（研究科様式　博７添付）

氏　　　　　　　名	王　碩（おう　せき）
授　与　学　位	博士（生物資源科学）
学位授与年月日	令和２年３月２４日
学位授与の根拠法規	学位規則第４条第１項
研　究　科　専　攻	秋田県立大学大学院生物資源科学研究科 博士後期課程　生物資源科学専攻
学　位　論　文　題　目	Studies on the quality characteristics of fermented Seasonings (Miso and Soy sauce) from Akita Prefecture and development of quality evaluation methods
指　導　教　員	教授　陳　介余
論　文　審　査　委　員	主査　教授　陳　介余 副査　教授　秋山　美展 副査　准教授　張　函

論　文　内　容　要　旨

Soy sauce and miso are two traditional Japanese fermented condiments that are derived from fermenting soybeans, grains, salt, and water, through microbial fermentation and enzymatic activity. Even though soy sauce is a liquid while miso is a paste, there is a close relationship between soy sauce and miso due to the raw materials used in the preparation and the fermentation process for both are very similar. They were essential condiments for the traditional Japanese cuisine, namely *washoku*, which was designated as UNESCO Intangible Cultural Heritage of Humanity in December 2013. They also had been spread to the foreign countries and integrated into many western cuisines owing to their intense umami tastes and distinct flavors. In present, there is a strong desire for high-quality products of both soy sauce and miso. However, the qualities of soy sauce and miso were mainly determined by sensory evaluation, which is subjective, time-consuming, and costly. The traditional sensory evaluation also could not meet those needs from manufacturer and consumers. Few studies have investigated the development of objective methods for assessing the quality of soy sauce or miso. Consequently, there is a lack of understanding of quality characteristics for Japanese fermented soy sauce and miso products as well as the efficient and rapid evaluation method to assess the final qualities of soy sauce and miso.

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; 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 comprehensive study is carried out to investigate the essential chemical characteristics for the quality improvement of soy sauce and miso products manufactured in the Akita area. 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. Results showed that the high-ranking soy sauce preferred a dark-reddish color and also possessed high levels of sugars, acids (except formic acid), and ethanol (**Figure 1**). Moreover, the level of amino acids, especially for those amino acids with sweet or sour taste, were positively correlated with high-ranking soy sauce. On the other hand, the high-ranking miso samples possessed high levels of $a*$, organic acids, and amino acids, than the low-ranking samples (**Figure 2**). In contrast, the detected sugars in high-ranking miso was lower than that of low-ranking samples, which proposed sugars were probably consumed during fermentation process.　The negative role played by $L*$ in miso quality were also observed. Moreover, the principal component analysis was performed on the measured compositions from soy sauce and miso, respectively. The correlation between the measured compositions and products was further investigated.

In Chapter 3, considering the flavor is a key index for the determination of the qualities of soy sauce and miso products, the tested soy sauce and miso samples were further subjected to the analysis of volatile compounds. The role of the individual flavor compound played in the final quality of soy sauce or miso was investigated. The volatile compounds were directly extracted by headspace analysis to reduce technical interference and to identify the most common compounds in soy sauce and miso. The qualitative results showed that 62 and 48 volatile compounds were detected in soy sauce and miso, respectively. The detected volatile compounds of soy sauce contained ten aldehydes, ten ketones, nine furan(one)s, seven alcohols, seven esters, six sulfur-containing compounds, five pyrazines, three phenols, and two others In the case of miso, the detected volatile compounds contained nineteen esters, nine aldehydes (including furan-2-carbaldehyde), eight alcohols, four ketones, three acids, two sulfur-containing compounds, and three others, no pyrazines and phenols were detected.

The correlation between the detected volatile compounds and the sensory evaluation were investigated. Results (**Figure 3**) showed that most of the detected aldehydes, ketones, furan(one)s, and alcohols had a certain effect on determining the quality of soy sauce product, positively and negatively. Particularly, the detected pyrazines, except 2,6-dimethylpyrazine, played a negative role in determining the quality of soy sauce. In total, 19 volatile compounds were found to positively contribute to the quality of soy sauce, while 23 volatile compounds were negative with the quality. With regarding to the flavor characteristic of miso, results (**Figure 4**) showed most of the detected volatile compounds showed a positive correlation with the final quality, and no volatile compounds showed a certain negative correlation. This indicated that one of the essential characteristics for the high-quality miso product is to have an intense flavor.

In Chapter 4, through the investigation of chemical composition and flavor compounds for soy sauce and miso, the measured compositions, which influenced the color, taste, and flavor of soy sauce and miso products, played a key role in determining the final quality of products. Therefore, it is promising to apply regression analysis to predict the final quality of soy sauce and miso products. The regression analysis for predicting the sensory scores of soy sauce and miso products is performed on the measured chemical composition and volatile compounds, respectively. Totally, 74 parameters from the tested soy sauce samples and 87 parameters from the tested miso samples were considered as predictive variables, respectively. However, these predictive variables might be complex and interconnected resulting in a decrease in accuracy. In order to solve this problem, a novel paradigm, based on the theory of Compressed Sensing (CS), is applied for better identifying and quantifying the contributing components in regression equations then selecting the proper number of the predictive variables. After calculation the contribution of each predictive variable to the sensory score (**Figure 5**), 30 and 32 variables were selected for developing the predictive models of soy sauce and miso, respectively. Subsequently, the models for predicting the final qualities of soy sauce and miso were established using partial least squares regression (PLSR), respectively. The validation test was carried out on the external samples. Results showed: the values of R^2 and RMSEP obtained for the soy sauce validation samples were 0.80 and 11.47 (**Figure 6**); and the values of R^2 and RMSEP for miso validation samples were 0.64 and 21.14 (**Figure 7**), respectively. Both the constructed models for soy sauce and miso were found to be consistent with the process of sensory evaluation.

In Chapter 5, in order to develop rapid detection methods for evaluating the final qualities of soy sauce and miso, respectively, the feasibility of near-infrared (NIR) spectroscopy in combination with PLSR were investigated. The NIR spectra of soy sauce were acquired using transmittance and transflectance modes in the range of 400-1800 nm and 680-2500 nm, respectively. The calibration models were constructed on the obtained full spectrum using PLSR with different pre-treatment methods. The models based on the transflectance spectra showed a better performance than those of transmittance spectra. Further optimization of the models was carried out by selecting the proper ranges in order to reduce interference signals. Finally, the optimized models were constructed based on the 2050 to 2400 nm transflectance spectra region using the pre-treatment of standard normal variate, with a R_P^2 value of 0.78 and a RMSEP value of 11.13 **(Figure 8)**. With regarding to the development of predictive models for miso products, the NIR reflection spectrums was acquired in the range of 400-2500 nm. On the basis of selecting the informative NIR regions, the optimal model of predicting the quality of miso products was constructed in the region from 400 to 1100 nm after pre-treatment with standard normal variate combined with the 1st derivative. The final model provided a R_P^2 value of 0.56 and a RMSEP value of 24.80 **(Figure 9)**. Both the obtained models for soy sauce and miso could predict the final qualities in a few seconds, thus were considered to be suitable for field used.

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.

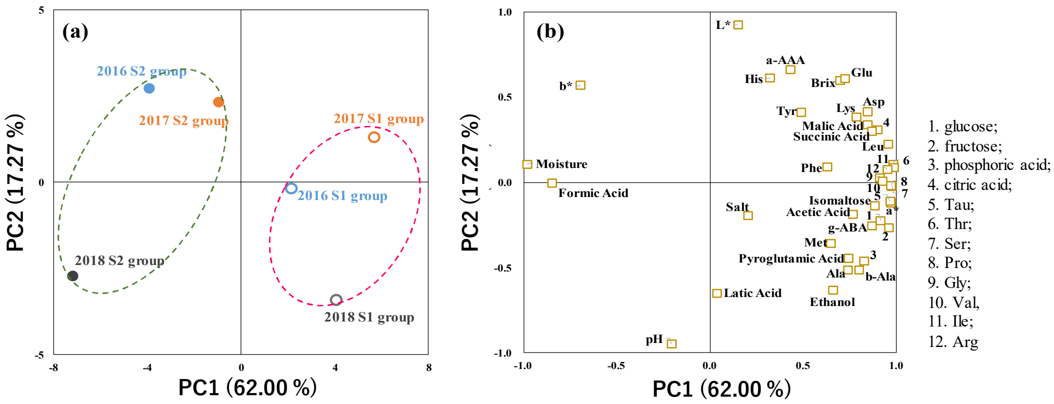

Figure 1 PCA performed on the chemical compositions for soy sauce samples produced from 2016 to 2018: (a) Scores and (b) loadings.

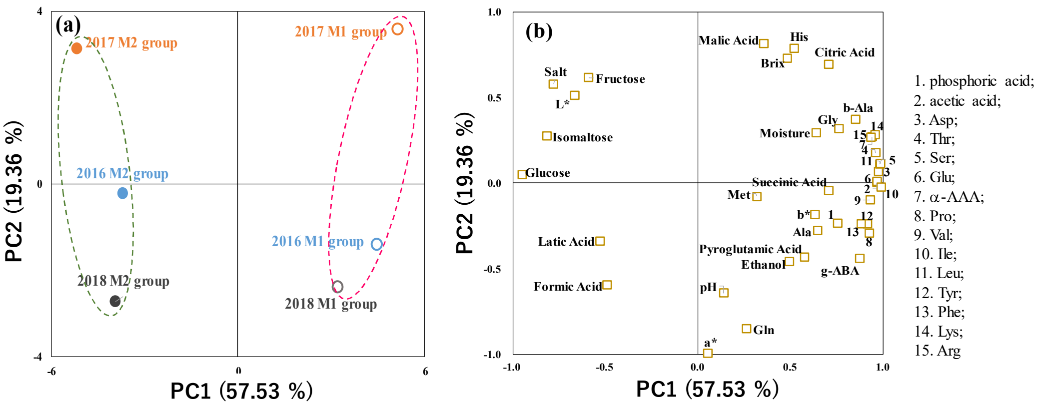

Figure 2 PCA performed on the chemical compositions for miso samples produced from 2016 to 2018: (a) Scores and (b) loadings.

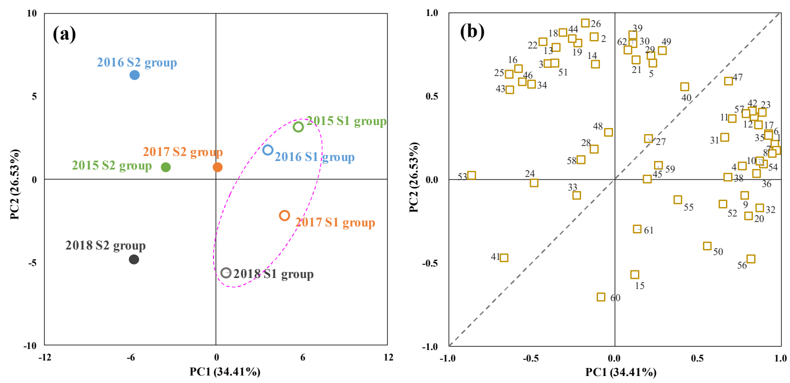

Figure 3 PCA of the average values for the volatile compounds in the

soy sauce groups S1 and S2 from 2015 to 2018: (a) scores; (b) loadings.

1	2-methylpropanal	16	1-methylsulfanylpropane	31	Heptanol	47	1-(1H-pyrrol-2-yl)ethanone
2	butane-2,3-dione	17	Hexanal	32	2-methyloctan-3-one	48	2-ethyl-3,5-dimethylpyrazine
3	butan-2-one	18	3-methylbut-2-enal	33	3-methyloxolan-2-one	49	methylsulfanylcyclohexane
4	ethyl acetate	19	butane-2,3-diol	34	5-methyloxolan-2-one	50	Nonanal
5	2-methylpropan-1-ol	20	ethyl 2-oxopropanoate	35	octane-2,3-dione	51	2-phenylethanol
6	acetic acid	21	ethyl (2S)-2-hydroxypropanoate	36	(5-methylfuran-2-yl)methanol	52	pentyl 2-methylbutanoate
7	3-methylbutanal	22	2-Methylpyrazine	38	Dimethyl Trisulfide	53	2-phenylprop-2-enal
8	2-methylbutanal	23	furan-2-carbaldehyde	39	3-methylsulfanylpropan-1-ol	54	diethyl butanedioate
9	pentan-2-one	24	3-methylpent-3-en-2-one	40	oct-1-en-3-ol	55	(methyltetrasulfanyl)methane
10	1-hydroxypropan-2-one	25	3-methylbutanoic acid	41	octamethylcyclotetrasiloxane	56	3-phenylfuran
11	pentane-2,3-dione	26	furan-2-ylmethanol	42	(5-methylfuran-2-yl)methanethiol	57	2-Phenylcrotonaldehyde
12	3-hydroxybutan-2-one	27	2-ethoxybutane	43	2-ethyl-6-methylpyrazine	58	4-ethyl-2-methoxyphenol
13	3-methylbutan-1-ol	28	furfuryl formate	44	2,3,5-trimethylpyrazine	59	4-ethenyl-2-methoxyphenol
14	2-methylbutan-1-ol	29	methional	45	1-(furan-2-yl)propan-1-one	60	Texanol
15	2-methylpropanoic acid	30	2,6-dimethylpyrazine	46	2-phenylacetaldehyde	61	2,4-ditert-butylphenol
						62	2,2,4-trimethylpentan-1,3-diol-diisobutyrate

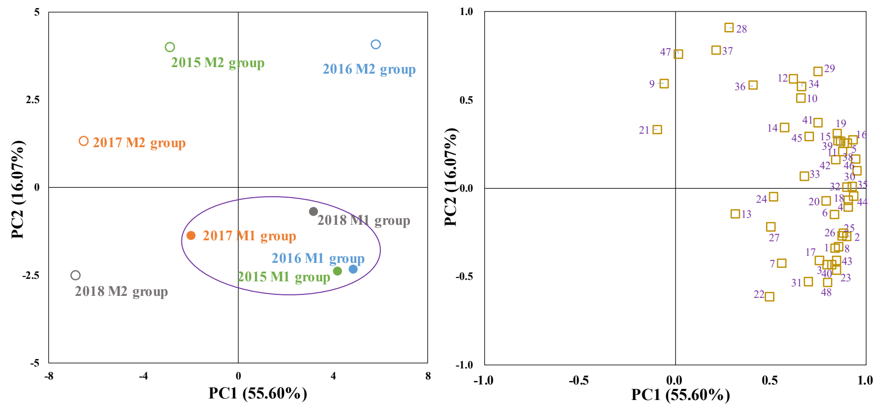

Figure 4 PCA of the average values for the volatile compounds in the miso groups M1 and M2 from 2015 to 2018: (a) scores; (b) loadings.

1	2-Methylpropanal	13	2-Methylbutan-1-ol	25	Ethyl 2-methylbutanoate	38	2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane
2	Butane-2,3-dione	14	2-Methylpropanoic acid	26	Ethyl 3-methylbutanoate	39	Ethyl hexanoate
3	Butan-2-one	15	Ethyl 2-methylpropanoate	27	Ethylbenzene	40	2-Phenylacetaldehyde
4	Ethyl acetate	16	2-Methylpropyl acetate	28	Hexan-1-ol	41	2-Phenylethanol
5	2-Methylpropan-1-ol	17	3-Methylbut-2-enal	29	3-Methylbutyl acetate	42	2,2,4,4,6,6,8,8,10,10-decamethyl-1,3,5,7,9,2,4,6,8,10-pentaoxapentasilecane
6	Acetic acid	18	Ethyl 2-hydroxypropanoate	30	2-Methylbutyl acetate	43	Ethyl benzoate
7	3-Methylbutanal	19	Butane-2,3-diol	31	3-Methylsulfanylpropanal	44	Diethyl butanedioate
8	2-Methylbutanal	20	Ethyl 2-oxopropanoate	32	Oxolan-2-one	45	Ethyl octanoate
9	Pentanal	21	Ethyl (2S)-2-hydroxypropanoate	33	Benzaldehyde	46	Ethyl 2-phenylacetate
10	Ethyl propanoate	22	Butyl acetate	34	Oct-1-en-3-one	47	2-Phenylethyl acetate
11	1,1-Diethoxyethane	23	Furan-2-carbaldehyde	35	3-Methylsulfanylpropan-1-ol	48	(E)-2-phenylbut-2-enal
12	3-Methylbutan-1-ol	24	3-Methylbutanoic acid	37	Octan-3-one		

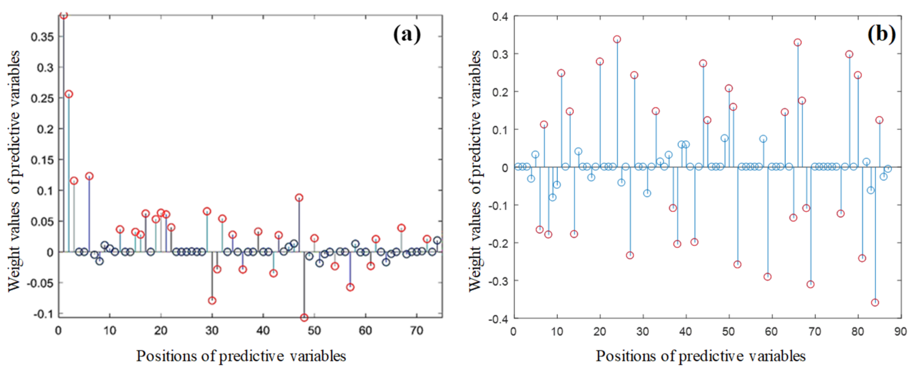

Figure 5 The results of the weight values for each predictive variable: (a) soy sauce; (b) miso. These selected predictive variables were differentiated by using red circles.

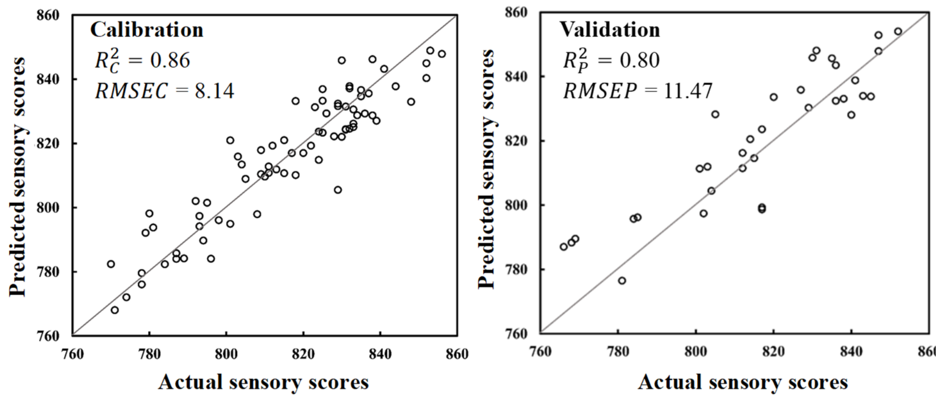

Figure 6 Scatter plot of the actual sensory scores versus the predicted sensory scores of soy sauce products.

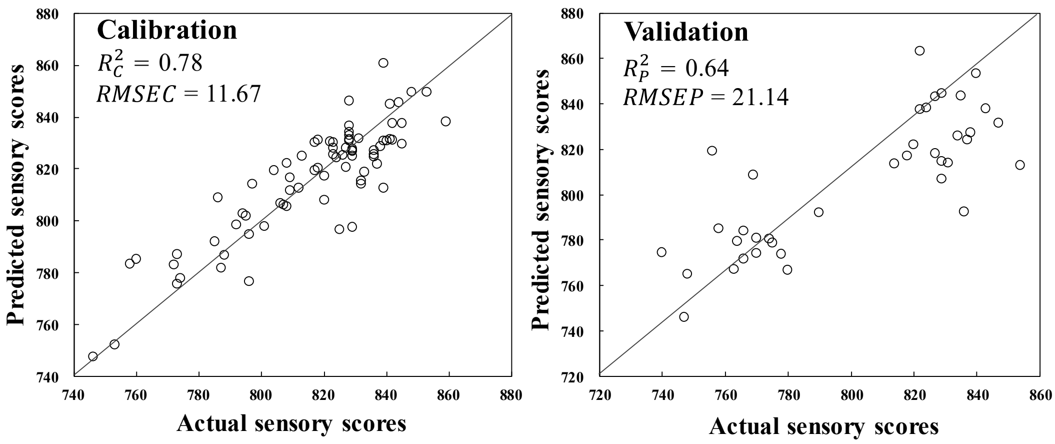

Figure 7 Scatter plot of the actual sensory scores versus the predicted sensory scores of miso products.

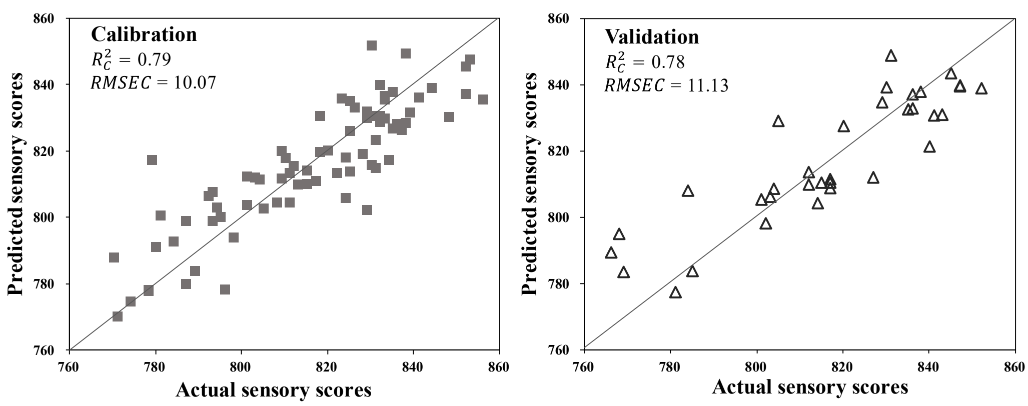

Figure 8 Scatter plot of the actual sensory scores versus the predicted sensory scores of soy sauce products based on the model using the spectra in the range of 2050–2400 nm.

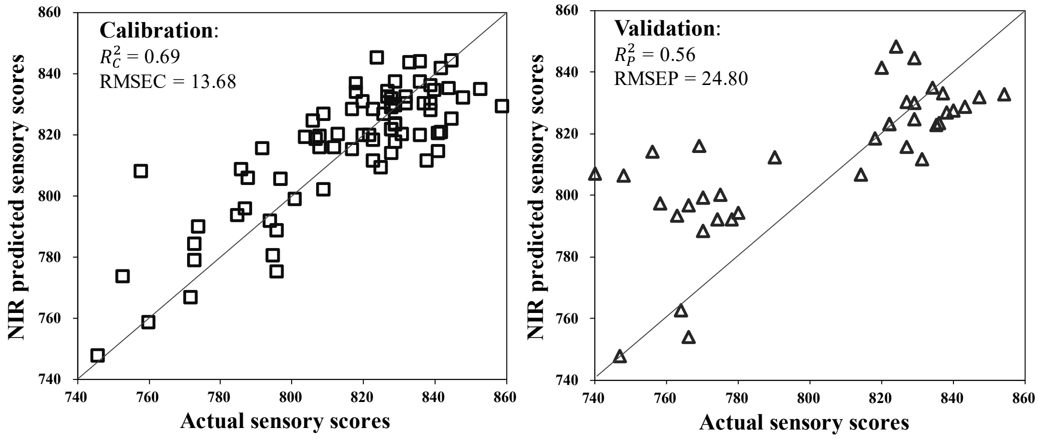

Figure 9 Scatter plot of the actual sensory scores versus the predicted sensory scores of miso products based on the model using the spectra in the range of 400–1100 nm.

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論 文 審 査 結 果 要 旨

地域産伝統調味料の高品質化およびその利用拡大は、地域経済活性化にとって重要である。その利用拡大には、地域産伝統調味料の品質特性を把握する必要だけではなく、客観的品質評価技術および現場でも利用可能な迅速的品質評価技術も必要である。本論文は、秋田県産味噌・醤油の高品質化及び利用拡大という地域発展のニーズに応えたもので、地域産味噌・醤油の品質特性（美味しさの秘密）の解明およびその客観的評価法の開発をめざしたものである。

本論文では、まず、秋田県品評会に出品した味噌・醤油に対し、機器分析法で理化学特性、呈味成分及び匂い成分を詳細に分析した。これらの理化学特性、呈味成分および匂い成分組成に基づいて、高評価されたものと低評価されたものの差異を相関分析や主成分分析などの様々な分析方法で検討を行い、秋田県産味噌・醤油の品質特性を明らかにした。高評価された味噌・醤油は、濃い赤みの色を呈し、高い有機酸含量とアミノ酸含量を有している一方、低評価されたものは逆に高いギ酸含量を有している。また味噌・醤油から検出された揮発成分は実に多く、おおむねアルデヒド類、ケトン類、フラン類、アルコール類、エステル類、およびピラジン類などに分類できた。いずれの種類には味噌・醤油の品質に関与するものが多く、高評価に関与する揮発成分と低評価に関与する揮発成分があった。

次に、品質評価モデル構築のために、従来画像処理で使われる「圧縮法」の利用を提案し、機器分析で得られた大量な理化学特性値、呈味成分値および匂い成分値からから、少数の有用な成分項目を抽出した。抽出された成分項目をベースにし、多変量解析法で最適な味噌・醤油の品質評価モデルの開発に成功しました。開発された予測モデルを用いて 2018 年のものの官能評価値を予測したところ、醤油の場合では予測誤差は 11.5 であり、味噌場合では予測誤差は 21.1 であり、いずれの品質予測モデルの実用性を示した。

さらに、近赤外分光法の迅速的性質や多成分同時分析可能等の利点を利用して、現場でも使える簡便な品質評価方法を提案し、迅速的な味噌・醤油の品質評価技術の開発を試みた。まず、味噌・醤油のそれぞれの性状を考慮して可視・近赤外スペクトルの測定方法を考案した上で、味噌・醤油の可視・近赤外スペクトルと官能評価値との関係を明らかにした。また、実用機器開発の観点から、波長領域の選択やスペクトルの前処理を施した上で、近赤外分光法を活用した迅速的な味噌・醤油の品質評価モデルの開発に成功した。

これらの結果より、本論文ではこれまで分かっていない秋田県産味噌・醤油の品質特性が明らかにしたもので、地域産味噌・醤油などの調味料の高品質化およびその利用拡大に資するものと考えられる。また従来画像処理で使われる「圧縮法」の利用を提案し、機器分析で得られた大量な理化学特性値および各種成分値から有用な成分項目を抽出し、それをベースにした最適な味噌・醤油の客観的な品質評価モデルの開発に成功した。さらに近赤外分光法の利点を利用して、迅速的な味噌・醤油の品質評価法を開発したことから、将来味噌・醤油の製造現場における品質向上や新しい製品の開発において貢献できる重要な結果が得られている。以上の審査結果から、審査員一同は本研究論文の学術的な新規性と実用的な有用性を評価し、博士学位を授与するに値すると判断した。