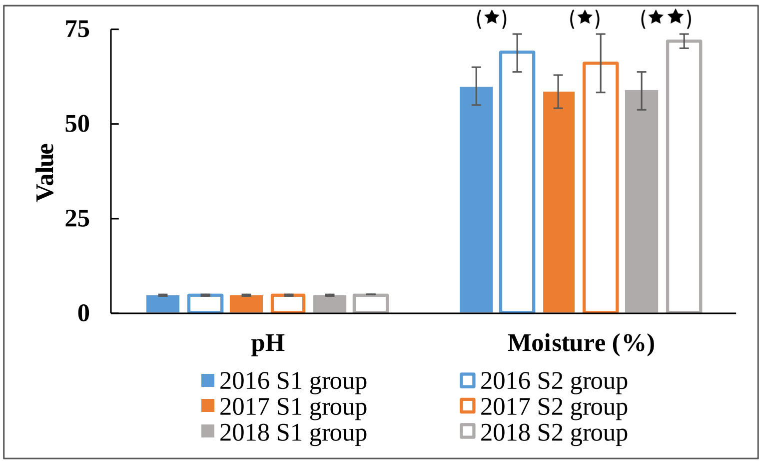


Figure 2-6 Comparison of the average pH value and moisture content in soy sauce products between groups S1 and S2 from 2016 to 2018. ★ 5% significant level. ★★ 1% significant level.

However, the significant difference in the moisture content for different groups that represented different sensory qualities, that is, the average moisture content of group S1 in 2016, 2017, and 2018, was clearly lower than that of S2. This indicated that the negative correlation between the moisture content and the sensory quality of soy sauce. The SSFSC in soy sauce also had a negative influence on the moisture level. According to the previously studies (Hamano & Sugimoto, 1978; Hamidi-Esfahani, Shojaosadati, & Rinzema, 2004), the moisture content had a great influence on the fermentation and texture formation of soy sauce. Therefore, the moisture content was considered to have an obvious relationship with the classification of soy sauce product.

#### 2.4.1.7 PCA Results

PCA is generally used to transform initial variables (sensory attributes) into a new set of variables as principal components that explain most of the variance observed. In the current study, PCA was performed on a dataset that included standardized values for a total of sensory attributes in soy sauce produced in Akita area from 2016 to 2018 to determine which compositions contributed most to the classification of soy sauce. A Principal component (PC) with eigenvalue greater than 1 was considered important and extracted due to the significant amount of variance they account for. Consequently, the first fourth principal components explain 97.97% of variance were extracted.

The first two PCs (PC1 and PC2) obtained from the original attribute measurements resulted in 79.27% total variance. PC1 had an eigenvalue of 24.18 and described 62.00% of the total variance, and covered as much of the variance as possible. PC2 had an eigenvalue of 6.74 and described 17.27% of the total variance. The PCA scores of samples for PC 1 versus PC2 are shown in Figure 2-7. As shown in the plot of PCA scores (Figure 2-7 (a)), the soy sauce samples assigned with different quality characteristics were clearly discriminated. The high-ranking samples in the groups S1 from 2016 to 2018, which represent the better sensory quality from sensory evaluation, were grouped together, located on the positive axis of PC1. In contrast, the soy sauce samples in S2 group from 2016 to 2018 were located on the negative axis of PC1.

PC1 was considered as a major contributor to the classification of the tested soy sauce. The main loadings of each principal component are shown in Figure 2-7 (b). It was found that PC1 was positively correlating with the value of a*, SSFSC, glucose, most of acids except for lactic acid and formic acid, and most of the detected amino acids, especially for proline, and negatively correlating with the formic acid, moisture, and salt content. These results are in agreement with the results above. PC2 is mainly involved in the *L**, pH, and lactic acid.

Moreover, the correlation matrix also showed some highly positively sensory attributes loaded on the positive axis of PC1: fructose, citric acid, malic acid, acetic acid, pyroglutamic acid, taurine, threonine, serine, glycine, valine and arginine. Although the related correlation between these compositions and the sensory quality of the tested soy sauce was not very significant previously, it can be deduced from the results based on PCA that there exist the synergistic relationship between the sensory quality and individual content of these compositions. Therefore, these compositions were also considered as an important contributor to involve in the assessment of the final quality for soy sauce products.

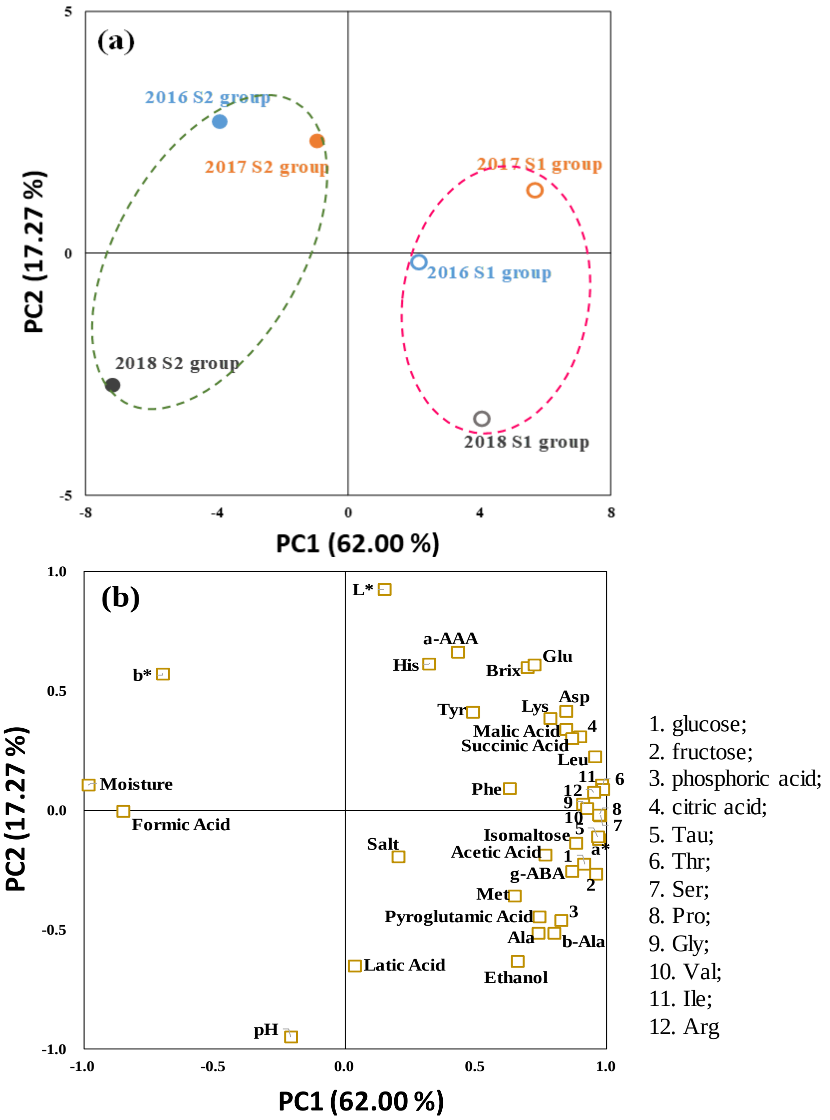


Figure 2-7 Principal component analysis (PCA) performed on the chemical compositions for soy sauce samples produced from 2016 to 2018: (a) Scores and (b) loadings.

### 2.4.2 Miso

As the same as the soy sauce samples, the miso products were also divided into two groups (M1 and M2) to well understand the effect of chemical compositions on the quality based on the results of the sensory evaluation (Table 2-2). The detail group information was as follows: the top 13 miso products which enter the second stage from 2016 and 2017 and the top 14 miso products which enter the second stage in 2018 were classified into group M1, and the remaining miso products which remained at the first stage were classified into groups M2, respectively. Consequently, the overall preference of the miso samples in group M1 was better than that of the miso samples in group M2. The average chemical parameters of the tested miso products from different groups were used to determine different sensory preferences for miso products.

Table 2-2 Sensory scores of miso products submitted to the Akita Prefectural Miso and Soy Sauce Products Competition from 2016 to 2018. The products from each year are divided into groups according to the ranking results.

| Rank | Sensory Score | | | Group | Information |
|---|---|---|---|---|---|
| | 2016 | 2017 | 2018 | | |
| 1 | 845 | 859 | 854 | M1 | Samples evaluated by both the first and the second stage of sensory evaluation. Ascendant ranking order based on sensory scores.. |
| 2 | 845 | 842 | 840 | | |
| 3 | 842 | 841 | 838 | | |
| 4 | 836 | 839 | 836 | | |
| 5 | 829 | 832 | 814 | | |
| 6 | 828 | 831 | 790 | | |
| 7 | 825 | 812 | 775 | | |
| 8 | 820 | 801 | 770 | | |
| 9 | 809 | 796 | 766 | | |
| 10 | 807 | 795 | 766 | | |
| 11 | 787 | 792 | 764 | | |
| 12 | 753 | 773 | 763 | | |
| 13 | 746 | 758 | 748 | | |
| 14 | 20 | 20 | 747 | | |
| 15 | 20 | 21 | 20 | M2 | Samples only evaluated by the first stage of sensory evaluation. Descending ranking order based on sensory scores. |
| 16 | 21 | 21 | 21 | | |
| 17 | 22 | 22 | 21 | | |
| 18 | 23 | 23 | 21 | | |
| 19 | 23 | 23 | 22 | | |
| 20 | 23 | 25 | 23 | | |
| 21 | 23 | 25 | 23 | | |
| 22 | 24 | 25 | 23 | | |
| 23 | 24 | 25 | 23 | | |
| 24 | 26 | 25 | 23 | | |
| 25 | 28 | 25 | 25 | | |
| 26 | 28 | 27 | 26 | | |

#### 2.4.2.1 Color

The average values of *a**, *b**, and *L** of the tested miso products in different groups are shown in Figure 2-8. As it can be seen that the average value of *a** ranged from 10.43 to 9.70 for groups M1 and M2 in 2016, from 12.78 to 11.42 for groups M1 and M2 in 2017, from 11.01 to 10.17 for groups M1 and M2 in 2018, respectively (Figure 2-8 (a)); the average value of *b** ranged from 18.40 to 21.60 for groups M1 and M2 in 2016, from 20.99 to 23.04 for groups M1 and M2 in 2017, from 19.14 to 21.29 for groups M1 and M2 in 2018, respectively (Figure 2-8 (b)); the average value of *L** ranged from 37.47 to 42.64 for groups M1 and M2 in 2016, from 43.99 to 48.89 for groups M1 and M2 in 2017, from 42.21 to 45.00 for groups M1 and M2 in 2018, respectively (Figure 2-8 (c)).

It is found that the miso products in group M1 had higher *a** values than those in M2 from the same year. This is well correlated with the preference of red color for the external appearance of miso. The *b** values of M1 were lower than those of M2. This showed that the *b** value had a negative influence on the external appearance of the miso products. Comparing the average *L** values of M1 and M2 from 2016 showed that an increased *L** value had a negative effect on the sensory quality. The same observation was also made for M1 and M2 from 2017 and 2018, suggesting that a lower brightness improved the external appearance of miso. In general, the color data in Figure 2-8 indicates that the parameters of color had a significant influence on the sensory quality of rice miso produced in the Akita area, with a reddish-brown color is preferred, in agreement with the previously published studies (Atsuko & Ichiro, 2008; Isao & Keiji, 1998).

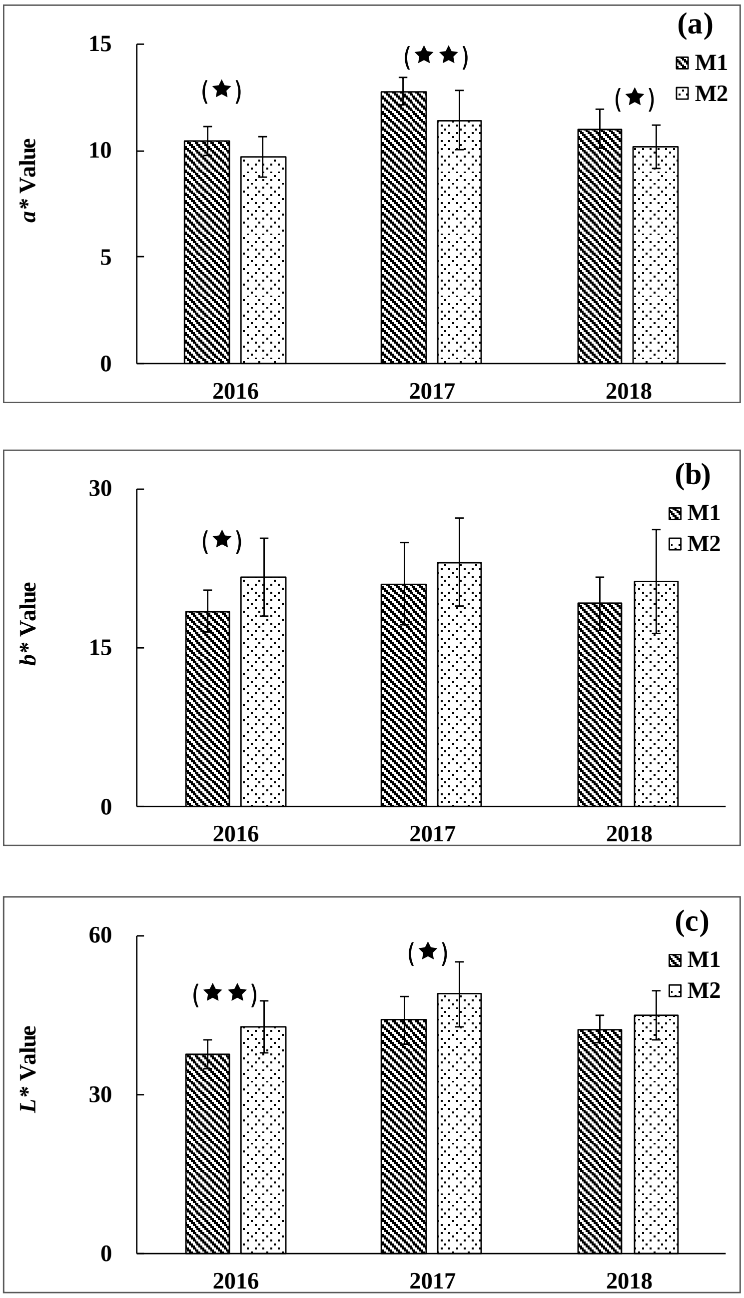


Figure 2-8 Average $a^*$, $b^*$, and $L^*$ values in miso products from 2016 to 2018 between groups M1 and M2: (a) $a^*$ value, (b) $b^*$ value, (c) $L^*$ value. ★ 5% significant level. ★★ 1% significant level.

#### 2.4.2.2 Salt Content and SSFSC

The analysis of salt and SSFSC in miso were conducted on the soluble fraction of miso. The average values of salt and SSFSC for miso samples evaluated from 2016 to 2018 were shown in Figure 2-9. The salt content for the miso samples was ranged greater than soy sauce samples with a range from 6.9% to 14.89 %, and also most of the miso samples were contained salt content about 12%. While the SSFSC were ranged smaller than soy sauce samples with an approximate range from 30% to 44%. As can be seen from Figure 2-9, most of the samples in group M1 which represented a better sensory quality were gathered together in the center of the figure, with the salt content was about 12% and the range of SSFSC was from 35% to 40%.

The salt in miso mainly comprises sodium chloride, which not only provides the typical salty taste and contributes to overall taste improvements of miso, but also affects the activity and growth of microorganisms (Park et al., 2012; Sugawara et al., 1994). Moreover, miso production in Akita Prefecture showed a preference for a heavy and salty taste, which had a good seasoning and freshening effect (Nowak & Kuligowski, 2017). Consequently, it was found that some miso samples (i.e. the samples ranked at 14th, 20th, 23th, and 26th in 2016, 14th, 17th, 19th, and 24th in 2017, 20th, 23th, and 26th in 2018) contained a salt content lower than 11% and ranked at a lower position based on sensory evaluation, indicating the improvement of miso quality needed a certain salt content. On the other hand, the sensory quality was also found to deteriorate in miso products with salt concentrations higher than 13%. For example, samples ranked 18th and 24th in 2016, 20th and 26th in 2017, and 19th in 2018 had salt concentrations of 13.21%, 14.89%, 13.13%, 14.66%, and 14.00%, respectively. These samples were classified as having low sensory quality during competitions, due in part to the large amount of salt destroying the mouthfeel and providing an undesirable saltiness. It was also believed that lactic acid bacteria growth, which is necessary to provide the desired environment for the next stage of fermentation, including alcoholic yeast fermentation, to give miso the desired flavor and aroma, was inhibited by excessive salt concentrations during miso fermentation (Klinke, Thomsen, & Ahring, 2004).

Although most of the miso samples annually ranked from 21st to 26th possessed a 12% salt content, the SSFSC for these samples was lower than 34%, indicating SSFSC also had an influence on the sensory classification of miso products. To sum up, unlike soy sauce, good quality of miso product requires an appropriate salt and SSFSC range, i.e., about 12% salt content and 35% to 40% SSFSC.

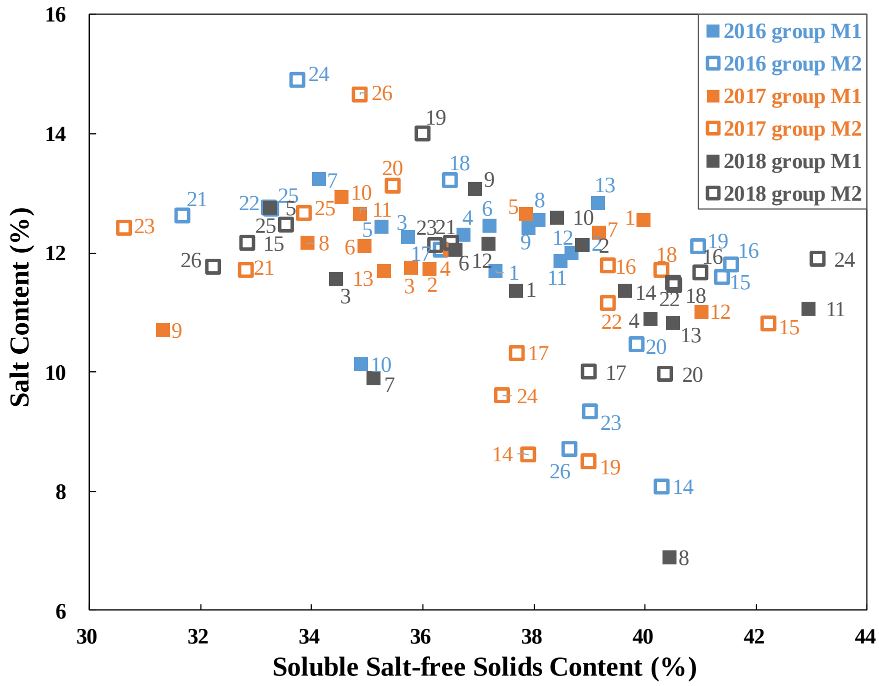


Figure 2-9 Plot of salt content versus SSFSC for soy sauce products from 2016 to 2018. The numbers in the figure represent the rankings of each sample during the annual competition.

### 2.4.2.3 Carbohydrates and ethanol

The main carbohydrates found in the tested miso were isomaltose, glucose, and fructose. The average carbohydrate compositions and ethanol content of the tested miso samples from each group are shown in Figure 2-10. The predominant carbohydrate composition was glucose owing to the majority of starch being saccharified to glucose (Anuradha, Suresh, & Venkatesh, 1999). A negative correlation was observed between the glucose level and sensory quality, which showed an opposite trend with soy sauce product: the average glucose level of group M1 from 2016 to 2018 was 135.21 g/kg, 133.57 g/kg, and 141.12 g/kg which was lower than that of group M2 (2016, 150.11 g/kg; 2017, 155.94 g/kg; 2018, 147.01 g/kg). Sugars in miso not only provide sweetness, but can be used as raw materials in other important reactions during fermentation, as follows (Chiou, 1999; Kwak & Lim, 2004): (i) Miso sugars are digested in lactic acid fermentation to produce lactic acid and acetic acid, which contributes to miso acidity; (ii) participate in alcoholic fermentation to produce ethanol, higher alcohols, and organic acids, which affects both the acidity and aroma of miso; and (iii) undergo amino-carbonyl reactions with amino acids to form brown pigments and a delicious flavor. Accordingly, the lower amount of glucose in group M1 than in group M2 might be related to the consumption of glucose for leading to a wide range of biochemical changes, which had a positive influence on the final product. Consequently, it is believed that the high rankings of miso samples imparted with lower glucose levels were due to good fermentation properties. Additionally, it is found that the glucose levels in M2 varied widely, with some miso samples showing obviously higher glucose levels, perhaps due to a lack of yeast activity, such as in 2016 miso samples ranked 19th (189.44 g/kg), 20th (177.62 g/kg), and 23rd (182.38 g/kg), 2017 miso samples ranked 15th (205.12 g/kg), 16th (176.20 g/kg), 17th (182.51 g/kg), 19th (174.99 g/kg), and 22nd (205.12 g/kg), and 2018 miso samples ranked 16th (175.26 g/kg), 20th (170.80 g/kg), 22th (184.25 g/kg) and 24nd (168.42 g/kg) In contrast, some tested miso samples in groups group M2 showed much lower glucose levels, which might be due to the components of rice being poorly digested by the enzymes produced by koji, such as 2016 miso samples ranked 21st (109.95 g/kg), 22nd (117.90 g/kg), and 24th (127.24 g/kg), 2017 miso samples ranked 21st (111.81 g/kg), 23rd (96.38 g/kg), and 25th (123.77 g/kg), and 2018 miso samples ranked 23st (101.68 g/kg), 25rd (117.64 g/kg), and 25th (120.55 g/kg).

Next, the average values of ethanol of group M1 in 2017 and 2018 were significantly higher than that of group M2, indicating the positive role of ethanol played in the miso quality. Moreover, it is also observed that the standard deviations in group M1 was smaller than those in group M2. This might be due to the technological maturity of fermentation used by indigenous companies. The companies that produced miso samples found in group M1 seemed to employ more sophisticated techniques for miso fermentation, which contributed to maintaining and improving beneficial miso components.

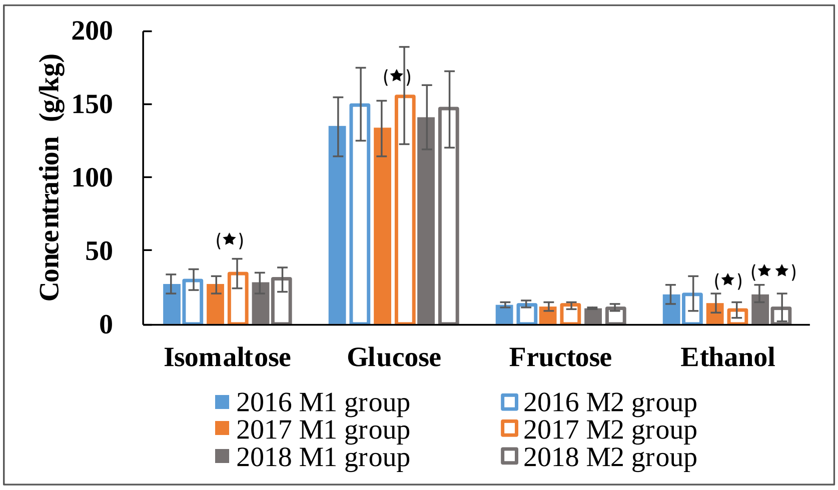


Figure 2-10 Average carbohydrates and ethanol content in miso products between groups M1 and M2 from 2016 to 2018. ★ 5% significant level. ★★ 1% significant level.

### 2.4.2.4 Acids

The acid compositions of the tested miso samples were further studied to understand the influence of acids on sensory quality. The major acid components detected in the miso samples tested were the same as the previous study of soy sauce, that is, phosphoric acid, citric acid, malic acid, succinic acid, lactic acid, formic acid, acetic acid, and pyroglutamic acid. Phosphoric acid in miso was derived from phytic acid in rice, while the other organic acids were produced by lactic acid fermentation and alcoholic fermentation. The average concentration of each group (M1 and M2) is shown in Figure 2-11. Phosphoric acid, citric acid, and pyroglutamic acid were the main acid components in the tested miso samples. The sensory quality increased with increasing acid concentration for all acids except formic acid and lactic acid, and especially phosphoric acid, citric acid, and pyroglutamic acid. The organic acids in miso reacted with ethanol and higher alcohols to form esters had a positive influence on miso aroma (Harada et al., 2018). It also had been reported that an increased amount of organic acid could improve the degree of perceived saltiness and taste preference (Yi, Tsai, & Liu, 2017). Phosphoric acid can also reduce the dephosphorylation activity of inosine monophosphate and contribute to the improved characteristic umami taste of glutamate in miso (Marui et al., 2013). Therefore, acids in miso seem to show a positive correlation with improved miso sensory quality. In contrast, Figure 2-11 also shows that the sensory quality was negatively correlated with the average level of lactic acid. Group M2 of ordinary sensory quality from 2017 and 2018, respectively, possessed a higher concentration of lactic acid than that of group M1. Moreover, a large amount of lactic acid in miso is likely to result in an undesired sour taste and should be avoided (Rhee, Lee, & Lee, 2011).

Notably, the lactic acid levels of the 10th (3.11 g/kg) and 20th (3.83 g/kg) ranked samples in 2016, the 9th (2.05 g/kg), 13th (3.18 g/kg), 17th (4.30 g/kg), and 21st (2.86 g/kg) ranked samples in 2017, and 7th (7.06 g/kg), 8th (0.87 g/kg), 17th (5.65 g/kg), and 26st (9.57 g/kg) ranked samples in 2018 were obviously higher than those of other samples. Conversely, the salt contents of these samples were 10.14%, 10.46%, 10.71%, 11.70%, 10.30%, 11.71%, 9.90%, 6.90%, 10.00%, and 11.76%, respectively, which were lower than those of other samples. This observation is reasonable to surmise that a low salt content in miso would not contribute to the condition for the halophilic yeast growth, and then affected the latter reaction of lactic acid. Additionally, the 23rd and 26th ranked samples from 2016 possessed salt contents of 9.34% and 8.70%, the 19th and 24th ranked samples from 2017 possessed salt contents of 8.50% and 9.61%, and the 20th ranked samples from 2018possessed salt contents of 9.96% respectively. As these samples had salt contents of less than 10% and lactic acid concentrations of less than 0.10 g/kg, and were ranked at a much lower position, it was suggested that a low salt concentration (lower than 10%) in miso might restrict the induction of halophilic yeasts growth, which has an inhibitory effect on the acid production (Onda et al., 2002). As a result, the latter formation of flavor and aroma also seemed to be impaired and then the sensory quality in miso was decreased.

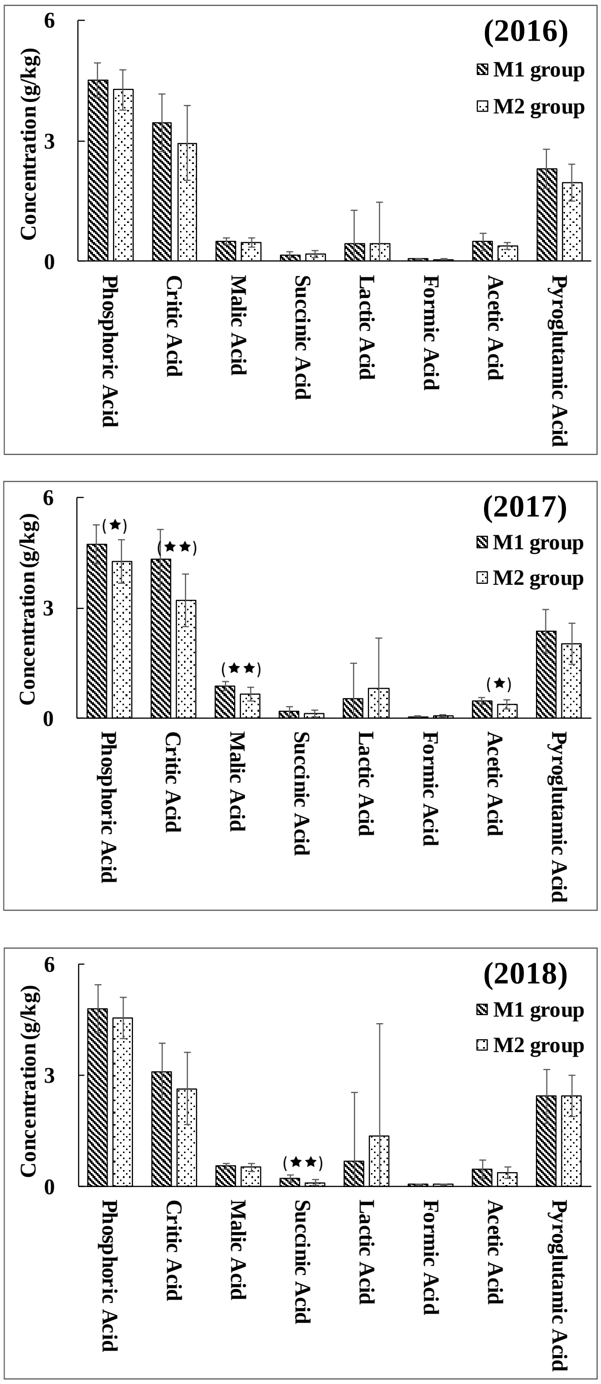


Figure 2-11 Average acids content in miso products from 2016 to 2018 between groups M1 and M2. ★ 5% significant level. ★★ 1% significant level.

2.4.2.5 Amino Acids

The major amino acid components detected in the tested miso samples were aspartic acid, threonine, serine, glutamic acid, α-aminoadipic acid, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, β-alanine, γ-aminobutyric acid, lysine, histidine, and arginine. In this work, the total amount of amino acids in the tested miso samples was calculated as the sum of these 20 amino acids. The average total amino acids of groups M1 and M2 are shown in Figure 2-12. A stable result was observed, with little difference in the total amounts of amino acids between groups with the same level of sensory quality from 2016 to 2018. However, group M1 which clearly showed the best sensory quality contained higher total amino acid concentrations than group M2. This evidence suggested that amino acids played an important role in improving miso sensory quality.

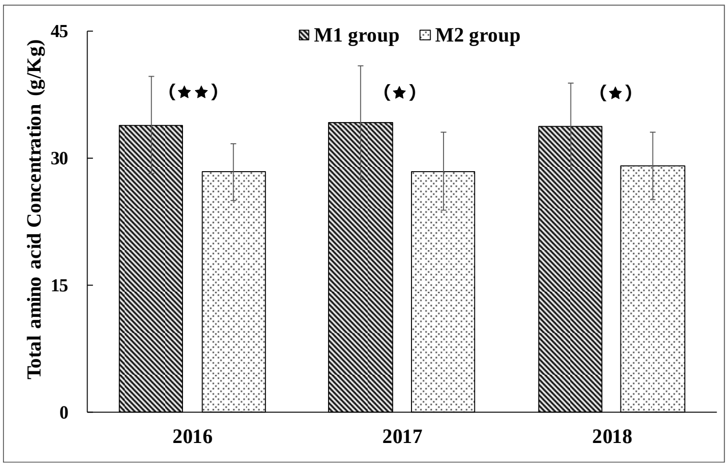


Figure 2-12 Average of the total amino acids concentration between groups M1 and M2 from 2016 to 2018. ★ 5% significant level. ★★ 1% significant level.

In this work, aspartic acid and glutamic acid were the two most abundant amino acids in the tested miso samples. Prior work has shown that aspartic acid and glutamic acid can be used to elicit the umami taste important for the quality of fermented soybean foods (Kato, Rhue, & Nishimura, 1989; Kurihara, 2009). The miso samples tested in group M1 were imparted with higher concentrations of aspartic acid and glutamic acid. The average aspartic acid concentrations in group M1 from 2016 to 2017 were 3.90 g/kg, 3.82 g/kg, and 3.72g/kg, respectively, and the aspartic acid concentrations in groups M2 (2016, 3.18 g/kg; 2017, 3.23 g/kg; 2018, 3.12 g/kg) decreased with decreasing sensory quality. The average concentrations of glutamic acid in group M1 from 2016 to 2017 were 5.83 g/kg, 5.78 g/kg, and 5.53 g/kg, respectively, which were much higher than those of group M2 (2016, 4.42 g/kg; 2017, 4.65 g/kg; 2018, 4.75 g/kg). The linear trends for the relationship between the concentration of glutamic acid and the final sensory scores of the samples classified into group M1 from 2016 to 2018 were calculated, and the relatively high correlation coefficients were also observed (2016, $r = 0.65$; 2017, $r = 0.55$; 2018, $r = 0.63$). It is suggested that the positive role of glutamic acid played on the sensory quality of miso.

In addition, the glutamic acid concentration was found to be obviously higher in samples ranked 3rd (2016, 8.42 g/kg; 2017, 9.24 g/kg; 2018, 7.86 g/kg) and 25th (2016, 6.44 g/kg; 2017, 8.58 g/kg; 2018, 9.61 g/kg). However, the concentrations of aspartic acid and other amino acids in these two samples were quite different, with the sample ranked 3rd containing higher concentrations of all amino acids, which may be due to the protein being better digested by protease from koji, while the sample ranked 25th in 2017 had a higher concentration of only glutamic acid and lower concentrations of the other amino acids. Comparing the sensory quality of these two samples showed that, although the concentrations of other amino acids were lower than those of aspartic acid and glutamic acid, they still had an important effect on the improved sensory quality.

### 2.4.2.6 pH and Moisture

The pH value and moisture content of the tested miso samples were determined, and also the average pH value and moisture content for the groups M1 and M2 were calculated and recorded in Figure 2-13. The little difference of these two compositions in the classification of tested miso samples was observed by pH values with a negligible difference between groups M1 and M2 in each year (as shown in Figure 2-6). This observation indicated that the measured pH value and moisture content had no direct relationship with the classification of miso.

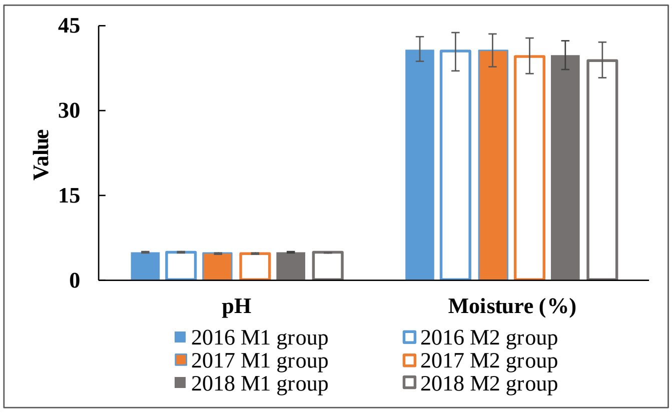


Figure 2-13 Comparison of the average pH value and moisture content in soy sauce products between groups M1 and M2 from 2016 to 2018.

2.4.2.7 PCA Results

The chemical compositions detected in all miso samples were further summarized and subjected to PCA to determine the chemical compositions that contributed mostly in sensory quality of miso. PCA result showed four PCs with eigenvalues greater than 1, which are considered important and accounted for 97.79% of total variation among the samples that were retained. The PC1 and PC2 obtained from the original attribute measurements resulted in 76.89% total variance. PC1 had an eigenvalue of 22.44 and described 57.53% of the total variance, and covered as much of the variance as possible. PC2 had an eigenvalue of 7.55 and described 19.36% of the total variance. The PCA scores of samples for PC1 versus PC2 are shown in Figure 2-14 (a). The miso samples from different groups M1 and M2 were clearly discriminated. The miso groups M1 from 2016 to 2018, which represent a better sensory quality, were grouped together in the positive axis of PC1, while the groups M2 from 2016 to 2018 were located on negative axis of PC2.

To explain the distribution of the tested miso samples, the correlation loadings plot of the chemical compositions in the first two PCs is also shown in Figure 2-14 (b). Overall, the amino acids, except glutamine, were positively loaded far from PC 1, which indicated the importance of these amino acids in miso for defining this PC. By comparison with the score plot (Figure 2-14 (a)), the positive loading indicating that the increased amino acid content in miso would contribute to improved sensory quality. Similarly, glucose and isomaltose were also far from PC1 and negatively loaded on this PC. Glucose and isomaltose were shown to be negatively correlated with the amino acids in miso. This could be attributed to glucose and isomaltose being digested for amino acid formation. Furthermore, $L^*$ value was located far from PC1 with a negative loading indicating a negative correlation with the miso sensory quality. The amounts of phosphoric acid, citric acid, malic acid, acetic acid, and pyroglutamic acid, had a positive contribution to the PC1, which were opposed to lactic acid and formic acid, indicating their different effects on the miso sensory quality. Lactic acid and formic acid were placed in negative side of PC1, indicating the negative correlation with the quality of miso. These findings agree well with the previous results.

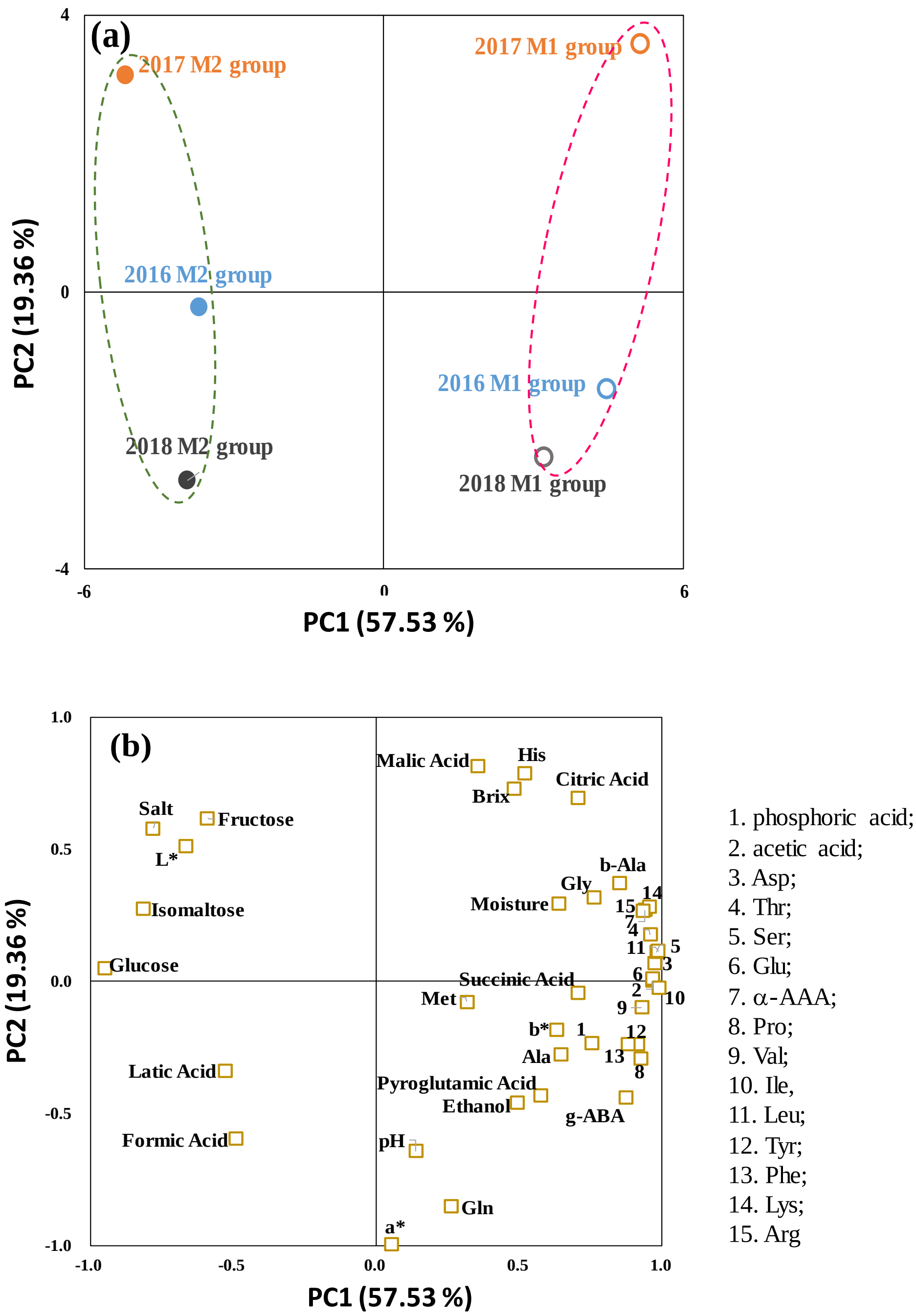


Figure 2-14 PCA performed on the chemical compositions for miso samples produced from 2016 to 2018: (a) Scores and (b) loadings.

## 2.5 Conclusions

The chemical analysis of soy sauce and miso products collected from the Akita Prefectural Miso Competition from 2016 to 2018 was conducted. Diversity in the chemical characteristics of both the tested soy sauce and miso samples was observed and resulted in different sensory qualities. The key attributes affecting product qualities were systematically studied in combination with the results of sensory evaluation: I) the good quality soy sauce should possess a color with relatively high +$a^*$ values. The soluble salt-free solids content of high-ranking soy sauce was much higher than that of low-ranking soy sauce, especially in the top three ranked soy sauces (at greater than 40%), while the salt content negatively affected soy sauce quality. The content of glucose, phosphoric acid, acetic acid, and the total amino acid, had a positive effect on the improvement of soy sauce quality. Particularly, proline showed an obviously positive relationship with the sensory quality. The PCA results confirmed the previous findings, and also highlighted the important role played by individual acids and amino acids which were not very obvious before in the assessment of soy sauce quality; II) color parameters $L^*$, $a^*$, and $b^*$ were significantly related to miso acceptance, with higher $a^*$ values found to improve the quality, while the other two parameters showed the opposite effect. During the whole fermentation process, carbohydrates can be consumed during fermentation to produce other beneficial substances, which has a positive impact on the classification of miso. Meanwhile, increased contents of acids and amino acids in miso were conducive to improving the sensory quality. The optimal salt content for miso was about 12%, while a soluble salt-free solids content ranging from 35% to 40% was generally considered acceptable. PCA results obtained from miso samples was agreed well with the previous results.

Considering correlations between the obtained chemical analysis parameters and sensory scores seems advisable to provide greater insight into the quality standardization of soy sauce and miso. Furthermore, because flavor is an important property that influences consumer preference and acceptance, further research is needed to combine the present work and the relationship between the flavor of these two kinds of condiments and the results of the sensory evaluation to provide a comprehensive understanding of the quality of fermented condiment products. This could provide an alternative approach to the objective sensory evaluation for quality control at the industrial level.

## 2.6 References


Anuradha, R., Suresh, A. K., & Venkatesh, K. V. (1999). Simultaneous saccharification and fermentation of starch to lactic acid. *Process Biochemistry, 35,* 367–375.

Atsuko, S., & Ichiro, N. (2008). Relationship between the color of soybean past and antioxidative activity. *Journal for the Integrated Study of Dietary Habits, 19,* 247–250.

Bonkohara, K., Fuji, M., Nakao, A., Igura, N., & Shimoda, M. (2016). Preparation of reminiscent aroma mixture of Japanese soy sauce. *Bioscience, Biotechnology, and Biochemistry, 80,* 533–539.

Chiou, R. Y. Y., Ferng, S., & Beuchat, L. R. (1999). Fermentation of low-salt miso as affected by supplementation with ethanol. *International Journal of Food Microbiology, 48,* 11–20.

Chiou, R. Y. Y. (1999). Salt-free Miso Fermentation Using Ethanol, Sugars, and Polyols. *Journal of Food Science, 64,* 918–920.

Fukushima, D. (2004). Industrialization of fermented soy sauce production centering around Japanese shoyu. In *Industrialization of Indigenous Fermented Foods, Revised and Expanded* (K.H. Steinkraus, pp. 1–98). New York: Marcel Dekker, Inc.

Giri, A., Okamoto, A., Okazaki, E., & Ohshima, T. (2010). Headspace Volatiles along with Other Instrumental and Sensory Analyses as Indices of Maturation of Horse Mackerel Miso. *Journal of Food Science, 75,* S406–S417.

Hamano, M., & Sugimoto, H. (1978). Water Sorption, Reduction of Caking and Improvement of Free Flowingness of Powdered Soy Sauce and Miso. *Journal of Food Processing and Preservation, 2,* 185–196.

Hamidi-Esfahani, Z., Shojaosadati, S. A., & Rinzema, A. (2004). Modelling of simultaneous effect of moisture and temperature on A. niger growth in solid-state fermentation. *Biochemical Engineering Journal, 21,* 265–272.

Harada, R., Yuzuki, M., Ito, K., Shiga, K., Bamba, T., & Fukusaki, E. (2018). Microbe participation in aroma production during soy sauce fermentation. *Journal of Bioscience and Bioengineering, 125,* 688–694.

Hitomi Y. (1994). Quality Evaluation of Rice Miso. *Brewing Society of Japan, 89,* 200–206.

Hoang, N. X., Ferng, S., Ting, C. H., Huang, W. H., Chiou, R. Y. Y., & Hsu, C. K. (2016). Optimizing the initial moromi fermentation conditions to improve the quality of soy sauce. *LWT, 74,* 242–250.

Isao, M., & Keiji, K. (1998). Salty Red Rice Miso (Echigo Miso—Sendai Miso). *Brewing Society of Japan*, *93*, 932–941.

Kaneko, S., Kumazawa, K., & Nishimura, O. (2011). Isolation and Identification of the Umami Enhancing Compounds in Japanese Soy Sauce. *Bioscience, Biotechnology, and Biochemistry*, 75, 1275-1282.

Kaoru O. (2006). A Comparison of the Analytical Data on Misos and Soysauces Submitted to Akita Prefectural Competitions during the Last 50 Years. *Akita Research of Food & Brewing*, *101*, 81–87.

Kato, H., Rhue, M. R., & Nishimura, T. (1989). Role of Free Amino Acids and Peptides in Food Taste. In R. Teranishi, R. G. Buttery, & F. Shahidi (Eds.), *Flavor Chemistry* (Vol. 388, pp. 158–174). Washington, DC: American Chemical Society.

Kitamura, Y., Kusumoto, K., Oguma, T., Nagai, T., Furukawa, S., Suzuki, C., & Tamaki, H. (2016). Ethnic Fermented Foods and Alcoholic Beverages of Japan. In P. T. Jyoti (Ed.), *Ethnic Fermented Foods and Alcoholic Beverages of Asia* (pp. 193–236). New Delhi: Springer.

Klinke, H. B., Thomsen, A. B., & Ahring, B. K. (2004). Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology*, *66*, 10–26.

Kobayashi, A., & Sugawara, E. (1999). Flavor Components of Shoyu and Miso Japanese Fermented Soybean Seasonings. In F. Shahidi & C. T. Ho (Eds.), *Flavor Chemistry of Ethnic Foods* (pp. 5–14). Boston, MA: Springer US.

Koyama, M., Nakamura, C., & Nakamura, K. (2013). Changes in phenols contents from buckwheat sprouts during growth stage. *Journal of Food Science and Technology, 50*, 86–93.

Kremer, S., Mojet, J., & Shimojo, R. (2009). Salt Reduction in Foods Using Naturally Brewed Soy Sauce. *Journal of Food Science*, *74*, S255–S262.

Kumazawa, K., Kaneko, S., & Nishimura, O. (2013). Identification and Characterization of Volatile Components Causing the Characteristic Flavor in Miso (Japanese Fermented Soybean Paste) and Heat-Processed Miso Products. *Journal of Agricultural and Food Chemistry*, *61*, 11968–11973.

Kurihara, K. (2009). Glutamate: From discovery as a food flavor to role as a basic taste (umami). *The American Journal of Clinical Nutrition*, *90*, 719S-722S.

Kwak, E. J., & Lim, S. I. (2004). The effect of sugar, amino acid, metal ion, and NaCl on model Maillard reaction under pH control. *Amino Acids*, *27*, 85–90.

Lioe, Hanifah N., Apriyantono, A., Takara, K., Wada, K., Naoki, H., & Yasuda, M. (2004). Low Molecular Weight Compounds Responsible for Savory Taste of Indonesian Soy Sauce. *Journal of Agricultural and Food Chemistry*, *52*, 5950–5956.

Lioe, Hanifah N., Apriyantono, A., Takara, K., Wada, K., & Yasuda, M. (2005). Umami Taste Enhancement of MSG/NaCl Mixtures by Subthreshold L-α-Aromatic Amino Acids. *Journal of Food Science*, *70*, s401–s405.

Lioe, Hanifah N., Selamat, J., & Yasuda, M. (2010). Soy Sauce and Its Umami Taste: A Link from the Past to Current Situation. *Journal of Food Science*, *75*, R71–R76.

Lioe, Hanifah N., Wada, K., Aoki, T., & Yasuda, M. (2007). Chemical and sensory characteristics of low molecular weight fractions obtained from three types of Japanese soy sauce (shoyu)–Koikuchi, tamari and shiro shoyu. *Food Chemistry*, *100*, 1669–1677.

Luh, B. (1995). Industrial production of soy sauce. *Journal of Industrial Microbiology*, *14*, 467–471.

Manta, C., Peralta-Altier, G., Gioia, L., Méndez, M. F., Seoane, G., & Ovsejevi, K. (2013). Synthesis of a Thiol-β-cyclodextrin, a Potential Agent for Controlling Enzymatic Browning in Fruits and Vegetables. *Journal of Agricultural and Food Chemistry*, *61*, 11603–11609.

Marui, J., Tada, S., Fukuoka, M., Wagu, Y., Shiraishi, Y., Kitamoto, N., & Kusumoto, K. I. (2013). Reduction of the degradation activity of umami-enhancing purinic ribonucleotide supplement in miso by the targeted suppression of acid phosphatases in the Aspergillus oryzae starter culture. *International Journal of Food Microbiology*, *166*, 238–243.

Miyagi, A. (2012). Research on purchase consciousness and color preference of Japanese soy sauce among general consumers in Chiba prefecture. *Journal for the Integrated Study of Dietary Habits*, *22*, 320–324.

Noda, F., Hayashi, K., & Mizunuma, T. (1980). Antagonism between osmophilic lactic acid bacteria and yeasts in brine fermentation of soy sauce. *Appl. Environ. Microbiol.*, *40*, 452–457.

Nowak, J., & Kuligowski, M. (2017). Functional Properties of Traditional Food Products Made by Mold Fermentation. In *Fermented Foods, Part II: Technological Interventions* (pp. 46–73).

Nunomura, N., Sasaki, M., & Yokotsuka, T. (1980). Shoyu (Soy Sauce) Flavor Components: Acidic Fractions and the Characteristic Flavor Component. *Agricultural and Biological Chemistry, 44*, 339–351.

Ogasawara, M., Yamada, Y., & Egi, M. (2006). Taste enhancer from the long-term ripening of miso (soybean paste). *Food Chemistry, 99*, 736–741.

Onda, T., Yanagida, F., Uchimura, T., Tsuji, M., Ogino, S., Shinohara, T., & Yokotsuka, K. (2002). Widespread distribution of the bacteriocin-producing lactic acid cocci in Miso-paste products. *Journal of Applied Microbiology, 92*, 695–705.

Osaki, K., Okamoto, Y., Akao, T., Nagata, S., & Takamatsu, H. (1985). Fermentation of Soy Sauce with Immobilized Whole Cells. *Journal of Food Science, 50*, 1289–1292.

O'toole, D. K. (2019). Chapter Two—The role of microorganisms in soy sauce production. In G. M. Gadd & S. Sariaslani (Eds.), *Advances in Applied Microbiology* (Vol. 108, pp. 45–113). Academic Press.

Pacyński, M., Wojtasiak, R. Z., & Mildner-Szkudlarz, S. (2015). Improving the aroma of gluten-free bread. *LWT - Food Science and Technology, 63*, 706–713.

Park, S. L., Lee, S. Y., Nam, Y. D., Yi, S. H., & Lim, S. I. (2012). Fermentation properties of low-salted Doenjang supplemented with licorice and mustard. *Food Science and Biotechnology, 21*, 91–99.

Ratnaningrum, D., Budiwati, T. A., Darsini, T., & Mawarda, P. C. (2018). The Production of Corn Kernel Miso Based on Rice-koji Fermented by Aspergillus oryzae and Rhizopus oligosporus. *Journal of Tropical Biodiversity and Biotechnology, 3*, 8–17.

Rhee, S., Lee, J. E., & Lee, C. H. (2011). Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories, 10*, S5.

Sasaki, M. (1996). Influence of Sodium Chloride on the Levels of Flavor Compounds Produced by Shoyu Yeast. *Journal of Agricultural and Food Chemistry, 44*, 3273–3275.

Steinhaus, P., & Schieberle, P. (2007). Characterization of the Key Aroma Compounds in Soy Sauce Using Approaches of Molecular Sensory Science. *Journal of Agricultural and Food Chemistry, 55*, 6262–6269.

Suezawa, Y., & Suzuki, M. (2007). Bioconversion of Ferulic Acid to 4-Vinylguaiacol and 4-Ethylguaiacol and of 4-Vinylguaiacol to 4-Ethylguaiacol by Halotolerant Yeasts Belonging to the Genus *Candida*. *Bioscience, Biotechnology, and Biochemistry*, *71*, 1058–1062.

Sugawara, E., Hashimoto, S., Sakurai, Y., & Kobayashi, A. (1994). Formation by Yeast of the HEMF (4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone) Aroma Component in Miso with Aging. *Bioscience, Biotechnology, and Biochemistry*, *58*, 1134–1135.

Yamaguchi, S., & Ninomiya, K. (2000). Umami and Food Palatability. *The Journal of Nutrition*, *130*, 921S-926S.

Yi, C., Tsai, M.-L., & Liu, T. (2017). Spray-dried chitosan/acid/NaCl microparticles enhance saltiness perception. *Carbohydrate Polymers*, *172*, 246–254.

Yokotsuka, T. (1961). Aroma and Flavor of Japanese Soy Sauce. In *Advances in Food Research* (Vol. 10, pp. 75–134). Elsevier.

Yoshida, H., Yamazaki, J., Ozawa, S., Mizukoshi, T., & Miyano, H. (2009). Advantage of LC-MS Metabolomics Methodology Targeting Hydrophilic Compounds in the Studies of Fermented Food Samples. *Journal of Agricultural and Food Chemistry*, *57*, 1119–1126.

Zhuang, M., Lin, L., Zhao, M., Dong, Y., Sun-Waterhouse, D., Chen, H., & Su, G. (2016). Sequence, taste and umami-enhancing effect of the peptides separated from soy sauce. *Food Chemistry*, *206*, 174–181.

# CHAPTER 3EFFECT OF VOLATILE COMPOUNDS OF SOY SAUCE AND MISO UPON THE SENSORY EVALUATION

## 3.1 Abstract

To understand the effects of volatile compounds on the evaluation of two traditionally famous fermented condiments, i.e. soy sauce and miso, so their quality could be classified, samples collected from 2015 to 2018 were evaluated then ranked. The volatile compounds of these evaluated samples were directly extracted by headspace analysis to reduce technical interference and to identify the most common compounds in soy sauce and miso. Sixty-two and forty-eight volatile compounds were identified in the evaluated soy sauces and miso, respectively. The correlation coefficients between the levels of volatile compounds detected and the sensory rankings of the evaluated samples were calculated. This enabled those volatile compounds having a greater measure of influence, both negatively and positively, on the quality of the soy sauce and miso to be identified. Finally, the obtained results on the basis of principal component analysis provided the specific understandings for the contribution of each compound detected in the present work to classify the soy sauce and miso products produced in Akita area, respectively.

## 3.2 Introduction

Measuring the quality of fermented condiments requires a comprehensive understanding of chemical composition, flavors, and sensory evaluation. The flavors for the products of fermented condiments are mainly produced during a long period of fermentation and are important distinguishing characteristics of fermented condiments. At present, the Japanese soy sauce and miso are indispensable for use in Japanese routine diet, and are also becoming popular after introduced to western countries as ethnic foods. One of the key indices thought necessary for the high-quality of soy sauce and miso products is an intense flavor. Nearly 300 and 200 volatile compounds have been found in Japanese fermented soy sauce and miso products, respectively, and the flavor of miso is difficult to evaluate due to this condiment was a paste that could not be pasteurized resulting in a higher instability than soy sauce (Honma, 1987; Sugawara, Saiga, & Kobayashi, 1994; T. Yokotsuka & Sasaki, 1997). The volatile compounds that had been detected in soy sauce and miso could be classified into different chemical classes, such as alcohols, esters, ketones, aldehydes, acids, furans, furanones, phenols, pyrazines, and sulfur-containing compounds (Feng et al., 2014; Sun, Jiang, & Zhao, 2010). The role that these compounds play in the flavor of both soy sauce and miso has been extensively studied (Kobayashi & Sugawara, 1999).

Many studies have used qualitative and/or quantitative analyses to investigate the volatile compounds responsible for the most important aromatic profiles of both soy sauce and miso. Some studies have investigated a single compound or several compounds which contribute to a particular note: for example, Meng et al. (2017) investigated how 2-methyl-3-furanthiol contributed to the cooked meat-like aroma of fermented soy sauce; The 4-Hydroxy-2 (or 5)-ethyl-5 (or 2)-3 (2H)-furanone imparted with a sweet aroma had been identified as a flavor component for miso (Sugawara, 1991b; Sugawara, Hashimoto, Sakurai, & Kobayashi, 1994).Other studies have investigated more complex situations, where those key volatile compounds were identified through comparing different indices, such as the threshold, and odor activity values (OVA), and FD factor (Kaneko, Kumazawa, & Nishimura, 2012; Kumazawa, Kaneko, & Nishimura, 2013; Wang, Fan, & Xu, 2014). However, these indices are mainly related to the perceived intensity of each volatile compound present in products. The composition of the overall aroma of soy sauce and miso products is very complicated and there is still little understanding of the relationships between the analytical data on the volatile compounds and the product qualities indicated by assessing the aroma.

Volatile compounds have usually been analyzed using gas chromatography-mass spectrometry (GC-MS). Sample preparation is necessary before GC-MS analysis by extracting the volatile compounds from products. The major advantages of different methods for the sample preparation, such as dynamic headspace (DHS), direct solvent extraction (DSE), and vacuum simultaneous steam distillation-solvent extraction (V-SDE), have been previously reported (Wanakhachornkrai, 2003). However, many new methods for extracting volatile compounds from soy sauce have emerged, such as the concentrating method and solid-phase micro-extraction (SPME) (Giri, Osako, & Ohshima, 2010; Kaneko, Kumazawa, & Nishimura, 2013; Lee, Seo, & Kim, 2006). For SPME technology in particular, the details of parameters affecting extraction have been systematically optimized (Feng et al., 2017). However, the different extraction methods led to different results for the detected volatile compounds. Therefore, there may be inconsistencies and contradictions: volatile compounds found in one study to have an important effect on the aroma of soy sauce may not be detected or rarely present in other studies (Feng et al., 2015; Harada et al., 2017). Despite the use of all of these extraction methods, more needs to be known about the volatile compounds commonly present in both soy sauce and miso, and how they influence the overall quality of products.

In the present study, to improve knowledge of the commonality of the detected substances and discriminate between them, the volatile compounds in the tested soy sauce and miso samples will be analyzed by extracting them directly from the headspace to reduce the steps in sample preparation and to minimize any interference. The aim of this study is thus to investigate the influence of these volatile compounds commonly detected in both the fermented soy sauce and miso on the final quality.

## 3.3 Materials and Methods

### 3.3.1 Materials and Reagents

#### 3.3.1.1 Soy Sauce Sample

Sixty-two soy sauce samples were collected from the Akita Prefectural Miso and Soy Sauce Competition held in 2015, 2016, 2017, and 2018 by the Akita Prefectural Miso and Soy Sauce Manufacturer Cooperative (Akita, Japan). The total consisted of 17, 16, 15, and 14 samples from the competitions held for these four consecutive years, respectively. These samples of traditionally fermented soy sauce were produced by different companies located in the Akita Prefecture. In general, the manufacturing processes consisted of three major steps: 1) *koji* production, cooked soybeans and roasted wheat were used as starting materials to get mixed together with *koji* mold (a type of fungus); 2) brine fermentation, after the molds grow over several days, salt brine was added to the cultured mold, and then the obtained mash was fermented for six months to one year; 3) refining, once the fermentation finished, the soy sauce was finally strained, pasteurized, and packed. All samples were immediately stored in a refrigerator at 3–6 °C before analysis.

2.3.1.2 Miso sample

Twenty-six Miso samples entered into Akita Prefectural Miso and Soy Sauce Competition each year (from 2015 to 2018), a total of 104 samples, were produced by different companies located in Akita Prefecture. The type of the evaluated miso samples belonged to rice miso, which has a similar fermentation process as Japanese fermented soy sauce with a high concentration of salt and many different microbial activities. All the evaluated miso products were directly collected from the competition each year and were immediately stored in a refrigerator at −25 °C prior to use.

2.3.1.3 Reagents

The custom alkanes standard mixture (C6 – C16, Restek, Bellefonte, PA, USA) were purchased and used to calculate the Kovats retention index (RI) of each peak.

### 3.3.2 Analytical Methods

3.3.2.1 Sampling of Volatile compounds for GC-MS Analysis

To ensure comparability of the measurements in this study, all samples were subjected to similar preconditioning and extraction parameters. Samples of 0.25 g of soy sauce or miso were placed directly in 20-mL vials then tightly capped. The vials were equilibrated at 80 °C for 30 min in a HS-20 headspace auto-sampler (Shimadzu Co., Kyoto, Japan). The extraction process was as follows: the temperatures of the oven, sample line and transfer line were 80, 180, and 180 °C, respectively; the pressure, pressure time, and pressure equilibration time were 50 kPa, 2 min, and 0.1 min, respectively; the vial shaking level was 2; the load and load equilibration times were 0.5 and 0.1 min, respectively; the extracted gas phase (1 μL) was automatically withdrawn from the headspace of each vial then transferred to the GC-MS.

2.3.2.2 GC-MS Analysis

The GC-MS analysis was performed using a Shimadzu GCMS-QP2020 system (Shimadzu Co.) equipped with an SH-Rxi-5Sil MS capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness, Shimadzu Co.). Injection for the GC-MS analysis was made in the split mode (split ratio, 1:10) with an injection time of 0.5 min. The analyses were made using helium as the carrier gas with a total flow of 15.0 mL $min^{-1}$ and a column flow of 1.50 mL $min^{-1}$. The column temperature was held at 40 °C for 5 min isothermally, ramped at 4 °C/min to 250 °C, then held there for 3 min. The ion source and interface temperatures were maintained at 200 and 230 °C, respectively. The mass spectrometer was operated in full scan mode and mass spectra in the 33-350 m/z range were recorded.

2.3.2.3 Identification

Individual volatile compound were identified by comparing the retention times (RT) and mass spectral data with those of standard compounds, and computer matching with the NIST 17 Mass Spectral Library, as well as by comparing the calculated Kovats retention index (RI) of the peaks with those reported in the literature. The RI values of the peaks were calculated using the C6 to C16 *n*-alkane series. The absolute concentrations of volatile compounds were determined as the average peak area from three replicate experiments.

### 3.3.3 Sensory Evaluation

The sensory evaluation process was the same as the description in Chapter 2 (2.3.3 Sensory Evaluation). Briefly, the Akita Prefectural Miso and Soy Sauce Competition has been held in October annually by the Akita Prefectural Miso and Soy Sauce Manufacturer Cooperative (Akita, Japan). Nine specially trained panelists working in the field of fermented foods and with expertise in food sensory evaluation were selected for assessing the soy sauce and miso samples. To be more effective, the process of sensory evaluation was conducted in two stages: in the first stage, assessors evaluated the initial preference of the samples using a 5-point hedonic scale, from 1 to 5 indicating the quality of the samples as very good, good, neither good nor poor, poor, and very poor, respectively. The sensory score of the sample was then obtained by summing the scores of all panelists. The best possible score of a sample was thus 9 points in the first stage. Any samples with scores above 20 did not continue to the second stage of sensory evaluation and were tentatively ranked in ascending order.

The second stage was classification where samples with scores under 20 were further evaluated using a 100-point scale to separately assess the quality characteristics, such as external appearance, texture, taste, flavor, and overall acceptance. The total score from the nine panelists was the sensory score for the sample at the second stage. The highest possible total score for each sample was 900 points with a better quality indicated by a higher score. The samples in the second round were ranked according to the descending order of the scores. Finally, the ranking of the samples for each year were determined: the rankings of the samples obtained from the second stage were first listed then followed by the rankings of the samples remaining from the first stage.

### 3.3.4 Statistical Analysis

Statistical procedures were carried out using IBM SPSS Statistics (v. 19.0, IBM Corp., Armonk, NY, USA). Univariate statistical analysis (one-way ANOVA) was applied to the data to determine significant differences between the levels of volatile compounds of soy sauce. Principal component analysis (PCA) was performed on the absolute concentrations of volatile compounds and to investigate the effect on the quality for both the soy sauce and miso samples.

## 3.4 Results and Discussion

### 3.4.1 Soy Sauce

#### 3.4.1.1 Identification of Volatiles in Soy Sauce

A total of 62 peaks were detected by GC-MS analysis from the tested soy sauce samples evaluated in the Akita Miso and Soy Sauce Competitions and all were positively identified (Table 3-1 and Figure 3-1). These compounds were classified into different groups based on their chemical structure: ten aldehydes, ten ketones, nine furan(one)s, seven alcohols, seven esters, six sulfur-containing compounds, five pyrazines, three phenols, and two others, most of which had been previously reported in soy sauce. Of these 62 volatile compounds, 35 were present in all the soy sauce samples tested (Table 3-1).

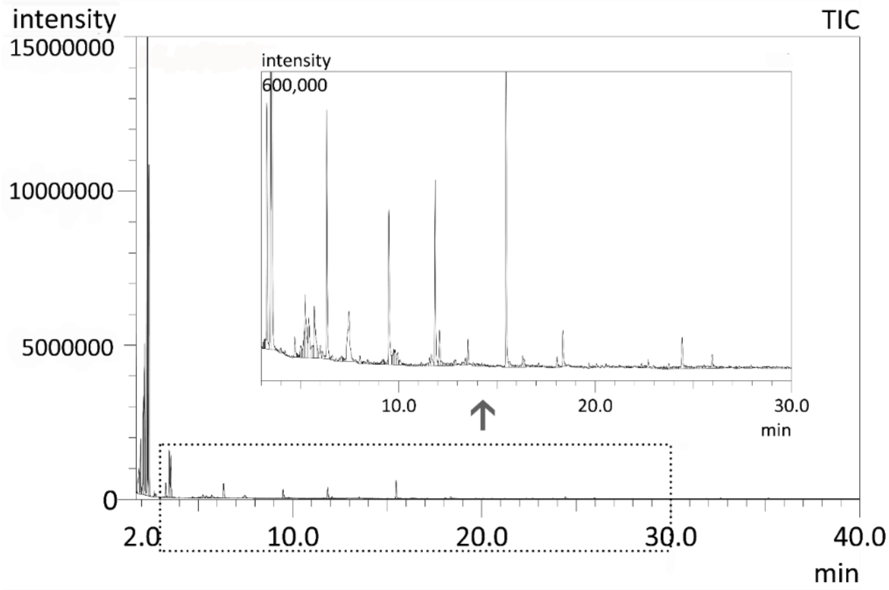


Figure 3-1 Total ion chromatogram of volatile compounds in soy sauce detected by direct extraction from the headspace.

Table 3-1 The volatile compounds identified in the headspace of the evaluated soy sauce samples

| No.[1] | RI[2] | $RI_{ref}$ [3] | Compounds | Quantifying ion (*m/z*) | Correlation coefficient (*r*)[4] | | | | Identification[5] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2015 | 2016 | 2017 | 2018 | |
| *Alcohols* | | | | | | | | | |
| 5* [6] | 622 | 628 | 2-Methylpropan-1-ol | 74 (33, 56) | 0.04 | 0.01 | 0.35 | 0.18 | AB |
| 13* | 727 | 738 | 3-Methylbutan-1-ol | 70 (42, 55) | 0.08 | 0.47 | 0.37 | 0.35 | AB |
| 14* | 731 | 742 | 2-Methylbutan-1-ol | 57 (41, 70) | -0.01 | 0.13 | 0.28 | 0.04 | AB |
| 19 | 802 | 815 | Butane-2,3-diol | 45 (56, 75) | 0.15 | 0.55 | 0.44 | 0.37 | AB |
| 31* | 914 | 935 | Heptanol | 108 (60, 40) | -0.42 | -0.50 | -0.52 | -0.39 | AC |
| 40 | 980 | 992 | Oct-1-en-3-ol | 57 (43, 72) | -0.16 | -0.14 | 0.15 | 0.32 | AB |
| 51* | 1108 | 1128 | 2-Phenylethanol | 91 (122, 65) | 0.32 | 0.61 | 0.53 | 0.34 | AB |
| *Aldehydes* | | | | | | | | | |
| 1* | <600 | <600 | 2-Methylpropanal | 43 (72, 41) | -0.79 | -0.79 | -0.80 | -0.92 | AB |
| 7* | 647 | 658 | 3-Methylbutanal | 58 (71, 44) | -0.68 | -0.79 | -0.68 | -0.85 | AB |
| 8* | 656 | 668 | 2-Methylbutanal | 41 (56, 57) | -0.60 | -0.75 | -0.78 | -0.69 | AB |
| 17 | 760 | 769 | Hexanal | 57 (44, 72) | -0.33 | -0.23 | -0.34 | -0.40 | AC |
| 18* | 779 | 781 | 3-Methylbut-2-enal | 84 (55, 41) | 0.71 | 0.51 | 0.35 | 0.80 | AC |
| 37* | 957 | 980 | Benzaldehyde | 106 (77, 51) | 0.50 | -0.10 | -0.03 | -0.37 | AB |
| 46* | 1039 | 1060 | 2-Phenylacetaldehyde | 91 (120, 65) | 0.49 | 0.58 | 0.61 | 0.44 | AB |
| 50 | 1103 | 1106 | Nonanal | 57 (41, 70) | -0.43 | -0.28 | 0.25 | -0.05 | AB |
| 53* | 1151 | 1161 | 2-Phenylprop-2-enal | 103 (132, 77) | 0.46 | 0.73 | 0.58 | 0.79 | AC |
| 57* | 1264 | 1292 | (*E*)-2-phenylbut-2-enal | 117 (146, 91) | -0.22 | -0.40 | -0.67 | -0.31 | AB |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Acids* | | | | | | | | | |
| 6* | 633 | 604 | Acetic acid | 60 (43, 45) | -0.46 | -0.57 | -0.48 | -0.62 | AB |
| 15 | 739 | 765 | 2-Methylpropanoic acid | 85 (57, 67) | 0.42 | 0.38 | 0.35 | -0.66 | AB |
| 25 | 843 | 862 | 3-Methylbutanoic acid | 60 (87, 43) | 0.47 | 0.60 | 0.58 | -0.51 | AB |
| *Esters* | | | | | | | | | |
| 4* | 610 | 612 | Ethyl acetate | 43 (61, 70) | -0.14 | -0.07 | 0.13 | 0.01 | AB |
| 20* | 806 | 785 | Ethyl 2-oxopropanoate | 43 (61, 116) | -0.55 | -0.67 | -0.69 | -0.41 | AC |
| 21 | 812 | 824 | Ethyl 2-hydroxypropanoate | 45 (56, 75) | 0.18 | 0.22 | 0.45 | 0.19 | AB |
| 52 | 1131 | 1136 | Pentyl 2-methylbutanoate | 103 (57, 39) | -0.14 | 0.10 | 0.09 | -0.17 | AC |
| 54 | 1178 | 1179 | Diethyl butanedioate | 129 (101, 73) | -0.13 | -0.12 | 0.27 | 0.28 | AC |
| 60 | 1368 | 1380 | (3-Hydroxy-2,2,4-trimethylpentyl) 2-methylpropanoate | 71 (43, 89) | -0.28 | 0.17 | -0.03 | 0.10 | AC |
| 62 | 1583 | 1588 | [2,2,4-Trimethyl-3-(2-methylpropanoyloxy)pentyl] 2-methylpropanoate | 71 (43, 159) | -0.09 | 0.22 | 0.52 | 0.23 | AC |
| *Ketones* | | | | | | | | | |
| 2* | <600 | 601 | Butane-2,3-dione | 86 (43) | 0.23 | 0.33 | 0.18 | 0.33 | AB |
| 3* | <600 | 603 | Butan-2-one | 72 (43) | 0.43 | 0.32 | 0.12 | 0.42 | AB |
| 9 | 682 | 700 | Pentan-2-one | 86 (43, 58) | -0.45 | -0.45 | -0.61 | -0.82 | AB |
| 10* | 689 | 694 | 1-Hydroxypropan-2-one | 43 (74) | -0.30 | -0.39 | -0.32 | -0.65 | AC |
| 11* | 695 | 706 | Pentane-2,3-dione | 100 (43, 57) | -0.41 | -0.45 | -0.60 | -0.64 | AB |
| 12* | 720 | 724 | 3-Hydroxybutan-2-one | 88 (45, 73) | -0.22 | -0.58 | -0.24 | -0.50 | AB |
| 24 | 833 | 848 | 3-Methylpent-3-en-2-one | 98 (55, 83) | 0.01 | 0.47 | 0.18 | 0.48 | AB |
| 32* | 941 | 947 | 2-Methyloctan-3-one | 99 (43, 71) | -0.40 | -0.43 | -0.48 | -0.81 | AB |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 35 | 951 | 966 | Octane-2,3-dione | 43 (71, 99) | -0.58 | -0.65 | -0.21 | -0.58 | AC |
| 47* | 1064 | 1092 | 1-(1*H*-pyrrol-2-yl)ethanone | 94 (109, 66) | -0.50 | -0.14 | -0.21 | -0.49 | AB |
| *Furan(one)s* | | | | | | | | | |
| 23* | 828 | 845 | Furan-2-carbaldehyde | 96 (39, 95) | -0.44 | -0.61 | -0.46 | -0.59 | AB |
| 26* | 856 | 864 | Furan-2-ylmethanol | 98 (81, 41) | 0.20 | 0.30 | 0.22 | 0.15 | AB |
| 28 | 898 | 904 | Furan-2-ylmethyl formate | 81 (53, 39) | 0.02 | 0.45 | 0.16 | 0.72 | AB |
| 33 | 947 | 957 | 3-Methyloxolan-2-one | 56 (41, 43) | 0.23 | 0.22 | 0.30 | 0.19 | AC |
| 34 | 951 | 950 | 5-Methyloxolan-2-one | 85 (41, 56) | 0.23 | 0.42 | 0.22 | 0.32 | AC |
| 36 | 954 | 953 | (5-Methylfuran-2-yl)methanol | 95 (43, 55) | -0.50 | -0.68 | -0.73 | -0.70 | AC |
| 42 | 994 | 1001 | (5-Methylfuran-2-yl)methanethiol | 95 (43, 94) | -0.82 | -0.67 | -0.43 | nd | AB |
| 45 | 1004 | 1028 | 1-(Furan-2-yl)propan-1-one | 95 (124, 39) | -0.11 | 0.02 | 0.05 | -0.12 | AB |
| 56* | 1218 | 1237 | 3-Phenylfuran | 115 (144, 116) | -0.47 | -0.74 | -0.81 | -0.65 | AB |
| *Sulfur-containing compounds* | | | | | | | | | |
| 16* | 750 | 752 | 1-Methylsulfanylpropane | 90 (61, 41) | 0.27 | 0.51 | 0.17 | 0.76 | AC |
| 29* | 904 | 906 | 3-Methylsulfanylpropanal | 104 (48, 76) | 0.56 | 0.35 | 0.61 | 0.38 | AB |
| 38* | 962 | 981 | (Methyltrisulfanyl)methane | 126 (79, 111) | -0.16 | -0.67 | 0.02 | -0.08 | AB |
| 39 | 978 | 993 | 3-Methylsulfanylpropan-1-ol | 106 (57, 61) | -0.26 | 0.13 | 0.06 | -0.21 | AB |
| 49* | 1100 | 1127 | Methylsulfanylcyclohexane | 82 (130, 39) | 0.25 | -0.16 | -0.55 | -0.09 | AB |
| 55* | 1208 | 1209 | (Methyltetrasulfanyl)methane | 158 (79, 94) | -0.28 | -0.19 | 0.36 | 0.13 | AC |
| *Phenols* | | | | | | | | | |
| 58 | 1270 | 1285 | 4-Ethyl-2-methoxyphenol | 137 (152, 77) | 0.54 | 0.26 | 0.33 | 0.18 | AB |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 59* | 1305 | 1317 | 4-Ethenyl-2-methoxyphenol | 150 (135, 107) | -0.18 | -0.37 | 0.20 | -0.27 | AB |
| 61 | 1502 | 1527 | 2,4-Di*tert*-butylphenol | 191 (57, 206) | nd [7] | -0.52 | 0.48 | -0.57 | AB |
| *Pyrazines* | | | | | | | | | |
| 22* | 821 | 834 | 2-Methylpyrazine | 94 (67, 53) | 0.50 | 0.46 | 0.32 | 0.85 | AB |
| 30* | 910 | 920 | 2,6-Dimethylpyrazine | 108 (39, 42) | 0.05 | 0.23 | -0.21 | -0.21 | AB |
| 43 | 995 | 1002 | 2-Ethyl-6-methylpyrazine | 121 (39, 94) | 0.30 | 0.57 | 0.55 | 0.61 | AB |
| 44 | 998 | 990 | 2,3,5-Trimethylpyrazine | 122 (42, 81) | 0.40 | 0.73 | 0.55 | 0.44 | AB |
| 48 | 1082 | 1084 | 2-Ethyl-3,5-dimethylpyrazine | 136 (42, 54) | 0.31 | 0.42 | 0.45 | 0.24 | AB |
| *Others* | | | | | | | | | |
| 27 | 890 | | 2-Ethoxybutane | 73 (45, 59) | 0.26 | 0.24 | 0.16 | 0.49 | A |
| 41* | 989 | 994 | 2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane | 281 (133, 265) | 0.55 | 0.49 | 0.44 | 0.30 | AC |

[1] The volatile compounds are arranged in order of their retention time within each chemical group.

[2] RI, retention indices, RI value were calculated for the SH-Rxi™-5SilMS capillary column.

[3] $RI_{ref}$, references retention indices.

[4] The correlation coefficients (*r*) were calculated between the peak area of each compound and the rankings of the evaluated soy sauces.

[5] Identification: (A) by comparison of the mass spectrum with the NIST 17 Mass Spectral Library; (B) by comparison of RI on a similar phase column for the study of soy sauce (Feng et al., 2014, 2017, 2015; Steinhaus & Schieberle, 2007; Sun et al., 2010); (C) by comparison of RI on a similar phase column reported in the literature (Alissandrakis et al., 2007; Beal & Mottram, 1994; Bonaťi et al., 2005; Dallüge et al., 2002; Kim & Chung, 2009; Leffingwell & Alford, 2005; Qiming et al., 2006; Radulović, Blagojević, & Palić, 2010; Raffo et al., 2009; Rychlik & Bosset, 2001).

[6] "*" means the related volatile compounds were detected in all soy sauce samples tested.

[7] nd, not detected.

#### 3.4.1.2 Relationship between the Level of Volatile Compounds and the Quality of Soy Sauce

Table 3-1 lists the calculated correlation coefficient (*r*) between the level of each volatile compounds and the sample rankings from 2015 to 2018. A negative value of *r* represents a positive correlation between the level of a volatile compound and the sensory quality of soy sauce, while a positive value represents a negative correlation, with a greater absolute value of *r* indicating a stronger influence.

In the alcohols group, In the alcohols group, 2-phenylethanol, previously reported as a rose-like odor-active compound (Lee et al., 2013), adversely affected the overall aroma of fermented soy sauce as indicated by the higher value of *r* (2015, 0.32; 2016, 0.61; 2017, 0.53; 2018, 0.34). 2-phenylethanol was generated from phenylalanine, which can intensify the umami taste of soy sauce (Lioe et al., 2007), indicating that the formation of 2-phenylethanol may reduce the quality of soy sauce. In previous studies on soy sauce flavor (Lee et al., 2006), the abundance of alcohols has been reported to have a high impact on the aromatic profile of soy sauce. However, it was observed in the present study that 3-methylbutan-1-ol and butane-2,3-diol had a clearly negative effect on the sensory quality of fermented soy sauces. Nunomura et al. (1980) investigated the flavor components of Japanese soy sauce by separating the flavor concentrates into different fractions, and pointed out that those fractions which exhibited unpleasant odors contained a great amount of 2-methylpropan-1-ol, 3-methylbutan-1-ol, 2-methylbutan-1-ol, or 4-ethyl-2-methoxyphenol. The results obtained from the present study agreed well with these previous findings. It has also been reported that higher alcohols were derived from amino acids through the Ehrlich pathway (Pires et al., 2014). The type and amount of amino acids formed during fermentation can affect the yeast to different extents and thus ultimately the final flavor profile. Therefore, the adverse effect of alcohols may also be associated with the formation and enzymatic hydrolysis of amino acids. Heptanol was the only alcohol component that had a positive influence on the flavor of soy sauce, possibly relating to its fragrant odor.

The aldehydes group comprised ten volatile compounds. 2-methylpropanal, 3-methylbutanal and 2-methylbutanal are mainly generated from the degradation of the corresponding amino acids (e.g., valine, isoleucine, and leucine) through the Ehrlich pathway (e.g., valine, isoleucine, and leucine) through the Enrlilch pathway (Smit, Engels, & Smit, 2009), and have been identified as having a high impact on the malty odor note (Steinhaus & Schieberle, 2007). Table 3-1 shows that these three components provided a significant and positive contribution to the overall aroma of soy sauce. The long-chain aldehyde, hexanal, and (*E*)-2-phenylbut-2-enal had a positive effect on the aromatic composition. The positive effect of hexanal might have been related to its interaction with hydrogen sulfide to generate fruit aroma compounds (Boelens et al., 1974). In contrast, the negative contribution of 3-methylbut-2-enal, 2-phenylacetaldehyde, and 2-phenylprop-2-enal to the final quality was also observed. 2-Phenylacetaldehyde has previously been considered as a key aroma compound contributing to the flavor of soy sauce (Kaneko et al., 2012). The data in Table 3-1 also showed the highly negative influence of 2-phenylacetaldehyde on the overall quality of soy sauce. This negative influence might be related to 2-phenylacetaldehyde masking or counteracting the perception of fruit odor (Felipe et al., 2011). There is a great amount of benzaldehyde detected in the consecutive three years of samples. This compound was generated by the degradation of the aromatic amino acid phenylalanine and microbial fermentation and had been regarded as one of the key volatiles with high odor activity values in Japanese soy sauce (Lee et al., 2006). However, it seemed like that this chemical had little effect on the final quality of soy sauce due to the positively or negatively small value of $r$.

For the acids group, acetic acid was dominant in the tested samples, followed by two branched-chain acids, 3-methylbutanoic acid and 2-methylpropanoic acid. Acetic acid produced by lactic acid bacteria during fermentation give the sour odor to soy sauce and contribute substantially to its aromatic profile. The positive effect of acetic acid on the aromatic profile was observed in all 4 consecutive years shown by the negative values of $r$ (2015, -0.46; 2016, -0.57; 2017, -0.48; 2018, -

0.62). Acetic acid has been reported to be one of the most intense odor-active compounds because of the high value of the flavor dilution factor tested using aroma extract dilution analysis (Lee et al., 2006). Acetic acid can react with alcohols to generate the corresponding acetate esters, which impart various fruity aromas. The influence of acetic acid on the formation of furan-2-carbaldehyde, 2-furanmethanol, and 3-methylsulfanylpropanal has been previously investigated (Harada et al., 2018). Therefore, it is reasonable to suggest that acetic acid has a positive effect on the overall aroma of soy sauce. 2-methylpropanoic acid and 3-methylbutanoic acid imparted an acidic note to soy sauce with their positive values of *r* indicating their negative effects on the final quality of soy sauce in 2015, 2016, and 2017, while the values of *r* in 2018 were negative indicating a positive effect on the final quality. The accumulation process of these two compounds involved the further oxidation of the aldehydes, 2-methylpropanal and 3-methylbutanal, which are two important and positive contributors to the flavor of soy sauce (Coleman & Chung, 2002). Therefore, the difference in the determination of the final quality of soy sauce for these two compounds was considered to be related to the specific fermentation process, including the used raw materials, climatic conditions, and so on.

In the present study, seven esters were identified in samples collected from 4 consecutive years with ethyl acetate and ethyl 2-oxopropanoate common to all samples. The positive relationship between the level of ethyl 2-oxopropanoate and the sample rankings indicated its positive effect on the aromatic composition of soy sauce but the influence of ethyl 2-hydroxypropanoate on the final quality was weakly negative. The relationship between the levels of ethyl acetate, pentyl 2-methylbutanoate, diethyl butanedioate, (3-hydroxy-2,2,4-trimethylpentyl) 2-methylpropanoate, and [2,2,4-trimethyl-3-(2-methylpropanoyloxy)pentyl] 2-methylpropanoate and their rankings of samples were similarly not very clear. This may have been related to the loss of esters by volatilization or hydrolysis during heating (Meng et al., 2017).

In the ketones group, there was a distinct separation of the different effects on the final quality of soy sauce which confirmed that their role in the aromatic composition of soy sauce was significant. Of the ten volatile compounds in the ketones group, three ketones, butane-2,3-dione, butan-2-one, and 3-metyl-3-penten-2-one, were regarded as aroma defects that contributed negatively to the final quality of soy sauce. It has previously been reported that 3-hydroxybutan-2-one could be oxidized to form butane-2,3-dione, which could be further oxidized to form butan-2-one (Baizer et al., 1984). Table 3-1 shows that 3-hydroxybutan-2-one was identified as a positive contributor to the final quality as indicated by its reliable and negative *r* values (2015, -0.22; 2016, -0.58; 2017, -0.24; 2018, -0.50). Thus, the apparent negative effects of butane-2,3-dione and butan-2-one on the final quality could be explained by the reduction in the level of 3-hydroxybutan-2-one. In contrast, ketones in soy sauce can produce a sweet smell as well as promoting the formation of pleasing aromas, such as grassy, fruity, and caramel (Zhao et al., 2018). Therefore, seven ketones, pentan-2-one, 1-hydroxypropan-2-one, pentane-2,3-dione, 3-hydroxybutan-2-one, 2-methyloctan-3-one, octane-2,3-dione, and 1-(1*H*-pyrrol-2-yl)ethanone exerted a positive effect on the final quality of soy sauce.

Regarding the furan(one)s group, most of these compounds except 1-(furan-2-yl)propan-1-one influenced the overall aroma of soy sauce. In particular, furan-2-carbaldehyde, recognized as a key aroma compound in soy sauce, is a major dehydration product of pentose through the Maillard reaction and can be further conversed to furan-2-ylmethanol (Taherzadeh et al., 1999). The significant influence of the interactions between furan-2-carbaldehyde and other reactive components on generating other important aroma compounds associated with roasted and boiled flavors has been previously reported (Mottram, 1998). This confirmed the positive and important role played by furan-2-carbaldehyde on the final quality of soy sauce, while the negative effect of furan-2-ylmethanol could have been related to the consumption of furan-2-carbaldehyde. 3-phenylfuran, another important aroma-active compounds, has also been identified by aroma extract dilution analysis (Ono et al., 2015). Interestingly, a different situation was observed regarding 3-methyloxolan-2-one, 5-methyloxolan-2-one, (5-methylfuran-2-yl)methanol, and (5-methylfuran-2-yl)methanethiol with the classification results for the tested samples subjecting to the 2015-2018 Akita Prefectural Soy Sauce

Competition. The absence of 3-methyloxolan-2-one was observed in most of the samples that ranked at a higher position: for example, in 2015, it was not detected in samples ranked from 1st to 4th, and 6th; in 2016, samples ranked from 1st to 3rd, and 6th; in 2017, samples ranked from 1st to 5th; and in 2018, samples ranked from 2nd to 6th. Similarly, 5-methyloxolan-2-one was not detected in the 2015 samples ranked from 1st to 10th (except 4th), in the 2016 samples ranked from 1st to 4th, in the 2017 samples ranked from 1st to 6th (except 4th), and in the 2018 samples ranked from 1st to 5th. Both of these compounds showed positive values of *r* indicating their negative effects on the final quality of soy sauce so can be regarded as aroma defects. In contrast, (5-methylfuran-2-yl)methanol and (5-methylfuran-2-yl)methanethiol were detected in those samples that ranked highly in the sensory evaluation of soy sauce from the 4 years. For example, (5-methylfuran-2-yl)methanol was detected in 2015 samples ranked from 1st to 3rd, in 2016 samples ranked from 1st to 5th, in 2017 samples ranked from 1st to 9th, and in 2018 samples ranked from 1st to 3rd and 7th; and (5-methylfuran-2-yl)methanethiol was detected in 2015 samples ranked from 1st to 9th, in 2016 samples ranked from 1st to 10th, and in 2017 samples ranked from 1st to 10th (in 2018 samples were not detected). Consequently, these two compounds positively influenced the final quality of soy sauce. In general, furan(one)s have been reported as important aromatic substances which supplement the pleasant odor of soy sauce (Zhao et al., 2015). However, the furan compounds, formed from amino acids and saccharides in soy sauce, have been reported to be strongly affected by the different dates of production, technological steps, and substrate consumptions (Huang & Barringer, 2016). In comparison, the apparent differences between 3-methyloxolan-2-one, 5-methyloxolan-2-one, (5-methylfuran-2-yl)methanol, and (5-methylfuran-2-yl)methanethiol on influencing the quality may be associated with the different processing conditions.

Six sulfur-containing compounds were identified in the samples tested, and detected in all samples except the 2015 sample ranked 10th and the 2018 sample ranked 13th where no 3-methylsulfanylpropan-1-ol was detected. Of these compounds, 3-methylsulfanylpropanal, (methyltrisulfanyl)methane, and 3-methylsulfanylpropan-1-ol have previously been recognized as key compounds affecting the flavor of soy sauce (Feng et al., 2017). 3-Methylsulfanylpropanal has been reported to have a cooked potato-like note originating from methionine, and could be further degraded to 3-methylsulfanylpropan-1-ol. In contrast, 3-methylsulfanylpropan-1-ol shows a more acceptable flavor characteristic than 3-methylsulfanylpropanal, which is not good for the aroma of soy sauce (Kobayashi & Sugawara, 1999). Table 3-1 shows that the correlation between the level of 3-methylsulfanylpropanal and the overall quality score was negative. In contrast, the effect of 3-methylsulfanylpropan-1-ol on the final quality of soy sauce was not very significant because the correlations for the 3 years were not consistently positive or negative, with much lower *r* values. The level of 1-methylsulfanylpropane was also negatively related to the sample rankings. Both (methyltrisulfanyl)methane and (methyltetrasulfanyl)methane can be generated from methanethiol through spontaneous secondary reactions (Landaud, Helinck, & Bonnarme, 2008). Although the levels of these two compounds in soy sauce have not been reported as high, they have been recognized as important aroma compounds because of their low odor threshold (Feng et al., 2014). However, the significant effect of these two compounds reported on the flavor of soy sauce was not observed in the present study.

In the phenols group, 4-ethenyl-2-methoxyphenol was detected in all the samples tested. Like 3/5-methyloxolan-2-one, no 4-ethyl-2-methoxyphenol was detected in the high-ranking samples: e.g. the 2015 samples ranked from 1st to 4th, the 2016 samples ranked from 1st to 4th, the 2017 samples ranked from 1st to 5th, and the 2018 samples ranked from 1st to 7th (except 5th). Several studies have identified 4-ethyl-2-methoxyphenol, 4-ethenyl-2-methoxyphenol, and 2,4-di*tert*-butylphenol as important aroma components contributing to the aroma of soy sauce (Feng et al., 2015; Kaneko et al., 2013; Lee et al., 2006). However, Table 3-1 shows the surprising correlation between the level of 4-ethyl-2-methoxyphenol and the sample rankings, implying that higher levels had led to soy sauce with a poor aromatic composition. The effect of 4-ethenyl-2-methoxyphenol on the aromatic composition of soy sauce was not very significant, and the effect of 2,4-di*tert*-butylphenol on the final quality of soy sauce was not clear due to the positive or negative value of *r*. The observation that 4-ethyl-2-methoxyphenol has an adverse effect agreed with previously reported findings (Nunomura et al., 1980). Some studies have reported the negative effect of phenols in masking the fruity aromas of red wine and soy sauce (Felipe et al., 2011; Meng, Imamura, et al., 2017). Consequently, it can be suggested that 4-ethyl-2-methoxyphenol played a role in decreasing the final quality of soy sauce.

Pyrazines have been regarded as important characteristic aroma compounds mainly associated with roasted and nutty aromatic notes (Müller & Rappert, 2010). However, Feng et al. (2015) have also investigated the aroma profiles of commercial soy sauces using odor activity value and omission test and pointed out that pyrazines did not contribute because of their high odor thresholds. In the present study, five pyrazines were identified in the samples tested. The *r* values between pyrazines and the rankings of the tested samples were positive and relatively high (Table 3-1), indicating that the quality of soy sauce was significantly and negatively influenced by the level of the detected pyrazines except for 2,6-dimethylpyrazine, which had no obvious effect on the quality of the soy sauce. This showed that these pyrazines might be considered important aroma defects in soy sauce. This negative effect has also been observed in the suppression of the fruity aroma of wine (Campo et al., 2005). The formation of pyrazines during high-temperature processing, and their accumulation has been reported to increase with heating time (Müller & Rappert, 2010). As a result, heat treatment would lead to a loss of beneficial aromas, thus weakening the aromatic profile of soy sauce.

For the group, labeled ‘others’, two compounds with a negative effect on the overall quality of soy sauce were observed: no 2-ethoxybutane was detected in the high-ranking samples, the 2015 samples ranked from 1st to 4th, the 2016 samples ranked from 1st to 3rd, and 6th, and the 2017 samples ranked from 1st to 4th, and the 2018 samples ranked from 1st to 6th (except 5th). Therefore this compound was considered to play a negative role on the quality of soy sauce. 2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane was detected in all the samples tested and its negative effect on the final quality was confirmed by the *r* values shown in Table 3-1. This volatile siloxane was converted by silicon-based organic polymers within raw materials and generally involved in the study of sewage gases (Salazar Gómez, Lohmann, & Krassowski, 2016). Therefore, although the amount of this compound appears to be relatively low, the negative effect of this compound was considered to reflect the fermentation conditions.

#### 3.4.1.3 PCA Results

PCA was used to gain a comprehensive understanding of the differences in volatile compounds between the soy sauces from 2015 to 2018. As the same with the previous, the tested soy sauce samples were divided into two groups based on the results of sensory evaluation obtained from 2015 and 2018, respectively. The products that entered the second stage during the evaluation were classified into the S1 group, and the remaining products were classified into the S2 group, respectively. Generally, the overall preference of the samples in S1 group was better than those in S2 group. Benzaldehyde with no obvious effect on the determination of the quality of soy sauce was used as an internal standard for the normalization of the obtained data. Each GC-MS peak area of the other detected 61 volatile compounds were normalized at its ratios to the peak area of benzaldehyde and then the average of the obtained ratios of each group was used as variables in PCA. The compounds which contributed most to the final quality of soy sauce were investigated. The two most important PCs (PC1 and PC2) extracted by PCA, explained 34.41% and 26.53% of the variance, respectively, and were sufficient to discriminate between the soy sauce samples tested.

The distinct difference in position between the high-quality soy sauces in S1 groups and the soy sauces in S2 groups is observed in Figure 3-2 (a). In general, the S1 groups from 2015 to 2018 were located in the positive PC1 region, indicating that the PC1 score was positively related to the quality of soy sauce. In contrast, the S2 groups from 2015 to 2018 were located in the negative PC1 region. Moreover, the S1 groups were positioned in the lower right position of the S2 groups. In this case, volatile compounds having large positive factor loadings on PC1 and negative factor loadings on PC2 tended to contribute to the final quality of soy sauce. Thus, it is understandable that the volatile compounds identified in the present study played an important role in discriminating the final quality of the soy sauce.

The corresponding loadings plot (Figure 3-2 (b)) also showed the different contributions of volatile compounds. The S1 groups from 2015 to 2018 were located at the positive PC1 region, and 2-methylpropanal (No. 1, malty), ethyl acetate (No. 4, fruity), acetic acid (No. 6, sour), 3-methylbutanal (No. 7, malty), 2-methylbutanal (No. 8, malty), pentan-2-one (No. 9, fruity), 1-hydroxypropan-2-one (No. 10, caramellic), 3-hydroxybutan-2-one (No. 12, buttery), hexanal (No. 17, green), ethyl 2-oxopropanoate (No. 20, fruity), furan-2-carbaldehyde (No. 23, bready), heptanol (No. 31, fragrant), 2-methyloctan-3-one (No. 32, buttery), octane-2,3-dione (No. 35, dill), (5-methylfuran-2-yl)methanol (No. 36, herbal), dimethyl trisulfide (No. 38, alliaceous), (5-methylfuran-2-yl)methanethiol (No. 42, sulfurous), 1-(1*H*-pyrrol-2-yl)ethanone (No. 47, roasty), pentyl 2-methylbutanoate (No. 52, fruity), diethyl butanedioate (No. 54, fruity), 3-phenylfuran (No. 56, jasmine), and (*E*)-2-phenylbut-2-enal (No. 57, roasty), were associated with this region. This result agreed well with the earlier findings in the present work.

For the S2 groups from 2015 to 2018 located in the negative PC1 region, the compounds, butane-2,3-dione (No. 2, creamy), butan-2-one (No. 3, fruity), 3-methylbutan-1-ol (No. 13, fruity), 2-methylbutan-1-ol (No. 14, roasted), 1-methylsulfanylpropane (No. 16, alliaceous), 3-methylbut-2-enal (No. 18, fruity), butane-2,3-diol (No. 19, creamy), 2-methylpyrazine (No. 22, nutty), 3-methylpent-3-en-2-one (No. 24, sweaty), 3-methylbutanoic acid (No. 25 sour), furan-2-ylmethanol (No. 26, bready), furan-2-ylmethyl formate (No.28 floral), 5-methyloxolan-2-one (No. 34, herbal), 2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane (No. 41, odorless), 2-ethyl-6-methylpyrazine (No. 43, nutty), 2,3,5-trimethylpyrazine (No. 44, nutty), 2-phenylacetaldehyde (No. 46, green), 2-ethyl-3,5-dimethylpyrazine (No. 48, nutty), 2-phenylethanol (No. 51, floral), 2-phenylprop-2-enal (No. 53, floral), and 4-ethyl-2-methoxyphenol (No. 58, spicy/burnt), were associated with this region. These negative compounds agreed with findings previously obtained in the present study.

Next, the compounds, 2-methylpropan-1-ol (No. 5, musty), ethyl 2-hydroxypropanoate (No. 21, fruity), 2-ethoxybutane (No. 27, berry), 3-methylsulfanylpropanal (No. 29, cooked potato-like), 2,6-dimethylpyrazine (No. 30, nutty), 3-methyloxolan-2-one (No. 33, woody), 3-methylsulfanylpropan-1-ol (No. 39, sulfurous), oct-1-en-3-ol (No. 40, green), methylsulfanylcyclohexane (No. 49, alliaceous), and [2,2,4-Trimethyl-3-(2-methylpropanoyloxy)pentyl] 2-methylpropanoate (No. 62, mild), were located on upper left side of the S1 groups from 2015 to 2018, also had a weak and negative influence on the quality of soy sauce.

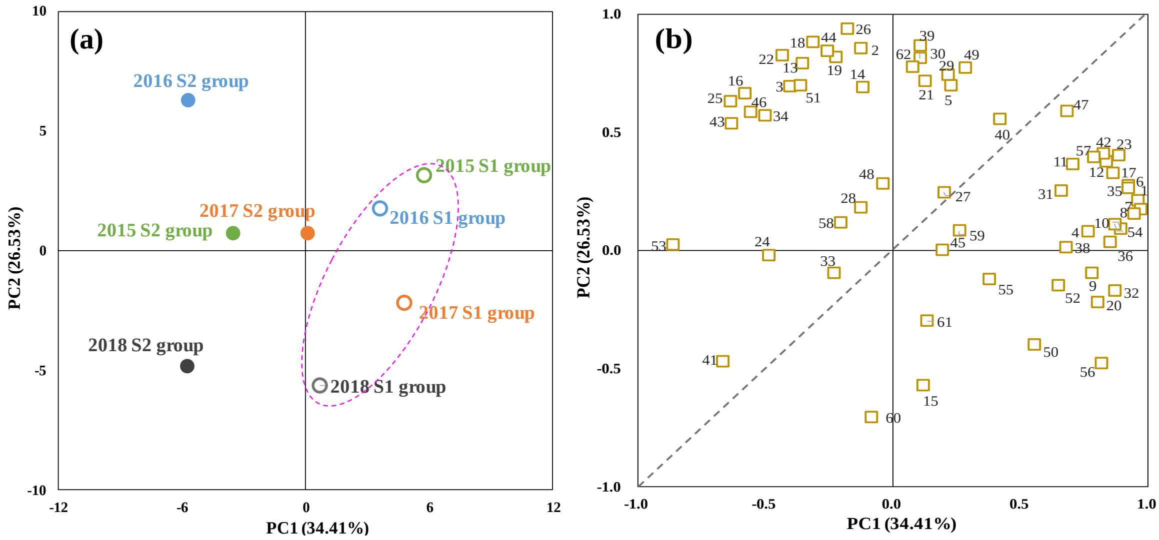


Figure 3-2 Principal component analysis (PCA) of the average values for the volatile compounds detected from soy sauce products in the groups S1 and S2 from 2015 to 2018: (a) scores; (b) loadings. The numbers in the loadings plot represent the order for each volatile compound shown in Table 3-1.

### 3.4.2 Miso

3.4.2.1 Identification of Volatiles in Miso

Based on the GC-MS analysis, a total of 48 peaks were detected from the tested miso samples evaluated in the annual competition from 2015 to 2018 and all were positively identified (Table 3-2 and Figure 3-3). These compounds were classified into different groups based on their chemical structure: nineteen esters, nine aldehydes (including furan-2-carbaldehyde), eight alcohols, four ketones, three acids, two sulfur-containing compounds, and three others. In comparison with soy sauce, no pyrazines and phenols were detected. The phenols in soy sauce were formed from the degradation of lignin glycosides, and lignins could be provided from cereal bran due to the materials consist of the whole wheat during soy sauce fermentation (Yokotsuka et al., 1980). While the fermentation materials of miso were different, that is polished white rice. The pyrazines formation were correlated to the pasteurization, which is a high-temperature processing (Müller & Rappert, 2010). On the other hand, the manufacturing process of miso did not include a high-temperature sterilization process. That is, there were no suitable conditions for the formation of phenols and pyrazines during miso fermentation. Of these 48 volatile compounds, 21 were present in all the miso samples tested (Table 3-2).

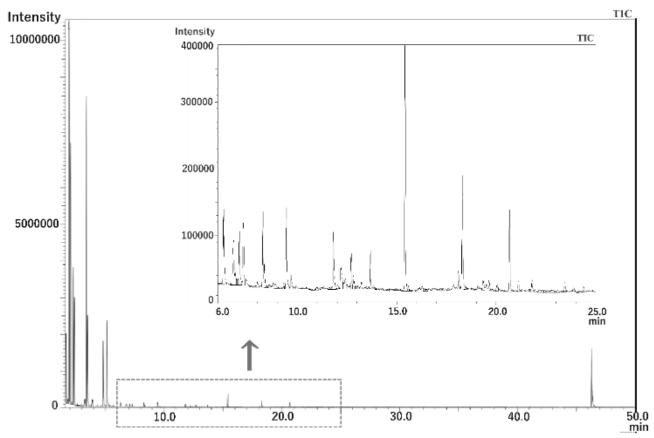


Figure 3-3 Total ion chromatogram of volatile compounds in miso detected by direct extraction from the headspace.

Table 3-2 The volatile compounds identified in the headspace of the evaluated miso samples

| No.[1] | RI[2] | $RI_{ref}$[3] | Compounds | Quantifying ion (*m/z*) | Correlation coefficient (*r*)[4] | | | | Identification[5] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2015 | 2016 | 2017 | 2018 | |
| *Alcohols* | | | | | | | | | |
| 5* [6] | 622 | 628 | 2-Methylpropan-1-ol | 43 (74) | -0.22 | -0.03 | -0.12 | -0.40 | AB |
| 11 | 721 | 730 | 1,1-Diethoxyethane | 73 (45, 103) | -0.53 | -0.05 | -0.22 | -0.20 | AC |
| 12* | 726 | 738 | 3-Methylbutan-1-ol | 55 (42, 70) | -0.28 | 0.04 | 0.03 | -0.35 | AB |
| 13* | 730 | 742 | 2-Methylbutan-1-ol | 57 (41, 70) | -0.28 | -0.05 | -0.10 | -0.38 | AB |
| 19 | 799 | 815 | Butane-2,3-diol | 45 (57) | -0.35 | 0.02 | -0.22 | -0.52 | AB |
| 28 | 869 | 869 | Hexan-1-ol | 56 (43, 69) | -0.22 | 0.25 | 0.18 | 0.04 | AC |
| 36* | 979 | 992 | Oct-1-en-3-ol | 57 (43, 72) | -0.21 | 0.14 | 0.08 | 0.03 | AB |
| 41 | 1107 | 1128 | 2-Phenylethanol | 91 (92, 122) | -0.49 | -0.02 | -0.12 | -0.49 | AB |
| *Aldehydes* | | | | | | | | | |
| 1* | <600 | <600 | 2-Methylpropanal | 43 (41, 72) | -0.41 | -0.35 | -0.32 | -0.34 | AB |
| 7* | 647 | 658 | 3-Methylbutanal | 44 (41, 58) | -0.44 | -0.46 | -0.44 | -0.38 | AB |
| 8* | 656 | 668 | 2-Methylbutanal | 41 (56, 57) | -0.44 | -0.36 | -0.32 | -0.28 | AB |
| 9* | 698 | 699 | Pentanal | 44 (58) | -0.06 | 0.07 | 0.27 | -0.06 | AC |
| 17* | 778 | 781 | 3-Methylbut-2-enal | 84 (41, 55) | -0.65 | -0.53 | -0.34 | -0.34 | AB |
| 23* | 828 | 845 | Furan-2-carbaldehyde | 96 (39, 95) | -0.15 | -0.47 | -0.62 | -0.56 | AB |
| 33* | 957 | 980 | Benzaldehyde | 106 (51, 77) | -0.50 | -0.39 | -0.18 | -0.31 | AB |
| 40* | 1039 | 1060 | 2-Phenylacetaldehyde | 91 (65, 120) | -0.73 | -0.59 | -0.62 | -0.56 | AB |
| 48* | 1263 | 1292 | (*E*)-2-phenylbut-2-enal | 115 (91, 146) | -0.53 | -0.36 | -0.37 | -0.24 | AB |
| *Acids* | | | | | | | | | |
| 6* | 625 | 604 | Acetic acid | 60 (43) | -0.34 | -0.26 | -0.41 | -0.21 | AB |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 14 | 744 | 765 | 2-Methylpropanoic acid | 43 (73, 88) | 0.22 | -0.11 | 0.08 | -0.25 | AB |
| 24 | 839 | 862 | 3-Methylbutanoic acid | 60 (43, 87) | -0.23 | -0.19 | -0.18 | 0.08 | AB |
| | | | | | | | | | |
| *Esters* | | | | | | | | | |
| 4* | 610 | 612 | Ethyl acetate | 43 (45, 61) | -0.16 | -0.35 | 0.43 | -0.58 | AB |
| 10 | 708 | 706 | Ethyl propanoate | 57 (74, 102) | -0.28 | -0.07 | -0.19 | -0.58 | AC |
| 15 | 748 | 747 | Ethyl 2-methylpropanoate | 43 (71, 116) | -0.13 | 0.13 | -0.05 | -0.55 | AC |
| 16 | 765 | 770 | 2-Methylpropyl acetate | 43 (56, 73) | -0.25 | -0.15 | -0.22 | -0.42 | AC |
| 18 | 787 | 787 | Ethyl 2-hydroxypropanoate | 45 (75) | -0.30 | -0.13 | -0.23 | -0.57 | AC |
| 20 | 806 | 785 | Ethyl 2-oxopropanoate | 43 (106) | -0.53 | -0.20 | -0.29 | -0.57 | AB |
| 21 | 812 | 824 | Ethyl (2S)-2-hydroxypropanoate | 45 (75) | 0.03 | -0.11 | 0.05 | -0.14 | AB |
| 22 | 813 | 812 | Butyl acetate | 43 (56, 73) | -0.39 | -0.29 | -0.32 | -0.28 | AC |
| 25 | 847 | 849 | Ethyl 2-methylbutanoate | 57 (85, 102) | -0.10 | -0.19 | -0.22 | -0.56 | AC |
| 26 | 852 | 858 | Ethyl 3-methylbutanoate | 88 (57, 85) | -0.15 | -0.33 | -0.26 | -0.60 | AC |
| 29 | 876 | 876 | 3-Methylbutyl acetate | 43 (55, 70) | -0.31 | -0.06 | -0.15 | -0.63 | AC |
| 30 | 878 | 880 | 2-Methylbutyl acetate | 70 (43, 55) | -0.33 | -0.23 | -0.29 | -0.43 | AC |
| 32 | 909 | 908 | Oxolan-2-one | 42 (56, 86) | -0.40 | -0.16 | -0.29 | -0.47 | AC |
| 39 | 998 | 999 | Ethyl hexanoate | 88 (43, 99) | -0.26 | -0.05 | -0.30 | -0.19 | AC |
| 43 | 1166 | 1171 | Ethyl benzoate | 105 (77, 122) | -0.26 | -0.51 | -0.60 | -0.60 | AC |
| 44 | 1178 | 1179 | Diethyl butanedioate | 101 (73, 129) | -0.37 | -0.12 | -0.27 | -0.37 | AB |
| 45 | 1195 | 1196 | Ethyl octanoate | 88 (101, 127) | -0.45 | 0.05 | -0.04 | -0.36 | AC |
| 46 | 1238 | 1243 | Ethyl 2-phenylacetate | 91 (65, 164) | -0.35 | -0.16 | -0.38 | -0.54 | AC |
| 47 | 1250 | 1256 | 2-Phenylethyl acetate | 104 (43) | -0.38 | -0.01 | 0.03 | -0.41 | AC |
| | | | | | | | | | |
| *Ketones* | | | | | | | | | |
| 2* | <600 | 601 | Butane-2,3-dione | 86 (43) | -0.63 | -0.28 | -0.08 | -0.36 | AB |
| 3* | 601 | 603 | Butan-2-one | 72 (43) | -0.69 | -0.57 | -0.40 | -0.39 | AB |
| 34 | 976 | 980 | Oct-1-en-3-one | 55 (70, 97) | -0.56 | 0.03 | 0.05 | -0.06 | AC |
| 37 | 984 | 988 | Octan-3-one | 43 (57, 99) | -0.38 | 0.28 | 0.24 | -0.03 | AC |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Sulfur-containing compounds* | | | | | | | | | |
| 31* | 904 | 906 | 3-Methylsulfanylpropanal | 48 (76, 104) | -0.59 | -0.64 | -0.39 | -0.36 | AB |
| 35 | 977 | 993 | 3-Methylsulfanylpropan-1-ol | 106 (58, 61) | -0.52 | -0.21 | -0.34 | -0.49 | AB |
| | | | | | | | | | |
| *Others* | | | | | | | | | |
| 27 | 855 | 855 | Ethylbenzene | 91 (65, 106) | -0.79 | 0.07 | -0.18 | -0.29 | AC |
| 38* | 989 | 994 | 2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane | 281 (133, 265) | 0.55 | 0.49 | 0.44 | 0.30 | AB |
| 42* | 1140 | 1171 | 2,2,4,4,6,6,8,8,10,10-decamethyl-1,3,5,7,9,2,4,6,8,10-pentaoxapentasilecane | 73 (267) | -0.43 | 0.29 | -0.20 | -0.26 | AC |

[1]The volatile compounds are arranged in order of their retention time within each chemical group.

[2]RI, retention indices, RI value were calculated for the SH-Rxi™-5SilMS capillary column.

[3]$RI_{ref}$, references retention indices.

[4] The correlation coefficients (*r*) were calculated between the peak area of each compound and the rankings of the evaluated miso samples.

[5] Identification: (A) by comparison of the mass spectrum with the NIST 17 Mass Spectral Library; (B) by comparison of the corresponding volatile compounds on the previous work for soy sauce (Wang et al., 2019); (C) by comparison of the RI value on a similar phase column reported in the literature (Baccouri et al., 2007; Bader et al., 2003; Beaulieu & Grimm, 2001; Bylaite & Meyer, 2005; Cho et al., 2008; Dall üge et al., 2002; Fan & Qian, 2006, 2005; Feng et al., 2014, 2017, 2015; Lucero, Estell, & Fredrickson, 2003; Methven et al., 2007; Morinaga et al., 1990; Pino, Almora, & Marbot, 2003; Rout et al., 2007; Steinhaus & Schieberle, 2007; Sun et al., 2010; Wu et al., 2007).

[6]"*" means the related volatile compounds were detected in all miso samples tested.

3.4.2.2 Relationship between the Level of Volatile Compounds and the Quality of miso

The correlation coefficients (*r*) between the intensity of detected 48 peaks and the sensory rankings of the samples by sensory evaluation from 2015 to 2018 are shown in Table 3-2. As for the soy sauce, the change of volatile compounds in the sensory rankings for miso products was further discussed: a negative value of *r* represents a positive correlation between the intensity of a volatile compound based on the GC-MS analysis and the quality of miso; reversely, a positive value represents a negative correlation; and also a greater absolute value of *r* indicating a stronger effect on the determination of miso quality.

The alcohols group comprised eight volatile compounds. Except for 1,1-diethoxyethane and hexan-1-ol, the other six compounds were also presented in soy sauce and therefore considered to be common to the fermentation process of soy sauce and miso products. As opposed to soy sauce, 2-phenylethanol showed a positive influence on the quality improvement of miso, especially in 2015 ($r = -0.49$) and 2018 ($r = -0.49$). The positive effect of 2-phenylethanol on determining miso quality had also been observed in previous study (Sugawara, Saiga, & Kobayashi, 1992). The different roles played in the quality improvement of soy sauce and miso with 2-phenylethanol could be assumed to be involved in the manufacturing process of soy sauce and miso. Moreover, 2-methylpropan-1-ol, 1,1-diethoxyethane, and 2-methylbutan-1-ol also had a weak positive effect on the quality of miso due to the smaller and negative *r* value from 2015 to 2018. The positive effect of 2-methylpropan-1-ol and 2-methylbutan-1-ol on miso quality was in agreement with previously reported (Sugawara, 1991a). The hexan-1-ol and oct-1-en-3-ol are the major aroma compounds of soybean, and these two compounds showed little potential for the determination of quality due to the *r* values, positively and negatively, were very smaller from 2015 to 2018.

In the aldehydes group, except for pentanal, the other eight compounds showed a relatively greater influence on miso with greater *r* values in comparison to the other compounds in different groups. The *r* value of these eight compounds was negative from 2015 to 2018 indicating their positive roles played in the quality improvement of miso products. Of all nine compounds in the aldehydes group, seven compounds, such as 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, 3-methylbut-2-enal, furan-2-carbaldehyde, benzaldehyde, 2-phenylacetaldehyde, and (*E*)-2-phenylbut-2-enal, were also found in soy sauce samples based on GC-MS analysis. These compounds were considered to be common products to both soy sauce and miso during fermentation. The different effects of 3methylbut-2-enal, 2-phenylacetaldehyde, and benzaldehyde on miso compared with on soy sauce were observed, where all these three compounds had a positive influence on the quality improvement of miso. This may have been related to the synergistic and antagonistic effects between these compounds and other constituents in soy sauce and miso, respectively.

As with the GC-MS analysis of soy sauce, there are three acids detected, including acetic acids, 2-methylpropanoic acid, and 3-methylbutanoic acid. Acetic acid was common in overall the tested miso samples, and its content was dominant among acid compounds. The positive relationship between the level of acetic acid and the sensory rankings in all 4 consecutive years demonstrated its positive effect on the quality improvement of miso products. However, it seemed like that the other two detected acids (i.e. 2-methylpropanoic acid and 3-methylbutanoic acid) had little effect on the quality of miso due to the *r* values for these two compounds were weakly positive or negative.

For the esters group, a total of 19 compounds were identified. In addition to the difference in the numbers of ester compounds, the detected esters have different effects on soy sauce and miso products. For soy sauce, only 2-oxopropanoate has a positive effect and ethyl 2-hydroxypropanoate has a weakly negative effect, and the remaining 5 esters have no clear effect on the quality of soy sauce. However, for miso products, the level of most esters is positively correlated with the quality of miso. The difference in esters maybe one of the important factors to the different flavor characteristics for soy sauce and miso. The newly detected esters in miso mainly belonged to the high fatty acid esters, which produced by using the whole soybean as fermentation materials during the early periods of the refining process. The period of fermentation had a positive effect on increasing the level of high fatty acid esters (Kobayashi & Sugawara, 1999). Accordingly, it is found that the absence of the related esters was mostly observed in the samples that ranked at a lower position, especially for ethyl octanoate, ethyl 2-phenylacetate, and 2-phenylethyl acetate. As a consequence, the samples that ranked at a lower position may be related to the short or poor fermentation process.

In the ketones group, butane-2,3-dione and butan-2-one showed a positive influence on increasing the quality of miso products. Moreover, the obtained data of the peak area of oct-1-en-3-one and octan-3-one were quite small. Kumazawa et al. (2013) found a decreasing mechanism of oct-1-en-3-one and octan-3-one occur with the presence of amino acids resulting in the loss of these two compounds during heat processing and assumed that the loss of these two compounds involved in the flavor change for miso. However, the important effect of these two compounds on the flavor of miso was not observed due to the unstable correlations to the sensory rankings of miso in all 4 consecutive years, as shown in Table 3-2.

There are two sulfur-containing compounds were detected in the tested miso samples, that is, 3-methylsulfanylpropanal and 3-methylsulfanylpropan-1-ol. 3-methylsulfanylpropanal was detected in all miso samples from 2015 to 2018. This unique sulfur-containing compound showed a relatively high influence on increasing the quality of miso products, with the *r* values of -0.59, -0.64, -0.39, and -0.36, in 2015, 2016, 2017, and 2018, respectively. The same effect on miso was observed in 3-methylsulfanylpropan-1-ol. 3-methylsulfanylpropanal is generated from methionine by Strecker degradation, and its reduced product was 3-methylsulfanylpropan-1-ol under the reducing conditions of fermentation. Both these two compounds were identified as an off-flavor compound due to their threshold values (Kobayashi & Sugawara, 1999). However, the presence of 3-methylsulfanylpropanal has an obviously negative effect on the sensory quality of soy sauce (Wang et al., 2019). The opposed effects of 3-methylsulfanylpropanal on the sensory quality for soy sauce and miso suggested that the synergistic and antagonistic effects between it and/or other components in miso and soy sauce during fermentation, respectively.

In addition to the above volatile compounds, three other components were also detected during GC-MS analysis and were classified to the group labeled "others". 2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane was also found in previous GC-MS analysis of soy sauce showing a negative effect on the sensory quality. However, in the case of miso, this compound and the other two compounds had little influence on the quality due to the positively or negatively small values of *r* in all 4 consecutive years.

### 3.4.2.3 PCA Results

In order to further simplify the interpretation of relationships between the sensory quality of miso and the obtained volatile compounds, the tested miso samples from 2015 to 2018 were divided into two groups M1 and M2 with reference to the previous work (as shown in Table 2-2). That is, the top 14 samples in 2015 and 2018, and the top 13 samples in 2016 and 2017 were classified into the group M1 representing for a better sensory quality of miso products, the remaining miso samples from 2015 to 2018 were classified into the group M2 representing for a relatively lower sensory quality. The average value of the detected volatile compounds was calculated for each group from 2015 to 2018, respectively, and then PCA was carried out on the average values of the groups. PCA result showed six principal components with eigenvalues greater than 1, which are considered important and accounted for 98.91% of total variation among the samples that were retained. The first two PCs, PC1 and PC2, resulted in 71.67% total variance. PC1 had an eigenvalue of 26.69 and described 55.60% of the total variance, and covered as much of the variance as possible. PC2 had an eigenvalue of 7.72 and described 16.07% of the total variance.

The PCA scores for PC1 versus PC2 are shown in Figure 3-4 (a). The miso samples from different groups M1 and M2 were clearly discriminated. The miso groups M1 from 2015 to 2018, which represent better sensory quality, were loaded closer to each other and positioned in/near the lower right quadrant, indicating the group M1 for all 4 consecutive years possess similar characteristics of flavor. The miso groups M2 were located far from the groups M1 and also the groups M2 from 2015 to 2018 showed distinct differences between each other. Both the PC1 and PC2 for the detected volatile compounds served to distinguish the different sensory quality of miso products. Therefore, it is reasonable to assume that the volatile compounds identified in the tested miso samples had an important influence on the discrimination of the quality.

The correlation loadings plot of the volatile compounds in the first two PCs is also shown in Figure 3-4 (b). Overall, all compounds except for pentanal, ethyl 2-hydroxypropanoate, and 2-phenylethyl acetate, contributed positively to the PC1, and no identified compounds had large negative factor loadings on the PC1. By comparison with the score plot (Figure 3-4 (a)), the low-quality miso products assigned to group M2 in 2017 and 2018 groups might be related to low intensities of volatile compounds. In contrast, the high-quality miso products were considered to have an in intense flavor. Moreover, the compounds that loaded on the upper right quadrant and the lower right quadrant, respectively, involved in the differences in sensory quality between the groups M1 and M2 in 2016. That is, the compounds located on the negative side of PC2, including 3-methylsulfanylpropanal, butyl acetate, and most aldehydes, such as 2-methyl-propan-1-ol, 3-methylbutanal, 2-methylbutanal, 3-methylbut-2-enal, furan-2-carbaldehyde, 2-phenylacetaldehyde, and (*E*)-2-phenylbut-2-enal, showed a more prominent contribution to improve the sensory quality of miso products than that on the positive side of PC2, such as ethyl propanoate, 3-methylbutan-1-ol, hexan-1-ol, 3-methylbutyl acetate, oct-1-en-3-one, and oct-1-en-3-ol.

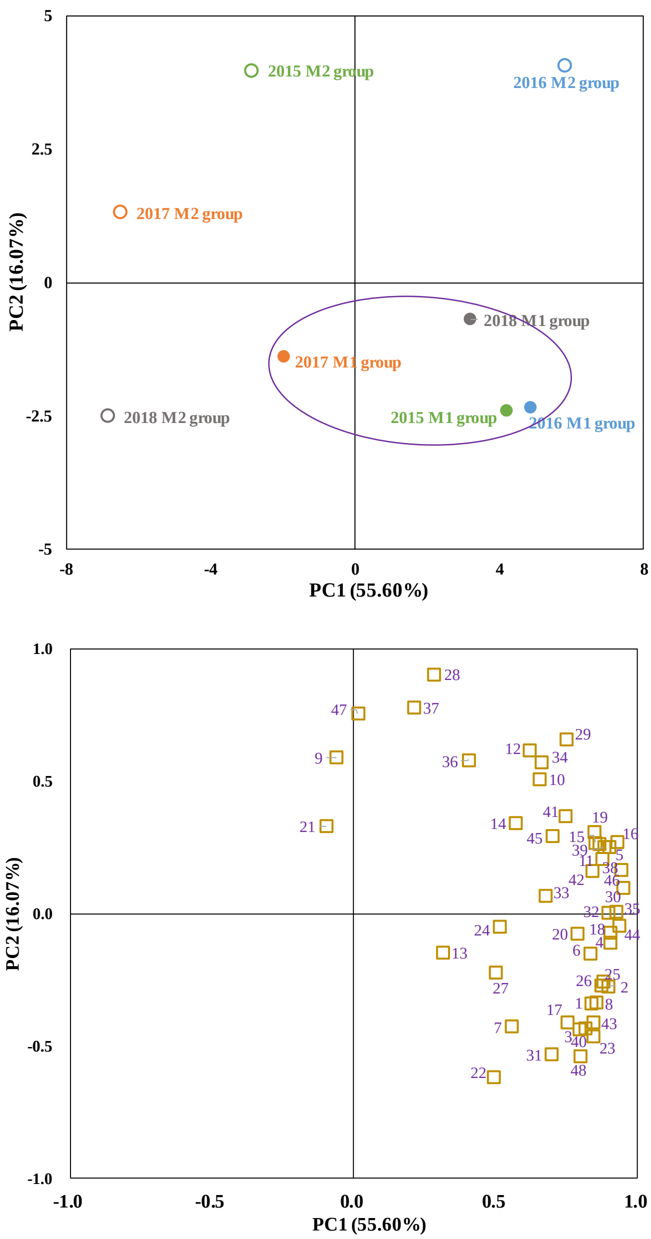


Figure 3-4 Principal component analysis (PCA) of the average values for the volatile compounds detected from miso products in the groups M1 and M2 from 2015 to 2018: (a) scores; (b) loadings. The numbers in the loadings plot represent the order for each volatile compound shown in Table 3-2.

## 3.5 Conclusions

Flavor as one of the key attributes in fermented condiments directly determined the final quality of the product. The present work was carried out to study the volatile compounds that extracted directly by headspace and to correlate directly to the sensory evaluation for soy sauce and miso.

The obtained results showed: for soy sauce, nineteen detected volatile compounds (2-methylpropanal, acetic acid, 3-methylbutanal, 2-methylbutanal, pentan-2-one, 1-hydroxypropan-2-one, pentane-2,3-dione, 3-hydroxybutan-2-one, hexanal, ethyl 2-oxopropanoate, furan-2-carbaldehyde, heptanol, 2-methyloctan-3-one, octane-2,3-dione, (5-methylfuran-2-yl)methanol, (5-methylfuran-2-yl)methanethiol, 1-(1*H*-pyrrol-2-yl)ethanone, 3-phenylfuran, (*E*)-2-phenylbut-2-enal) were found to be positively correlated with the increasing sensory quality of soy sauce, whereas twenty-three detected volatile compounds (butane-2,3-dione, butan-2-one, 3-methylbutan-1-ol, 1-methylsulfanylpropane, 3-methylbut-2-enal, butane-2,3-diol, ethyl 2-hydroxypropanoate, 2-methylpyrazine, 3-methylpent-3-en-2-one, furan-2-ylmethanol, 2-ethoxybutane, furan-2-ylmethyl formate, 3-methylsulfanylpropanal, 3-methyloxolan-2-one, 5-methyloxolan-2-one, 2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, 2-phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine, 2-phenylethanol, 2-phenylprop-2-enal, 4-ethyl-2-methoxyphenol) showed a negative correlation. In the case of miso, most detected compounds showed a positive effect on the determination of quality, and barely had an obviously negative correlation with the determination of quality, indicating the high-quality miso products were correlated to an intense flavor, The statistical analysis of the data using principal component analysis confirmed the effect of the volatile compounds suggested by the values of $r$, and further classified the contribution of each compound with the assessed quality of samples. The obtained results can contribute for the flavor optimization research of soy sauce and miso products and provide data support for developing their quality standards.

## 3.6 References


Alissandrakis, E., Tarantilis, P. A., Harizanis, P. C., & Polissiou, M. (2007). Comparison of the volatile composition in thyme honeys from several origins in Greece. *Journal of Agricultural and Food Chemistry*, *55*, 8152–8157.

Baccouri, B., Temime, S. B., Campeol, E., Cioni, P. L., Daoud, D., & Zarrouk, M. (2007). Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. *Food Chemistry*, *102*, 850–856.

Bader, A., Flamini, G., Cioni, P. L., & Morelli, I. (2003). Essential oil composition of Achillea santolina L. and Achillea biebersteinii Afan. Collected in Jordan. *Flavour and Fragrance Journal*, *18*, 36–38.

Baizer, M. M., Nonaka, T., Park, K., Saito, Y., & Nobe, K. (1984). Electrochemical conversion of 2,3-butanediol to 2-butanone in undivided flow cells: A paired synthesis. *Journal of Applied Electrochemistry*, *14*, 197–208.

Beal, A. D., & Mottram, D. S. (1994). Compounds contributing to the characteristic aroma of malted barley. *Journal of Agricultural and Food Chemistry*, *42*, 2880–2884.

Beaulieu, J. C., & Grimm, C. C. (2001). Identification of Volatile Compounds in Cantaloupe at Various Developmental Stages Using Solid Phase Microextraction. *Journal of Agricultural and Food Chemistry*, *49*, 1345–1352.

Boelens, M., Van der Linde, L. M., De Valois, P. J., Van Dort, H. M., & Takken, H. J. (1974). Organic sulfur compounds from fatty aldehydes, hydrogen sulfide, thiols, and ammonia as flavor constituents. *Journal of Agricultural and Food Chemistry*, *22*, 1071–1076.

Bylaite, E., & Meyer, A. S. (2005). Characterisation of volatile aroma compounds of orange juices by three dynamic and static headspace gas chromatography techniques. *European Food Research and Technology*, *222*, 176.

Campo, E., Ferreira, V., Escudero, A., & Cacho, J. (2005). Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. *Journal of Agricultural and Food Chemistry*, *53*, 5682–5690.

Cho, I. H., Namgung, H. J., Choi, H. K., & Kim, Y. S. (2008). Volatiles and key odorants in the pileus and stipe of pine-mushroom (Tricholoma matsutake Sing.). *Food Chemistry*, *106*, 71–76.

Coleman, W. M., & Chung, H. L. (2002). Pyrolysis GC–MS analysis of Amadori compounds derived from selected amino acids and glucose. *Journal of Analytical and Applied Pyrolysis*, *62*, 215–223.

Dallüge, J., Van Stee, L. L. P., Xu, X., Williams, J., Beens, J., Vreuls, R. J. J., & Brinkman, U. A. T. (2002). Unravelling the composition of very complex samples by comprehensive gas chromatography coupled to time-of-flight mass spectrometry: Cigarette smoke. *Journal of Chromatography A*, *974*, 169–184.

Fan, W., & Qian, M. C. (2006). Characterization of Aroma Compounds of Chinese "Wuliangye" and "Jiannanchun" Liquors by Aroma Extract Dilution Analysis. *Journal of Agricultural and Food Chemistry*, *54*, 2695–2704.

Fang, Y., & Qian, M. (2005). Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). *Flavour and Fragrance Journal*, *20*, 22–29.

Felipe, S.-J., Ferreira, V., Cacho, J., & Escudero, A. (2011). Quality and Aromatic Sensory Descriptors (Mainly Fresh and Dry Fruit Character) of Spanish Red Wines can be Predicted from their Aroma-Active Chemical Composition. *Journal of Agricultural and Food Chemistry*, *59*, 7916–7924.

Feng, Y., Cai, Y., Su, G., Zhao, H., Wang, C., & Zhao, M. (2014). Evaluation of aroma differences between high-salt liquid-state fermentation and low-salt solid-state fermentation soy sauces from China. *Food Chemistry*, *145*, 126–134.

Feng, Y., Su, G., Sun-Waterhouse, D., Cai, Y., Zhao, H., Cui, C., & Zhao, M. (2017). Optimization of Headspace Solid-Phase Micro-extraction (HS-SPME) for Analyzing Soy Sauce Aroma Compounds via Coupling with Direct GC-Olfactometry (D-GC-O) and Gas Chromatography-Mass Spectrometry (GC-MS). *Food Analytical Methods*, *10*, 713–726.

Feng, Y., Su, G., Zhao, H., Cai, Y., Cui, C., Sun-Waterhouse, D., & Zhao, M. (2015). Characterisation of aroma profiles of commercial soy sauce by odour activity value and omission test. *Food Chemistry*, *167*, 220–228.

Giri, A., Osako, K., & Ohshima, T. (2010). Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chemistry*, *120*, 621–631.

Harada, R., Yuzuki, M., Ito, K., Shiga, K., Bamba, T., & Fukusaki, E. (2017). Influence of yeast and lactic acid bacterium on the constituent profile of soy sauce during fermentation. *Journal of Bioscience and Bioengineering*, *123*, 203–208.

Harada, R., Yuzuki, M., Ito, K., Shiga, K., Bamba, T., & Fukusaki, E. (2018). Microbe participation in aroma production during soy sauce fermentation. *Journal of Bioscience and Bioengineering*, *125*, 688–694.

Honma, N. (1987). Aroma and aroma components of miso (2) (in Japanese). *Journal of the Society of Brewing, Japan*, *82*, 547–553.

Huang, X., & Barringer, S. A. (2016). Kinetics of furan formation during pasteurization of soy sauce. *LWT - Food Science and Technology*, *67*, 200–205.

Kaneko, S., Kumazawa, K., & Nishimura, O. (2012). Comparison of Key Aroma Compounds in Five Different Types of Japanese Soy Sauces by Aroma Extract Dilution Analysis (AEDA). *Journal of Agricultural and Food Chemistry*, *60*, 3831–3836.

Kaneko, S., Kumazawa, K., & Nishimura, O. (2013). Studies on the Key Aroma Compounds in Raw (Unheated) and Heated Japanese Soy Sauce. *Journal of Agricultural and Food Chemistry*, *61*, 3396–3402.

Kim, J.-S., & Chung, H. Y. (2009). GC-MS analysis of the volatile components in dried boxthorn (Lycium chinensis) fruit. *Journal of the Korean Society for Applied Biological Chemistry*, *52*, 516–524.

Kobayashi, A., & Sugawara, E. (1999). Flavor Components of Shoyu and Miso Japanese Fermented Soybean Seasonings. In F. Shahidi & C. T. Ho (Eds.), *Flavor Chemistry of Ethnic Foods* (pp. 5–14). Boston, MA: Springer US.

Kumazawa, K., Kaneko, S., & Nishimura, O. (2013). Identification and Characterization of Volatile Components Causing the Characteristic Flavor in Miso (Japanese Fermented Soybean Paste) and Heat-Processed Miso Products. *Journal of Agricultural and Food Chemistry*, *61*, 11968–11973.

Landaud, S., Helinck, S., & Bonnarme, P. (2008). Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food. *Applied Microbiology and Biotechnology*, *77*, 1191–1205.

Lee, K. E., Lee, S. M., Choi, Y. H., Hurh, B. S., & Kim, Y. S. (2013). Comparative Volatile Profiles in Soy Sauce According to Inoculated Microorganisms. *Bioscience, Biotechnology, and Biochemistry*, *77*, 2192–2200.

Lee, S. M., Seo, B. C., & Kim, Y. S. (2006). Volatile Compounds in Fermented and Acid-hydrolyzed Soy Sauces. *Journal of Food Science*, *71*, C146–C156.

Leffingwell, J. C., & Alford, E. D. (2005). Volatile Constituents of Perique Tobacco. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *4*, 899–915.

Lioe, H. N., Wada, K., Aoki, T., & Yasuda, M. (2007). Chemical and sensory characteristics of low molecular weight fractions obtained from three types of Japanese soy sauce (shoyu)–Koikuchi, tamari and shiro shoyu. *Food Chemistry*, *100*, 1669–1677.

Lucero, M. E., Estell, R. E., & Fredrickson, E. L. (2003). The Essential Oil Composition of Psorothamnus scoparius (A. Gray) Rydb. *Journal of Essential Oil Research*, *15*, 108–111.

Meng, Q., Imamura, M., Katayama, H., Obata, A., & Sugawara, E. (2017). Key compounds contributing to the fruity aroma characterization in Japanese raw soy sauce. *Bioscience, Biotechnology, and Biochemistry, 81,* 1984–1989.

Meng, Q., Kitagawa, R., Imamura, M., Katayama, H., Obata, A., & Sugawara, E. (2017). Contribution of 2-methyl-3-furanthiol to the cooked meat-like aroma of fermented soy sauce. *Bioscience, Biotechnology, and Biochemistry*, *81*, 168–172.

Methven, L., Tsoukka, M., Oruna-Concha, M. J., Parker, J. K., & Mottram, D. S. (2007). Influence of Sulfur Amino Acids on the Volatile and Nonvolatile Components of Cooked Salmon (Salmo salar). *Journal of Agricultural and Food Chemistry*, *55*, 1427–1436.

Morinaga, M., Hara, K., Kageura, M., Hieda, Y., Takamoto, M., & Kashimura, S. (1990). A simple, rapid and simultaneous analysis of complex volatile hydrocarbon mixtures in blood using gas chromatography/mass spectrometry with a wide-bore capillary column. *Zeitschrift Für Rechtsmedizin*, *103*, 567–572.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry*, *62*, 415–424.

Müller, R., & Rappert, S. (2010). Pyrazines: Occurrence, formation and biodegradation. *Applied Microbiology and Biotechnology*, *85*, 1315–1320.

Nunomura, N., Sasaki, M., & Yokotsuka, T. (1980). Shoyu (Soy Sauce) Flavor Components: Acidic Fractions and the Characteristic Flavor Component. *Agricultural and Biological Chemistry*, *44*, 339–351.

Ono, T., Usami, A., Nakaya, S., Shinpuku, H., Yonejima, Y., Ikeda, A., & Miyazawa, M. (2015). Agroecosystem Development of Industrial Fermentation Waste—Characterization of Aroma-active Compounds from the Cultivation Medium of Lactobacillus brevis—. *Journal of Oleo Science*, *64*, 585–594.

Pino, J. A., Almora, K., & Marbot, R. (2003). Volatile components of papaya (Carica papaya L., Maradol variety) fruit. *Flavour and Fragrance Journal*, *18*, 492–496.

Pires, E. J., Teixeira, J. A., Brányik, T., & Vicente, A. A. (2014). Yeast: The soul of beer's aroma—a review of flavour-active esters and higher alcohols produced by the brewing yeast. *Applied Microbiology and Biotechnology*, *98*, 1937–1949.

Qiming, X., Haidong, C., Huixian, Z., & Daqiang, Y. (2006). Chemical composition of essential oils of two submerged macrophytes, Ceratophyllum demersum L. and Vallisneria spiralis L. *Flavour and Fragrance Journal*, *21*, 524–526.

Radulović, N., Blagojević, P., & Palić, R. (2010). Comparative study of the leaf volatiles of Arctostaphylos uva-ursi (L.) Spreng. And Vaccinium vitis-idaea L. (Ericaceae). *Molecules (Basel, Switzerland)*, *15*, 6168–6185.

Raffo, A., Kelderer, M., Paoletti, F., & Zanella, A. (2009). Impact of innovative controlled atmosphere storage technologies and postharvest treatments on volatile compound production in cv. Pinova apples. *Journal of Agricultural and Food Chemistry*, *57*, 915–923.

Rout, P. K., Rao, Y. R., Sree, A., & Naik, S. N. (2007). Composition of essential oil, concrete, absolute, wax and headspace volatiles of Murrarya paniculata (Linn.) Jack flowers. *Flavour and Fragrance Journal*, *22*, 352–357.

Rychlik, M., & Bosset, J. O. (2001). Flavour and off-flavour compounds of Swiss Gruyère cheese. Evaluation of potent odorants. *International Dairy Journal*, *11*, 895–901.

Salazar Gómez, J. I., Lohmann, H., & Krassowski, J. (2016). Determination of volatile organic compounds from biowaste and co-fermentation biogas plants by single-sorbent adsorption. *Chemosphere*, *153*, 48–57.

Smit, B. A., Engels, W. J. M., & Smit, G. (2009). Branched chain aldehydes: Production and breakdown pathways and relevance for flavour in foods. *Applied Microbiology and Biotechnology*, *81*, 987–999.

Steinhaus, P., & Schieberle, P. (2007). Characterization of the Key Aroma Compounds in Soy Sauce Using Approaches of Molecular Sensory Science. *Journal of Agricultural and Food Chemistry*, *55*, 6262–6269.

Sugawara E. (1991a). Change in Aroma Components of Miso with Aging. *NIPPON SHOKUHIN KOGYO GAKKAISHI*, *38*, 1093–1097.

Sugawara, E. (1991b). Identification of 4-Hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3 (2 H)-furanone as a Flavor Component in Miso. *Nippon Shokuhin Kogyo Gakkaishi*, *38*, 491–493.

Sugawara, E., Hashimoto, S., Sakurai, Y., & Kobayashi, A. (1994). Formation by Yeast of the HEMF (4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone) Aroma Component in Miso with Aging. *Bioscience, Biotechnology, and Biochemistry*, *58*, 1134–1135.

Sugawara, E., Saiga, S., & Kobayashi, A. (1992). Relationships between Aroma Components and Sensory Evaluation of Miso. *Nippon Shokuhin Kogyo Gakkaishi*, *39*, 1098–1104.

Sugawara, E., Saiga, S., & Kobayashi, A. (1994). Multiple Regression Analysis of Aroma Components and Sensory Evaluation of Miso. *Nippon Shokuhin Kogyo Gakkaishi*, *41*, 844–846.

Sun, S. Y., Jiang, W. G., & Zhao, Y. P. (2010). Profile of Volatile Compounds in 12 Chinese Soy Sauces Produced by a High-Salt-Diluted State Fermentation. *Journal of the Institute of Brewing*, *116*, 316–328.

Taherzadeh, M. J., Gustafsson, L., Niklasson, C., & Lidén, G. (1999). Conversion of furfural in aerobic and anaerobic batch fermentation of glucose by Saccharomyces cerevisiae. *Journal of Bioscience and Bioengineering*, *87*, 169–174.

Wanakhachornkrai, P. (2003). Comparison of determination method for volatile compounds in Thai soy sauce. *Food Chemistry*, *83*, 619–629.

Wang, S., Tamura, T., Kyouno, N., Liu, X., Zhang, H., Akiyama, Y., & Chen, J. Y. (2019). Effect of volatile compounds on the quality of Japanese fermented soy sauce. *LWT*, *111*, 594–601.

Wang, X., Fan, W., & Xu, Y. (2014). Comparison on aroma compounds in Chinese soy sauce and strong aroma type liquors by gas chromatography–olfactometry, chemical quantitative and odor activity values analysis. *European Food Research and Technology*, *239*, 813–825.

Wu, S., Zorn, H., Krings, U., & Berger, R. G. (2007). Volatiles from submerged and surface-cultured beefsteak fungus, Fistulina hepatica. *Flavour and Fragrance Journal*, *22*, 53–60.

Yokotsuka, T., & Sasaki, M. (1997). Fermented protein foods in the Orient: Shoyu and miso in Japan. In B. J. B. Wood (Ed.), *Microbiology of Fermented Foods* (pp. 351–415). Boston, MA: Springer US.

Yokotsuka, Tamotsu, Sasaki, M., Nunomura, S., & Asao, Y. (1980). Flavor of shoyu (1) (in Japanese). *Journal of the Society of Brewing, Japan*, *75*, 516-522,457.

Zhao, G., Ding, L.-L., Yao, Y., Cao, Y., Pan, Z.-H., & Kong, D.-H. (2018). Extracellular Proteome Analysis and Flavor Formation During Soy Sauce Fermentation. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01872

Zhao, G., Yao, Y., Hao, G., Fang, D., Yin, B., Cao, X., & Chen, W. (2015). Gene regulation in Aspergillus oryzae promotes hyphal growth and flavor formation in soy sauce koji. *RSC Advances*, *5*, 24224–24230.

# CHAPTER 4MODEL DEVELOPMENT FOR THE QUALITY OF SOY SAUCE AND MISO

## 4.1 Abstract

The present study focused on selecting relevant variables for developing a flexible and objective model for predicting the quality of soy sauce and miso samples, respectively. The sensory parameters with the potential to influence the overall acceptability of soy sauce and miso samples were measured and regarded as potential variables for predicting the sensory score. The variable selection approach was inspired by Compressed Sensing (CS) theory and has been used for the first time on the calibration set of soy sauce and miso samples to calculate the contribution of each predictive variable to the sensory score. Consequently, those predictive variables shown to affect the quality of soy sauce and miso were successfully selected, respectively. Subsequently, the model for predicting quality was established using partial least squares regression (PLSR). The validity of the models for predicting the quality of soy sauce and miso, respectively, was evaluated using validation sample sets and the values of coefficient of determination ($R^2$) and the root-mean-square error of prediction (RMSEP) were calculated. The constructed models were thus considered to be suitable for predicting the sensory quality of soy sauce and miso during their production processes.

## 4.2 Introduction

The objective of increasing fermented condiment quality to satisfy consumer product acceptance in today's highly innovative and global food environment has brought new challenges on how to quantitatively assess its final quality. Soy sauce and miso are now widely consumed not only in Asian countries, but also around the world so its quality is receiving more attention (Chavasit & Photi, 2018; Steinhaus & Schieberle, 2007). In general, soy sauce and miso are manufactured by a series of processes: the treatment of raw materials, *koji* making, and brine fermentation (Cho et al., 2018; Ogasawara, Yamada, & Egi, 2006). At present, soy sauce and miso are graded into different quality classes by sensory evaluation using a panel of human assessors so the quality is described in subjective terms and thus difficult to define (Xu et al., 2013).

To classify the quality of condiments based on their compositions, Aishima and Nobuhara (1977) used multiple regression analysis to examine the aroma of soy sauce by looking for a good linear relationship between the patterns from gas chromatography (GC) and the results of sensory tests. They then quantified how the area of each peak from GC contributed to the whole aroma of the sauce. Feng et al. (2013) used principal component analysis to establish a model for evaluating the flavor quality of soy sauce. They found that the rankings of six types of soy sauce were consistent with the results of sensory evaluation. Recently, four types of Southeast Asian soy sauce (sweet, salty, light and dark) have been classified using the profile of their chemical components and Quantitative Descriptive Analysis (QDA) based on their sensory attributes (Syifaa et al., 2016). The relationship between the sensory attributes and the chemical profiles thus provided information for evaluating the quality of soy sauce. The findings from these studies suggest that an objective evaluation of quality should be used to link the contents of the components of the tested sample to sensory evaluation performed by human assessors. Generally, it is believed that an objective model would predict the quality of food products better than traditional sensory evaluation performed by assessors which are relatively subjective, costly and time-consuming (Xu et al., 2019). An objective model would help improve food quality and the economic benefits to producers. However, to the best of our knowledge, there is no objective evaluation system for determining the final quality of soy sauce and miso.

Regression analysis has previously been used to measure and model changes in the quality of various foods (Pan et al., 2016; Song et al., 2013). At present, selecting the relevant variables for predicting the quality of soy sauce and miso for use in regression analysis is very complicated because so many parameters measured in there have been shown to play an important role or have a significant effect on their qualities (Jeong et al., 2004; Kim & Lee, 2008). Within a group of predictive variables, those related to different characteristics of quality can be complex and interconnected. Therefore, the approach to selecting variables is a critical step influencing the feasibility of the regression model developed. The processing procedure of variables has generally been based on previous findings and chemometric methods, such as principal component regression, partial least squares regression (PLSR), and support vector machine. Recently, the theory of Compressed Sensing (CS) has been devised for better formulating the problem of identifying and quantifying the contributing components in regression equations whose unknowns are the weights of the corresponding quality parameters (Luo, Zhang, & Xiong, 2018).

The number of samples is often much smaller than the number of parameters so there are fewer equations than unknowns. Hence, the regression equation is often undetermined. There are many solutions for the equations, and it is difficult to find the real solution. According to previous studies (Wang et al., 2019a, 2018), the number of parameters making a significant contribution to quality is much smaller than the total number. Hence, most of the weightings of the parameters are zero, and only a few are large. This solution with the above characteristics (often referred to as a sparse solution in CS) is the real solution. The CS-based method can find the sparse solution by $L_1$-minimization. Thus, the high inferred data quality can be achieved by the CS-based method (Wang et al., 2016). The present study will use this novel paradigm for selecting the proper number of parameters as predictive variables.

In the present study, we will select predictive variables based on CS theory to develop an objective model to predict the quality of Japanese fermented soy sauce and miso samples using PLSR. The tested samples collected from 2016 to 2018 will be used for calibration and validation, respectively. Specifically, this study aims to explore the validity of the predictive model established by integrating a wide range of sensory factors (including $^{o}$Brix, color, and contents of moisture, salt, carbohydrates, organic acids, amino acids, and volatile compounds).

## 4.3 Materials and Methods

### 4.3.1 Materials and Reagents

#### 4.3.1.1 Soy Sauce Sample

A total of 110 soy sauce samples were collected directly from the second trial of Akita Prefectural Miso and Soy Sauce Competitions held in 2016, 2017, and 2018 (39, 37, and 34 samples, respectively). Overall, the soy sauce samples were produced by commercial companies and artisan workshops located in Akita Prefecture, Japan. The typical form of these samples are *koikuchi*-type soy sauce characterized by a strong aroma and a dark reddish-brown color. After collection, all the soy sauce samples were immediately stored at –25 °C before use.

#### 4.3.1.2 Miso sample

A total of 115 miso samples that entered into the second trial of the Akita Prefectural Miso and Soy Sauce Competition from 2016 to 2018 were collected simultaneously with soy sauce samples. The sample numbers of 2016, 2017, and 2018 were 37, 40, and 38, respectively. The type of these tested miso samples manufactured by different companies located in Akita area belong to rice miso. All the evaluated miso products were directly collected from the competition each year and were immediately stored in a refrigerator at –25 °C prior to use.

#### 4.3.1.3 Reagents

The chemicals and standards used in the present study for the chemical analyysis are of the highest commercial grade and were purchased from FUJIFILM Wako Pure Chemical Industries Corp. (Osaka, Japan), Kanto Chemical Co. Inc., (Tokyo, Japan), and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The custom alkanes standard mixture (C6 – C16) were purchased from Restek (Bellefonte, PA, USA).

### 4.3.2 Analytical Methods

#### 4.3.2.1 Physical and Chemical Analysis

The composition of the soy sauce and miso samples has been reported previously (Chapter 2, 2.3.2 Analytical Methods). In brief, the moisture and color were analyzed on the raw material using the freeze-drying method and a Minolta CM-700d/600d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan), respectively. The color information from the samples ($L$*, $a$* and $b$* values) was recorded using the Commission Internationale de l'Eclairage L* a *b* (CIELAB) color space. The pH of the raw samples was measured using a HI99161N pH-meter (Hanna Instruments, Padova, Italy). The diluted soy sauce and miso samples were prepared into an appropriate concentration using distilled water before determining the content of total soluble solids (°Brix), salt, carbohydrates, ethanol, organic acids, and amino acids. The °Brix and salt content were determined using a digital refractometer (model PR-101) and a salt meter (model ES-421) from the Atago Co. (Tokyo, Japan), respectively. The difference between the °Brix and salt content represents the soluble salt-free solids content (SSFSC). The contents of carbohydrates and ethanol were analyzed using a high-performance liquid chromatograph (HPLC) equipped with a Sugar KS-801 column (Shodex, Tokyo, Japan). The content of organic acids was measured using a Nexera UHPLC/HPLC System chromatograph (Shimadzu Co., Kyoto, Japan) equipped with an RSpak KC-811 column (Shodex). The contents of amino acids were measured using an L-8900 high-speed amino acid analyzer (Hitachi, Tokyo, Japan).

#### 4.3.2.2 Volatile compounds analysis

The analysis of the volatile compounds for soy sauce and miso were previously reported (Chapter 3, 3.3.2 Analytical Methods). Briefly, Samples of soy sauce or miso (0.25 g) were equilibrated at 80 °C for 30 min in an HS-20 headspace auto-sampler (Shimadzu Co.). The extracted gas phase (1 µL) was automatically withdrawn from the headspace then transferred for GC-MS analysis using a Shimadzu GCMS-QP2020 system (Shimadzu Co.) equipped with an SH-Rxi-5Sil MS capillary column (30 m length × 0.25 mm i.d. × 0.25 µm film thickness, Shimadzu Co.). The oven temperature was programmed as follows: 40 °C (5 min) then raised at 4 °C/min to 250 °C (3 min). The mass spectrometer was operated in full scan mode and mass spectra in the 33-350 m/z range were recorded.

The individual volatile compounds were identified by matching their mass spectra with the NIST 17 Mass Spectral Library and comparing the Kovats retention index (RI) of the peaks with those reported in the literature. The RI value of each peak was calculated using the $C_6$ to $C_{16}$ *n*-alkane series under the same chromatographic conditions.

For quantification, benzaldehyde, found in soy sauce, but with no obvious effect on determining the quality of Japanese fermented soy sauce (Wang et al., 2019b), was used as an internal standard for semi-quantification. With respect to miso case, oct-1-en-3-ol was used as an internal standard for semi-quantification. The respective quantified values of the identified volatile compounds were calculated as the ratio of the peak area of volatile compounds of soy sauce or miso to the peak area of internal standard detected at the same time.

#### 4.3.2.3 Sensory Scores

The sensory scores for the tested soy sauce and miso samples were evaluated by using the sensory evaluation in the Akita Prefectural Miso and Soy Sauce Competition from 2016 to 2018. Nine assessors, working in the related fermentation field, with a deep understanding of soy sauce products and expertise in sensory evaluation were selected. The procedure of the sensory evaluation consisted of two trials: the first trial selected qualifying soy sauce samples from all the samples submitted by different producers located in the Akita area to move on to the second trial. The results from the first trial had no influence in the second trial. During the first trial, a 5-point scoring scale (satisfactory 1 $\rightarrow$ disagreeable 5) was used to discriminate between the qualities of the soy sauce products. After summing the scores from the nine assessors, only a product with a total score less than 20 could advance to the second trial. For the second trial, the assessors judged the intensity of the appearance, taste, aroma, and acceptability of the soy sauce samples separately. The final score of each sample was on a 100-point scale (excellent 100 $\rightarrow$ inferior 0) therefore the highest total score possible for each sample was 900 points from the nine assessors, with a better quality indicated by a higher score.

#### 4.3.2.4 Determination of the Weights of the Predictive Variables

Regression analysis is a method of determining the relationship between sample data collected on several independent variables, to predict the value of a dependent variable, and analyzing the magnitude of the influence of each of these independent predictive variables. In the present study, in view of the reasonable simplicity and effectiveness of linear regression analysis, we use the following multiple linear regression analysis model to ascertain the weights of the predictive variables:

$$y_m = \sum_{n=1}^{N} \beta_n x_{m,n} + w_m, \ \text{for } m = 1 \dots M, \ n = 1 \dots N \tag{4-1}$$

where $y_m$ denotes the sensory scores of the *m*-th sample, $x_{m,n}$ denotes the measured value of the *n*-th predictive variable of the *m*-th sample, $\beta_n$ is the weight of the *n*-th predictive variable, $w_m$ is the measurement error and/or noise associated with the *m*-th sample, and *M* and *N* are the numbers of samples and predictive variables respectively. Since measurement error and noise are usually caused by random factors, it is reasonable to assume that $w_m, \ m = 1, \dots, M$ satisfy the normal distribution according to the central limit theorem (Berger & Casella, 2001). The goal of the regression analysis is to solve $\beta_n$ from $y_m$ and $x_{m,n}$. Since $y_m$ and $x_{m,n}$ are real number, there is always real-valued $\beta_n$ for equation (4-1) (Donoho, 2006). Then, equation (4-1) can be simply expressed in the following matrix form:

$$\boldsymbol{y} = \mathbf{A}\boldsymbol{s} + \boldsymbol{w} \tag{4-2}$$

where $\boldsymbol{y} = [y_1, \dots, y_M]^T \in \mathbb{R}^{M\times 1}$ denotes the sample vector,

$$\mathbf{A} = [\mathbf{A}_1 \quad \cdots \quad \mathbf{A}_N] = \begin{bmatrix} x_{1,1} & \cdots & x_{1,N} \\ \vdots & \ddots & \vdots \\ x_{M,1} & \cdots & x_{M,N} \end{bmatrix} \in \mathbb{R}^{M\times N} \tag{4-3}$$

denotes the measured matrix, $\boldsymbol{s} = [\beta_1, \dots, \beta_N]^T \in \mathbb{R}^{N\times 1}$ denotes the weight vector to be found, and $\boldsymbol{w} = [w_1, \dots, w_m]^T \in \mathbb{R}^{M\times 1}$ denotes the noise vector, $\mathbb{R}$ denotes the set of real numbers, and the operator $(\cdot)^T$ denotes the transpose.

In view of the facts that the number of parameters *N* is rather large, and the cost for taking samples for all these parameters is usually high, it is necessary in practice to restrict the sampling number M to a reasonable region. Therefore, we assume here that *M* is smaller than *N*. Thus, there are an infinite number of solutions for equation (4-2), and it is difficult to find the actual solution we wanted. Fortunately, only a few of the predictive variables made a significant contribution to the soy sauce quality (Wang et al., 2019a, 2018). This means that most elements in ***s*** are zeros, and only a few elements, say *K* elements, are non-zeros, where $K \ll N$. In the CS theory, such a vector is called a *K*-sparse vector.

The CS theory is thus an effective and robust method to find the values, positions and number of these non-zero elements.

A natural method to construct a sparse vector, $\boldsymbol{s}$, is to solve the following optimization problem using $L_0$-norm to constrain the number of non-zero elements:

$$\text{minimize } ||\boldsymbol{s}||_0, \text{ subject to } ||\mathbf{A}\boldsymbol{s} - \boldsymbol{y}|| \leq \epsilon \tag{4-4}$$

where $\epsilon > 0$ is the tolerable representation error; and $||\cdot||$ and $||\cdot||_0$ denote the $L_2$-norm and $L_0$-norm, respectively. The $L_2$-norm of $\boldsymbol{x}$ is defined as $||\boldsymbol{x}|| = \sqrt{\sum_i x_i^2}$. The $L_0$-norm of $\boldsymbol{x}$ means the total number of non-zero elements in $\boldsymbol{x}$, and is defined by

$$||\boldsymbol{x}||_0 = \sum_i \delta(x_i)$$

where

$$\delta(x_i) = \begin{cases} 1, & x_i \neq 0; \\ 0, & x_i = 0. \end{cases}$$

In other words, the sparsest vector satisfying $\boldsymbol{y} = \mathbf{A}\boldsymbol{s}$ is searched within the within the representation error, $\epsilon$. Unfortunately, $L_0$-minimization is non-deterministic polynomial-time hardness (NP-hard) in general (Rachlin & Baron, 2008). To avoid this difficulty, the CS-based method uses the convex function $L_1$-norm instead of $L_0$-norm. The $L_1$-norm of $\boldsymbol{x}$ is defined as $||\boldsymbol{x}||_1 = \sum_i |x_i|$. Thus, the optimization problem

$$\text{minimize } ||\boldsymbol{s}||_1 \text{ subject to } ||\mathbf{A}\boldsymbol{s} - \boldsymbol{y}|| \leq \epsilon \tag{4-5}$$

can be solved by efficient methods of convex optimization. Note that equation (4-2) has an infinite number of real-valued solutions, and a sparse solution can always be found by solving the optimization problem of (4-5) from these real-valued solutions (Donoho, 2006). For the purpose of illustration, we consider a 2D example ($\mathrm{y} = \begin{bmatrix} x_{1,1} & x_{1,2} \\ x_{2,1} & x_{2,2} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix}$). The solution is searched by an $L_1$-norm ball. Result is shown in Figure 4-1. The intersection takes place at a corner of the ball, leading to a sparse solution. In this study, the Matlab toolbox function, CVX, was used to solve equation (4-5) directly. The data processing of this part was performed using Matlab R2018a (The MathWorks Inc, Natick, MA, USA).

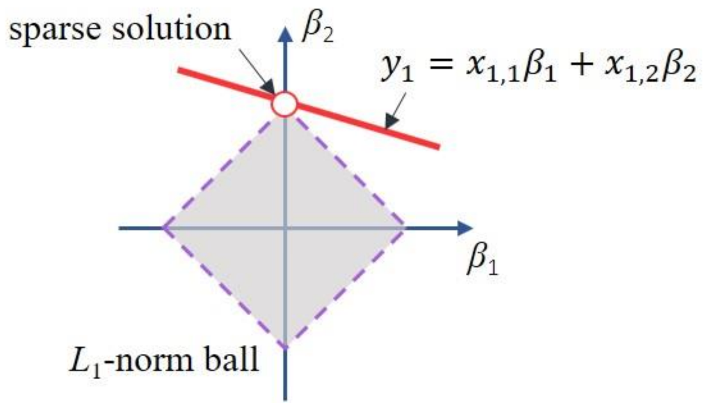


Figure 4-1 The intersection between L1-norm ball and the solutions of $\boldsymbol{y} = \mathbf{A}\boldsymbol{s}$ in the 2D plane.

Because the units of the indicators for data in the experiment are not uniform, the data are standardized before using the CS-based method. $\dot{\boldsymbol{y}}$ and $\dot{\mathbf{A}}_n$ denote the original sample data, and they are standardized, which will be used in the CS-based method, as follows:

$$\boldsymbol{y} = \frac{\dot{\boldsymbol{y}}}{\max(|\dot{\boldsymbol{y}}|)}, \text{and } \mathbf{A}_n = \frac{\dot{\mathbf{A}}_n}{\max(|\dot{\mathbf{A}}_n|)} \tag{4-6}$$

where $\max(\cdot)$ denotes the operator for finding the maximum value of a vector. Thus, each value of $\boldsymbol{y}$ and $\mathbf{A}_n$ is mapped into the range from -1 to 1, and the weighting of each predictive variable can be calculated fairly.

### 4.3.3 Statistical Analysis

The data for soy sauce or miso collected from the products over these three consecutive years were divided into calibration and validation sample sets, respectively. The significant data on the predictive variables and the sensory scores of the collected samples are shown in Table 4-1 and Table 4-2. The calibration and validation sample sets showed similar means and standard deviations for the predictive variables. The calibration set were used to establish the calibration model between the predictive variables and sensory scores, while the validation set were used to evaluate the calibration model results.

PLSR analysis was used to develop the model for predicting the quality of the tested soy sauce and miso products. The regression analysis was performed using Minitab 17 statistical software (Minitab Inc., State College, PA, USA). The optimum number of latent variables (PLS factors) with the smallest prediction error sum of squares was used in the calibration model. The validation set was then used to check the established calibration model. The coefficient of determination ($R^2$), the root mean square error of calibration (RMSEC), and the root mean square error of prediction (RMSEP) were calculated to evaluate the ability of the model to provide accurate predictions. These parameters are defined as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \tilde{y}_i)^2} \qquad (4\text{-}7)$$

$$RMSEC = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \qquad (4\text{-}8)$$

$$RMSEP = \sqrt{\frac{1}{m}\sum_{i=1}^{m}(y_i - \hat{y}_i)^2} \qquad (4\text{-}9)$$

where the $y_i$ is the actual value and the $\hat{y}_i$ is the predicted value for the *i*th sample; the $\tilde{y}_i$ stands for the mean of all the actual samples; *n* is the number of calibration samples; *m* is the number of validation samples. In general, the model with high accuracy should have a high value of $R^2$ and lower values of RMSEC and RMSEP.

## 4.4 Results and Discussion

### 4.4.1 Soy Sauce

#### 4.4.1.1 Predictive variables of Soy Sauce

To establish an accurate evaluation model for objectively assessing the quality of soy sauce products, as many predictive variables related to the final quality as possible should be collected. In the present study, a wide range of parameters of soy sauce was measured from both the calibration and validation samples: pH, contents of moisture, salt,

°Brix, SSFSC, and ethanol, three color parameters, and the contents of three carbohydrates, eight acids, 20 amino acids, and 34 volatile compounds, to give a total of 74 parameters. The details of these 74 parameters are shown in Table 4-1. For ease of observation, the 74 parameters were classified into two groups: the conventional physiochemical properties group; and the group of 34 volatile compounds commonly detected in all soy sauce samples. All 74 parameters were used as predictive variables potentially responsible for differences in the quality of soy sauce. They were further selected for developing the model to predict soy sauce quality by calculating the weighting of each predictive variable.

The sensory scores of the soy sauce samples were in a range of 770-856 points for the calibration set (soy sauces produced in 2016 and 2017) and 766-852 points for the validation set (soy sauces produced in 2018, as shown in Table 4-1). The sensory score was used as the dependent variable for both the calibration and validation sample sets.

Table 4-1 Mean, range, and SD of 74 parameters measured in soy sauce and corresponding sensory scores for the calibration and validation sample sets.

| No. | quality factors | calibration (n=76) | | | validation (n=34) | | |
|---|---|---|---|---|---|---|---|
| | | mean | range (min - max) | SD | mean | range (min - max) | SD |
| Physicochemical properties | | | | | | | |
| 1* | moisture (%) | 57.44 | 48.63 - 69.94 | 4.64 | 57.31 | 49.14 - 66.31 | 4.54 |
| 2* | pH | 4.61 | 4.40 - 4.89 | 0.12 | 4.70 | 4.46 - 4.98 | 0.13 |
| 3* | °Brix (%) | 47.65 | 35.46 - 55.72 | 4.70 | 48.43 | 39.04 - 56.37 | 4.98 |
| 4 | salt (%) | 12.79 | 10.71 - 15.68 | 1.12 | 12.71 | 11.00 - 14.76 | 1.10 |
| 5 | SSFSC (%) | 35.25 | 21.37 - 43.52 | 5.44 | 35.72 | 24.28 - 43.09 | 5.49 |
| 6* | *L** | 24.18 | 23.75 - 28.73 | 0.57 | 20.83 | 18.04 - 21.85 | 0.97 |
| 7 | *a** | 0.42 | 0.18 - 2.18 | 0.23 | 0.18 | 0.00 - 0.34 | 0.08 |
| 8 | *b** | 0.42 | -0.15 - 5.16 | 0.65 | 0.44 | -0.17 - 0.84 | 0.27 |
| 9 | isomaltose (g/kg) | 10.09 | 0.23 - 32.27 | 9.66 | 11.18 | 0.67 - 36.05 | 10.95 |
| 10 | glucose (g/kg) | 35.43 | 3.31 - 70.53 | 15.49 | 34.48 | 8.95 - 63.74 | 15.56 |
| 11 | fructose (g/kg) | 12.13 | 3.56 - 26.18 | 5.88 | 13.73 | 5.11 - 27.88 | 7.35 |
| 12* | ethanol (g/kg) | 20.77 | 6.50 - 36.12 | 7.29 | 27.64 | 12.84 - 43.47 | 8.38 |
| 13 | phosphoric acid (g/kg) | 7.15 | 3.58 - 12.06 | 1.82 | 9.04 | 5.68 - 13.03 | 2.22 |
| 14 | citric acid (g/kg) | 3.95 | 1.30 - 7.32 | 1.34 | 3.52 | 0.48 - 6.58 | 1.80 |
| 15* | malic acid (g/kg) | 1.16 | 0.23 - 2.36 | 0.49 | 0.87 | 0.31 - 1.82 | 0.42 |
| 16* | succinic acid (g/kg) | 0.52 | 0.09 - 2.27 | 0.42 | 0.41 | 0.16 - 0.93 | 0.18 |
| 17* | lactic acid (g/kg) | 7.17 | 0.21 - 31.39 | 6.82 | 8.92 | 0.07 - 33.67 | 10.64 |
| 18 | formic acid (g/kg) | 0.23 | 0.08 - 1.26 | 0.14 | 0.19 | 0.08 - 0.30 | 0.06 |
| 19* | acetic acid (g/kg) | 2.05 | 0.78 - 5.08 | 1.02 | 1.96 | 0.72 - 4.31 | 1.11 |
| 20* | pyroglutamic acid (g/kg) | 5.38 | 2.41 - 8.87 | 1.69 | 6.30 | 2.97 - 9.32 | 1.92 |
| 21* | taurine (g/kg) | 0.33 | 0.05 - 0.73 | 0.18 | 0.33 | 0.08 - 0.77 | 0.18 |
| 22* | aspartic acid (g/kg) | 6.50 | 0.77 - 9.87 | 2.06 | 5.71 | 0.22 - 9.32 | 2.92 |
| 23 | threonine (g/kg) | 3.05 | 2.36 - 4.11 | 0.38 | 2.96 | 2.14 - 3.87 | 0.43 |
| 24 | serine (g/kg) | 4.31 | 3.30 - 6.07 | 0.58 | 4.26 | 3.26 - 5.55 | 0.60 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | glutamic acid (g/kg) | 11.26 | 1.08 - 17.03 | 2.82 | 10.93 | 7.23 - 15.15 | 2.08 |
| 26 | α-aminoadipic acid (g/kg) | 0.17 | 0.00 - 0.36 | 0.08 | 0.12 | 0.00 - 0.22 | 0.07 |
| 27 | proline (g/kg) | 4.51 | 2.98 - 6.77 | 0.85 | 4.45 | 3.14 - 6.14 | 0.91 |
| 28 | glycine (g/kg) | 2.35 | 1.71 - 3.40 | 0.33 | 2.35 | 1.76 - 3.21 | 0.43 |
| 29* | alanine (g/kg) | 5.04 | 3.22 - 9.72 | 1.72 | 5.41 | 3.26 - 10.54 | 2.47 |
| 30* | valine (g/kg) | 4.28 | 3.33 - 5.65 | 0.51 | 4.37 | 3.30 - 5.53 | 0.55 |
| 31* | methionine (g/kg) | 0.71 | 0.45 - 1.22 | 0.12 | 0.87 | 0.54 - 1.72 | 0.25 |
| 32* | isoleucine (g/kg) | 3.53 | 2.80 - 4.08 | 0.33 | 3.40 | 2.50 - 4.68 | 0.47 |
| 33 | leucine (g/kg) | 5.16 | 3.90 - 6.25 | 0.45 | 4.94 | 3.51 - 6.99 | 0.72 |
| 34* | tyrosine (g/kg) | 0.60 | 0.00 - 1.72 | 0.36 | 0.60 | 0.00 - 1.96 | 0.54 |
| 35 | phenylalanine (g/kg) | 3.32 | 1.11 - 4.77 | 0.61 | 3.35 | 1.59 - 5.37 | 0.98 |
| 36* | β-alanine (g/kg) | 1.36 | 0.31 - 2.42 | 0.50 | 1.90 | 0.82 - 3.28 | 0.77 |
| 37 | γ-aminobutyric acid (g/kg) | 1.42 | 0.15 - 10.68 | 1.84 | 1.56 | 0.16 - 7.19 | 2.11 |
| 38 | lysine (g/kg) | 3.90 | 2.99 - 5.17 | 0.46 | 3.69 | 2.78 - 4.70 | 0.52 |
| 39* | histidine (g/kg) | 0.87 | 0.25 - 1.38 | 0.24 | 0.70 | 0.00 - 1.19 | 0.31 |
| 40 | arginine (g/kg) | 4.25 | 2.30 - 6.63 | 0.88 | 4.02 | 1.77 - 5.73 | 0.82 |
| Volatile compounds | | | | | | | |
| 41 | 2-methylpropanal | 18.45 | 9.02 - 30.52 | 5.26 | 13.46 | 7.57 - 20.65 | 3.37 |
| 42* | butane-2,3-dione | 0.88 | 0.43 - 1.59 | 0.21 | 0.79 | 0.47 - 2.56 | 0.46 |
| 43* | butan-2-one | 2.18 | 1.44 - 3.38 | 0.39 | 1.70 | 0.95 - 2.50 | 0.38 |
| 44 | ethyl acetate | 6.91 | 1.70 - 25.88 | 4.77 | 6.97 | 1.51 - 11.13 | 2.60 |
| 45 | 2-methylpropan-1-ol | 11.32 | 0.00 - 26.90 | 6.35 | 8.44 | 3.29 - 21.05 | 4.55 |
| 46 | acetic acid | 17.81 | 4.17 - 42.39 | 9.56 | 14.02 | 2.43 - 28.06 | 7.30 |
| 47* | 3-methylbutanal | 39.85 | 21.37 - 62.23 | 10.38 | 33.58 | 21.69 - 51.03 | 7.79 |
| 48* | 2-methylbutanal | 24.63 | 13.74 - 40.19 | 6.88 | 20.41 | 12.60 - 28.66 | 4.44 |
| 49 | 1-hydroxypropan-2-one | 0.36 | 0.12 - 0.65 | 0.12 | 0.33 | 0.16 - 0.63 | 0.13 |
| 50* | pentane-2,3-dione | 0.32 | 0.19 - 0.48 | 0.06 | 0.27 | 0.17 - 0.50 | 0.07 |
| 51 | 3-hydroxybutan-2-one | 0.64 | 0.09 - 1.96 | 0.44 | 0.14 | 0.02 - 0.33 | 0.09 |
| 52 | 3-methylbutan-1-ol | 6.67 | 0.34 - 21.70 | 4.93 | 4.99 | 0.15 - 21.73 | 4.63 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 53 | 2-methylbutan-1-ol | 4.63 | 0.44 - 15.68 | 3.70 | 3.25 | 0.43 - 8.99 | 2.01 |
| 54* | 1-methylsulfanylpropane | 0.04 | 0.00 - 0.13 | 0.02 | 0.03 | 0.01 - 0.07 | 0.02 |
| 55 | 3-methylbut-2-enal | 0.18 | 0.10 - 0.29 | 0.04 | 0.12 | 0.08 - 0.18 | 0.04 |
| 56 | ethyl 2-oxopropanoate | 0.20 | 0.04 - 0.35 | 0.07 | 0.23 | 0.09 - 0.57 | 0.10 |
| 57* | 2-methylpyrazine | 0.04 | 0.02 - 0.14 | 0.02 | 0.02 | 0.01 - 0.05 | 0.01 |
| 58 | furan-2-carbaldehyde | 0.62 | 0.17 - 2.03 | 0.46 | 0.59 | 0.18 - 1.88 | 0.52 |
| 59 | furan-2-ylmethanol | 0.67 | 0.17 - 2.05 | 0.35 | 0.38 | 0.15 - 0.67 | 0.12 |
| 60 | 3-methylsulfanylpropanal | 1.24 | 0.63 - 2.05 | 0.32 | 0.72 | 0.33 - 1.25 | 0.21 |
| 61* | 2,6-dimethylpyrazine | 0.11 | 0.02 - 0.22 | 0.05 | 0.09 | 0.02 - 0.17 | 0.04 |
| 62* | heptan-1-ol | 0.03 | 0.01 - 0.05 | 0.01 | 0.03 | 0.01 - 0.05 | 0.01 |
| 63 | 2-methyloctan-3-one | 0.02 | 0.01 - 0.05 | 0.01 | 0.02 | 0.00 - 0.05 | 0.01 |
| 64 | (methyltrisulfanyl)methane | 0.15 | 0.00 - 0.06 | 0.06 | 0.09 | 0.05 - 0.16 | 0.02 |
| 65 | 2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane | 0.01 | 0.07 - 0.34 | 0.01 | 0.03 | 0.01 - 0.04 | 0.01 |
| 66 | 2-phenylacetaldehyde | 2.01 | 0.00 - 0.03 | 0.72 | 2.00 | 0.42 - 3.43 | 0.84 |
| 67* | 1-(1H-pyrrol-2-yl)ethanone | 0.08 | 0.02 - 0.31 | 0.06 | 0.05 | 0.01 - 0.20 | 0.05 |
| 68 | methylsulfanylcyclohexane | 0.04 | 0.01 - 0.09 | 0.02 | 0.02 | 0.01 - 0.04 | 0.01 |
| 69 | 2-phenylethanol | 0.79 | 0.07 - 2.57 | 0.60 | 0.62 | 0.03 - 2.25 | 0.56 |
| 70 | 2-phenylprop-2-enal | 0.02 | 0.00 - 0.09 | 0.02 | 0.03 | 0.00 - 0.06 | 0.02 |
| 71 | (methyltetrasulfanyl)methane | 0.01 | 0.00 - 0.03 | 0.01 | 0.01 | 0.00 - 0.03 | 0.00 |
| 72* | 3-phenylfuran | 0.04 | 0.01 - 0.06 | 0.01 | 0.05 | 0.02 - 0.08 | 0.02 |
| 73 | (E)-2-phenylbut-2-enal | 0.10 | 0.04 - 0.17 | 0.03 | 0.08 | 0.04 - 0.13 | 0.02 |
| 74 | 4-ethenyl-2-methoxyphenol | 0.05 | 0.01 - 0.19 | 0.03 | 0.03 | 0.01 - 0.07 | 0.01 |
| Sensory evaluation | | | | | | | |
| | sensory points (maximum 900) | 815.97 | 770 - 856 | 21.87 | 816.74 | 766 - 852 | 24.28 |

"*" denotes variables selected using the CS-based method;

The respective quantified values of the identified volatile compounds were calculated as the ratio of the peak area of volatile compounds to the peak area of oct-1-en-3-ol detected at the same time

4.4.1.2 Selection of the Relevant Predictive Variables

The CS-based method was used to solve the sparse solution of equation (4-2). Once the non-zero elements in $\boldsymbol{s}$ is known, the important predictive variables could be selected from the 74 parameters. Because the measured noise $\boldsymbol{n}$ will affect the sensory score, the sparse solution was searched within the tolerated error $\epsilon$. However, a too-large $\epsilon$ will result in a solution too sparse while a too-small $\epsilon$ will result in overfitting. In the present study, $\epsilon$ was set as 0.05 to balance between the error and the sparsity of the solution (Figure 4-2). The weighting values of 44 predictive variables were close to zero because they did not contribute to the sensory score while the other variables were significantly larger than zero and thus contributed to the sensory score. This last group of 30 predictive variables (Figure 4-2) were: moisture, pH, $^{o}$Brix, $L$*, and the contents of ethanol, malic acid, succinic acid, lactic acid, acetic acid, pyroglutamic acid, taurine, aspartic acid, α-aminoadipic acid, valine, methionine, isoleucine, tyrosine, β-alanine, histidine, butane-2,3-dione, butane-2-one, 3-methylbutanal, 2-methylbutanal, petane-2,3-dione, 1-methylsulfanylpropane, 2-methylpyrazine, 2,6-dimethylpyrazine, heptan-1-ol, 1-(1H-pyrrol-2-yl)ethanone, and 3-phenylfuran.

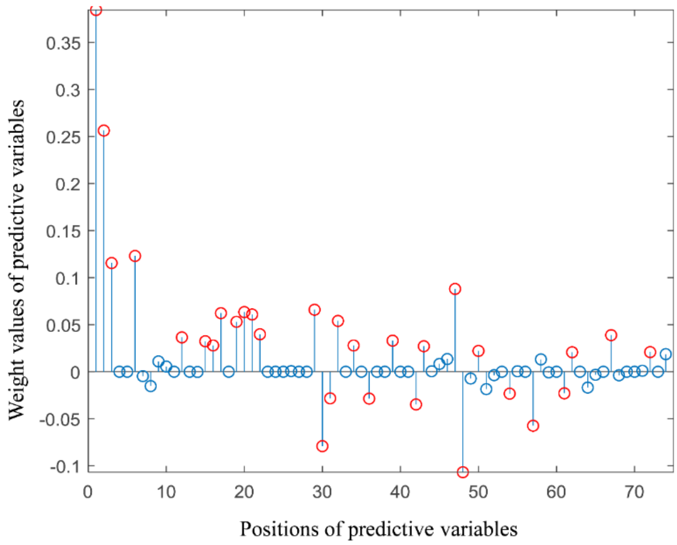


Figure 4-2 The results of the weight values for each predictive variable of soy sauce when $\epsilon$ was set as 0.05. These 30 selected predictive variables were differentiated in the figure by using red circles.

4.4.1.3 Development of the Model for Predicting Soy Sauce Quality

In the present study, PLSR was used to develop a model for predicting the sensory scores of commercial soy sauce products based on the predictive variables analyzed. To select the relevant latent variables, PLSR was applied to 76 calibration samples of soy sauce products collected in 2016 and 2017. From this, 30 predictive variables selected based on CS theory were specified as the X-matrix, and the sensory scores of the soy sauce products were specified as the Y-matrix. The calibration results showed that the correlation between the actual and predicted sensory scores of soy sauce products reached a maximum ($r$ = 0.93) with the number of latent variables 5 of the model. Subsequently, the model for predicting the sensory score of a soy sauce product was obtained using the following formula:

Soy sauce sensory score = 773.696 – 0.360[moisture] + 2.107[pH] + 0.706[ºBrix] – 1.285[$L$*] + 0.226[ethanol] – 0.715[malic acid] + 8.959[succinic acid] + 0.354[lactic acid] + 5.314[acetic acid] + 2.031[pyroglutamic acid] + 23.962[taurine] + 0.449[aspartic acid] + 1.903[alanine] – 0.771[valine] – 26.038[methionine] + 11.981[isoleucine] + 9.681[tyrosine] – 4.362[β-alanine] – 17.560[histidine] – 9.070[butane-2,3-dione] – 2.072[butan-2-one] + 0.283[3-methylbutanal] – 0.114[2-methylbutanal] – 50.035[pentane-2,3-dione] – 245.964[1-methylsulfanylpropane] – 175.454[2-methylpyrazine] – 21.176[2,6-dimethylpyrazine] + 27.875[heptan-1-ol] + 18.436[1-(1H-pyrrol-2-yl)ethanone] – 89.670[3-phenylfuran]

Figure 4-3 shows the predicted scores plotted against the actual sensory scores of soy sauce products. A strong linearity was observed ($R^2$ = 0.86), with a RMSEC value of 8.14. Therefore, the calibration model could predict the sensory score of soy sauce fairly well.

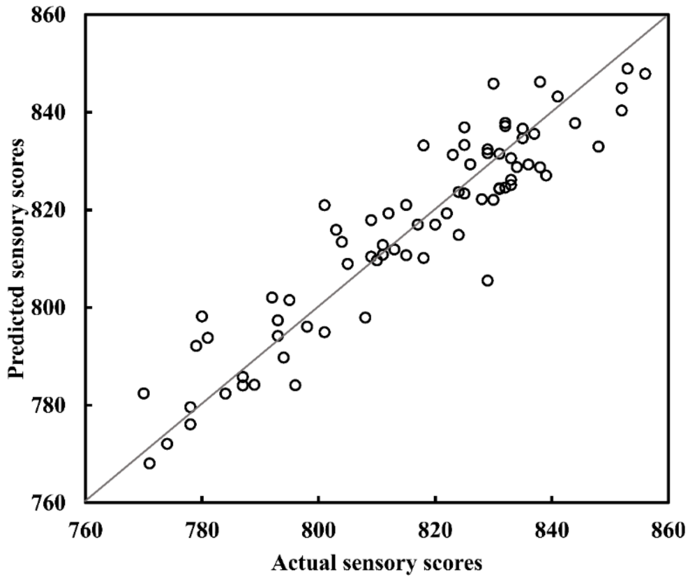


Figure 4-3 Scatter plot of the actual sensory scores versus the predicted sensory scores of soy sauce products based on the calibration sample set (*n* = 76).

The relationship shown in the obtained formula was consistent those previously studied (**Chapter 2 & 3**). Regarding the physicochemical properties: *L**, with its negative coefficient, was consistent with the deep reddish-brown color of soy sauce; the positive coefficients of °Brix, ethanol, and aspartic acid agreed with their positive effects on the sensory quality of soy sauce; the moisture content was inversely related to the °Brix content and, therefore, its coefficient was negative; four organic acids, succinic, lactic, acetic, and pyroglutamic, with their positive coefficients, have also been reported as desirable characteristics of soy sauce (Kim et al., 2013; Fu & Kim, 2011). Regarding the volatile compounds, the coefficients were positive for the predictive variables, 3-methylbutanal, pentane-2,3-dione, heptan-1-ol, and 1-(1H-pyrrol-2-yl)ethanone, which had a positive effect on the aroma of soy sauce; in contrast, the negative coefficients for butane-2,3-dione, butan-2-one, 1-methylsulfanylpropane, and 2-methylpyrazine for the aroma of soy sauce agreed with a previous study (Wang et al., 2019b).

#### 4.4.1.4 Model Evaluation

The validation samples (Table 4-1) were used to check the validity of the predictive model. When the predictive model was used to predict the sensory score of soy sauce manufactured in 2018 (*n* = 34), good validation results were obtained. Figure 4-4 shows the predicted and actual values for the sensory scores of the validation samples. The values of $R^2$ and RMSEP obtained for the validation samples were 0.80 and 11.47, respectively. The mean of the absolute differences between the actual and predicted values was 8.83. Consequently, these results showed that the predictive model developed was suitable for predicting the sensory quality of Japanese fermented soy sauce.

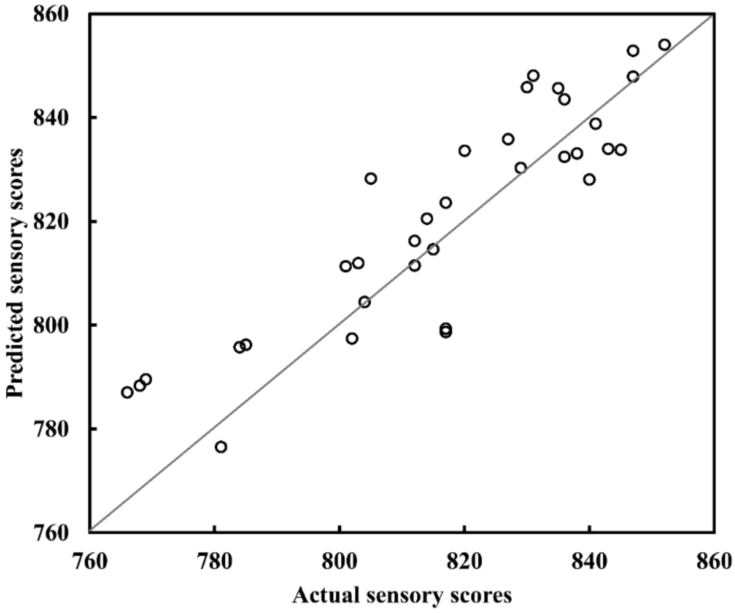


Figure 4-4 Scatter plot of the actual sensory scores versus the predicted sensory scores of soy sauce products using the established model on the validation sample set ($n = 34$).

Surprisingly, glucose, which is generally considered to be important for the quality of soy sauce based on experience, was not selected as a predictive variable. Therefore, the model was developed again with glucose as a new variable in addition to the 30 originally selected predictive variables. For this new predictive model, the optimized component number was 5 and the results are shown in Figure 4-5. The results were very good: for the calibration results (Figure 4-5 (a)), the values of $R^2$ at 0.86 and RMSEC at 8.05 were very similar to the previous calibration results; and for the validation results (Figure 4-5 (b)), the values of $R^2$ and RMSEP were 0.78 and 11.47, respectively, which were also very similar to the previous validation results. However, the mean of the absolute difference between the actual values and those predicted by the new predictive model at 9.32, was higher than the 8.83 from the previous model. Therefore, adding glucose as a predictive variable made the model more complex and did not improve its accuracy. This confirmed that the 30 predictive variables selected using the CS-based method could be considered to be optimal for developing the predictive model. The obtained predictive model provides a reasonable accuracy for determining the sensory score of soy sauce products.

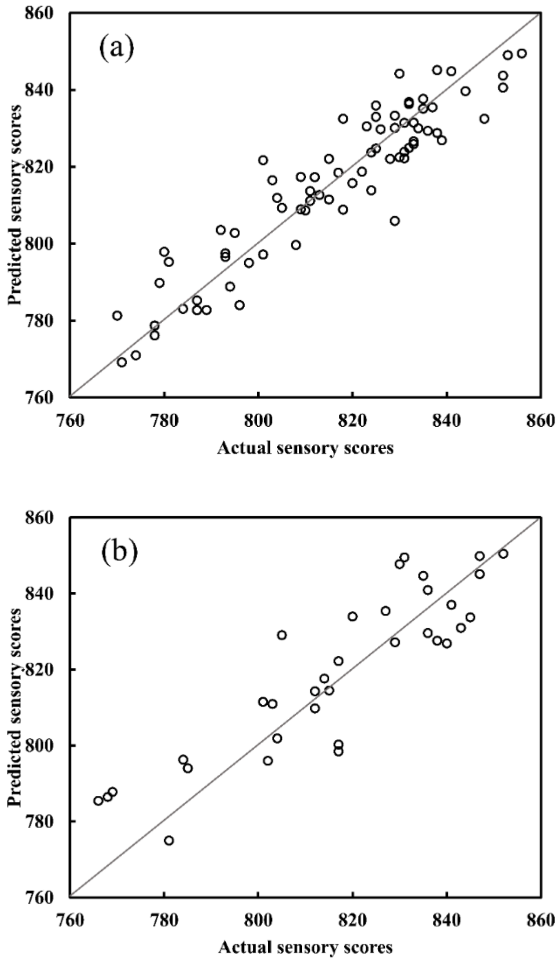


Figure 4-5 Scatter plots of actual versus predicted sensory scores based the 30 selected quality factors and glucose as predictive variables. (a) Plot from the calibration sample set ($n$ = 76); (b) Plot from the validation sample set ($n$ = 34).

### 4.4.2 Miso

#### 4.4.2.1 Predictive variables of Miso

Proper development of an analytical methodology for assessing the final quality of miso entails using samples representative of that to be analyzed and spanning an adequate range. On the basis of previous works (Chapter 2 & 3), a wide range of parameters of miso was measured from both the calibration and validation samples: pH, contents of moisture, salt, $^{o}$Brix, SSFSC, and ethanol, three color parameters, and the contents of three carbohydrates, eight acids, 20 amino acids, and 47 volatile compounds, to give a total of 87 parameters. The detail data for these 87 parameters was shown in Table 4-2. Like the procedure of soy sauce, the 87 parameters were classified into two groups: the conventional physiochemical properties group; and the group of 47 volatile compounds commonly detected in all miso samples. All 87 parameters were used as predictive variables potentially responsible for differences in the quality of miso products. They were further selected for developing the model to predict miso quality by calculating the weighting of each predictive variable.

The sensory score was used as the dependent variable for both the calibration and validation sample sets. The sensory scores of the miso samples were in a range of 746-859 points for the calibration set (miso products produced in 2016 and 2017) and 740-854 points for the validation set (miso products produced in 2018, Table 4-2).

Table 4-2 Mean, range, and SD of 87 parameters measured in miso and corresponding sensory scores for the calibration and validation sample sets.

| No. | quality factors | calibration (n=77) | | | validation (n=38) | | |
|---|---|---|---|---|---|---|---|
| | | mean | range (min - max) | SD | mean | range (min - max) | SD |
| Physicochemical properties | | | | | | | |
| 1 | moisture (%) | 41.01 | 35.51 – 48.09 | 2.03 | 40.29 | 36.08 – 45.61 | 2.21 |
| 2 | pH | 4.83 | 4.58 – 5.12 | 0.11 | 4.86 | 4.55 – 5.02 | 0.09 |
| 3 | ºBrix (%) | 48.63 | 42.03 – 52.77 | 2.06 | 49.05 | 44.01 – 54.01 | 2.27 |
| 4 | salt (%) | 12.10 | 10.14 – 13.24 | 0.52 | 11.73 | 6.90 – 13.06 | 1.03 |
| 5 | SSFSC (%) | 36.53 | 31.32 – 41.02 | 1.95 | 37.31 | 33.23 – 42.94 | 2.27 |
| 6* | $L^*$ | 40.48 | 34.48 – 57.40 | 4.32 | 40.99 | 37.74 – 47.79 | 2.39 |
| 7* | $a^*$ | 11.86 | 9.18 - 14.18 | 1.31 | 11.35 | 9.10 – 12.59 | 0.74 |
| 8* | $b^*$ | 19.60 | 14.64 – 31.60 | 2.94 | 18.44 | 14.51 – 23.32 | 2.19 |
| 9 | isomaltose (g/kg) | 25.70 | 17.15 – 40.70 | 5.10 | 25.87 | 14.82 – 37.28 | 5.91 |
| 10 | glucose (g/kg) | 131.54 | 82.20 – 176.38 | 14.82 | 133.97 | 84.21 – 165.69 | 16.82 |
| 11* | fructose (g/kg) | 11.19 | 7.59 – 19.05 | 2.02 | 10.97 | 9.17 – 14.41 | 1.33 |
| 12 | ethanol (g/kg) | 16.77 | 5.20 – 31.17 | 7.16 | 17.49 | 6.26 –31.20 | 5.27 |
| 13* | phosphoric acid (g/kg) | 4.65 | 3.74 – 5.63 | 0.42 | 5.13 | 3.67 – 5.89 | 0.53 |
| 14* | citric acid (g/kg) | 3.88 | 1.44 – 5.54 | 0.93 | 3.23 | 0.80 – 4.25 | 0.89 |
| 15 | malic acid (g/kg) | 0.67 | 0.31 – 1.12 | 0.21 | 0.55 | 0.40 – 0.70 | 0.06 |
| 16 | succinic acid (g/kg) | 0.18 | 0.03 – 0.50 | 0.09 | 0.19 | 0.04 - 0.39 | 0.09 |
| 17 | lactic acid (g/kg) | 0.33 | 0.02 – 3.28 | 0.64 | 0.60 | 0.03 – 9.71 | 1.89 |
| 18 | formic acid (g/kg) | 0.04 | 0.01 – 0.09 | 0.02 | 0.04 | 0.02 - 0.07 | 0.01 |
| 19 | acetic acid (g/kg) | 0.46 | 0.26 – 1.10 | 0.12 | 0.45 | 0.22 – 1.68 | 0.26 |
| 20* | pyroglutamic acid (g/kg) | 2.32 | 1.56 – 3.52 | 0.53 | 2.58 | 0.72 – 3.64 | 0.68 |
| 21 | aspartic acid (g/kg) | 4.00 | 2.83 – 5.82 | 0.77 | 3.91 | 1.84 – 5.53 | 0.78 |
| 22 | threonine (g/kg) | 1.17 | 0.77 – 1.66 | 0.21 | 1.09 | 0.53 – 1.62 | 0.24 |
| 23 | serine (g/kg) | 1.88 | 1.30 – 2.76 | 0.36 | 1.82 | 0.90 – 2.61 | 0.37 |
| 24* | glutamic acid (g/kg) | 6.15 | 3.78 – 9.24 | 1.21 | 5.89 | 2.83 – 8.43 | 1.29 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | glutamine | 1.14 | 0.37 – 30.34 | 3.38 | 1.30 | 0.00 – 1.70 | 0.44 |
| 26 | α-aminoadipic acid (g/kg) | 0.17 | 0.07 - 0.30 | 0.05 | 0.15 | 0.00 - 0.24 | 0.05 |
| 27* | proline (g/kg) | 1.60 | 1.08 – 2.15 | 0.24 | 1.67 | 0.70 – 2.20 | 0.32 |
| 28* | glycine (g/kg) | 0.88 | 0.61 – 1.33 | 0.17 | 0.79 | 0.35 - 1.21 | 0.17 |
| 29 | alanine (g/kg) | 1.83 | 1.40 – 2.41 | 0.25 | 1.78 | 0.91 – 2.38 | 0.28 |
| 30 | valine (g/kg) | 1.76 | 1.25 – 2.56 | 0.31 | 1.79 | 1.04 – 2.40 | 0.32 |
| 31 | methionine (g/kg) | 0.32 | 0.06 – 0.67 | 0.11 | 0.41 | 0.16 – 0.89 | 0.19 |
| 32 | isoleucine (g/kg) | 1.45 | 0.97 - 2.08 | 0.26 | 1.42 | 0.69 – 2.11 | 0.31 |
| 33* | leucine (g/kg) | 2.65 | 1.74 – 3.63 | 0.45 | 2.59 | 1.34 – 3.68 | 0.49 |
| 34 | tyrosine (g/kg) | 1.56 | 0.93 – 2.49 | 0.35 | 1.67 | 0.69 – 2.67 | 0.43 |
| 35 | phenylalanine (g/kg) | 2.04 | 1.26 – 3.01 | 0.39 | 2.21 | 1.10 – 3.35 | 0.46 |
| 36 | β-alanine (g/kg) | 1.47 | 0.66 - 2.35 | 0.35 | 1.44 | 0.77 – 2.65 | 0.33 |
| 37* | γ-aminobutyric acid (g/kg) | 0.51 | 0.34 – 0.81 | 0.10 | 0.53 | 0.00 – 0.81 | 0.16 |
| 38* | lysine (g/kg) | 2.09 | 1.51 – 2.96 | 0.37 | 2.87 | 1.12 – 2.87 | 0.38 |
| 39 | histidine (g/kg) | 0.33 | 0.21 – 0.56 | 0.09 | 0.27 | 0.11 – 0.46 | 0.09 |
| 40 | arginine (g/kg) | 2.66 | 1.87 – 3.94 | 0.54 | 2.55 | 1.37 – 3.70 | 0.54 |
| Volatile compounds | | | | | | | |
| 41 | 2-methylpropanal | 167.62 | 34.58 – 592.40 | 126.58 | 214.01 | 24.74 – 775.49 | 169.92 |
| 42* | butane-2,3-dione | 6.28 | 1.13 – 20.57 | 4.28 | 7.63 | 0.96 – 37.62 | 6.65 |
| 43 | butan-2-one | 9.21 | 0.97 – 28.41 | 6.60 | 9.84 | 0.78 – 30.60 | 7.03 |
| 44* | ethyl acetate | 1649.64 | 183.68 – 6195.12 | 1445.07 | 1714.59 | 185.77 – 6996.88 | 1413.27 |
| 45* | 2-methylpropan-1-ol | 506.25 | 131.07 – 1778.36 | 356.23 | 526.80 | 67.53 – 1361.05 | 349.83 |
| 46 | acetic acid | 184.55 | 45.89 – 633.93 | 116.59 | 234.61 | 22.60 – 1683.68 | 274.01 |
| 47 | 3-methylbutanal | 288.72 | 53.00 – 1131.94 | 228.31 | 361.19 | 41.12 – 1334.77 | 292.96 |
| 48 | 2-methylbutanal | 189.89 | 38.79 – 581.93 | 118.70 | 222.73 | 29.02 – 685.26 | 149.59 |
| 49 | pentanal | 4.08 | 0.55 – 18.86 | 4.54 | 4.53 | 0.23 – 14.99 | 4.10 |
| 50* | ethyl propanoate | 9.66 | 1.22 – 37.92 | 7.54 | 10.13 | 1.76 – 39.29 | 7.68 |
| 51* | 1,1-diethoxyethane | 4.17 | 0.16 – 15.37 | 3.16 | 3.56 | 0.47 – 8.33 | 2.35 |
| 52* | 3-methylbutan-1-ol | 333.28 | 46.33 – 1369.20 | 285.52 | 322.07 | 50.50 – 1017.09 | 233.87 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 53 | 2-methylbutan-1-ol | 124.88 | 23.38 – 455.67 | 92.46 | 127.73 | 16.74 – 413.33 | 92.10 |
| 54 | 2-methylpropanoic acid | 4.32 | 0.37 - 17.21 | 3.65 | 6.81 | 0.50 – 43.53 | 7.11 |
| 55 | ethyl 2-methylpropanoate | 19.92 | 3.48 – 107.09 | 17.89 | 29.78 | 3.73 – 223.25 | 36.52 |
| 56 | 2-methylpropyl acetate | 4.86 | 0.60 – 22.53 | 4.70 | 4.67 | 0.28 – 26.02 | 4.84 |
| 57 | 3-methylbut-2-enal | 3.11 | 0.36 – 11.21 | 4.70 | 3.32 | 0.19 – 10.84 | 2.50 |
| 58 | ethyl 2-hydroxypropanoate | 44.71 | 3.63 – 220.39 | 45.12 | 49.23 | 8.35 – 140.68 | 33.48 |
| 59* | butane-2,3-diol | 109.34 | 0.00 – 738.40 | 137.09 | 96.69 | 0.00 – 384.44 | 81.31 |
| 60 | ethyl 2-oxopropanoate | 3.74 | 0.39 – 14.00 | 2.61 | 3.86 | 0.54 – 9.30 | 1.88 |
| 61 | ethyl (2S)-2-hydroxypropanoate | 17.58 | 0.00 – 433.80 | 56.58 | 54.20 | 0.00 – 1456.63 | 245.40 |
| 62 | butyl acetate | 0.71 | 0.00 – 4.03 | 1.17 | 0.89 | 0.00 – 3.76 | 1.02 |
| 63* | furan-2-carbaldehyde | 15.94 | 2.57 – 46.21 | 11.11 | 18.84 | 1.18 – 62.53 | 15.56 |
| 64 | 3-methylbutanoic acid | 2.08 | 0.00 – 7.86 | 2.39 | 2.67 | 0.00 – 10.48 | 2.98 |
| 65* | ethyl 2-methylbutanoate | 2.63 | 0.00 – 15.06 | 2.74 | 6.45 | 0.51 – 34.58 | 6.54 |
| 66* | ethyl 3-methylbutanoate | 1.59 | 0.11 – 6.69 | 1.30 | 1.87 | 0.06 – 5.30 | 1.51 |
| 67* | ethylbenzene | 0.11 | 0.00 – 0.67 | 0.12 | 0.09 | 0.00 – 0.47 | 0.10 |
| 68* | hexan-1-ol | 0.83 | 0.11 – 4.52 | 0.60 | 0.87 | 0.09 – 4.75 | 0.74 |
| 69* | 3-methylbutyl acetate | 5.21 | 0.64 – 31.02 | 5.67 | 5.03 | 0.25 – 29.62 | 5.56 |
| 70 | 2-methylbutyl acetate | 0.99 | 0.13 – 4.49 | 0.91 | 0.88 | 0.00 – 5.29 | 0.96 |
| 71 | 3-methylsulfanylpropanal | 11.50 | 2.14 – 37.45 | 7.82 | 14.07 | 1.22 – 45.26 | 9.76 |
| 72 | oxolan-2-one | 0.94 | 0.16 – 2.88 | 0.63 | 0.94 | 0.00 – 2.03 | 0.60 |
| 73 | benzaldehyde | 8.20 | 1.24 – 27.60 | 6.17 | 7.69 | 0.79 – 25.73 | 5.58 |
| 74 | oct-1-en-3-one | 0.21 | 0.00 – 0.81 | 0.20 | 0.04 | 0.00 – 0.23 | 0.07 |
| 75 | 3-methylsulfanylpropan-1-ol | 1.21 | 0.18 – 3.44 | 0.79 | 1.17 | 0.00 – 4.26 | 0.98 |
| 76* | octan-3-one | 0.09 | 0.00 – 0.66 | 0.16 | 0.09 | 0.00 – 0.34 | 0.12 |
| 77 | 2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane | 0.96 | 0.22 – 2.85 | 0.61 | 0.94 | 0.09 – 2.97 | 0.65 |
| 78* | ethyl hexanoate | 2.18 | 0.23 – 5.61 | 1.35 | 1.98 | 0.19 – 3.82 | 1.03 |
| 79 | 2-phenylacetaldehyde | 59.14 | 10.74 – 189.27 | 42.47 | 61.05 | 4.57 – 208.88 | 45.30 |
| 80* | 2-phenylethanol | 18.93 | 1.39 – 93.46 | 21.26 | 14.77 | 0.73 – 53.17 | 12.62 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 81* | 2,2,4,4,6,6,8,8,10,10-decamethyl-1,3,5,7,9,2,4,6,8,10-pentaoxapentasilecane | 1.75 | 0.43 – 6.17 | 1.25 | 1.81 | 0.14 – 5.76 | 1.22 |
| 82 | ethyl benzoate | 8.73 | 0.25 – 41.77 | 8.54 | 7.77 | 0.27 – 31.42 | 8.04 |
| 83 | diethyl butanedioate | 0.98 | 0.00 – 7.37 | 1.25 | 0.71 | 0.00 – 2.89 | 0.64 |
| 84* | ethyl octanoate | 1.02 | 0.09 – 8.47 | 1.27 | 0.95 | 0.07 – 2.91 | 0.68 |
| 85* | ethyl 2-phenylacetate | 1.57 | 0.19 – 6.40 | 1.12 | 1.55 | 0.20 – 3.99 | 1.14 |
| 86 | 2-phenylethyl acetate | 1.16 | 0.00 – 9.67 | 1.61 | 0.90 | 0.00 – 3.64 | 0.90 |
| 87 | (E)-2-phenylbut-2-enal | 0.56 | 0.12 – 1.91 | 0.39 | 0.58 | 0.05 – 2.51 | 0.52 |
| Sensory evaluation | | | | | | | |
| | sensory points (maximum 900) | 816.53 | 746-859 | 24.80 | 801.84 | 740-854 | 34.94 |

"*" denotes variables selected using the CS-based method;

The respective quantified values of the identified volatile compounds were calculated as the ratio of the peak area of volatile compounds to the peak area of oct-1-en-3-ol detected at the same time

4.4.2.2 Selection of the Relevant Predictive Variables

The CS-based method was used to calculate the weighting values of these 87 measured parameters for the selection of the important predictive variables. The results showed that the weighting values 32 predictive variables were significantly larger than zero when the tolerated error $\epsilon$ were set as 2.00 (Figure 4-6). Therefore, these 32 predictive were considered to be important to contribute to the sensory score, namely, $L^*$, $a^*$, $b^*$, fructose, phosphoric acid, critic acid, pyroglutamic acid, glutamic acid, proline, glycine, leucine, γ-aminobutyric acid, lysine, butane-2,3-dione, ethyl acetate, 2-methylpropan-1-ol, ethyl propanoate, 1,1-diethoxyethane, 3-methylbutan-1-ol, butane-2,3-diol, furan-2-carbaldehyde, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethylbenzene, hexan-1-ol, 3-methylbutyl acetate, octan-3-one, ethyl hexanoate, 2-phenylethanol, 2,2,4,4,6,6,8,8,10,10-decamethyl-1,3,5,7,9,2,4,6,8,10-pentaoxapentasilecane, ethyl octanoate, and ethyl 2-phenylacetate. The remaining 53 predictive variables with a weighting value close to zero were not selected to establish the predictive model of miso quality.

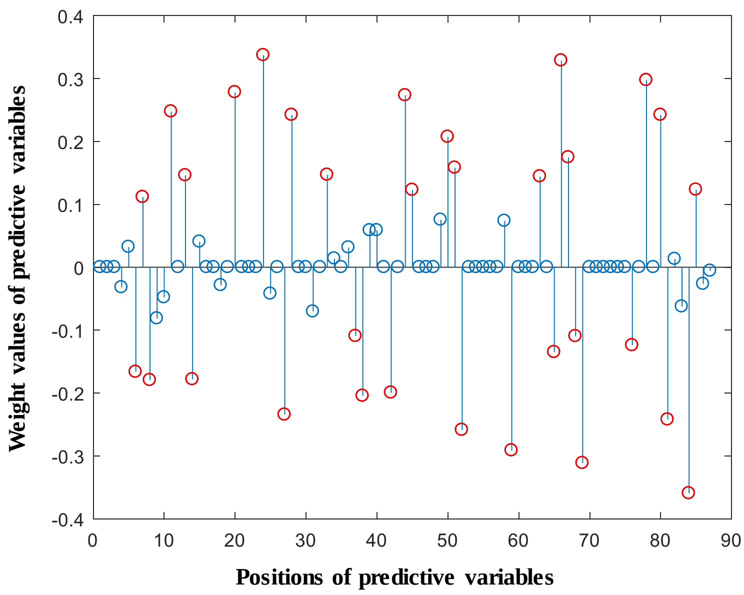


Figure 4-6 The results of the weight values for each predictive variable of miso when $\epsilon$ was set as 2.00. These 32 selected predictive variables were differentiated in the figure by using red circles.

4.4.2.3 Development of the Model for Predicting Miso Quality

Next, the miso products from 2016 to 2018 were split into the calibration and validation sets to construct and evaluate the model. The miso products produced in 2016 and 2017 (sample number $n$ = 77) were selected into calibration sample set. The 32 selected predictive variables based on CS theory were specified as X-matrix, and the sensory scores for samples from 2016 and 2017 were specified as Y-matrix. The calibration models were constructed by PLSR on the calibration sample set. The calibration results showed that the model present a maximum $R^2$ value of 0.78 and a minimum RMSEC value of 11.67 when the number of latent variables reached 9 (Figure 4-7). This obtained calibration model provided a good accuracy considerably for assessing the sensory score of miso products. Subsequently, this model was obtained using the following formula:

Miso sensory score = 746.379 – 4.058[$L$*] + 9.608[$a$*] + 0.501[$b$*] + 1.549[fructose] + 0.036[phosphoric acid] + 4.375[critic acid] – 3.251[pyroglutamic acid] + 9.994[glutamic acid] + 9.365[proline] + 34.933[glycine] + 1.714[leucine] – 16.226[γ-aminobutyric acid] – 21.24[lysine] – 0.089[butane-2,3-dione] – 0.001[ethyl acetate] + 0.009[2-methylpropan-1-ol] +0.329[ethyl propionate] + 0.826[1,1-diethoxyethane] + 0.014[3-methylbutan-1-ol] – 0.002[butane-2,3-diol] + 0.361[furan-2-carbaldehyde] + 0.803 [ethyl 2-methylbutanoate] + 2.742[ethyl 3-methylbutanoate] – 0.408[ethylbenzene] – 2.852[hexan-1-ol] – 1.176[3-methylbutyl acetate] – 12.050[octan-3-one] + 8.393[ethyl hexanoate] + 0.006[2-phenylethanol] – 3.885[2,2,4,4,6,6,8,8,10,10-decamethyl-1,3,5,7,9,2,4,6,8,10-pentaoxapentasilecane] + 0.614[ethyl octanoate] – 0.377[ethyl 2-phenylacetate]

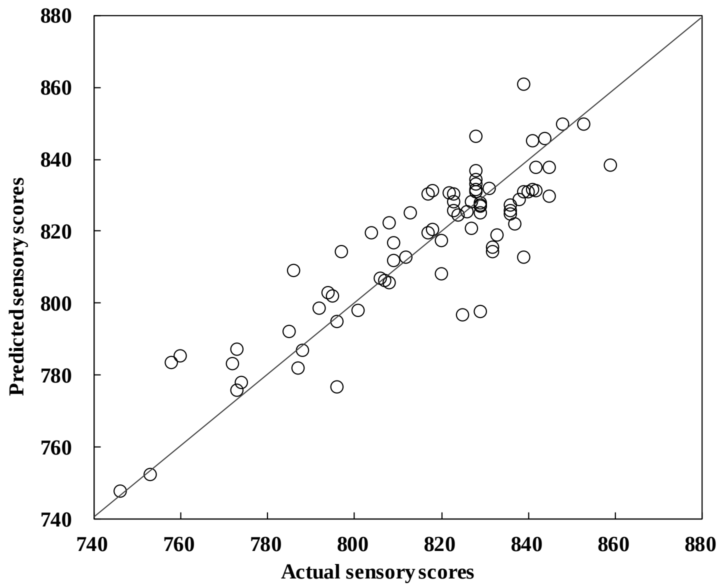


Figure 4-7 Scatter plot of the actual sensory scores versus the predicted sensory scores of miso based on the calibration sample set ($n$ = 77).

For the obtained formula, the parameters $L$*, $a$*, and $b$*, denoted the contribution of color to the quality of miso, the negative coefficient of $L$* and positive coefficient of $a$* indicated the different effects of these two parameters on the miso quality that had been previously reported (**Chapter 2**); The negative coefficients of fructose agreed with the content of sugars negatively correlated to the miso quality; pshosphoric acid, critic acid, and pyroglutamic aicd, were dominant acids in miso, they were also selected into the formula ; glutamic acid was closely related to the umami taste of miso, and also proline, glycine, and leucine, were desired for the quality improvement of miso. Regarding to the volatile compounds, the coefficients were positive for most of the detected esters, except for 3-methylbutyl acetate and ethyl 2-phenylacetate, indicating the key contribution to the quality of miso.

#### 4.4.2.4 Model Evaluation

The obtained predictive model was validated in order to determine whether it would allow the accurate quantitation of the sensory score of miso products. The miso products produced in 2018 ($n$ = 38, Table 4-2) were used to compare the sensory scores provided by the model with the actual sensory scores. The results showed that the constructed model provided an accurate prediction for the sensory score of miso productions (Figure 4-8). The values of $R^2$ and RMSEP for the obtained model with 2018 miso products were 0.64 and 21.14, respectively. Moreover, it is observed that three samples with a lower sensory score located on the left and upper position in Figure 4-8, indicating the predictive model was not very sufficient, may be related to the lack of predictive variables of color. Therefore, further work entails using more samples representative to improve the accuracy of the model.

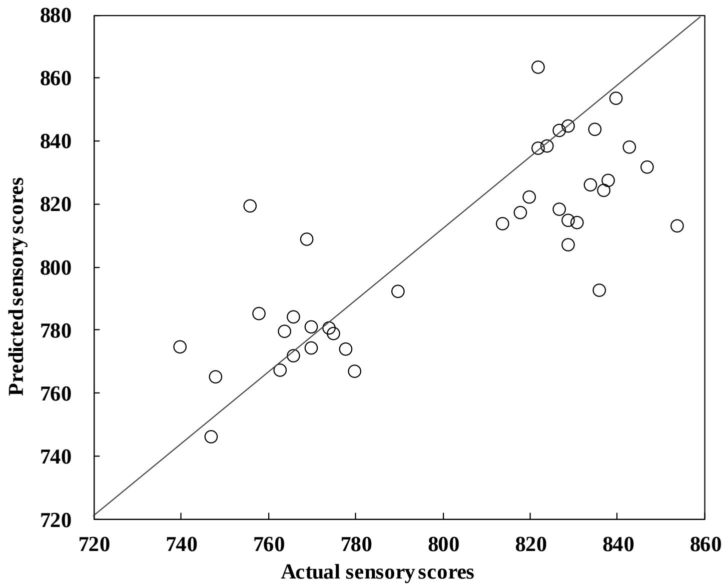


Figure 4-8 Scatter plot of the actual sensory scores versus the predicted sensory scores of miso using the established model on the validation sample set ($n$ = 38).

## 4.5 Conclusions

In summary, based on our studies of Japanese fermented condiments (i.e. soy sauce and miso) produced in the Akita prefecture, the models for predicting the sensory score of soy sauce and miso have been developed. Initially, a wide range of parameters related to the quality of soy sauce and miso was measured. These parameters were regarded as potential variables in a model for predicting the sensory score. The CS-based method was used to select the most contribution variables to the sensory score and then the predictive models for the quality of soy sauce and miso were constructed using the selected variables, respectively. The evaluation of the predictive models, developed using PLSR, showed that it could provide a suitable accuracy for determining the sensory quality of soy sauce and miso products produced in Akita area, respectively.

## 4.6 References


Aishima, T., & Nobuhara, A. (1977). Flavour evaluation by multiple regression analysis of gas chromatographic patterns in soy sauce. *Food Chemistry*, *2*, 161–175.

Baccouri, B., Temime, S. B., Campeol, E., Cioni, P. L., Daoud, D., & Zarrouk, M. (2007). Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. *Food Chemistry*, *102*, 850–856.

Bader, A., Flamini, G., Cioni, P. L., & Morelli, I. (2003). Essential oil composition of Achillea santolina L. and Achillea biebersteinii Afan. Collected in Jordan. *Flavour and Fragrance Journal*, *18*, 36–38.

Beaulieu, J. C., & Grimm, C. C. (2001). Identification of Volatile Compounds in Cantaloupe at Various Developmental Stages Using Solid Phase Microextraction. *Journal of Agricultural and Food Chemistry*, *49*, 1345–1352.

Berger, R. L., & Casella, G. (2001). *Statistical inference*. Duxbury.

Bylaite, E., & Meyer, A. S. (2005). Characterisation of volatile aroma compounds of orange juices by three dynamic and static headspace gas chromatography techniques. *European Food Research and Technology*, *222*, 176.

Chavasit, V., & Photi, J. (2018). Chapter 15—Condiments and Sauces. In M. G. V. Mannar & R. F. Hurrell (Eds.), *Food Fortification in a Globalized World* (pp. 153–158). Academic Press.

Cho, I. H., Namgung, H. J., Choi, H. K., & Kim, Y. S. (2008). Volatiles and key odorants in the pileus and stipe of pine-mushroom (Tricholoma matsutake Sing.). *Food Chemistry*, *106*, 71–76.

Cho, J. H., Zhao, H. L., Kim, J. S., Kim, S. H., & Chung, C. H. (2018). Characteristics of fermented seasoning sauces using Tenebrio molitor larvae. *Innovative Food Science & Emerging Technologies*, *45*, 186–195.

Dallüge, J., van Stee, L. L. P., Xu, X., Williams, J., Beens, J., Vreuls, R. J. J., & Brinkman, U. A. T. (2002). Unravelling the composition of very complex samples by comprehensive gas chromatography coupled to time-of-flight mass spectrometry: Cigarette smoke. *Journal of Chromatography A*, *974*, 169–184.

Donoho, D. L. (2006). Compressed sensing. *IEEE Transactions on Information Theory*, *52*, 1289–1306.

Fan, W., & Qian, M. C. (2006). Characterization of Aroma Compounds of Chinese "Wuliangye" and "Jiannanchun" Liquors by Aroma Extract Dilution Analysis. *Journal of Agricultural and Food Chemistry*, *54*, 2695–2704.

Fang, Y., & Qian, M. (2005). Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). *Flavour and Fragrance Journal, 20*, 22–29.

Feng, Y., Cai, Y., Su, G., Zhao, H., Wang, C., & Zhao, M. (2014). Evaluation of aroma differences between high-salt liquid-state fermentation and low-salt solid-state fermentation soy sauces from China. *Food Chemistry, 145*, 126–134.

Feng, Y., Cui, C., Zhao, H., Gao, X., Zhao, M., & Sun, W. (2013). Effect of koji fermentation on generation of volatile compounds in soy sauce production. *International Journal of Food Science & Technology, 48*, 609–619.

Feng, Y., Su, G., Sun-Waterhouse, D., Cai, Y., Zhao, H., Cui, C., & Zhao, M. (2017). Optimization of Headspace Solid-Phase Micro-extraction (HS-SPME) for Analyzing Soy Sauce Aroma Compounds via Coupling with Direct GC-Olfactometry (D-GC-O) and Gas Chromatography-Mass Spectrometry (GC-MS). *Food Analytical Methods, 10*, 713–726.

Feng, Y., Su, G., Zhao, H., Cai, Y., Cui, C., Sun-Waterhouse, D., & Zhao, M. (2015). Characterisation of aroma profiles of commercial soy sauce by odour activity value and omission test. *Food Chemistry, 167*, 220–228.

Fu, X. T., & Kim, S. M. (2011). Salt-Tolerant Acid Proteases: Purification, Identification, Enzyme Characteristics, and Applications for Soybean Paste and Sauce Industry. In D. Krezhova (Ed.), *Soybean—Genetics and Novel Techniques for Yield Enhancement* (pp. 311–326). Rijeka: IntechOpen.

Jeong, S.-Y., Chung, S.-J., Suh, D.-S., Suh, B.-C., & Kim, K. O. (2004). Developing a Descriptive Analysis Procedure for Evaluating the Sensory Characteristics of Soy Sauce. *Journal of Food Science, 69*, S319–S325.

Kim, H.-W., Choi, Y.-S., Choi, J.-H., Kim, H.-Y., Lee, M.-A., Hwang, K.-E., & Kim, C.-J. (2013). Tenderization effect of soy sauce on beef M. biceps femoris. *Food Chemistry, 139*, 597–603.

Kim, J.-S., & Lee, Y.-S. (2008). A study of chemical characteristics of soy sauce and mixed soy sauce: Chemical characteristics of soy sauce. *European Food Research and Technology, 227*, 933–944.

Kobayashi, A., & Sugawara, E. (1999). Flavor Components of Shoyu and Miso Japanese Fermented Soybean Seasonings. In F. Shahidi & C.-T. Ho (Eds.), *Flavor Chemistry of Ethnic Foods* (pp. 5–14). Boston, MA: Springer US.

Kumazawa, K., Kaneko, S., & Nishimura, O. (2013). Identification and Characterization of Volatile Components Causing the Characteristic Flavor in Miso (Japanese Fermented Soybean Paste) and Heat-Processed Miso Products. *Journal of Agricultural and Food Chemistry*, *61*, 11968–11973.

Lucero, M. E., Estell, R. E., & Fredrickson, E. L. (2003). The Essential Oil Composition of Psorothamnus scoparius (A. Gray) Rydb. *Journal of Essential Oil Research*, *15*, 108–111.

Luo, R., Zhang, Z., & Xiong, W. (2018). Research on Compressed Sensing Data Acquisition Method in Cold Chain Logistics. *Proceedings of the 8th International Conference on Management and Computer Science (ICMCS 2018)*. Presented at the 8th International Conference on Management and Computer Science (ICMCS 2018), Shenyang, China.

Methven, L., Tsoukka, M., Oruna-Concha, M. J., Parker, J. K., & Mottram, D. S. (2007). Influence of Sulfur Amino Acids on the Volatile and Nonvolatile Components of Cooked Salmon (Salmo salar). *Journal of Agricultural and Food Chemistry*, *55*, 1427–1436.

Morinaga, M., Hara, K., Kageura, M., Hieda, Y., Takamoto, M., & Kashimura, S. (1990). A simple, rapid and simultaneous analysis of complex volatile hydrocarbon mixtures in blood using gas chromatography/mass spectrometry with a wide-bore capillary column. *Zeitschrift Für Rechtsmedizin*, *103*, 567–572.

Müller, R., & Rappert, S. (2010). Pyrazines: Occurrence, formation and biodegradation. *Applied Microbiology and Biotechnology*, *85*, 1315–1320.

Ogasawara, M., Yamada, Y., & Egi, M. (2006). Taste enhancer from the long-term ripening of miso (soybean paste). *Food Chemistry*, *99*, 736–741.

Pan, T. T., Sun, D. W., Cheng, J. H., & Pu, H. (2016). Regression Algorithms in Hyperspectral Data Analysis for Meat Quality Detection and Evaluation. *Comprehensive Reviews in Food Science and Food Safety*, *15*, 529–541.

Pino, J. A., Almora, K., & Marbot, R. (2003). Volatile components of papaya (Carica papaya L., Maradol variety) fruit. *Flavour and Fragrance Journal*, *18*, 492–496.

Rachlin, Y., & Baron, D. (2008). The secrecy of compressed sensing measurements. *2008 46th Annual Allerton Conference on Communication, Control, and Computing*, 813–817. Monticello, IL, USA: IEEE.

Rout, P. K., Rao, Y. R., Sree, A., & Naik, S. N. (2007). Composition of essential oil, concrete, absolute, wax and headspace volatiles of Murrarya paniculata (Linn.) Jack flowers. *Flavour and Fragrance Journal*, *22*, 352–357.

Song, S., Yuan, L., Zhang, X., Hayat, K., Chen, H., Liu, F., & Niu, Y. (2013). Rapid measuring and modelling flavour quality changes of oxidised chicken fat by electronic nose profiles through the partial least squares regression analysis. *Food Chemistry*, *141*, 4278–4288.

Steinhaus, P., & Schieberle, P. (2007). Characterization of the Key Aroma Compounds in Soy Sauce Using Approaches of Molecular Sensory Science. *Journal of Agricultural and Food Chemistry*, *55*, 6262–6269.

Sugawara E. (1991). Change in Aroma Components of Miso with Aging. *NIPPON SHOKUHIN KOGYO GAKKAISHI*, *38*, 1093–1097.

Sugawara, E., Saiga, S., & Kobayashi, A. (1992). Relationships between Aroma Components and Sensory Evaluation of Miso. *Nippon Shokuhin Kogyo Gakkaishi*, *39*, 1098–1104.

Sun, S. Y., Jiang, W. G., & Zhao, Y. P. (2010). Profile of Volatile Compounds in 12 Chinese Soy Sauces Produced by a High-Salt-Diluted State Fermentation. *Journal of the Institute of Brewing*, *116*, 316–328.

Syifaa, A. S., Jinap, S., Sanny, M., & Khatib, A. (2016). Chemical Profiling of Different Types of Soy Sauce and the Relationship with its Sensory Attributes: CHEMICAL PROFILING AND SENSORY OF SOY SAUCES. *Journal of Food Quality*, *39*, 714–725.

Wang, L., Zhang, D., Wang, Y., Chen, C., Han, X., & M'hamed, A. (2016). Sparse mobile crowdsensing: Challenges and opportunities. *IEEE Communications Magazine*, *54*, 161–167.

Wang, S., Tamura, T., Kyouno, N., Liu, X., Zhang, H., Akiyama, Y., & Chen, J. Y. (2019a). Effect of the Chemical Composition of Miso (Japanese Fermented Soybean Paste) Upon the Sensory Evaluation. *Analytical Letters*. Retrieved from https://www.tandfonline.com/doi/abs/10.1080/00032719.2019.1570244

Wang, S., Tamura, T., Kyouno, N., Liu, X., Zhang, H., Akiyama, Y., & Chen, J. Y. (2019b). Effect of volatile compounds on the quality of Japanese fermented soy sauce. *LWT*, *111*, 594–601.

Wang, S., Zhang, H., Liu, X., Tamura, T., Kyouno, N., & Chen, J. Y. (2018). Relationship between Chemical Characteristics and Sensory Evaluation of Koikuchi Soy Sauce. *Analytical Letters*, *51*, 2192–2204.

Wu, S., Zorn, H., Krings, U., & Berger, R. G. (2007). Volatiles from submerged and surface-cultured beefsteak fungus, Fistulina hepatica. *Flavour and Fragrance Journal, 22*, 53–60.

Xu, L., Wang, X., Huang, Y., Wang, Y., Zhu, L., & Wu, R. (2019). A predictive model for the evaluation of flavor attributes of raw and cooked beef based on sensor array analyses. *Food Research International*, *122*, 16–24.

Yokotsuka, T., Sasaki, M., Nunomura, S., & Asao, Y. (1980). Flavor of shoyu (1). *Journal of the Society of Brewing, Japan*, *75*, 516-522,457.

# CHAPTER 5QUICK QUALITY EVALUATION FOR SOY SAUCE AND MISO PRODUCTS BY NEAR-INFRARED SPECTROSCOPY

## 5.1 Abstract

This study investigated the feasibility of rapidly evaluating the final quality of Japanese fermented soy sauce and miso using NIR spectroscopy and partial least-squares (PLS) regression. In total, 110 soy sauce samples and 115 miso samples that had been entered in the annual soy sauce competition from 2016 to 2018 were collected and analyzed. The transmittance spectra and the transflectance spectra of soy sauce samples and the reflection spectra of miso samples were acquired and processed by different pre-treatments, respectively. The results showed that the models constructed using the full spectra region performed a certain accuracy for the prediction of the sensory quality of soy sauce and miso samples. Comparing the influence of different regions in the acquired spectra enabled the accuracy of the models to be improved. The model constructed from transflectance spectra from the 2050 to 2400 nm region using pre-treatments based on standard normal variate (SNV) and first derivative was superior to the other models for soy sauce samples, with a coefficient of determination ($R_P^2$) value of 0.78 and the lowest root mean square error of prediction (RMSEP) value of 11.13 in the validation set. Similarly, the optimal model for the quality prediction of miso samples was constructed in the region of reflection spectra from 400 to 1100 nm with the SNV and first derivative, resulting in the values of $R_P^2$ and RMSEP were 0.59 and 24.80, respectively. This study has demonstrated that the NIR spectroscopy technique could be used as an alternative routine quality control procedure, which can rapidly and economically estimate the quality of soy sauce and miso products.

## 5.2 Introduction

Soy sauce and miso are important fermented seasonings that are widely used in the cuisine of East and Southeast Asian countries, especially in Japan. Generally, soy sauce and miso products are manufactured using a natural fermentation process for between six and twelve months under controlled temperature conditions (Noda, Hayashi, & Mizunuma, 1980). The long period of fermentation provides products with a better aroma and umami taste as well as more functional substances than products manufactured chemically or by limited fermentation, which are sold globally except in Japan (Kataoka, 2005). Because of their high quality and functional properties, soy sauce and miso products have been used as an all-purpose seasoning and have also been developed through cultural fusion, as shown by their popularity in many other countries in the USA and Europe (Moon & Rhee, 2016; Song et al., 2008).

According to the Ministry of Agriculture, Forestry and Fisheries, Japan, based on the Ministry of Finance's trade statistics, the amount of soy sauce products exported in 2013 was 42.7 tons, subsequently increasing each year to 51.8, 61.9, 66.1 and 71.5 tons by 2017, thus illustrating how the demand for soy sauce products has increased rapidly. This rapid increase in the amount exported has brought huge economic benefits and also an increasing demand for product quality to be improved. This has now brought new challenges on how to quantitatively and rapidly assess the final quality of soy sauce and miso products. Usually the quality of these two products has been assessed using sensory evaluation, combining the human perceptions of color, taste, flavor, and aroma (Lioe et al., 2007; Steinhaus & Schieberle, 2007). As the process of sensory evaluation is often time-consuming and expensive, it cannot be used frequently. For this reason, sensory evaluation has not been thought suitable for rapidly assessing the quality of new products. A panel of assessors can also be susceptible to changes in the environment leading to their judgments being subjective (Xu et al., 2013). Therefore, a rapid and objective method needs to be developed for assessing the quality of soy sauce and miso products to further satisfy both the routine requirements of food production and to improve quality.

Near-infrared (NIR) spectroscopy has become a well-accepted method that has been widely applied to the quantitative and qualitative analysis of food constituents (Kazeminy et al., 2009; Nicolaï et al., 2014). NIR spectroscopy is a rapid, accurate, and non-destructive technique, requires little preparation of samples, and is environmentally friendly; therefore, it has become increasingly important for quality and process control in the food industry (Chen, Zhang, &

Matsunaga, 2006; Ma, Zhang, Tuchiya, Miao, & Chen, 2014; Ye, Gao, Li, Yuan, & Yue, 2016). Several studies have reported the use of NIR spectroscopy for determining the chemical components of soy sauce (Katayama et al. 2013; Ogawa et al., 2001; Ouyang et al., 2013; Ouyang et al., 2012; Xu et al., 2016; Zhao et al., 2013). Both the quantitative and qualitative results obtained from previous studies have highlighted the good performance of NIR spectroscopy for predicting the physicochemical parameters of soy sauce. However, NIR spectroscopy has been little used for developing a model for predicting soy sauce and miso quality. Recently, Ritthiruangdej and Suwonsichon (2007) investigated the correlation between sensory attributes and NIR data from fish sauce, finding that the different sensory attributes of fish sauces obtained from 12 assessors were highly correlated with the different spectral regions of NIR spectroscopy. The quality of miso products were also proved to be well related with the different spectral regions of NIR spectroscopy (Chen et al., 2018). These findings indicated the potential for linking NIR spectroscopy to sensory analysis thus developing a rapid objective model for assessing the final quality of soy sauce and miso.

The main aim of the present study was to use the NIR spectroscopy technique to quickly determine the final quality of soy sauce and miso, specifically, to establish a predictive model using the products produced in 2016 and 2017, then to evaluate the model's performance using the products produced in 2018. The present study thus attempts to investigate the feasibility of a predictive model based on NIR spectroscopy for rapidly assessing the final quality of soy sauce and miso products.

## 5.3 Materials and Methods

### 5.3.1 Materials

#### 5.3.1.1 Soy Sauce Sample

In total, 110 soy sauce samples were collected from the Akita area (Japan) from 2016 to 2018 by Akita Prefectural Miso and Soy Sauce Manufacturer Cooperative. All these samples were entered in the second trial of the Akita Prefectural Miso and Soy Sauce Competition held in October annually for the assessment of quality. The type of these samples is known as *Koikuchi-shoyu* in Japan. Of all soy sauce consumed in Japan, 85% is of the *Koikuchi* type.

The sensory scores of these samples were evaluated the total score for each sample was 900 points. All samples were collected directly from the competition then stored immediately in a freezer at –25 °C before analysis.

#### 5.3.1.2 Miso sample

As with soy sauce, a total of 115 miso samples entered into the second trial of the Akita Prefectural Miso and Soy Sauce Competition each year (from 2016 to 2018) were produced by different companies located in Akita Prefecture. The sensory scores for these miso samples were evaluated then the quality were classified. All the evaluated miso products were directly collected from the competition each year and were immediately stored in a refrigerator at –25 °C prior to use.

### 5.3.2 NIR Methods

#### 5.3.2.1 NIR Spectra Acquisition

This study compared the transmittance (*T*) spectra and the transflectance spectra of soy sauce as well as the reflection spectra of miso to quantify the final quality of soy sauce. For soy sauce, a model NIRSystems 6500 spectrophotometer (Foss NIRSystems, Silver Spring, MD, USA) was used to measure the *T* spectra. The samples were placed in disposable glass test tubes (5 mL, 13 mm diameter) as the sample cell and scanned over the range of 400 to 1800 nm at 2-nm intervals. The absorbances were recorded as log (1/*T*). The acquired *T* spectra of each sample were obtained from the average of 32 scans. The transflectance spectra of soy sauce were obtained over the range of 680 to 2500 nm at 1-nm intervals using a Unity SpectraStar 2500XL-R NIR spectrometer (Unity Scientific, Brookfield, CT, USA). A few drops of each soy sauce sample were placed onto the liquid cup, and then the transflectance insert with a thickness of 0.1 mm was lowered onto the cup base to compress the sample between the backing surface and the window. The transflectance spectra were obtained from 24 scans in the sample compartment mode. Both the measurement of the *T* spectra and the transflectance spectra were made in a constant temperature environment of 25 °C.

For miso samples, the NIR spectra was obtained over the range of 680 to 1900 nm at 1-nm intervals using a Unity SpectraStar 2500XL-R NIR spectrometer. The visible/near infrared spectra of miso samples were qcquired with the NIRSystems 6500 spectrophotometer using the reflection mode. The spectrophotometer had a range of 400 to 2500 nm at 2-nm intervals. For acquisition, miso samples were filled with the sample cups, where is a metal cup (inner diameter: 39 mm; height: 12mm) for the Unity SpectraStar 2500XL-R NIR spectrometer and a plastic cup (inner diameter: 40 mm; height: 12mm) for the NIRSystems 6500 spectrophotometer, and then the prepared samples were irradiated from the window of these two instruments by the light source, the reflection spectrum was obtained from the average of 24 scans and 32 scans, respectively.

#### 5.3.2.2 Reference Measurement

The sensory scores of the soy sauce and miso samples were obtained by sensory evaluation. The process of sensory evaluation used has been reported previously. Briefly, nine assessors from the Akita Integrated Food Research Center, Japan Soy Sauce Technology Center, who worked in the related fermentation field, participated in the soy sauce competition as assessors and performed the sensory evaluation. This procedure of sensory evaluation consists of two trials: the first trial used a 5-point method (satisfied = 1 to disagreeable = 5) to provide an overall assessment of the samples that had been entered in the competition. The sensory score of the tested samples from the first trial was then calculated by adding the total scores from the nine assessors. A tested sample with a total score less than 20 then continued into the second trial. During the second trial, assessors used a 100-point method (excellent = 100 to inferior = 0) to evaluate the differences in the quality of these superior samples. The final score for these samples was calculated by summing the scores from the nine assessors, so the highest total score possible for each sample was 900 points.

### 5.3.3 Statistical Analysis

The raw spectroscopic data for soy sauce and miso was processed using the Unscrambler X software (version 10.3, CAMO Software, Oslo, Norway). Different combinations of pre-treatment method (1st derivative, 2nd derivative, multiplicative scatter correction (MSC), and standard normal variate (SNV)) were used to optimize the development of the calibration model (Blanco et al., 2000; Dhanoa et al., 1994). The Savitsky-Golay algorithm with ten points of smoothing was used for processing both the 1st derivative and 2nd derivative spectra. The calibration models were established using the partial least-square regression (PLSR) technique on the raw NIR spectra and their optimized data.

The soy sauce and miso samples were separated into two groups: the soy sauce and miso samples from 2016 and 2017 were used as the calibration set, and the soy sauce and miso samples from 2018 were used as the validation set (Table 5-1 & Table 5-2). The values of the means and standard deviations for the calibration and validation sets were very similar for the sensory scores of the tested samples. The soy sauce and miso samples in the calibration set were used to develop the calibration models, while the soy sauce and miso samples in the validation set were used to evaluate the model performance.

The calibration models were constructed using PLSR from the raw and processed spectroscopic data of the calibration set. The coefficient of determination for calibration ($R_C^2$) and the root mean square error of calibration (RMSEC) were used to compare the accuracy of the generated calibration models. The developed models were further validated using the validation set. The coefficient of determination for prediction ($R_P^2$) and the root mean square error of prediction (RMSEP) were used to judge how well the calibration model could predict the quality of the tested soy sauce and miso samples in the validation set. The calculations of $R^2$, RMSEC, and RMSEP were as follows:

$$R_{C,P}^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \tilde{y}_i)^2} \quad (5\text{-}1)$$

$$\text{RMSEC} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \quad (5\text{-}2)$$

$$\text{RMSEP} = \sqrt{\frac{1}{m}\sum_{i=1}^{m}(y_i - \hat{y}_i)^2} \quad (5\text{-}3)$$

where the $y_i$ is the sensory score of the soy sauce or miso samples from the sensory evaluation and the $\hat{y}_i$ is the predicted value for the *i*th soy sauce or miso sample; $\tilde{y}_i$ represents the mean of all the sensory scores of the soy sauce or miso samples from sensory evaluation; *n* is the number of calibration samples; *m* is the number of validation samples. In general, a model with high accuracy should have a high value of $R^2$ and a lower value of RMSEC and RMSEP, as well as a small difference between the RMSEC and RMSEP values.

## 5.4 Results and Discussion

### 5.4.1 Soy Sauce

#### 5.4.1.1 Sensory Evaluation Results

The sensory scores of soy sauce samples originating from the competitions in 2016, 2017 and 2018 that participated in the second trial of sensory evaluation are summarized in Table 5-1.

Table 5-1 Reference data of soy sauce for calibration and validation sets.

| | Sample number (*n*) | Point range (min - max) | Mean | Standard deviation |
|---|---|---|---|---|
| Calibration set | 76 | 770 - 856 | 815.97 | 21.87 |
| Validation set | 34 | 766 - 852 | 816.74 | 24.28 |

#### 5.4.1.2 Performance of Different Acquisition Models

A calibration set consisting of 76 soy sauce samples from 2016 and 2017 was used to establish the PLS calibration models, and a validation set comprising 34 samples from 2018 was used to validate the models. A total of nine different pre-treatment algorithms were used to remove sampling variation from the spectra to improving the accuracy of the corresponding PLS calibration models. The PLSR results of the models established for predicting the sensory scores of soy sauce samples based on the *T* spectra and transflectance spectra are shown in Table 5-2. It was found that the direct application of PLSR to the *T* spectra with no pre-treatment provided the minimum values of $R_C^2$ and $R_P^2$ for predicting the sensory scores of the soy sauce samples. If the pre-treatment using the 1st or 2nd derivative was applied to the *T* spectra, the values of both $R_C^2$ and $R_P^2$ increased and those of RMSEC and RMSEP both decreased, indicating that the ability of these models were optimized. However, the performance parameters for the models obtained using the MSC or SNV pre-treatment methods alone, where the RMSEP value increased, were no better than those for a model with no pre-treatment.

Table 5-2 Statistical parameters for the PLSR models for the calibration and validation sets of sensory scores of soy sauce samples based on transmittance and transflectance spectra.

| Spectral mode | Wavelength (nm) | Pretreatment | Calibration | | Validation | |
|---|---|---|---|---|---|---|
| | | | $R_C^2$ | RMSEC | $R_P^2$ | RMSEP |
| Transmittance ($T$) | 400-1800 | None | 0.44 | 16.35 | 0.43 | 18.13 |
| | | 1st derivative | 0.57 | 14.41 | 0.44 | 17.88 |
| | | 2nd derivative | 0.69 | 12.14 | 0.54 | 16.29 |
| | | MSC | 0.45 | 16.21 | 0.35 | 19.26 |
| | | MSC + 1st derivative | 0.56 | 14.48 | 0.44 | 17.88 |
| | | MSC + 2nd derivative | 0.69 | 12.19 | 0.54 | 16.21 |
| | | SNV | 0.45 | 16.19 | 0.36 | 19.19 |
| | | SNV + 1st derivative | 0.56 | 14.49 | 0.44 | 17.93 |
| | | SNV + 2nd derivative | 0.69 | 12.19 | 0.54 | 16.24 |
| Transflectance | 680-2500 | None | 0.70 | 11.97 | 0.63 | 14.57 |
| | | 1st derivative | 0.60 | 13.76 | 0.62 | 14.75 |
| | | 2nd derivative | 0.58 | 14.18 | 0.56 | 15.90 |
| | | MSC | 0.67 | 12.54 | 0.62 | 14.78 |
| | | MSC + 1st derivative | 0.73 | 11.47 | 0.65 | 14.09 |
| | | MSC + 2nd derivative | 0.72 | 11.68 | 0.64 | 14.33 |
| | | SNV | 0.67 | 12.54 | 0.62 | 14.78 |
| | | SNV + 1st derivative | 0.72 | 11.50 | 0.65 | 14.13 |
| | | SNV + 2nd derivative | 0.71 | 11.71 | 0.64 | 14.36 |

The performance of the models obtained from the transflectance spectra was clearly better than those obtained from the *T* spectra, with higher values of $R_C^2$ and $R_P^2$ and lower values of RMSEC and RMSEP. This difference in the performance of the models based on these two spectral modes may have been caused by the sample cells used during the acquisition of the NIR spectra. When acquiring the *T* spectra, minute differences in the diameter and circular shape of the individual glass test tubes could have caused baseline offset and slopes in the spectra. In contrast, spectra acquired in the transflectance mode used the same sample cell. The optical path through the transflectance insert (thickness 0.1 mm) was less than that through the glass test tubes used when acquiring the *T* spectra, thus leading to a more stable optical path. Therefore, the better results for PLS models were found by using transflectance mode (NIR spectra was shown in Figure 5-1). For the nine PLS calibration models based on transflectance, the model constructed using the pre-treatment method of MSC combined with the 1st derivative provided the best results for predicting the sensory scores of soy sauce samples: the results for calibration were an $R_C^2$ value of 0.73 and an RMSEC value of 11.40 and those for validation were an $R_P^2$ value of 0.65 and an RMSEC value of 14.09. These results also showed that NIR spectroscopy had promise as a rapid tool for predicting the sensory quality of soy sauce samples. However, there was still a need to further optimize the development of the calibration models.

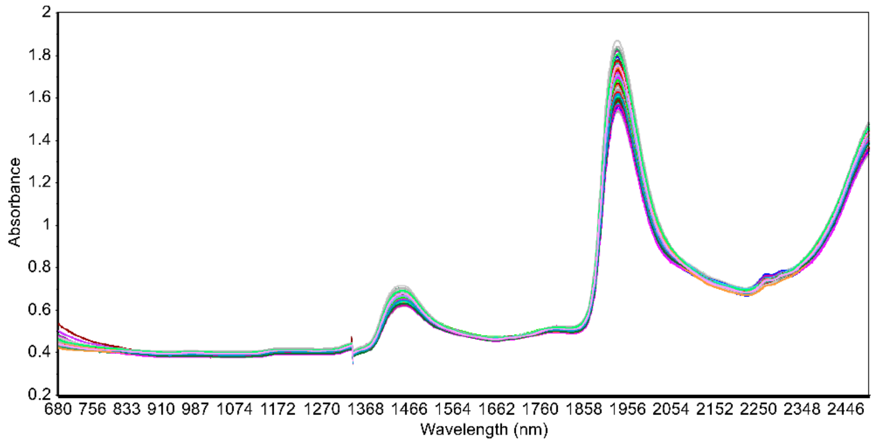


Figure 5-1 Original spectra of soy sauce samples obtained over the range of 680 to 2500 nm using a Unity SpectraStar 2500XL-R NIR spectrometer.

#### 5.4.1.3 Model Optimization

For the PLS models constructed using the full spectrum (680–2500 nm), much unwanted information such as irrelevant or collinear variables was included, which would inevitably weaken the performance of the resulting models. The careful selection of variables is one of the effective ways for improving model performance. Choosing only ranges that contain the most systematic variation related to the modeled phenomenon would not only enhance the accuracy of the model, but also improve its practical application (Heinz et al., 2007). Therefore, calibration models based on different regions of transflectance spectra were constructed and tested.

In the present study, the transflectance spectra obtained from each soy sauce sample were first divided into three wavelength regions: 680–1100, 1100–1800, and 1800–2500 nm. The region of 680-1100 nm mainly represents bands resulting from the second and third overtones (680-1100 nm); the region of 1100-1800 nm, where the bands arising from the first and second overtones could be observed; and the region of 1800-2500 nm was a combination tone mode region (Ozaki, 2012; Tasumi, 2014). Table 5-3 shows the results from the model constructed using these three different regions over the range from 1800 to 2500 nm to highlight the best performance. In particular, the application of the 1st derivative to this wavelength range established a model which predicted the sensory score of soy sauce samples, with $R_C^2$ and $R_P^2$ values of 0.80 and 0.75, respectively. These values were much higher than those providing the best model performance using all the transflectance spectral regions ($R_C^2$ of 0.73 and $R_P^2$ of 0.65). The values of RMSEC (9.70) and RMSEP (12.08) using the 1800 to 2500 nm range were also much lower than those provided using all the spectral regions: RMSEC (11.40) and RMSEP (14.09). Generally, the wavelength region from 1800 to 2500 nm was a combination of the tone and spectral region that covered the NIR spectral features. Most of the absorption band in this region is related to features ascribed to the food constituents and this region also exhibits a larger absorption coefficient so is important for developing an accurate model (Delwiche & Graybosch, 2014). The models constructed using spectra from this wavelength region (1800 to 2500 nm) may mainly be associated with quality attributes in soy sauce samples, making their performance much better than those based on the full spectrum.

Table 5-3 Statistical parameters for the PLSR models for the calibration and validation sets of sensory scores of soy sauce samples based on transflectance spectra with the given spectral regions.

| Wavelength (nm) | Pretreatment | Calibration | | Validation | |
|---|---|---|---|---|---|
| | | $R_C^2$ | RMSEC | $R_P^2$ | RMSEP |
| 680-1100 | None | 0.59 | 14.00 | 0.62 | 14.75 |
| | 1st derivative | 0.68 | 12.28 | 0.61 | 14.95 |
| | 2nd derivative | 0.62 | 13.36 | 0.29 | 20.12 |
| | MSC | 0.62 | 13.41 | 0.60 | 15.17 |
| | MSC+1st derivative | 0.63 | 13.20 | 0.58 | 15.61 |
| | MSC+2nd derivative | 0.84 | 8.81 | 0.37 | 18.94 |
| | SNV | 0.62 | 13.41 | 0.60 | 15.23 |
| | SNV+1st derivative | 0.63 | 13.19 | 0.57 | 15.63 |
| | SNV+2nd derivative | 0.84 | 8.80 | 0.38 | 18.92 |
| 1100-1800 | None | 0.70 | 11.90 | 0.70 | 13.04 |
| | 1st derivative | 0.61 | 13.61 | 0.62 | 14.84 |
| | 2nd derivative | 0.62 | 13.33 | 0.53 | 16.45 |
| | MSC | 0.69 | 12.19 | 0.68 | 13.59 |
| | MSC+1st derivative | 0.69 | 12.17 | 0.67 | 13.85 |
| | MSC+2nd derivative | 0.72 | 11.49 | 0.63 | 14.50 |
| | SNV | 0.69 | 12.19 | 0.68 | 13.58 |
| | SNV+1st derivative | 0.68 | 12.20 | 0.66 | 13.93 |
| | SNV+2nd derivative | 0.72 | 11.48 | 0.63 | 14.64 |
| 1800-2500 | None | 0.76 | 10.71 | 0.67 | 13.77 |
| | 1st derivative | 0.80 | 9.70 | 0.75 | 12.08 |
| | 2nd derivative | 0.70 | 11.87 | 0.70 | 13.05 |
| | MSC | 0.74 | 11.03 | 0.66 | 13.91 |
| | MSC+1st derivative | 0.75 | 10.76 | 0.69 | 13.44 |
| | MSC+2nd derivative | 0.72 | 11.51 | 0.59 | 15.34 |
| | SNV | 0.74 | 11.03 | 0.66 | 13.91 |
| | SNV+1st derivative | 0.76 | 10.76 | 0.68 | 13.47 |
| | SNV+2nd derivative | 0.72 | 11.49 | 0.59 | 15.31 |

Next, the method of spectral interval selection was applied in the NIR regions of 1800-2500 nm in order to further reduce the interference signals and improve the performance of the model. A moving window with a size of 350 nm is set and an interval of 50 nm was set, and the window was moved from the first spectral point to the endpoint over the given region from 1800 to 2500 nm. Accordingly, the performance of the models constructed by using these eight sub-regions, that is, 1800-2150 nm region, 1850-2200 nm region, 1900-2250 nm region, 1950-2300 nm region, 2000-2350 nm region, 2050-2400 nm region, 2100-2450 nm region, and 2150-2500 nm region, was compared. It is found that the model constructed in the region of 2050-2400 nm with a pre-treatment of SNV provided the best performance for predicting the sensory scores of soy sauce products. This model reduced the RMSEP value from 12.08 to 11.13 for predicting the sensory scores of the soy sauce samples (Figure 5-2). The further division of the spectral region from 2050 to 2400 did not improve the results for estimating the sensory scores of soy sauce samples.

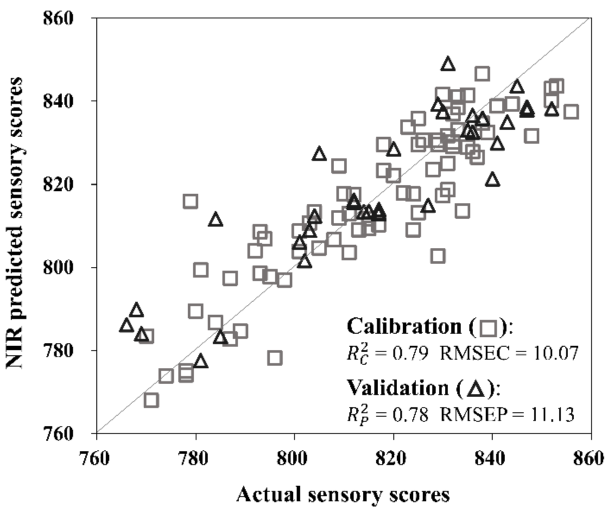


Figure 5-2 Scatter plots of actual sensory scores (x-axis) for soy sauce samples versus the predicted values (y-axis) based on transflectance spectra from the 2050 to 2400 nm regions.

The non-zero regression coefficients using the spectra in the range of 2050–2400 nm after pre-treatment with SNV for predicting soy sauce sample quality are shown in Figure 5-3, which shows the influence of each wavelength on the sensory scores. The absorptions at around 2110 nm and 2245 nm were associated with the N-H stretching and the $NH_3^+$ deformation vibrations, which may be relevant for the amino acids in the soy sauce samples (Tran & Kong, 2000). The absorption at around 2280 nm correspond to the C-C stretching vibration and the O-H stretching vibration. These spectral ranges were likely related to the carbohydrates and acids present in the soy sauce samples (Salgó & Gergely, 2012; Yano et al., 2000). Moreover, the absorptions at around 2350 nm correspond to the $CH_2$ symmetric stretch and = $CH_2$ deformation vibrations which were likely related to the fatty acids from the soybean in soy sauce samples (Ozaki et al., 2006; Westad et al., 2008). Overall, the spectral range from 2050 to 2400 nm contained much information on the sensory constituents of the soy sauce samples that were related to the results of sensory evaluation. Thus, using the spectral regions from 2050 to 2400 nm to build a model helped to estimate the final quality of the soy sauce samples.

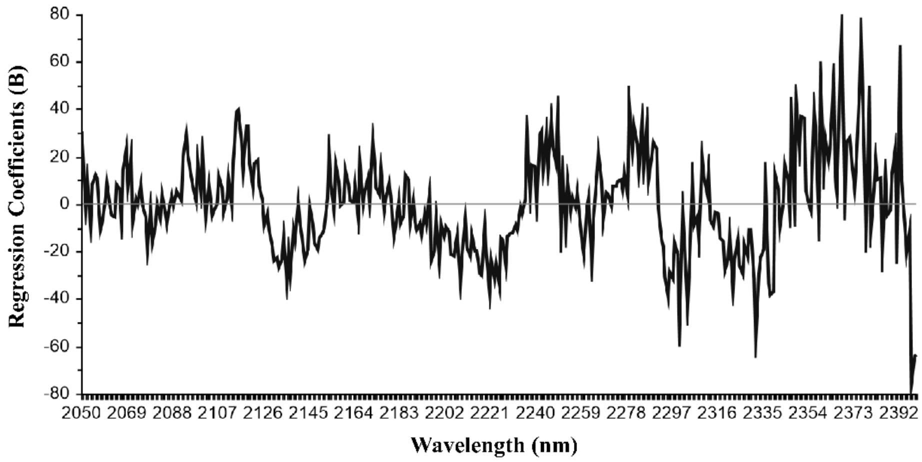


Figure 5-3 Regression (beta-) coefficients for the model constructed using spectra from the region of 2050 to 2400 nm using an SNV as pre-treatment.

### 5.4.2 Miso

#### 5.4.2.1 Sensory Evaluation Results

The sensory scores of miso samples originating from the competitions in 2016, 2017 and 2018 that participated in the second trial of sensory evaluation are summarized in Table 5-4.

Table 5-4 Reference data of miso for calibration and validation sets.

| | Sample number (*n*) | Point range (min - max) | Mean | Standard deviation |
|---|---|---|---|---|
| Calibration set | 77 | 746 - 859 | 816.53 | 24.80 |
| Validation set | 38 | 740 - 854 | 801.84 | 34.94 |

5.4.2.2 Acquisition of Miso NIR Spectroscopy

Considering that the Japanese miso is a soybean paste, light cannot pass through easily. The reflectance mode was applied for the acquisition of NIR spectroscopy for miso products. The acquisition was conducted on a NIRSystems 6500 spectrophotometer with a spectral range of 400-2500 nm, including the visible region. Like the building of soy sauce predictive model, nine different pre-treatment algorithms were used to improve the accuracy of the corresponding PLS calibration models. The PLSR results of the models established for predicting the sensory scores of miso samples over the full spectrum using the NIRSystems 6500 spectrophotometer are shown in Table 5-5. It was found that the maximum values of $R_C^2$ and $R_P^2$ were 0.75 and 0.57, respectively. Comparing the RMSEC and RMSEP of different models, the minimum values of RMSEC and RMSEP obtained were 12.42 and 24.60, respectively. On the basis of the obtained results, these two models showed a certain potential for developing a model to predict the sensory quality of miso products, while the performance of the model needed to be further optimized.

Table 5-5 Statistical parameters for the calibration and validation of miso samples using the full spectrum from 400 nm to 2500 nm.

| Instrument | Pretreatment | Calibration | | Validation | |
|---|---|---|---|---|---|
| | | $R_C^2$ | RMSEC | $R_P^2$ | RMSEP |
| NIRSystems 6500 spectrophotometer | None | 0.67 | 14.06 | 0.48 | 27.06 |
| | 1st derivative | 0.75 | 12.45 | 0.55 | 25.07 |
| | 2nd derivative | 0.48 | 17.77 | 0.46 | 27.58 |
| | MSC | 0.71 | 13.39 | 0.49 | 26.87 |
| | MSC + 1st derivative | 0.75 | 12.42 | 0.57 | 24.60 |
| | MSC + 2nd derivative | 0.52 | 17.15 | 0.47 | 27.41 |
| | SNV | 0.71 | 13.34 | 0.48 | 27.06 |
| | SNV + 1st derivative | 0.75 | 12.41 | 0.57 | 24.60 |
| | SNV + 2nd derivative | 0.51 | 17.18 | 0.47 | 27.43 |

5.4.2.3 Model Optimization

The selection of informative NIR regions where one can obtain an optimized calibration model for predicting the sensory quality of miso was performed. Specifically, the NIR reflectance spectra obtained using the NIRSystems 6500 spectrophotometer were first divided into three wavelength regions: 400–1100 nm region, 1100–1800 nm region, and

1800–2500 nm. The 400-1100 nm region included the visible region (400-700 nm) and the short-wavelength NIR region (700-1100 nm) where dominated by bands ascribed to second and third overtones. The region of 1100-1800 is concerned with bands due to the first and second overtones. The region of 1800-2500 nm is a combination-mode region.

Table 5-6 shows the results from the model constructed using these three different regions. The range from 400 to 1100 nm showed the best performance than the other two regions. In particular, the application of the pre-treatment of SNV combined with 1st derivative to this wavelength range enables to establish the best performance for predicting the sensory score of miso samples. The minimum RMSEP value of 24.80 was similar with the RMSEP value, which is 24.60, provided using all the spectral regions. And also, the values of $R_C^2$, $R_P^2$, and RMSEC were similar for these two models. Consequently, the obtained models using the full spectrum and the given region from 400-1100 nm provided the similar performance. For the obtained model using the region of 400-1100 nm, the range of 700 to 1100 assigned to short-wave NIR provided a possible indication of changes in chemical components, for example, carbohydrates, organic acids, and amino acids, and which related to the quality change of miso products (Fernández-Novales et al., 2009; González-Caballero et al., 2010). Moreover, the wavelength range from 400 to 700 assigned to the visible range was considered to relate with the diversity in the color of miso products. Consequently, the application of NIR technology for the development of the model using the wavelength region from 400 to 1100 nm was concerned with most sensory compositions that affect the quality of miso, therefore, the obtained predictive model was feasible for predicting the quality of miso products and lower the cost. On the other hand, it is noted that the obtained model for miso products showed lower performance in comparison with that of soy sauce. This may be caused by the inhomogeneity of miso samples.

Table 5-6 Statistical parameters for the calibration and validation of sensory scores of miso samples using the NIRSystems 6500 spectrophotometer with the given spectral regions.

| Wavelength (nm) | Pretreatment | Calibration | | Validation | |
|---|---|---|---|---|---|
| | | $R_C^2$ | RMSEC | $R_P^2$ | RMSEP |
| 400-1100 | None | 0.65 | 14.52 | 0.49 | 26.67 |
| | 1st derivative | 0.73 | 12.92 | 0.56 | 25.02 |
| | 2nd derivative | 0.88 | 8.70 | 0.56 | 24.97 |
| | MSC | 0.65 | 14.49 | 0.51 | 26.25 |
| | MSC+1st derivative | 0.69 | 13.71 | 0.55 | 25.06 |
| | MSC+2nd derivative | 0.87 | 8.74 | 0.49 | 26.76 |
| | SNV | 0.62 | 15.27 | 0.46 | 27.54 |
| | SNV+1st derivative | 0.69 | 13.68 | 0.56 | 24.80 |
| | SNV+2nd derivative | 0.91 | 7.50 | 0.56 | 25.00 |
| 1100-1800 | None | 0.51 | 17.29 | 0.47 | 27.33 |
| | 1st derivative | 0.37 | 19.58 | 0.43 | 28.38 |
| | 2nd derivative | 0.37 | 19.49 | 0.33 | 30.62 |
| | MSC | 0.44 | 18.37 | 0.48 | 27.07 |
| | MSC+1st derivative | 0.40 | 19.12 | 0.43 | 28.38 |
| | MSC+2nd derivative | 0.42 | 18.84 | 0.38 | 29.43 |
| | SNV | 0.43 | 18.67 | 0.45 | 27.85 |
| | SNV+1st derivative | 0.40 | 19.13 | 0.43 | 28.39 |
| | SNV+2nd derivative | 0.41 | 18.87 | 0.38 | 29.44 |
| 1800-2500 | None | 0.40 | 19.01 | 0.22 | 33.03 |
| | 1st derivative | 0.33 | 20.16 | 0.30 | 31.47 |
| | 2nd derivative | 0.43 | 18.64 | 0.39 | 29.36 |
| | MSC | 0.29 | 20.77 | 0.25 | 32.38 |
| | MSC+1st derivative | 0.24 | 21.42 | 0.29 | 31.56 |
| | MSC+2nd derivative | 0.46 | 18.18 | 0.41 | 28.88 |
| | SNV | 0.29 | 20.75 | 0.26 | 32.34 |
| | SNV+1st derivative | 0.28 | 20.91 | 0.13 | 35.01 |
| | SNV+2nd derivative | 0.46 | 18.17 | 0.40 | 28.95 |

## 5.5 Conclusions

The present study investigated the feasibility of using NIR spectra for predicting the final quality of soy sauce and miso samples collected from major manufacturing companies and small workshops located in the Akita area of Japan. The overall results showed that NIR spectra can be used to develop a rapid model for predicting the sensory scores of soy sauce and miso samples. PLS models based on different spectral regions were constructed then compared to identify the important systematic variations related to sensory quality. The model producing the optimal performance for soy sauce was based on transflectance spectra in the region from 2050 to 2400 nm after the SNV pre-treatment with a $R_P^2$ value of 0.78, and an RMSEC value of 11.13. The optimal model for predicting the sensory quality of miso product was constructed in the region from 400 to 1100 nm after pre-treatment with SNV combined with the 1st derivative. Moreover, NIR technology also has the advantages of being non-destructive, rapid, easy-to-use, and with no need to use chemical reagents or solvents. It can be concluded that the application of NIR spectra has great potential as an alternative quality control method to assess the final quality of soy sauce and miso production rapidly and economically.

## 5.6 References


Blanco, M., Coello, J., Iturriaga, H., Maspoch, S., & Pagès, J. (2000). NIR calibration in non-linear systems: Different PLS approaches and artificial neural networks. *Chemometrics and Intelligent Laboratory Systems, 50*, 75–82.

Chen, J. Y., Zhang, H., & Matsunaga, R. (2006). Rapid Determination of the Main Organic Acid Composition of Raw Japanese Apricot Fruit Juices Using Near-Infrared Spectroscopy. *Journal of Agricultural and Food Chemistry, 54*, 9652–9657.

Chen J. Y., Zhang H., Tamura T., & Kyouno N. (2018). Visible/Near Infrared Spectroscopy for Miso Quality Assessment. *Journal of Japanese Society of Taste Technology, 16*, 23–29.

Delwiche, S. R., & Graybosch, R. A. (2014). Measurement of Blend Concentrations of Conventional and Waxy Hard Wheats Using NIR Spectroscopy. *Cereal Chemistry Journal, 91*, 358–365.

Dhanoa, M. S., Lister, S. J., Sanderson, R., & Barnes, R. J. (1994). The Link between Multiplicative Scatter Correction (MSC) and Standard Normal Variate (SNV) Transformations of NIR Spectra. *Journal of Near Infrared Spectroscopy, 2*, 43–47.

Fernández-Novales, J., López, M. I., Sánchez, M. T., Morales, J., & González-Caballero, V. (2009). Shortwave-near infrared spectroscopy for determination of reducing sugar content during grape ripening, winemaking, and aging of white and red wines. *Food Research International, 42*, 285–291.

González-Caballero, V., Sánchez, M. T., López, M. I., & Pérez-Marín, D. (2010). First steps towards the development of a non-destructive technique for the quality control of wine grapes during on-vine ripening and on arrival at the winery. *Journal of Food Engineering, 101*, 158–165.

Heinz, A., Savolainen, M., Rades, T., & Strachan, C. J. (2007). Quantifying ternary mixtures of different solid-state forms of indomethacin by Raman and near-infrared spectroscopy. *European Journal of Pharmaceutical Sciences, 32*, 182–192.

Kataoka, S. (2005). Functional effects of Japanese style fermented soy sauce (shoyu) and its components. *Journal of Bioscience and Bioengineering, 100*, 227–234.

Katayama, N., Kobayashi, Y., Katsuragawa, Y., & Sakai, E. (2013). Near Infrared spectroscopy and Chemometrics study on ingredient composition change of fermented foods.pdf. *Japanese NIR Forum, 28*, 121.

Kazeminy, A., Hashemi, S., Williams, R. L., Ritchie, G. E., Rubinovitz, R., & Sen, S. (2009). A Comparison of near Infrared Method Development Approaches Using a Drug Product on Different Spectrophotometers and Chemometric Software Algorithms. *Journal of Near Infrared Spectroscopy*, *17*, 233–244.

Lioe, H. N., Wada, K., Aoki, T., & Yasuda, M. (2007). Chemical and sensory characteristics of low molecular weight fractions obtained from three types of Japanese soy sauce (shoyu)–Koikuchi, tamari and shiro shoyu. *Food Chemistry*, *100*, 1669–1677.

Ma, J., Zhang, H., Tuchiya, T., Miao, Y., & Chen, J. Y. (2014). Rapid Determination of Degradation of Frying Oil Using Near-Infrared Spectroscopy. *Food Science and Technology Research*, *20*, 217–223.

Moon, H., & Rhee, M. S. (2016). Synergism between carvacrol or thymol increases the antimicrobial efficacy of soy sauce with no sensory impact. *International Journal of Food Microbiology*, *217*, 35–41.

Nicolaï, B. M., Defraeye, T., De Ketelaere, B., Herremans, E., Hertog, M. L. A. T. M., Saeys, W., & Verboven, P. (2014). Nondestructive Measurement of Fruit and Vegetable Quality. *Annual Review of Food Science and Technology*, *5*, 285–312.

Noda, F., Hayashi, K., & Mizunuma, T. (1980). Antagonism between osmophilic lactic acid bacteria and yeasts in brine fermentation of soy sauce. *Appl. Environ. Microbiol.*, *40*, 452–457.

Ogawa, T., Tahata, K., Nagai, K., & Inoue, Y. (2001). Rapid Determination of Miso Quality Using Near Infrared Spectroscopy.pdf. *Bull. Hyogo Pre. Agri. Inst. (Agriculture)*, *49*, 9–13.

Ouyang, Q., Chen, Q., Zhao, J., & Lin, H. (2013). Determination of Amino Acid Nitrogen in Soy Sauce Using Near Infrared Spectroscopy Combined with Characteristic Variables Selection and Extreme Learning Machine. *Food and Bioprocess Technology*, *6*, 2486–2493.

Ouyang, Q., Zhao, J., Chen, Q., Lin, H., & Sun, Z. (2012). Rapid measurement of antioxidant activity in dark soy sauce by NIR spectroscopy combined with spectral intervals selection and nonlinear regression tools. *Analytical Methods*, *4*, 940.

Ozaki, Y. (2012). Near-infrared spectroscopy—Its versatility in analytical chemistry. *Analytical Sciences*, *28*, 545–563.

Ozaki, Y., McClure, W. F., & Christy, A. A. (Eds.) 2006. Near-infrared spectroscopy in food science and technology. *John Wiley & Sons.*

Ritthiruangdej, P., & Suwonsichon, T. (2007). Relationships between NIR Spectra and Sensory Attributes of Thai Commercial Fish Sauces. *Analytical Sciences*, *23*, 809–814.

Salgó, A., & Gergely, S. (2012). Analysis of wheat grain development using NIR spectroscopy. *Journal of Cereal Science*, *56*, 31–38.

Song, Y. S., Frias, J., Martinez-Villaluenga, C., Vidal-Valdeverde, C., & de Mejia, E. G. (2008). Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. *Food Chemistry*, *108*, 571–581.

Steinhaus, P., & Schieberle, P. (2007). Characterization of the Key Aroma Compounds in Soy Sauce Using Approaches of Molecular Sensory Science. *Journal of Agricultural and Food Chemistry*, *55*, 6262–6269.

Tasumi, M. (2014). Introduction to experimental infrared spectroscopy: Fundamentals and practical methods. *John Wiley & Sons.*

Tran, C. D., & Kong, X. (2000). Near-Infrared Spectrophotometric Determination of Tri- and Tetrapeptides. *Analytical Biochemistry*, *286*, 67–74.

Westad, F., Schmidt, A., & Kermit, M. (2008). Incorporating Chemical Band-Assignment in near Infrared Spectroscopy Regression Models. *Journal of Near Infrared Spectroscopy*, *16*, 265–273.

Xu, D., Li, C., Zhao, M., Fengc, Y., Sun, L., & Wang, Y. (2013). Assessment on the improvement of soy sauce fermentation by Aspergillus oryzae HG76. *Biocatalysis and Agricultural Biotechnology*, *2*, 344–351.

Xu, J., Huang, F., Li, Y., Chen, Z., & Wang, Y. (2016). Rapid detection of total nitrogen content in soy sauce using NIR spectroscopy. *Czech Journal of Food Sciences*, *33*, 518–522.

Yano, T., Matsushige, H., Suehara, K. I., & Nakano, Y. (2000). Measurement of the concentrations of glucose and lactic acid in peritoneal dialysis solutions using near-infrared spectroscopy. *Journal of Bioscience and Bioengineering*, *90*, 540–544.

Ye, M., Gao, Z., Li, Z., Yuan, Y., & Yue, T. (2016). Rapid detection of volatile compounds in apple wines using FT-NIR spectroscopy. *Food Chemistry*, *190*, 701–708.

Zhao, J., Ouyang, Q., Chen, Q., & Lin, H. (2013). Simultaneous determination of amino acid nitrogen and total acid in soy sauce using near infrared spectroscopy combined with characteristic variables selection. *Food Science and Technology International*, *19*, 305–314.

# CHAPTER 6OVERALL CONCLUSIONS, LIMITATIONS AND IMPLICATIONS FOR FUTURE WORK

## 6.1 Conclusions

Based on the results from the studies presented in this dissertation, the following conclusions were made:

1. Color, generated in fermentation and heat process, is one of the most important factors determining the qualities of soy sauce and miso. The *a** value contributed to the quality improvement for both soy sauce and miso products, whereas the other two parameters *L** and *b** were negatively correlated to the quality of miso products (Chapter 2).
2. Soluble salt-free solids content had a positive effect on soy sauce, while the effect of salt was negative. There was an optimal salt content of 12% and a range of soluble salt-free solids content from 35% to 40% for the high quality of miso products (Chapter 2).
3. The results of the present study indicate that glucose is one of the major determinants of final qualities for soy sauce and miso products. The different effects on the qualities of soy sauce and miso were observed, that is, positively related to the soy sauce quality and negative related to miso quality (Chapter 2).
4. The levels of phosphoric acid and organic acids in soy sauce and miso products had a considerable effect on the final quality, especially for phosphoric acid, critic acid, acetic acid and, pyroglutamic acid (Chapter 2).
5. Amino acids found in soy sauce and miso products contributed significantly to their qualities (Chapter 2).
6. The moisture content had a negative effect on the quality of soy sauce produced in Akita area, while the effect of moisture on miso was negligible (Chapter 2).
7. A total of 62 volatile compounds were found in soy sauce products produced in the Akita area, aldehydes and ketones were the most detected. Among them, 19 compounds were positively correlated to the final quality of soy sauce. In contrast, 23 compounds showed a negative correlation with the final quality (Chapter 3).

8. A total of 48 volatile compounds were found in miso products produced in the Akita area, esters are the most detected. Most of the detected compounds had a positive correlation with the final quality of miso, indicating the high-quality miso was imparted with an intense flavor (Chapter 3).
9. The mathematical models for evaluating the sensory quality of both soy sauce and miso was constructed through Compressed Sensing (CS) theory (Chapter 4). The constructed model could provide details for quality control of soy sauce and miso products produced in Akita area.
10. The CS-based method provided a new approach to selecting variables of practical importance for developing a predictive model of foods (Chapter 4).
11. The feasibility of near-infrared spectroscopy for rapidly determining the final qualities of soy sauce and miso products were also investigated. The obtained NIR models showed great potential in estimating the final quality of both soy sauce and miso as a rapid and accurate quality control tool (Chapter 5).

## 6.2 Limitations and Implications for Future Work

The results of the current research were focused on the chemical and flavor characteristics of both soy sauce and miso products produced in Akita area. The presence of chemical compositions and volatile compounds could descript some of the significant differences between the quality characteristics of foods produced in the traditional Japanese fermentation process. However, there are varieties of soy sauce or miso available in Japan, and the sensory quality of each fermented soybean food depends on the raw materials and production method used by the manufacturers. Moreover, the people living in different areas of Japan might also have different preferences due to the different traditions of cooking. These made the application of the models obtained in this work may be limited. Therefore, future work is needed to collect the products of both soy sauce and miso that manufactured in other places in Japan, even worldwide, in order to find the common features of soy sauce or miso, overcome geographical limitations, and then get a more widely used model for the quality control of soy sauce and miso.

In addition to amino acids, the peptides are also degraded from soybean proteins by enzymatic activities during the fermentation of both soy sauce and miso. These peptides had an umami-enhancing effect on the palatable tastes of soy sauce and miso products. Therefore, the peptides may play a role in the determination of the final qualities for both soy sauce and miso. In order to get a more comprehensive understanding of quality characteristics for soy sauce and miso, a future study of peptides should be set up to investigate the relationship between the concentration of peptides and the sensory evaluation.

In the current study, the semi-quantitative analysis carried out based on the GC-MS peak area of each volatile compound, then the correlations between the level of each volatile compound and the sensory evaluation were studied. However, the threshold value was one of the important indexes for the aroma of foods. One high level of volatile compound detected in current research might possess a low threshold value, resulting in the difficult to perceive. Therefore, there is a need to carry out additional experiments to investigate the more detail impact of volatile compounds on the final quality of both soy sauce and miso in combination with their threshold values.

The current research was focused on the final quality of the commercial products, which are finished the fermentation and had fixed properties. In general, soy sauce and miso are mainly prepared by koji making, moromi fermentation, and refining. Moromi fermentation takes a long period and is the key process for the quality of fermented soybean foods. In order to manufacture high-quality commercial products, future work is necessary to provide additional scientific data for the purpose of standardization and quality improvement of soy sauce or miso production. Based on the results of the current research, it is promising to develop the flexible models by selecting relevant variables, including pH, salt concentrations, sugars, acids, amino acids, and certain microbes, for monitoring the conditions of moromi fermentation to produce the desired commercial products.

Finally, although the two types of the model obtained from the current research have good prediction preferences for assessing the final qualities of both soy sauce and miso, there is still a need for further investigation to validate the applicability of these models on a variety of soy sauce and miso products produced in other Japan's region. In addition, further work for both two types of the models is needed to expand the sample capacity and optimize the selection of variables, or reduce the number of variables, for increasing the accuracy and range of use of the models, especially for miso products.