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Taste enhancer from the long-term ripening of miso (soybean paste)

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Abstract

Long-term ripened miso has a characteristic mouthfulness and continuity of flavour, which is heightened from 11 months of ripening. Focussing on its main components, changes of protein and sugar were investigated from 10 days up to 20 months of ripening. Protein and sugar changes during ripening did not correlate with sensory evaluation results. From visual observation of miso colour and colourimetric analysis of the water-soluble fraction, it was apparent that the Maillard reaction was occurring during 5–11 months of ripening. By fractionation and evaluation of umami, mouthfulness and continuity, we were able to identify a water soluble fraction of 20 month ripened miso with a molecular weight of 1000–5000 which was coloured and appeared to be a peptide that has undergone the Maillard reaction. From these results, the Maillard-reacted peptide was considered to be a key substance which gives the characteristic flavour (mouthfulness and continuity) of long-ripened miso.

Keywords: Soybean paste; Ripening; Hydrolysis; Peptide; Maillard reaction

1. Introduction

Fermented agricultural products which have been converted by enzymatic activities or microorganisms are considered to be more attractive than the original raw materials. In eastern and western countries, fermentation has been conventionally used to improve sensory quality and storage characteristic of foods. In Japan, miso (a fermented soybean paste) has been used primarily as a soup base. Miso is made from ''koji'' which is prepared from steamed rice and wheat, together with inoculated koji fungi. The proteins in the mixture are hydrolyzed to amino acids and peptides by the hydrolytic enzymes in koji. This hydrolysis continues for about 50 days and then terminates (Mochizuki, Rokugawa, Hondo, Ouchi, & Matsuki, 1968). It has been reported that peptides formed during the ripening process of miso are composed of 3–20 amino acids and also include amino acids such as glutamic acid, aspartic acid and proline (Hondo & Mochizuki, 1968; Hondo, Ouchi, & Mochizuki, 1969; Mochizuki et al., 1968). However, the role of peptides produced during the ripening process of miso for the formation of flavour is not clear. In the Shinshu area in Japan, miso, ripened for 12–24 months, is used as a seasoning (Okumura, 2003; Yoshii, 1999). Although it is known that well-ripened miso has a characteristic flavour (mouthfulness and taste continuity in the mouth), the taste expression mechanism and the responsible compounds have not been reported. This study investigates the key substances that give the characteristic flavour of long-ripened miso.

2. Materials and methods

2.1. Miso samples

Miso was obtained from a number of stages of ripening (10 days, 3 months, 5 months, 11 months, 15 months and 20 months). It was made by Miharaya Co. Ltd., in Nagano Prefecture, Japan. The analytical data of the miso were as follows: total sugar: about 45%, total protein: about 20%, total ash: about 25%, and total fat: about 10%, by dry weight.

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2.2. Preparation of the water-soluble fraction

The method used by Hondo and Mochizuki (1968) was used to prepare the water-soluble fraction. Distilled water (80 g) was added to 20 g of miso, which was then homogenized. The resulting slurry was heated at 100 °C for 10 min. After cooling, the slurry was filtered, using filter paper Toyo Roshi No. 2 (Toyo Roshi Kaisha Ltd., Tokyo, Japan). The resultant filtrate was made up to 100 ml and was used as the water-soluble fraction. The water soluble fraction was freeze-dried and stored at −18 °C until used.

2.3. Chemical analysis

Total nitrogen (T-N) was measured using the Dumas method (AOAC, 1984; Schmitter & Rihs, 1989), by an automatic nitrogen analysis system (Thermo Electron Corp., Waltham, USA). Formol nitrogen (F-N) was determined using the formol titration method (AOAC, 1970; Beveridge & Harrison, 1984). F-N is an index of free amino acids. Direct sugar (D-S) is measured by the Somogyi method(Nelson, 1944; Somogyi, 1960). D-S is an index of monosaccharides. Total sugar (T-S) measurement was performed after the sample was hydrolyzed in boiling water for 2.5 h with 2.5 N HCl. Chemical analysis was repeated three times. The soluble nitrogen rate (%) was calculated to express a ratio of T-N of water-soluble fraction to T-N of the miso, and the protein hydrolysis rate (%) was expressed as a ratio of F-N to T-N of the water-soluble fraction. The soluble sugar rate (%) and the polysaccharide hydrolysis rate (%) were expressed as the ratios of T-S of water-soluble fraction to T-S of miso and of D-S to T-S water-soluble fraction, respectively. The peptides were analyzed by HPLC (Shimadzu LC10, Shimadzu Co. Ltd., Kyoto, Japan) on a gel filtration column (TSKgelG2000SW, Tosoh Corp., Tokyo, Japan). The filtrate was applied to the column and eluted with an aqueous solution of 45% acetonitrile containing 0.1% trifluoroacetic acid. It was conducted at a flow rate of 0.8 ml/min and 40 °C. The UV detector was set at 215 nm and the fluorometer was set to measure total Maillard fluorescence at λ_{ex} 350 nm and λ_{em} 440 nm (Yeboah, Alli, & Yaylayan, 1999; Yeboah & Yaylayan, 2001). Aprotinin (MW 6500), oxidized glutathione (MW 612), glycylglycylglycine (MW 189), and glycine (MW 75) were used to create a calibration curve and to calculate molecular weight.

2.4. Preparation of MW1000–5000 fraction of maillard reaction products

The water-soluble fraction powders of 5 and 20 months-ripened miso were dissolved in de-ionized water. The solution was ultrafiltered through MW 1000 and 5000 cut-off membranes (RemolinoTM UF&RO system, Milipore Ltd., Milford, MA, USA) to obtain the MW 1000–5000 fraction and then freeze-dried. To this freeze-dried powder, 2.5% (w/v) of xylose was added and a solution containing 25% solid was prepared. This solution was adjusted to pH 6.0 with 1.0 N HCl and heated to 90 °C for 3 h. After the reaction, the solution was fractionated with 1000 and 5000 MW filters to obtain the MW 1000–5000 fraction.

2.5. Sensory evaluation

To ascertain the flavour characteristics of ripened miso, the sensory evaluation of miso soup was carried out. Miso soup was prepared using 5 g of miso in 45 g of hot water. To ascertain the flavour characteristics of the MW1000–5000 fraction, the sensory evaluation was carried out by the modified method of Ueda, Yonemitsu, Tsubuku, and Sakaguchi (1997). A dextrin (0.025%) (DE8-10, Nissi Co., Ltd., Tokyo, Japan) treated umami solution which, containing 0.5% monosodium glutamate (MSG) and 0.5% NaCl, was used as a control for the sensory evaluation. The MW 1000–5000 fraction (0.025%) was dissolved in the umami solution as a sample and warmed to 60 °C in a water bath. About 60 ml of sample and control solutions were served in opaque disposable plastic cups. Sensory evaluation was performed in separated sensory booths. The evaluation was conducted on a scale of 1–7, giving three points to a sample to which 0.025% dextrin had been added being used as a control; the samples were awarded points on the basis of umami, mouthfulness and continuity. The panel consisted of six males and four females (aged 25–45 years). All the panel members had extensive experience in tasting and made a consensus for the intensities of umami, mouthfulness, and continuity in the umami solution.

2.6. Statistical analysis

Statistical analysis was performed using Microsoft Excel 2000. The *t*-test was used to determine significant differences between samples. Trends were considered significant when the mean of compared sets differed at $p < 0.05$.

3. Results and discussion

3.1. Sensory evaluation of long-ripened miso

Fig. 1 shows photographs of the miso ripened from 10 days to 20 months. From the photographs, it is quite obvious that the degree of darkness of the miso was dependent on its degree of ripening; these changes being particularly prominent after 11 months of ripening. Table 1 shows the sensory evaluation results of ripened miso. The sensory evaluation revealed that the miso started to develop its typical flavour after 11 months of ripening and the taste treated to show mouthfulness and continuity. Miso is manufactured in many provinces throughout Japan. Generally, the production of miso is started in the spring or late autumn,it is allowed to ripen and is consumed after about 12–24 months (Okumura, 2003; Yoshii, 1999). This ripening process is an essential step for the development of a good miso flavour profile as we have confirmed in our sensory tests.

Fig. 1. Photographs of ripened miso from 10 days to 20 months.

Table 1
Sensory evaluation of various ripened miso samples

	Taste profile
10 days	Flavour not typical of miso soup, beany flavour
3 months	Somewhat like miso soup taste, salty
5 months	Close to the miso soup taste but plain and flat
11 months	Typical miso soup taste , has continuity and slight mouthfulness
15 months	Typical miso soup taste, has continuity and mouthfulness
20 months	Typical miso soup taste, has continuity and mouthfulness and more intense taste

3.2. Changes in the sugar and protein components during ripening

Fig. 2 presents the change in the sugar and protein components and Fig. 3 illustrates the gel filtration chromatogram of the water soluble fraction. The soluble sugar rate increased linearly during storage up to 5 months, reached about 90%, and then remained constant thereafter. In addition, the polysaccharide hydrolysis rate increased up to 3

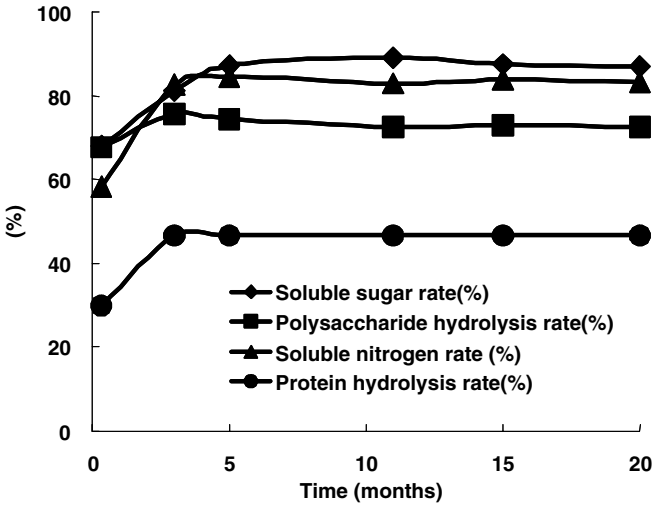

Fig. 2. The change in the nitrogen and sugar components during ripening of miso.

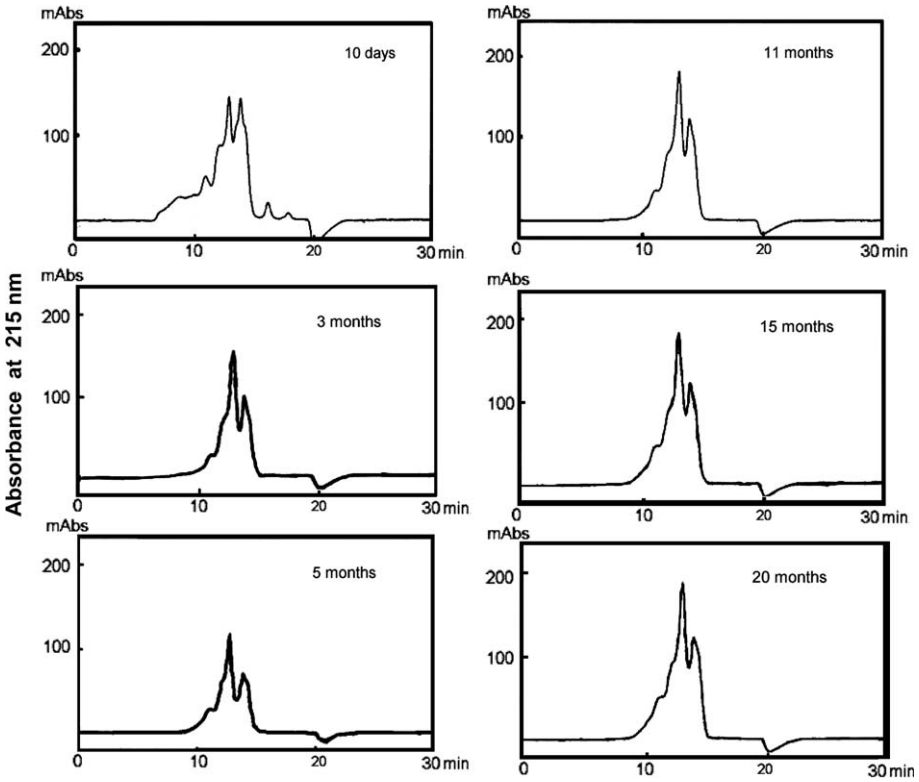

Fig. 3. Gel filtration chromatogram of the water-soluble fraction.

months of ripening, reached about 70%, and then remained constant thereafter. The soluble nitrogen rate increased linearly during storage up to 5 months, reached about 80%, and then remained constant thereafter. In addition, the protein hydrolysis rate also increased during 3 months of ripening, reached about 45%, and remained constant thereafter. From the gel filtration chromatogram of the water soluble fraction, it can be also seen that protein hydrolysis progressed from 10 days to 3 months of ripening, and remained constant thereafter. From these results, it can be concluded that sugars and proteins in the miso were hydrolyzed by koji enzymes during 3–5 months of ripening and then terminated. The termination of hydrolysis might be due to the inactivation of enzymes over 5 months of storage or due to the substrate specificity of enzymes. Further study is needed in order to reveal this phenomenon. The changes in the sugar or protein components, which are important in ripening, reached their maximum in the first 5 months and plateaued out thereafter. These results were not consistent with mouthfulness and continuity of miso that became pronounced from 11 months of maturation, suggesting that there are some other factors contributing to characteristic mouthfulness and continuity of the miso.

3.3. Coloured components generated during ripening

As seen in Fig. 1, the colour of the miso became darker during ripening. Absorbance of the water-soluble fraction was measured at 450 nm and is depicted in Fig. 4. T-N concentration of the water-soluble fraction was 0.05% (dry base). Absorbance of the water-soluble fraction increased after 11 months of ripening. To analyze the colouration of the water-soluble fraction in more detail, gel filtration chromatography was performed. Detection wavelengths at λ_{ex} 350 nm and λ_{em} 440 nm were used to detect Maillard reaction products. The chromatogram is shown in Fig. 5. Up to 5 months of ripening, Maillard reaction products with molecular weights of less than 350 were detected. After 11 months of ripening, Maillard reaction products with a molecular weight range of 350–5000 were observed. From these results, it was ascertained that the Maillard reaction mostly progressed after 11 months of ripening.

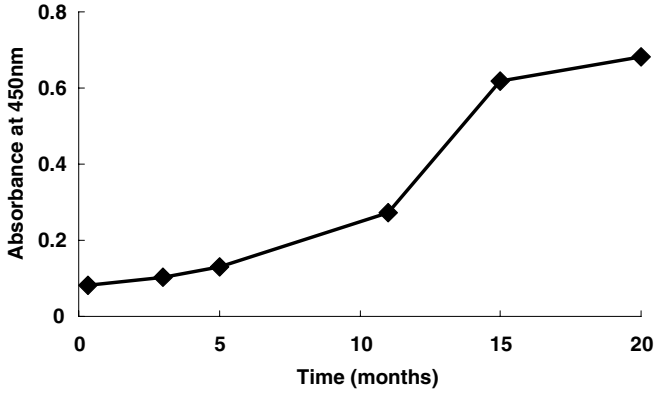

Fig. 4. Change in the colour during ripening of miso.

The results pertaining to the changes in the protein and sugar components during ripening suggest that peptides and sugars were generated during the first 5 months and the Maillard reaction followed. More detailed investigations may be required to elucidate this process. The generation of amino acids and peptides reached a constant level during the first 5 months, which led to the conclusion that the changes in flavour after 5 months of ripening could not be explained only by the generation of amino acids and peptides. There was a correlation between the changes in the miso flavour after 11 months and the formation of Maillard peptides in the water-soluble fraction, suggesting that the peptides that have undergone Maillard reactions contribute to the mouthfulness and continuity.

3.4. Taste characteristic of water soluble fraction MW 1000–5000 from ripened miso

A fraction was separated, with a molecular weight of 1000–5000, from the water-soluble fraction of the miso that had been ripened for 5 and 20 months and taste effects of the umami solution on this fraction were investigated. Umami solution was added to Fraction 1 (MW 1000–5000 of the water-soluble fraction from the miso ripened for 5 months) and Fraction 2 (MW 1000–5000 of water-soluble fraction from the miso ripened for 20 months). Fractions 1 and 2 had little aroma or basic taste in distilled water at a concentration of 0.025%. Fig. 6 shows the results of the addition test. The addition of Fraction 2 significantly increased the intensities of mouthfulness and continuity compared to the control and the addition of Fraction 1. From these results, it was suggested that the water-soluble fraction of the miso after 20 months of ripening, with a molecular weight of 1000–5000, could be a flavour enhancer. It can be concluded that the peptides that had undergone Maillard reactions contributed to the mouthfulness and continuity. We have the following hypothesis. During the ripening process of the miso, the enzymes in koji are responsible for the formation of peptides, amino acids and sugars. These hydrolyses are terminated within 3–5 months. The ripening process after 3–5 months seems to be dependent on complex reactions involving the formation of Maillard reaction products. The characteristic flavour of ripened miso is based on the formation of the Maillard peptide products with MW 1000–5000. To verify this hypothesis, Fraction 1 (MW 1000–5000, water-soluble fraction from the miso ripened for 5 months) was heated with xylose to form Fraction 1 Maillard products. Xylose was chosen, because it is present in a large quantity in the miso (Honma & Akitagawa, 1955). Fraction 1 and Fraction 1 Maillard products were added to umami solution and subjected to sensory evaluation. Fractions 1 and Fraction 1 Maillard products had little aroma or basic taste in distilled water at a concentration of 0.025%. Fig. 7 shows the results of an addition test. The addition of Fraction 1 Maillard products significantly increased the intensities of mouthfulness and continuity compared to the

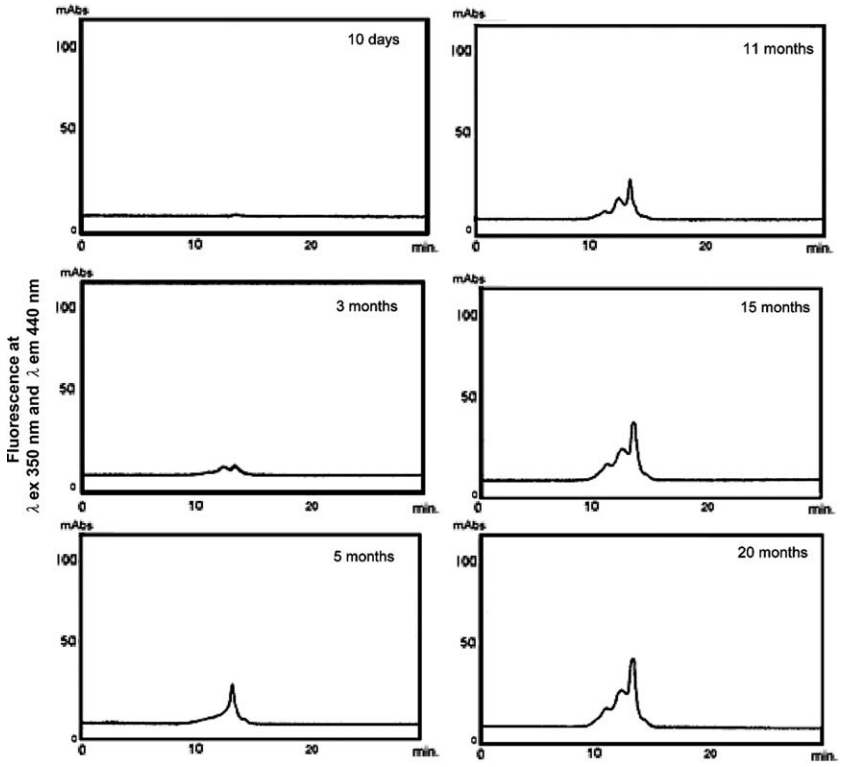

Fig. 5. Gel filtration chromatogram of the water-soluble fraction.

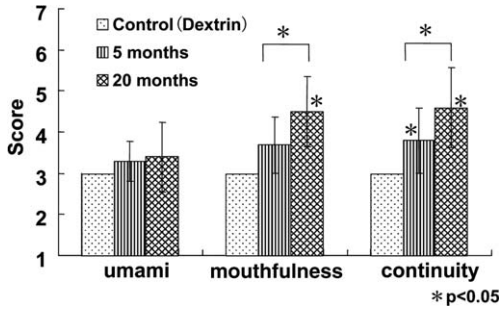

Fig. 6. Sensory evaluation of the water-soluble fraction of MW1000–5000 from 5 months- to 20 months-ripened miso.

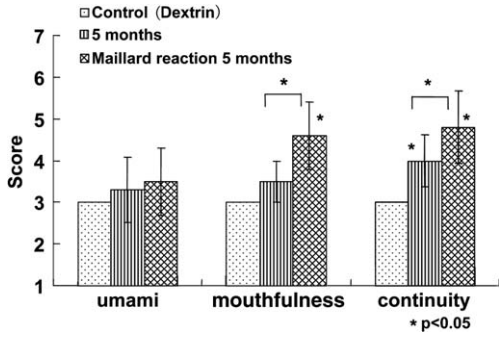

Fig. 7. Sensory evaluation of the Maillard reaction products prepared from the MW1000–5000 fraction of 5 months-ripened miso.

addition of the MW 1000–5000 fraction of water soluble fraction from the miso ripened for 5 months. From these results, it was concluded that the Maillard peptide products from the MW 1000–5000 peptides are behaving as a flavour enhancer and play an important role in the formation of the characteristic flavour of long-ripened miso.

With regard to the ripening of the miso, it has been reported that 30–50 days ripened miso, warmed at 45 °C for 1–3 days had good flavour properties (Matumoto, Akimoto, & Imai, 1991). However, nothing was mentioned about the substance involved in this flavour improvement. With regard to the flavour enhancer, it has been reported that a beefy meaty peptide added to beef extract gave a more intensive meaty or savoury taste (Wang, Maga, & Bechtel, 1996), and that glutathione had a characteristic flavour such as continuity, mouthfulness, and thickness in umami solution (Ueda et al., 1997). With regard to the Maillard reaction of soy protein, it has been reported that emulsification and antibacterial properties are improved as a result of the conjugation of galactomannan with soy protein (Babiker et al., 1998), and the conjugation of chitosan with soy protein (Usui et al., 2004). However, these chemicals and processes, which contribute to changes in flavour as a result of the ripening of foodstuffs, have not been reported. It can be suggested that the Maillard peptide products from the MW 1000–5000 peptides behave as a flavour enhancer, and peptides which have undergone Maillard reactions have an important role in the characteristic flavour of long-ripened miso.

4. Conclusions

Long-ripened miso had a characteristic mouthfulness and continuity of flavour especially increasing from 11 months of ripening. Protein and polysaccharide hydrolyses were terminated after 3–5 months of ripening and remained constant thereafter. There was a significant change in colour from 11 months of ripening and it was evident that Maillard reaction products were progressing. It can be suggested that peptides and sugars are generated as a result of the ripening of the miso, with further ripening causing the Maillard reaction to progress among the peptides and sugars, which then contribute to the characteristic flavour of ripened miso.

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