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α-Glucosidase Inhibitory Activity in Rice Miso Supplementary with Black Soybean

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Abstract In the present study, we processed rice miso supplementary with black soybean (RM-BS), and analyzed α-glucosidase inhibitory activity, polyphenol and melanoidin content at different fermentation periods (3, 6, 24, 36 months). The results showed α-glucosidase inhibitory activity, polyphenol and melanoidin content in RM-BS were increased with prolonging the fermentation periods. The α-glucosidase inhibitory activity in RM-BS was significantly stronger at different fermentation periods than those of RM, respectively. There were high positive relationships between α-glucosidase inhibitory activity and polyphenol content, and melanoidin content, respectively. Moreover, the ratio of melanoidin and polyphenol content was increased with prolonging the fermentation periods. So thus, we speculated that melanoidins were the main α-glucosidase inhibitory activity component in RM-BS. These results could be useful on researching and developing of rice miso products.

Keywords: *rice miso, black soybean, α-glucosidase inhibitory activity, polyphenol, melanoidin*

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1. Introduction

In recent years, dietary life of people is more inclined to meat [1], dairy products [2], vegetable oils, tobacco, sugary foods, Coca-Cola, and alcoholic beverages. These unhealthy dietary habits are at high risk of developing life-style diseases, such as diabetes, obesity, cancer, and metabolic syndrome [3,4,5,6] for middle aged and elderly people. Moreover, more and more young people are suffering from these diseases [7].

Soybeans and their fermented products such as touchi, natto, tempeh played significant roles in the Asian diets [8,9,10]. As a traditional fermentation food in Japan, rice miso demonstrated some beneficial effects, such as lipid peroxidation-inhibiting action, anti-hypertension, anti- mutagen action [11,12].

In the Compendium of Materia Medica, black soybeans were used as a traditional Chinese medicine. Yoshida, 2013 [13] reported that black soybeans have blood-activating, detoxifying and diuresis-promoting. Moreover, black soybean contained a large amount of dietary fiber, flavonoid and anthocyanin, and these components have with strong antioxidant activity, glycolysis-inhibitory activity, anti-obesity, anti-cancer and hepato-protective effect [14,15,16,17,18].

In this research, we processed rice miso supplementary with black soybean (RM-BS), and detected α-glucosidase inhibitory activity, polyphenol and melanoidin content at different fermentation periods.

2. Materials and Methods

2.1. Materials and Chemicals

Black soybean (*Glycine max*), soybean (*Glycine max*), rice-malt, salt, and seed miso were purchased from supermarket (Obihiro, Japan). Rice-malt was purchased from the Salt Industry Center (Tokyo, Japan). The rice miso products were manufactured and sampled for analysis at 3, 6, 24, 36 Months (M) of fermentation.

Folin-Ciocalteau reagent was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). DNS (3,5-dinitrosalicylic acid) were purchased from Sigma-Aldrich Co., LLC. (Tokyo, Japan). Glucose and glycine were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). The other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Extraction and Fractionation

The RM-BS was manufactured by industrial producing rice miso and extracted by 80% ethanol and 70% acetone [19]. The extracts of RM-BS were concentrated and dissolved by distilled water. The distilled water fraction was delaminated by n-hexane and ethyl acetate. Then, the water layer was fractioned by HP-20 column. After HP-20

column, the methanol fractions were analyzed as the following experiments.

2.3. α-Glucosidase Inhibitory Activity Assay

α-Glucosidase inhibitory activity was determined by the improved DNS method [15]. A mixture of 0.5 mL of sample extract with different concentration (0.05, 0.1, 0.2 g) and 0.5 mL of the α-glucosidase solution was pre- incubated at 37°C for 10 min to prepare solution I. A mixture of 50 μL of 0.4% sucrose solution, 625 μL of 0.1M Na_2PO_4 buffer (pH 6.8) and 125 μL of 1% NaCl was pre-incubated at 37°C for 10 min to prepare solution II. Then, 200 μL of solution I was mixed with solution II and incubated at 37°C for 30 min. The enzyme reaction was stopped by adding 125 μL of 2 N NaOH (added 2 N NaOH before incubation for blank). DNS solvent (1%, 125 μL) was added and reacted in boiling water bath for 10 min. Absorbance was measured at 540 nm. Standard curves were using glucose calibration, and expressed as α-glucosidase inhibitory activity (%).

$$\alpha - \text{Glucosidase inhibitory activity (\%)} = \left[1 - \left(\frac{A_{sample} - A_{blank}}{A_{control} - A_{test}}\right)\right] \times 100\%$$

where A_{sample} is the absorbance of the mixture of sample, sucrose solution, enzyme and DNS solvent; A_{blank} is the absorbance of the mixture of sample, sucrose solution and DNS solvent without enzyme; $A_{control}$ is the absorbance of the mixture of buffer (instead of sample), sucrose solution, enzyme and DNS solvent; A_{test} is the absorbance of the mixture of buffer (instead of sample), sucrose solution and DNS solvent without enzyme.

2.4. Polyphenol and Melanoidin Content Determination

Polyphenol content was determined using the method of Folin-Ciocelteau [22] with slight modifications. Each sample (100 μL) was mixed with 300 μL of distilled water, 400 μL of 50% Folin-Ciocelteu reagent, and 400 μL of 10% Na_2CO_3 aqueous solution. The reaction solution was incubated at 30°C for 30 min, and centrifuged at 1,006 ×g for 10 min. Thereafter, the absorbance was measured at 760 nm, and results were expressed as the mg catechin per gram DW miso ($y = 10.709x - 0.9949$, $r^2 = 0.9931$).

Melanoidin content was determined by the method of Martins [23]. Firstly, glucose and glycine was mixed as melanoidin standard. Then, melanoidin standard with different concentration (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg / mL) and each sample (100 μL) was mixed with 900 μL of distilled water. The absorbance was measured at 450 nm. The results were expressed as the mg melanoidin per gram DW miso ($y = 61.333x + 1.0356$, $r^2 = 0.9994$).

2.5. Statistical Analysis

The experiments were repeated at least three times. Data were expressed as means ± standard deviation. Significant differences were determined by one-way ANOVA and Fisher's test (SAS v. 7.1, SAS Institute Inc., Cary, NC, USA). Differences were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1. α-Glucosidase Inhibitory Activity in RM-BS

α-Glucosidase inhibitory activity of RM-BS and RM were shown in Figure 1. α-Glucosidase inhibitory activity in RM-BS and RM were significantly increasing with prolonging fermentation period, and the strongest inhibitory activity was shown at 24 Months fermentation. Moreover, α-glucosidase inhibitory activity at 3, 6, 24, 36 Months fermentation in RM-BS were significantly higher than those of RM (control; rice miso), respectively. α-Glucosidase inhibitory activity in rice miso supplementary with kidney bean at different fermentation periods were also significantly higher than those of rice miso [24]. There are also reported that soybean paste, natto, and touchi have α-glucosidase inhibitory activity [25,26,27].

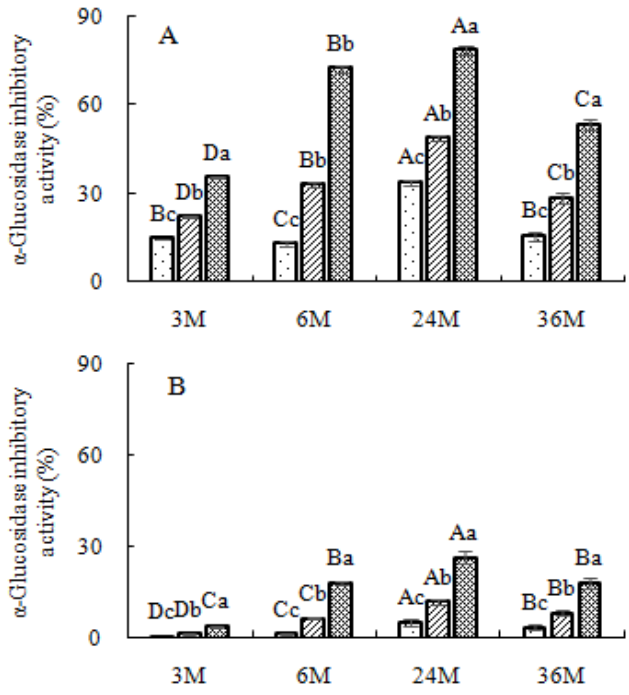

Figure 1. α-Glucosidase inhibitory activity contained in rice miso products with different fermentation periods. A, RM-BS. B, RM. □ for the 0.05 g DW miso; ▨ for the 0.1 g DW miso; ▩ for the 0.2 g DW miso. Values within a column followed by different small letters and values within a row followed by different capital letters are significant at $p < 0.05$.

3.2. Polyphenol and Melanoidin Content in RM-BS

The results of polyphenol and melanoidin content in RM-BS were shown in Table 1. Polyphenol content was increasing with prolonging the fermentation periods. The highest polyphenol value of RM-BS was shown at 36 Months fermentation (3.4 mg/g DW miso). The polyphenol content of RM-BS was increased by 3.9 folds

from 3 months to 36 months. There are similar results of total phenolic contents in soybean fermented products were increased with extension of fermentation [28,29].

We found positive relationships between α-glucosidase inhibitory activity and polyphenol content, and melanoidin content, respectively. The correlation coefficients were 0.8148 for former, and 0.7686 for latter, respectively (Figure 2). Glucose-amino acid model MRPs showed α-glucosidase inhibitory activity [30]. Polyphenolic compounds are strong inhibitors of α-glucosidase inhibitory activity, and serve as a potent alternative to avoid the adverse effects [31]. The ratio of melanoidin and polyphenol content was approximately 3:1 at 3 Months fermentation, and that of 6:1 at 36 Months fermentation. Melanoidin ratio was increased with the prolonging fermentation period. We speculated that melanoidins were the main α-glucosidase inhibitory activity component rather than polyphenols in RM-BS.

Table 1. Polyphenol and melanoidin content in RM-BS with different fermentation periods

Sample	Polyphenol content (mg/g DW miso)	Melanoidin content (mg/g DW miso)
RM-BS-3M	0.9 ± 0.1 Bd	3.1 ± 0.1 Ad
RM-BS-6M	2.1 ± 0.3 Bc	11.4 ± 0.5 Ac
RM-BS-24M	2.9 ± 0.3 Bb	15.6 ± 0.3 Ab
RM-BS-36M	3.4 ± 0.4 Ba	19.9 ± 0.8 Aa

Abbreviations: M, month; DW, dry weight; RM-BS, rice miso supplementary with black soybean. Data represent the mean ± SD from at least three independent studies. Values within a row followed by different capital letters and values within a column followed by different small letters are significant at $p < 0.05$.

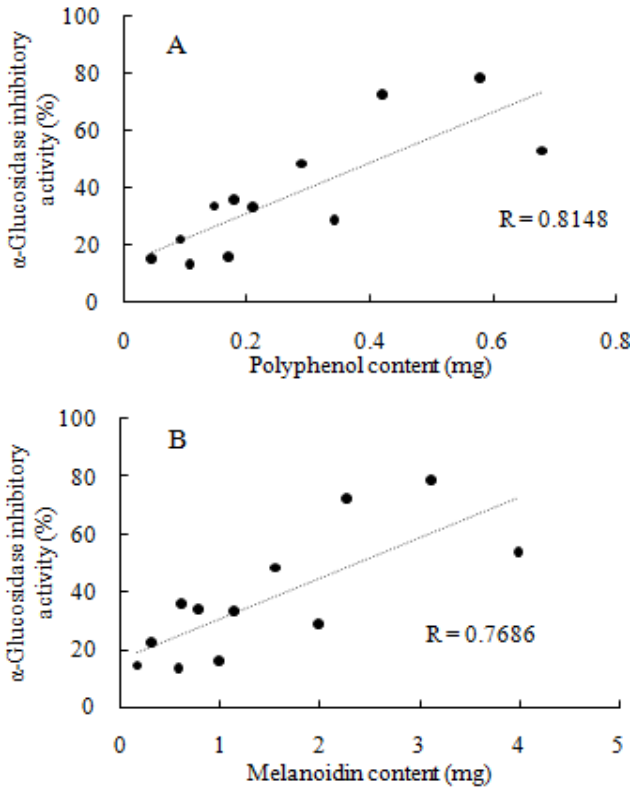

Figure 2. Relationship between α-glucosidase inhibitory activity and polyphenol content, melanoidin content in RM-BS

4. Conclusion

In the present study, we detected α-glucosidase inhibitory activity, polyphenol and melanoidin content in RM-BS were increased with prolonging fermentation periods. Moreover, the α-glucosidase inhibitory activity in RM-BS was significantly higher than RM. There were high positive relationships between α-glucosidase inhibitory activity and polyphenol content, and melanoidin content, respectively. Therefore, we speculated that the supplementation of black soybean in rice miso could improve α-glucosidase inhibitory activity with prolonging fermentation periods. As comparing with polyphenols, melanoidins maybe the main component for α-glucosidase inhibitory activity in RM-BS.

Acknowledgments

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

M: month; RM: rice miso; RM-BS: rice miso supplementary with black soybean; DW: dry weight; DNS: 3, 5-dinitrosalicylic acid.

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