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Miso production using koji mold isolated from *Rhus javanica* nurude leaves

Abstract

In this study, we isolated a koji mold from urushi (*Rhus javanica*) leaves and used it to produce soybean miso. The general component quantification of the produced soybean miso indicated moisture, protein, lipid, ash, and carbohydrate contents of 45.2, 16.0, 8.6, 19.0, and 11.2g/100g, respectively. Judging from the components and color, this soybean miso was sufficiently matured. Moreover, despite the very short maturation period of 3 months, the components did not significantly differ from those of general miso, and the decomposition of the raw materials was good. We confirmed that koji mold decomposes the raw materials well and that miso could be produced using urushi leaves. Based on these results, we inferred that the koji mold isolated from *Rhus javanica* display a high protease activity, as the protein content was high despite the short maturation period of 3 months.

Keywords: koji mold, *Aspergillus oryzae, Rhus javanica*, soybean mis

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Introduction

Certain documents[1–3] have been found in Japan during the Kamakura period, describing that soy sauce was made from *Rhus javanica* (hereafter referred to as Nurude) leaves. Based on these references, we isolated koji mold *Aspergillus oryzae* from Nurude and produced soybean miso.

Nurude is a member of the *Rhus* genus of the *Anacardiaceae* family, also known as Katsunoki, Kachinoki, and Fushinoki.[4] This Nurude is a deciduous shrub found throughout Japan. The leaf axis is winged, and the galls that grow on the leaves are rich in tannin, it was thus used as a mordant for herbal medicines and for blackening teeth. In certain regions, it was revered as a tree with magical powers believed to be held by ancient and primitive societies, and was considered a tree that protects against illnesses and disasters.[5]

Natural koji mold grows on the leaves of Nurude trees, and although this koji mold is said to have long been used for miso and soy sauce preparation, such production is currently unknown. Although the "Illustrated Guide to Native Trees"[6] mentions that koji mold grows on Nurude, this is the only currently available reference, and no references could be found that describe Nurude leave use in miso or soy sauce production.

Therefore, in this study, we aimed to produce soybean miso using Nurude leaves.

Material and methods

Raw materials

We collected Nurude leaves from a tree growing in the Atsugi campus of Tokyo University of Agriculture (Figures 1 & 2). We used Tsukui local soybeans from Atsugi City, Kanagawa Prefecture (Figure 3).

Soybean miso production

The koji mold was isolated from the collected Nurude leaves as follows. We lightly washed the leaves with water, soaked them in physiological saline for 20h, inoculated them onto a standard agar medium (meat extract, peptone, sodium chloride, and agar; pH 7) and cultured at 35°C for 2 days (Figure 4). A testing agency certified that the isolated koji mold was not producing aflatoxins.

Figure 1 *Rhus javannica* nurude.

Figure 2 Leaves of *Rhus javanica* nurude.

Figure 3 Tsukui local soybeans from Atsugi City, Kanagawa prefecture.

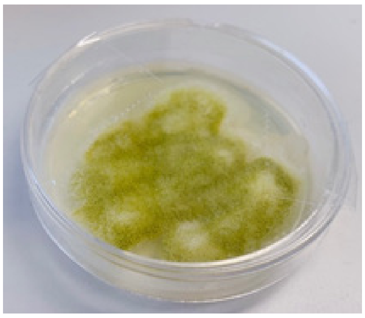

Figure 4 Koji mold *Aspergillus oryzae* : cultured at 35℃ for 2 days.

We placed the isolated koji mold spores on 500g of steamed soybeans, mixed them well, and cultured them at 35°C for 2 days to produce soybean koji, which we then mixed with 200g of salt to make shio koji, which, in turn, we minced, supplemented with 500g of minced steamed soybeans, and mixed well. The total amount of the preparation was 1200g with a salt concentration of 16%. We packed the container airtight and let the mixture age at 35°C for 3 months to produce soybean miso (Figure 5).

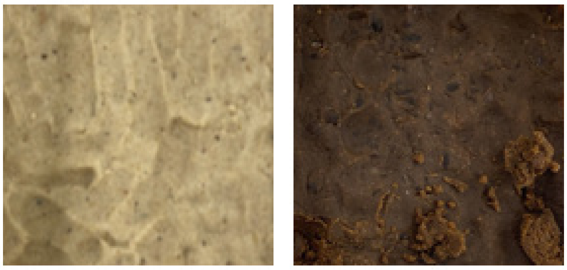

Figure 5 Nurude soybean miso.

Right: Immediately after production, Left: Matured for 3 months at 35℃.

Soybean miso general component analysis

We performed the general analysis of soybean miso according to the measurements in the Standard Tables of Food Composition in Japan, measuring moisture, protein, lipids, ash, and carbohydrates.

Moisture:[7] We applied the normal pressure heat drying method as follows. We weighed 2g of miso accurately, dried it at 135°C for 3 h, and then cooled it. We considered the weight lost as the moisture content.

Protein:[8] We measured the protein content using the Kjeldahl nitrogen method, placing 2g of miso in a digestion flask, supplemented it with 2g of decomposition accelerator and 30 mL of concentrated sulfuric acid, and let the mixture decompose by heating in a fume hood. After the digestion was completed, we diluted the mixture to 250 mL with water. We used a Parnas–Wagner distillation apparatus to distill 10 mL of the digestion dilution solution and 10 mL of 30% sodium hydroxide, using 10 mL of 0.1 M sulfuric acid with an indicator as the receiver. We terminated the distillation when the distillate reached 80 mL.

We titrated the excess sulfuric acid that was not neutralized by the distilled ammonia with 0.1M sodium hydroxide solution, and determined the titration volume, using green color as the end point. We set the nitrogen–protein conversion to 5.71.

Lipid:[9] We measured the lipid content using the Soxhlet extraction method, dissolving 5g of miso in 100 mL of hot water, which we mixed with diatomaceous earth, placed in a cylindrical filter paper, extracted with ether for 10 h, and dried the extract at 105°C and determined the constant weight.

Ash:[10] We measured the ash content by precisely weighing 2g of miso, completely incinerating it at 550°C for 5h, allowing it to cool, then weighing it to determine a constant weight.

Carbohydrate:[11] To calculate the carbohydrate content, we subtracted the moisture, protein, fat, and ash contents from 100.

Measurement of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Antioxidant activity was assessed by measuring DPPH radical scavenging activity. The sample solution was a 10- fold diluted miso solution, centrifuged at 3000 rpm for 5 minutes, and the supernatant was used. A mixture of 12 ml of 400 µM DPPH, 12 ml of 200 mM 2-morpholinoethanesulfonic acid buffer (pH 6.0), and 12 ml of 20% ethanol was prepared. 0.3 ml of the sample solution diluted with 80% ethanol was added to 0.9 ml of this mixture and allowed to react for 20 minutes. The reaction solution was measured at 520 nm, and the antioxidant activity was calculated from the rate of decrease in absorbance. A calibration curve was prepared using Torolox, and the antioxidant activity was evaluated using the Torolox conversion method.

Results and discussion

The general analysis of the nurude soybean miso

The results of the general analysis of the soybean miso we produced are as follows (Table 1).

Table 1 General component analysis of nurude soybean miso (g/100g miso)

Moisture	Protein	Fat	Ash	Carbohydrate
45.2	16	8.6	19	11.2

Moisture content

The moisture content was 45.2 g/100 g, while the average value of the National Miso Evaluation Association of Japan is 43.0 g/100 g. Therefore, the miso produced in this study exhibited a high moisture content. Although soybean miso is generally hard and difficult to dissolve, due to its high moisture content, this miso was easy to dissolve.

Protein content

The nitrogen content was 2.80 g/100 g, and the protein content was 16.0 g/100 g, representing only a 1-g difference from the average value of 17.0 g/100 g of the National Miso Evaluation Association, potentially due to the nitrogen content of the soybeans used as raw materials.

Fat content

The fat content was 8.6 g/100 g, representing a low value compared to the average 10.5 g/100 g of the National Miso Evaluation Association, also confirming that we produced a low-calorie miso.

Ash content

The ash content was 19.0 g/100 g, being approximately 1.5 times higher than the average value of 12.9 g/100 g from the National Miso Evaluation Association. We inferred that the mineral content was high, resulting in a functional miso.

Carbohydrate content

The carbohydrate content was 11.2 g/100 g, which was approximately 1.5 times lower than the average value of

16.6 g/100 g from the National Miso Evaluation Association.

Antioxidant activity of nurude soybean miso

Recently, the antioxidant activity of foods has been attracting a lot of attention as a food functionality. When the antioxidant activity of Nurude soybean miso was measured, it was confirmed that Nurude soybean miso had 0.307 mol Trolox per 100g, which was about 2.3 times higher than that of commercially available soybean miso (0.134 mol Trolox/100g miso). From these results, it was confirmed that Nurude soybean miso is a functional miso with antioxidant activity.

Although miso is generally aged for 6 months to 1 year, we let this miso age for only 3 months, representing a very short time. However, we confirmed that the components of this miso were not markedly different from those of regular miso, and that the koji mold had broken down the raw materials well.

In the future, we plan to measure the glutamic acid content to confirm whether this miso exhibits a high umami content. We also plan to examine the mineral content to verify the functionality of the miso, and to investigate the amylase and protease activities of the koji mold.

Conclusion

In this study, we isolated koji mold from the leaves of Rice Nurde and used it to produce soybean miso. The general component measurements revealed that the moisture, protein, lipid, ash, and carbohydrate content of the produced soybean miso was 45.2 16.0, 8.6, 19.0, and 11.2 g/100g, respectively. This soybean miso was fully matured, and despite the very short aging period of 3 months, the components were not significantly different from those of regular miso, with the raw materials being well-decomposed. Therefore, we successfully confirmed that Nurde leaves could be used for miso production. From these results, we inferred that the koji mold isolated from Nurde displayed high protease activity, as the protein content was high, despite the short aging period of 3 months. In addition, Nurude miso had approximately 2.3 times the antioxidant activity of commercially available soybean miso, making it a functional miso.

Currently, miso is produced by using controlled spores to make koji. However, if the Nurude (*Rhus javanica*) plant is available, miso could be produced overseas as well. Japanese food is gaining attention around the world. Miso has been a traditional Japanese food used in popular Japanese dishes such as miso soup and miso ramen. Its production overseas is very difficult because Koji mold cannot be exported, and miso export as a processed product also encounters challenges. However, Nurude is lived both in Japan and overseas, it might thus be potentially used for overseas miso production. Finally, in the future, we also plan to produce rice miso (Figure 6).

Figure 6 Nurude soybean miso and cutted leaves.

Acknowledgments

None

Conflicts of interest

The authors declare that there are no conflicts of interest.

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