博士論文要約（Summary）

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タイトル	Studies on the characterizations of functionalities in the rice miso products supplementary with beans and buckwheat at different fermentation periods

「Introduction and Purpose」

In recent years, prevention of lifestyle-related diseases such as obesity, diabetes mellitus is noted, the development of new rice miso products for beneficial and healthy has been expected. As a processed soybean fermented product, rice miso is a traditional Japanese seasoning food which not only prolongs the consumption period, but also has good sensory characteristics. Therefore, we fermented rice miso products by supplementary with black soybean, kidney bean, adzuki bean and buckwheat to improve the potential functionality, and clarify the nutritional components, antioxidant activity and enzyme inhibitory activity of improved rice miso products with different fermentation period.

「Materials and Methods」

For the experiment, we purchased black soybean (*Glycine max*), kidney bean (*Phaseolus vulgaris* L.), buckwheat (*Fagopyrum esculentum*), adzuki bean (*Vigna angularis*), soybean (*Glycine max*), rice-malt (*Aspergillus oryzae*), salt, seed miso from supermarket (Obihiro, Japan). Rice-malt was purchased from the Salt Industry Center (Tokyo, Japan).

The rice miso products were manufactured by a method for industrial producing rice miso. Firstly, yellow soybeans were soaked in water for 16 hours at room temperature (beans: distilled water = 1: 3 (w/w)). Then, autoclaved soaked bean (KT-3045, ALP Co., Ltd., Japan) for 20 min at 110°C. After preparing paste by crushing the steamed beans, mixed (KN1500, Taisho electric MFG. Co., Ltd., Japan) with rice-malt (2.5 kg), salt (1 kg), some seed water, and seed miso (400 g). The mixture was packed in pickle barrels (Shinkigosei Co., Ltd. Japan) and fermented at 30°C.Thereafter, the soaked beans were heated at 110 ° C. for 20 minutes under high pressure. The steamed beans were crushed and mixed with 2500 g rice-malt, 1000 g salt, 400 g season miso and seed water. The 10 000 g mixture was packed in a barrel and fermented at 30 ° C to prepare rice miso (RM; as a control). As mentioned above, rice miso supplementary with black soybean (RM-BS), rice miso supplementary with kidney bean (RM-KB), rice miso supplementary with adzuki bean (RM-AB), rice miso supplementary with buckwheat (RM-BW) were manufactured in the same way. The rice miso products were fermented for 3 months, 6 months, 24 months and 36 months, and a part of the fermented products was cooled at -20°C freezer for chemical analysis.

Subsequently, the peptide, reducing sugar, melanoidin, and polyphenol content were quantified and DPPH radical scavenging activity, ABTS radical scavenging activity, lipase inhibitory

activity and α-glucosidase inhibitory activity of rice miso products with different fermentation period were evaluated. We investigated the influence of these functional components in fermented rice miso products on the antioxidant activity, the effect on the anti-obesity action, and the effect on the inhibition of glucose metabolism.

Each rice miso sample (5 g) were mixed with 20 mL of 80% v/v ethanol, vortexed, and ultrasonicated for 30 min. The suspension was then centrifuged at 1,006 ×g for 10 min. As mentioned above, the same operation was repeated twice. Then, mixed with 20 mL of 70% v/v acetone and the aforementioned process was repeated thrice to take the extract as mixture extract. After that, the mixture extract was concentrated by rotary evaporation in vacuum, and constant volume to 20 mL with distilled water. Then, added the same amount of n-hexane and ethyl acetate to delaminate the solution. Subsequently, a part of water-soluble fraction was purified by chromatography through Diaion HP-20 column. The column was washed by distilled water and then eluted by methanol. The methanol solution was concentrated by rotary evaporation in vacuum, and dissolved in 2 mL of methanol. Furthermore, the eluate was fractionated by Sephadex LH-20 column chromatography. The column was successively eluted with ethanol, methanol, and 60% acetone to collect Fra. I, Fra. II, and Fra. III, respectively.

Melanoidin, peptide, reducing sugar, and polyphenol content, DPPH radical scavenging activity, ABTS radical scavenging activity, lipase inhibitory activity, and α-glucosidase inhibitory activity contained in each fraction were quantified.

「Results and Discussion」

The results showed that the melanoidin and polyphenol content was increased since the fermentation period of rice miso products were processed, and the ratio between the melanoidin and polyphenol content was increased from about 3 folds to 6 folds. Moreover, since the protein and carbohydrate are decomposed by rice koji malt (*Aspergillus oryzae*), peptide and reducing sugar content were increased up, and then for the melanoidin is synthesized, peptide and reducing sugar content were sharply decreased. In addition, the peptide, reducing sugar, melanoidin, and polyphenol content contained in rice miso products supplementary with black soybeans, kidney bean, adzuki bean, and buckwheat were significantly higher than that of rice miso (control) ($p < 0.05$).

DPPH radical scavenging activity and ABTS radical scavenging activity contained in the rice miso product were also increased with the prolongation of the fermentation period, and the RM-BS, RM-KB, RM-AB, and RM-BW were significantly higher than those of rice miso (RM) ($p < 0.05$). Furthermore, the melanoidin content and the polyphenol content have a high positive correlation between DPPH radical scavenging activity and ABTS radical scavenging activity, respectively, and since the melanoidin content was about 6 folds higher than the polyphenol content, it was recognized that the substances contributed to antioxidant activity in rice miso products were mainly melanoidin.

The lipase inhibitory activity and α-glucosidase inhibitory activity of rice miso products were also increased with the prolongation of the fermentation period. however, and the maximum value of RM-BS was detected at fermented after 36 months, and the highest value of RM-KB, RM-AB, RM-BW, and RM were detected at fermented after 24 months. Moreover, the lipase inhibitory activity and α-glucosidase inhibitory activity of RM-BS, RM-KB, RM-AB, and RM-BW were

significantly higher than that of RM (p <0.05). There was also a correlation between melanoidin content, polyphenol content and lipase inhibitory activity the correlation coefficient was 0.7405 and 0.7754 at 0.2g DW miso and a correlation between melanoidin content, polyphenol content and α-glucosidase inhibitory activity with the correlation coefficient 0.6206 and 0.6314 at 0.2g DW miso, respectively.

「Conclusion」

From the above results, melanoidin and polyphenol content, DPPH radical scavenging activity, ABTS radical scavenging activity, lipase inhibitory activity, and α-glucosidase inhibitory activity in various rice miso products increased with prolonging fermentation periods, and RM-BS, RM-KB, RM-AB and RM-BW were significant higher than those of RM, respectively. Moreover, RM-BS has the highest value at different fermentation period.

Both melanoidin and polyphenol content have high positive correlations with DPPH radical scavenging activity, ABTS radical scavenging activity, lipase inhibitory activity, and α-glucosidase inhibitory activity, respectively. Due to the ratio of melanoidin and polyphenol content was from approximately 3:1 (3 Months fermentation) to 6:1 (36 Months fermentation) with the prolonging fermentation period, and melanoidin ratio was increased, we speculated that melanoidins were the mainly functional component rather than polyphenols in the rice miso products.

We suggest that rice miso supplementary with high-performance raw materials, such as black soybean, kidney bean, adzuki bean, and buckwheat could increase useful components and improve the functionality, which may exert health beneficiary, in particular, potential applications for high antioxidant activity, and suppression of diabetes and body weight decrease.