

# Production and component analysis of miso-like seasoning using kidney beans

## Abstract

As part of a study on the processing and use of beans as functional foods, this study attempted to develop a new type of miso using kidney beans, red kidney beans, pinto beans, and large white kidney beans as ingredients. This study has been the first to attempt producing miso using kidney beans given the absence of related studies. Our results showed that miso made using kidney beans, pinto beans, and large white kidney beans all had higher peptide and amino acid contents, which are umami components, than did rice miso and were strong-tasting and highly nutritious. Furthermore, we were able to produce functional miso with antioxidant effects, high gamma-aminobutyric acid content, and high polyphenol content, which was not possible with rice miso. These miso were highly evaluated for its color, aroma, and taste. Based on our findings, new products using kidney beans can be expected in the future.

**Keywords:** miso-like seasoning, kidney beans, Miso production, component analysis, Analysis of miso components with an antioxidant effect

Volume 12 Issue 3 - 2024

Akiko Yamada Taniguchi, Machiko Kazami, Haruko Noguchi

Department of Agricultural Innovation for Sustainability, Faculty of Agriculture, Tokyo University of Agriculture, Japan

**Correspondence:** Akiko Yamada Taniguchi, Department of Agricultural Innovation for Sustainability, Faculty of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 243-0034, Japan, Email akiko@nodai.ac.jp

**Received:** November 23, 2024 | **Published:** December 18, 2024

## Introduction

The current study, which forms part of a study on the processing and use of beans in functional foods, developed a new type of miso using kidney beans as the carbohydrate component instead of rice or barley. Miso<sup>1</sup> is traditionally made from *koji* (a type of rice or barley with *koji* mold), salt, and soybeans, without using kidney beans. Considering their high starch and low lipid content, kidney beans can be used as an alternative to rice or barley. Moreover, kidney beans are rich in minerals, polyphenols, and dietary fiber; hence, miso produced from kidney beans can be considered functional. This study aimed to improve the taste of miso, shorten its aging period, and improve food functionality by using kidney beans and rice koji.

## Material and methods

### Experimental samples

Three types of kidney beans were used for miso production: red kidney beans, pinto beans, and large white kidney beans. All kidney beans were purchased from Ninohe City, Iwate Prefecture (Figure 1). Japanese soybeans (Yuzuru, from Akita Prefecture) were used as the soybean raw material. Regular salt was used during the miso production. The *koji* mold used was rice miso seed *koji* (manufactured by Bio'c Co., Ltd.) and *Aspergillus oryzae*.



**Figure 1** Three types of kidney beans used as samples.

Left: Red kidney bean, Right: Pinto bean, Top: Large white kidney bean.

### Miso production.<sup>2-4</sup>

Kidney beans, red kidney beans, pinto beans, and large white kidney beans were washed, soaked, drained, steamed, cooled to around 37°C, pressed, and mixed with steamed soybeans. After producing rice koji, salt was added, and the *koji* and raw materials were mixed together and aged for 6 months to produce the product. Three types of miso were produced using each kidney bean (Figure 2). The ingredients used were kidney beans, soybeans, and rice *koji*, each mixed in equal amounts (1/3 each). Specifically, 2 kg of kidney beans, 2 kg of soybeans, 2 kg of rice *koji*, and 1.2 kg of salt were used.



**Figure 2** Kidney bean miso manufacturing process.

## General components of miso<sup>5-7</sup>

General analysis of the produced miso was performed according to the following methods.

### Moisture

Moisture was measured using the normal pressure heating and drying method. In particular, 2g of miso was weighed out, heated, dried at 135°C for 3h, and then cooled, with the weight lost being taken as the moisture content.

### Ash content

Ash content was measured by weighing out 2g of miso, completely incinerating it at 550°C for 5h, and then weighing and determining a constant weight after cooling.

### pH

After grinding the miso, an equal amount of water was added to it, after which the pH was measured using a glass electrode.

### Acidity I, II

After diluting 5g of miso in 50 mL of water, 40 mL of water was added to 10 mL of the sample solution and titrated with N/10 sodium hydroxide while measuring the pH. The amount of titration solution (in milliliters) required to reach a pH of 7.0 was defined as titratable acidity I. After further titration was performed, the amount of 0.1 M sodium hydroxide solution (in milliliters) required to reach a pH of 8.3 from 7.0 was defined as titratable acidity II.

### Total nitrogen

Total nitrogen was measured using the Kjeldahl nitrogen method. Accordingly, 2g of miso was placed in a digestion flask to which 2g of decomposition accelerator and 30 mL of concentrated sulfuric acid were added. Thereafter, the mixture was heated and decomposed in a fume hood. After the digestion was completed, the mixture was diluted to 250 mL with water. A Parnas–Wagner distillation apparatus was used for distillation. Afterward, 10 mL of the digestion dilution solution and 10 mL of 30% sodium hydroxide were distilled, and 10 mL of 0.1 M sulfuric acid with an indicator was used as the receiver. Distillation was completed once the distillate reached 80 mL. Excess sulfuric acid not neutralized by the distilled ammonia was titrated with 0.1M sodium hydroxide solution to obtain the value.

### Water-soluble nitrogen

After diluting 5g of miso in 50 mL of water, the extract was measured for water-soluble nitrogen using the Kjeldahl method as described in the measurement of total nitrogen.

### Formol nitrogen

After diluting 5g of miso in 50 mL of water, 1% phenolphthalein solution was added to 10 mL of the sample solution and titrated with 0.1M sodium hydroxide solution. Afterward, 5 mL of neutral formalin solution was added, and the liberated acid was titrated again with 0.1M sodium hydroxide solution.

### Salt

Salt content was measured using the Mohr method. After diluting 2g of miso with 200 mL of water, 1 mL of 10% potassium chromate solution was added to 10 mL of this diluted solution and titrated with 0.02M silver nitrate solution.

## Total sugar

After accurately weighing 5g of miso, 200 mL of water followed by 10 mL of 25% hydrochloric acid were added, and the solution was hydrolyzed in a boiling water bath for 3h. After neutralizing with 10% sodium hydroxide solution, 250 mL of water was added to increase the volume of the solution, and the reducing sugar content was measured using the Somogyi–Nelson method.

### Direct reducing sugar<sup>8</sup>

The sample solution was prepared by diluting 5g of miso with 50 mL of hot water. After adding 0.5 mL of alkaline copper reagent to 0.5 mL of the sample solution, the solution was left in a boiling water bath for 10 min and then cooled to room temperature. Thereafter, 0.5 mL of Nelson's reagent was added to the solution, which was then stirred, and 2.5 mL of water was added. The absorbance at 660 nm was then measured. A calibration curve was created using glucose.

### Amount of $\gamma$ -aminobutyric acid in miso<sup>9</sup>

The  $\gamma$ -amino acid sample was prepared by preparing miso extract with a protein content of 0.5 mg/100 mL and passing it through a 0.2- $\mu$ L aqueous filter. The column used was ShimPack AMINO-Na (6 mm I.D.  $\times$  100 mm L.), the mobile phase was 20mm citrate sodium buffer pH 5.9, the column temperature was 40°C, the flow rate was 0.4 mL/min, and the sample injection volume was 10  $\mu$ L. The detector used was a spectro fluorometric RF-10AXL, and detection was performed using NaClO and OPA reagents.

### Amount of polyphenols in miso<sup>10</sup>

The amount of polyphenols was measured via colorimetric quantification using the Folin–Denis method. The sample was prepared by adding 50 mL of water to 5g of miso, stirring in a boiling bath for 60 min, adjusting the volume to 50 mL, and centrifuging at 3,000 rpm for 5 min. The supernatant was used. We then added 1 mL of Folin–Denis reagent to 1 mL of the sample and left it for 3 min. Thereafter, 1 mL of 10% sodium carbonate solution was added to the sample, which was then left for 30 min. The absorbance of this solution was measured at 700 nm. A calibration curve was created using gallic acid as the standard substance, and the amount of polyphenols was measured.

### Antioxidant effects of miso<sup>11</sup>

The antioxidant effects of the produced miso were examined by measuring its 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability. The sample was prepared by adding 50 mL of 80% ethanol to 5 g of miso, stirring for 60 min, and centrifuging at 3,000 rpm for 5 min. The supernatant was used. A mixture of 12 mL of 400  $\mu$ M DPPH, 12 mL of 200 mM MES buffer (pH 6.0), and 12 mL of 20% ethanol was prepared, and 0.3 mL of the sample solution diluted with 80% ethanol was added to 0.9 mL of the mixture. After 20 min of reaction, the reaction solution was measured at 520 nm. A calibration curve was then prepared using Torolox, and the antioxidant activity was examined using the Torolox conversion method.

### Miso color

As an indicator of miso maturation, the color of the extract was measured at 470 nm. After adding 50 mL of water to 5 g of miso, the sample was stirred for 60 min, and centrifuged at 3,000 rpm for 5 min, with the supernatant being subsequently used. The  $L^*$ ,  $a^*$ , and  $b^*$  values were measured using a colorimeter (Olympus CR-20).

## Results and discussion

The current study produced miso using red kidney beans, pinto beans, and large white kidney beans, which were aged for 6 months using a natural fermentation method.

### General components of kidney bean miso

Table 1 summarizes the measurements of the general components of miso produced from kidney beans, red kidney beans, pinto beans, and large white kidney beans. Notably, miso produced using kidney beans, pinto beans, and large white kidney beans had a total nitrogen content of 1.58%, 1.54%, and 1.48%, respectively, with kidney bean miso having the highest total nitrogen content. The water-soluble protein content of kidney bean, pinto bean, and large white kidney bean miso was 0.85%, 0.74%, and 0.92%, respectively, with large white kidney bean miso having 1.2 to 1.1 times higher water-soluble protein content than the other types of miso. Protein solubility rates of kidney bean, pinto bean, and large white kidney bean miso were approximately 54%, 48%, and 62%, respectively. The high protein solubility rate of large white kidney beans is believed to indicate that a large amount of low molecular weight proteins and a large amount of umami and functional components are produced. The high proportion

of water-soluble proteins in each miso indicates that the miso is highly nutritious. Hence, our findings suggest that the use of kidney beans in miso can accelerate protein decomposition and increase nutritional value. Large white kidney bean miso had the highest protein solubility rate and formol nitrogen content, which suggests they had the most advanced protein decomposition, the highest amino acid and peptide content, and a high maturation rate. In addition, both total sugar and reducing sugar content were higher in large white kidney bean miso than in commercially available miso, with red kidney bean miso having the highest direct reducing sugar content at 15.5%, followed by pinto bean miso at 19.6%, and large white kidney bean miso at 20.8%. The average degree of polymerization was calculated to be 1.50, 1.20, and 1.14 for red kidney bean, pinto bean, and large white kidney bean miso, respectively, indicating that large white kidney bean miso had the lowest molecular weight sugars and advanced sugar decomposition. Large white kidney bean miso had the lowest pH among the three types but had the highest acidity II value. These results suggest that the content of amino acids (basic amino acids such as lysine and arginine) and peptides was high. The ash content of red kidney bean, pinto bean, and large white kidney bean miso was 19.32%, 18.76%, and 18.44%, respectively, with red kidney bean miso containing the most minerals.

**Table 1** Components of kidney bean (Red kidney bean, Pinto bean, large white kidney bean) miso

	Red kidney bean	Pinto bean	Large white kidney bean
Total nitrogen (%)	1.58	1.54	1.48
Water-soluble nitrogen (%)	0.85	0.74	0.92
Formol nitrogen (%)	0.35	0.33	0.38
Protein solubility (%) <sup>1</sup>	53.8	48.1	62.2
Proteolysis rate (%) <sup>2</sup>	22.2	21.4	25.7
Total sugar (%)	23.2	23.5	23.7
Direct reducing sugar (%)	15.5	19.6	20.8
pH	5.20	5.22	5.15
Acidity I (ml)	7.5	7.6	7.4
Acidity II (ml)	6.2	6.0	8.0
Salt (%)	12.0	12.3	11.2
Moisture (%)	2.17	2.23	2.25
Ash (%)	19.32	18.76	18.44

<sup>1</sup>Water-soluble nitrogen/ Total nitrogen (%)

<sup>2</sup>Formol nitrogen/ Total nitrogen (%)

### Amount of $\gamma$ -aminobutyric acid in kidney bean miso

In recent years,  $\gamma$ -aminobutyric acid has been attracting attention for its supposed is said to be effectiveness in improving brain blood flow and function and ameliorating the aftereffects of stroke. As such, the current study examined the amount of  $\gamma$ -aminobutyric acid in kidney bean miso (Table 2), with our findings showing that red kidney bean, pinto beans, and large white kidney bean miso having 15, 15 and 18 mg of  $\gamma$ -aminobutyric acid/100g, which is 1.7 to 1.2 times higher than those present in commercially available miso. Reports have shown that  $\gamma$ -aminobutyric acid is abundant in koji;<sup>12</sup> however, using rice koji instead of miso koji supposedly results in a higher amount of  $\gamma$ -aminobutyric acid. The  $\gamma$ -aminobutyric acid values of kidney bean miso were quite high for a food product, suggesting that kidney bean miso warrants attention as a functional food product.

**Table 2** Amount of  $\gamma$ -aminobutyric acid in kidney bean miso

Red kidney bean	Pinto bean	Large white kidney bean
15	15	18

### Amount of polyphenols in kidney bean miso

An examination of the amount of polyphenols in kidney bean miso (Table 3) revealed that red kidney bean, pinto bean, and large white kidney bean miso had 260, 279, and 313 mg of polyphenols per 100g, respectively. This finding suggests that kidney beans contain a large amount of polyphenols. Large white kidney bean miso had the highest amount of polyphenols, which indicated that the amount of polyphenols was not associated with the pigments in the bean skin alone. We believe that the pigments in beans may have decomposed



by steaming and heating during miso production, suggesting that the amount of polyphenols was associated with substances generated during aging.

**Table 3** Amount of polyphenols in kidney bean miso (mg/100g miso)

Red kidney bean	Pinto bean	Large white kidney bean
260	279	313

### Antioxidant effects of kidney bean miso

Recently, the antioxidant activity of foods has been attracting considerable attention in hopes of improving food functionality. In line with this, the current study measured the antioxidant activity of kidney bean miso (Table 4). Notably, we found that red kidney bean, pinto bean, and large white kidney bean miso had 0.186, 0.170, and 0.157 mol Trolox per 100g, respectively, which was confirmed to be similar to or higher than the values in rice miso (0.158 mol Trolox/100g miso). These results confirm that kidney bean miso was a functional food product with good antioxidant properties.

**Table 4** Antioxidant effect of kidney bean miso (mol Trolox/100g miso)

Red kidney bean	Pinto bean	Large white kidney bean
0.186	0.170	0.157

(1,1-Diphenyl-2-picrylhydrazyl radical scavenging ability)

### Color tone of the miso extract

Red kidney bean, pinto bean, and large white kidney bean miso were measured using a color difference meter (Table 5-1). As observed through the naked eye, large white kidney bean miso had the brightest color and the highest  $L^*$  value. Moreover, pinto bean miso was the reddest among the samples, whereas large white kidney bean miso was the yellowest. This was thought to be due to the pigments in the bean skins. Furthermore, measurement of the color of the extracts from red kidney bean, pinto bean, and large white kidney bean miso at 470 nm (Table 5-2) revealed values of 1.64, 1.10, and 1.52, respectively. These values were similar to those observed in rice miso. These results confirmed that red kidney bean miso had a darker finished color and that large white kidney bean miso had a deep brown color despite using the skin of large white kidney beans, which is white in color. This phenomenon was presumably attributed to the involvement of reactions related to pigments during maturation, such as aminocarbonyl reactions and tyrosinase, and the effects of the products produced during maturation.

**Table 5-1** Color of kidney bean miso

	Red kidney bean	Pinto bean	Large white kidney bean
$L^*$	37.14	39.01	47.73
$a^*$	9.83	11.51	9.68
$b^*$	12.74	18.09	23.73

**Table 5-2** Color of kidney bean miso extract

Red kidney bean	Pinto bean	Large white kidney bean
1.64	1.10	1.52

※470nm measurement

## Conclusion

As part of a study on the processing and use of beans in functional foods, the current study attempted to develop a new type of miso by

using kidney beans, red kidney beans, pinto beans, and large white kidney beans instead of rice or wheat. Our results showed that all types of kidney beans contained a large amount of peptides and amino acids that are umami components and were highly nutritious with a strong umami flavor. Our findings suggest that the maturation period could be shortened because protein decomposition proceeded quickly. In addition, compared to general rice miso, we were able to produce functional miso with good antioxidant effects and high gamma-aminobutyric acid and polyphenol contents. We confirmed that the produced miso had good color, aroma, and taste; were well-matured; and had a high sensory evaluation. To date, no reports have been available on the production of miso using kidney beans. Hence, the current study has been the first to attempt producing miso using kidney beans, as well as investigate the functionality of the produced food. The current study confirmed that producing kidney bean miso products is possible, and new uses for kidney beans are expected in the future. We also confirmed that kidney bean miso had a unique umami taste not found in rice miso and is also functional with high mineral and polyphenol content and antioxidant properties. The current study produced miso using rice koji and a one-third mixture of kidney beans, rice koji, and soybeans. Consequently, a functional miso product was produced. The use of kidney beans provided a rich flavor and a unique umami taste, while the use of rice koji provided a sweet taste, making it a miso with good commercial value.<sup>13</sup>

Demand for miso is growing worldwide, and it is currently a popular seasoning.<sup>13-16</sup> Miso is recognized for its functionality, such as its ability to lower blood pressure.<sup>17,18</sup> We believe that by developing new types of miso, it will become a food that attracts even more attention in the future.

## Acknowledgments

None

## Conflicts of interest

The authors declare that there are no conflicts of interest.

## References

- Jyozo Y. *The history of miso* (in Japanese). 2013.
- Akinori I. *Manufacturing of soybean miso*. In: New miso technology handbook. National miso technology association. 2006:78–89.
- Hisao Y. *Miso, revised brewing science*. In: Kikuo N, editor. Kodansha Scientific. 2005:151–181.
- Akiko T. *Fermented seasonings*. In: Miura H, editor. Food preservation and distribution technology handbook. Kenpakusha; 2006:638–663.
- Toji Y. *Food analysis experiments, brewing analysis experiments, brewing and food science experiment book*. In: Toji Y, editor. Food Research Society. 1990:211–361.
- National miso technology association. *Standard miso analysis method*. In: New miso technology handbook. National miso technology association. 2006:1–53.
- Toshio M. *Quantitative experiments of the main components of foods, applied experiments*. In: Food and nutrition experiments from the basics. 2005:58–134.
- Sakuzo F. Quantitative analysis of reducing sugars using copper reagent, quantitative analysis of sugars based on sulfuric acid treatment. In: Quantitative methods of reducing sugars. *Academic Press Center*. 1990:7–80.

9. Masami O. *Quantitative analysis of amino acids*. In: Tatsuyuki S, Akio MK, editors. *New food analysis handbook*. 2000:33–44.
10. Fujijiro T. *Separation and structural determination of functional food components*. In: Kazuki Shinohara et al., *Food function research methods*. Korin. 2000:318–346.
11. Ikuo S. *Antioxidant function*. In: Kazuki S. et al., editor. *Food function research methods*. Korin. 2000:218–223.
12. Kimi T, Kenryo N, Masayoshi I. Production of  $\gamma$ -aminobutyric acid (GABA) by *Aspergillus oryzae* and the separation of GABA using electric dialysis. *J Brew Soc Japan*. 2002;97(12):878–882.
13. Global Miso Market 2018-2022 (Technical report). Research and Markets. IRTNTR21132. 2018.
14. Khintan (2020-12-02). All about miso & miso soup recipe. Indoindians.com. 2021.
15. Yabai. Everything you need to know about miso paste. YABAI - The modern, vibrant face of Japan. YABAI. 2021.
16. Yutaka I, Kato S, Saikusa M. Analysis of the cooked aroma and odorants that contribute to umami aftertaste of soy miso (Japanese soybean paste). *Food Chemistry*. 2016;213:521–528.
17. Koji I. Review of the health benefits of habitual consumption of miso soup: focus on the effects on sympathetic nerve activity, blood pressure, and heart rate. *Environ Health Prevent Med*. 2020;25(1):45.
18. Du DD, Yoshinaga M, Sonoda M, et al. Blood pressure reduction by Japanese traditional Miso is associated with increased diuresis and natriuresis through dopamine system in Dahl salt-sensitive rats. *Clin Exp Hypertens*. 2014;36(5):359–366.