Food Sci. Technol., Int., **2** (2), 127-130, 1996

Changes in ^{23}Na Nuclear Magnetic Resonance Signal, Water Activity and Saltiness of Miso during Fermentation

Sayuki NIKKUNI,[1] Takayuki KUROSAWA[2] and Nobuaki ISHIDA[1]

[1] *National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Kannondai 2-1-2, Tsukuba, Ibaraki 305, Japan*
[2] *Asari-Sasuke Shoten Co., Hanawa, Kazuno-shi, Akita 018-52, Japan*

Received December 6, 1995

Changes in saltiness evaluated by sensory analysis, ^{23}Na nuclear magnetic resonance (NMR) signal and water activity (a_w) of miso during fermentation were investigated. The line width (full width at half maximum intensity) of the ^{23}Na NMR signal of the miso extract increased with fermentation time, while the a_w and the saltiness decreased with fermentation time. The saltiness correlated with the line width ($p<0.001$) and the a_w ($p<0.001$). The line width was not much affected by NaCl concentration, but it increased on addition of glucose, casamino acid, ethanol and lactic acid. The line width of ^{23}Na NMR and the a_w of the miso model solution consisting of sodium chloride and these substances were not much changed during storage over 100 days. This suggests that the increase in the line width and the decreases in the a_w and the saltiness of miso during fermentation were caused by the increase in water-soluble substances such as glutamic acid.

Keywords: ^{23}Na NMR signal, water activity, saltiness, miso

Miso is a semisolid fermented food made from soybeans, rice or barley, and salt is mainly used for preparing miso-soup in Japan (Ebine, 1989). To make rice miso, the most popular miso of Japan, cooked soybeans are mixed with koji (steamed rice on which *Aspergillus oryzae* is cultured), salt and a small amount of water to control the moisture level. The mixture is then allowed to ferment (Ebine, 1989).

During miso fermentation, carbohydrates, proteins and lipids, the major components in the ingredients are hydrolyzed by the enzymes produced by *A. oryzae* into sugars, free amino acids and fatty acids (Shibasaki & Hesseltine, 1962; Mochizuki & Imai, 1982; Ebine, 1989). Halotolerant yeasts and halophilic lactic acid bacteria contribute to developing the flavor of miso by producing alcohols and organic acids (Mochizuki, 1978; Mochizuki & Imai, 1982; Ebine, 1989). It is known that the saltiness, which is very strong in the first stage of fermentation, becomes mild during fermentation progress (Ebine, 1989). This phenomena is called "Shio-nare" and it plays an important role in miso-soup making. Therefore, it can be said that to evaluate the saltiness of miso is to estimate the fermentation state. It is known that protein digestibility (the ratio of water-soluble nitrogen to total nitrogen, %) is one of the indicators for estimating the fermentation state (Mochizuki & Imai, 1982). It increases with the progress of fermentation and reaches a level of approximately 60% at the end of fermentation (Mochizuki, 1978). Titratable acidity also increases with the progress of fermentation (Mochizuki, 1978). The value of the pH decreases with fermentation time, and the state of fermentation can be estimated using it (Ebine, 1958).

Recently, Ishida *et al.* (1991) investigated the distribution and mobilities of sodium ions in foods using a ^{23}Na nuclear magnetic resonance (NMR) imaging probe and reported that ^{23}Na NMR imaging is useful for monitoring food quality in detail such as in the sub-tissue level during storage and processing. Aishima & Matsushita (1984) and Matsushita (1990) reported that the line width (full width at half maximum intensity) of the ^{23}Na NMR signal can be used to evaluate the saltiness of soy sauce during its fermentation. On the other hand, water activity (a_w) is one of the methods for investigating the physical state of water in food (Troller & Christian, 1978). Yoshii (1975), Osawa *et al.* (1983), Matsumoto *et al.* (1992) and Matsui *et al.* (1994) measured the a_w of misos. However, they used the a_w value mainly for the regulation of microorganisms.

We designed this experiment in order to determine whether the NMR signal and a_w can be used for evaluation of saltiness and the state of miso fermentation and investigated the changes in the ^{23}Na NMR signal, a_w and saltiness evaluated by sensory analysis during miso fermentation.

Materials and Methods

Preparation of miso sample The Tachinagaha variety of soybeans harvested in Ibaraki prefecture in Japan was used for the preparation of the miso sample. The soybeans (5 kg) were soaked overnight and cooked in an autoclave under 0.75 kg/cm^2 steam pressure for 30 min. Koji was prepared by the conventional method using a "Tane-koji" (M-1, Nihon Jozo Co.), which is a koji starter consisting of a number of spores of *A. oryzae*, and the Koshihikari variety of rice harvested in Ibaraki prefecture in Japan. The miso sample was prepared by mixing the cooked soybeans, the koji, NaCl (12% at the final concentration) and sterilized water (46% at the final concentration) containing cultivated *Zygosaccharomyces rouxii* NFRI 3401(=S96=ATCC42981) cells (10^5 cells/g miso in the mixing) as a starter. The ratio of the cooked soybeans and the koji was 10 : 8 (w/w, raw material). The mixture (approximately 18 kg) was placed in a stainless steel vessel (20 *l* capacity) with a weight (2.7 kg) for pressing and incubated at 30°C for 80 days followed by incubating at 25°C.

Preparation of miso extract Each miso sample (100 g) taken just after mixing (0-day miso) and after 5, 10, 30, 50 and 112 days of fermentation was mixed with water, homogenized by a food mill IFM-140 (Iwatani) and centrifuged at 20,000× *g* for 15 min. The NaCl concentration of the supernatant was adjusted to 7% (w/v) by dilution with deionized water.

Preparation of the model solution A NaCl solution (15%, w/v) was prepared with 15 g NaCl (guaranteed reagent, Wako) and highly purified water prepared using a reagent water system (Super-Q, Millipore). The model solution (pH 5.0) of miso was prepared by dissolving NaCl 15 g, glucose 10 g, casamino acid (Difco) 10 g, ethanol 2 g, lactic acid (88%) 0.23 ml and diluting to 100 ml with deionized water. The solution was filtered through a 0.22 μm filter (Millex-GV, Millipore). The filtrate was kept at 30°C.

Chemical analysis

Water-soluble nitrogen, reducing sugar, acidity I, glutamic acid, ethanol and Na Water-soluble nitrogen, reducing sugar and acidity I were determined according to the methods of the Official Methods of Miso Analysis (Institute of Miso Technologists, 1968). Glutamic acid was determined by the colorimetric method using a kit for glutamic acid determination (L-glutamic acid, Boehringer Mannheim Biochemica) according to the kit manual. Ethanol was determined using a gas chromatography. The miso sample (5 g) was suspended in 100 ml water containing 1 ml *n*-propanol as an internal standard. The suspension was centrifuged at 20,000×*g* for 15 min. Ten μl of the supernatant was injected to a gas chromatography (GC-4CPF, Shimadzu) with an FID detector. A Uniport R 80/100 column (3 m×3 mm i.d., GL Sciences Co.) coated with 10% PEG 1000 was employed using a nitrogen gas as a carrier gas at 90°C.

Sodium in solution was determined with an atomic absorption spectrophotometer (AA-781, Nippon Jarrell-Ash Co.) using a wavelength of 330.3 nm after diluting the sample solution with 1% HCl. NaCl was determined by the method of argentometry (Tsutsumi *et al.*, 1968).

Physical analysis

pH pH was measured according to the methods of the Official Methods of Miso Analysis (Institute of Miso Technologists, 1968) using a pH meter (HM-30V, Toa Denpa Co.).

^{23}Na NMR ^{23}Na NMR signals of the miso extracts, the model solution and NaCl solutions were obtained from a JEOL GSX-270WB spectrometer at 71.46 MHz and 25°C with 10 mm NMR tubes. A capillary tube with D_2O was inserted in the NMR tube for a lock. The pulse angle was 90° and the repetition time was 1.0 s with four accumulated transients. Each signal was drawn using 0.1 Hz of a line broadening factor.

a_w The a_w values of miso samples, the miso extracts, the model solution and NaCl solution were measured by a model a_w Center EEJA-3 (Novasina) at 25°C.

Sensory analysis The miso extracts contained 7% NaCl were diluted to 1.2% NaCl concentration with deionized water and judged sensorily by a panel of 10 members from the National Food Institute who were experienced in sensory evaluations and familiar with miso. The panel was asked to evaluate the NaCl concentrations of the sample solutions at room temperature (approximately 25°C) in a comparison of a standard series of NaCl solutions (0.4, 0.6, 0.8, 1.0, 1.2 and 1.4%). Water was available to the panelists. Means and standard deviations of scores (evaluated NaCl concentration) judged by the 10-member panel were calculated.

Data analysis Means, standard deviations and correlation of coefficients determined by a simple linear regression analysis were calculated using the Microsoft Excel software (Ver. 5.0, Microsoft Co.).

Results and Discussion

Changes in chemical and physical characteristics, line width of the ^{23}Na NMR signal and a_w during miso fermentation The reducing sugar increased remarkably within the first 10 days (Fig. 1) as Mochizuki *et al.* (1978) reported. The contents of water-soluble nitrogen, glutamic acid and ethanol and the value of acidity I increased with the fermentation time (Fig. 1). The pH value decreased with the fermentation time (Fig. 1). The line width of the ^{23}Na NMR signal increased with fermentation time (Fig. 2). The a_w of the

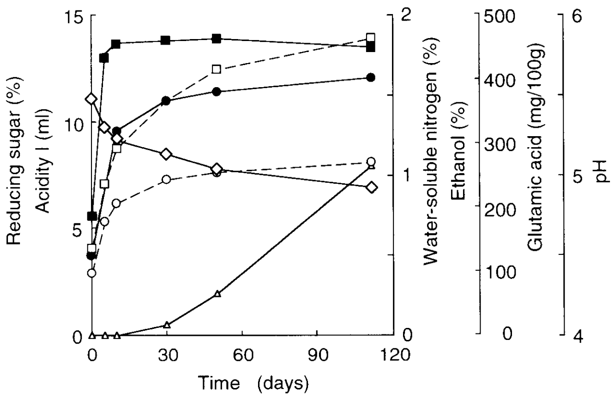

Fig. 1. Changes in chemical and physical characteristics of miso during fermentation. The miso sample was fermented at 30°C for 80 days followed by incubation at 25°C. Symbols: ○ water-soluble nitrogen content (%), ● glutamic acid content (mg/100 g), △ ethanol content, ■ reducing sugar content (%), □ acidity I (ml), ◇ pH value.

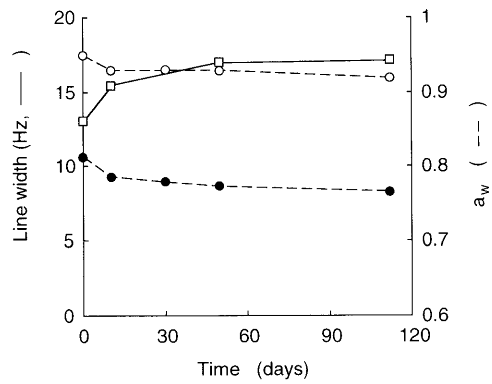

Fig. 2. Changes in line width of ^{23}Na NMR signal and a_w of miso during fermentation. Symbols: □ line width (Hz) of miso extract, ○ a_w of miso extract, ● a_w of miso.

miso and the miso extract decreased with fermentation time (Fig. 2).

Relation between saltiness by sensory test, line width and a_w The NaCl concentration of the 1.2% NaCl solution was evaluated as 1.17±0.07(mean±standard deviation)% by the 10 taste panelists. The NaCl concentration of the diluted miso extract prepared from the miso just after mixing (0-day miso) was 1.04±0.15%, and those fermented for 10, 50 and 112 days were evaluated to be 0.95±0.20, 0.90±0.15 and 0.86±0.17%, respectively. These results indicate that the saltiness evaluated by the sensory test panel decreased with fermentation time, although all the NaCl concentrations of the samples were adjusted to a level of 1.2%.

When these evaluated values were plotted with the line widths of the NMR signals of the miso extracts during fermentation, a linear relationship was observed at the 0.1% probability level ($p<0.001$) with a correlation coefficient of −0.596 (Fig. 3). The a_w of the miso extracts also correlated with the evaluated NaCl concentrations ($r=0.586$, $p<0.001$) (Fig. 4). These results suggested that both the NMR signal and a_w can be used as tools to estimate the saltiness.

Effect of NaCl concentration on NMR signal The line widths of the ^{23}Na NMR signals of various concentrations of NaCl solutions were measured. The widths of 7% NaCl, 15% NaCl and 20% NaCl solutions were 7.17 Hz, 8.6 Hz and 8.59 Hz, respectively. This result shows that the width was not much affected by NaCl concentration. Ishida *et al.* (1991) already reported that the spin-spin relaxation times (T2) of NaCl solution (from 0.1 to 1 M) were constant. However, the line width of the 15% NaCl solution (5.9% Na) increased to 18.0 Hz with the addition of casamino acids, glucose, ethanol and lactic acid (the model solution, 6.6% Na).

Effect of storage time on NMR signal and a_w Fermentation is carried out for long time for making a salty rice miso, the most popular miso in Japan (Ebine, 1989), although a controlled temperature favorable to fermentation can accelerate the fermentation process and shorten the time. Therefore, an experiment was designed to determine the effect of time for fermentation on saltiness using the model solution as a model of salty type rice miso. The model solution was stored at 30°C, and its ^{23}Na NMR signal and a_w were measured during storage. The average values of the line widths of ^{23}Na NMR signals of the 15% NaCl solution and the model solution over 103 days were 8.58±0.14 (mean±standard deviation, $n=5$) Hz and 18.05±0.08 Hz, respectively (Fig. 5). The average value of the a_w of the 15% NaCl and the model solution over 112 days were 0.896±0.006 (mean±standard deviation, $n=12$) and 0.845±0.003, respectively (Fig. 5). These results show that the line width of ^{23}Na NMR and the a_w of the model solution were not much changed during storage over 100 days. This suggests that time alone does not affect the saltiness of miso during fermentation, because significant correlation was observed between the saltiness and the line width or the a_w.

Because the values of chemical characteristics such as water-soluble nitrogen content changed during miso fermentation, the increase in the line width, the decrease in the a_w and the decrease in the saltiness during miso fermentation were considered to be caused by the changes in the values of chemical characteristics.

Yamaguchi and Takahashi (1984) investigated the sensory interactions of monosodium glutamate (MSG) and NaCl on the saltiness and palatability of a clear soup. They have

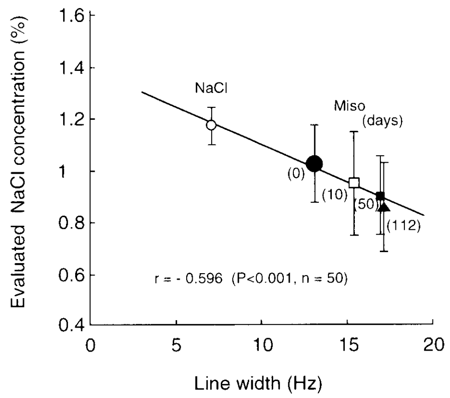

Fig. 3. Relation between line width of ^{23}Na NMR signal and saltiness evaluated by sensory test. Symbols: ○ 1.2% NaCl solution, ● miso extract from the 0-day miso, □ miso extract from the 10-day miso, ■ miso extract from the 50-day miso, ▲ miso extract from the 112-day miso.

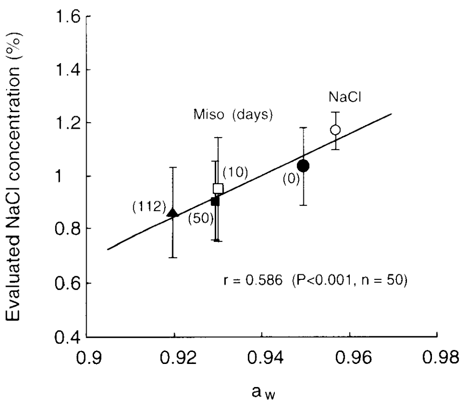

Fig. 4. Relation between a_w of miso extract and saltiness evaluated by sensory test. Symbols are the same as those in Fig. 3.

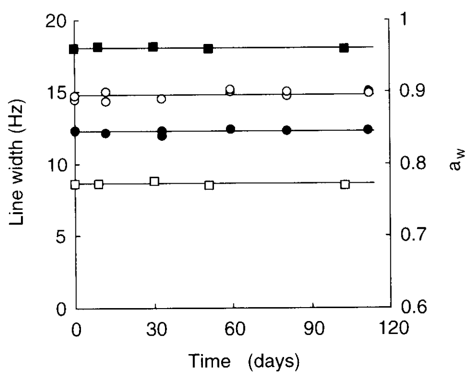

Fig. 5. Effect of storage time on line width of ^{23}Na NMR signal and a_w. Fifteen % NaCl solution (○ and □) and the model solution (● and ■) were stored at 30°C and the line widths of their ^{23}Na NMR signals (□ and ■)and a_w (○ and ●) were measured during storage.

revealed that there is a relationship between the two taste substances, MSG and NaCl, for palatability. In our experiments, we used a sample which contained 1.2% NaCl for the sensory analysis. The palatability score of the soup which contains 1.2% NaCl increases with an increase in MSG concentration up to 0.28%, according to Yamaguchi & Takahashi (1984). The concentration of glutamic acid in the diluted miso extract for the sensory analysis increased with fermentation time. However, the miso extract prepared from the 112-day miso which showed the highest glutamic acid content contained only 0.04% glutamic acid (Fig. 1). Because this value of 0.04% is smaller than 0.28%, the optimal level of MSG for 1.2% NaCl, it can be estimated that the saltiness of miso decreased with the increase in glutamic acid concentration in the sample for the sensory analysis during miso fermentation. Moreover, the line width increased with the addition of water-soluble substances such as glucose, casamino acid, ethanol and lactic acid as described above. Therefore, it is concluded that the increase in the line width and the decreases in the a_w and the saltiness of miso during fermentation were caused by the increase in water-soluble substances such as glutamic acid, not by time alone.

Acknowledgments We thank Drs. Chise Suzuki, Shigehiro Naito, National Food Research Institute, and Kamaljit S. Vilkhu, Australian Food Industry Centre, for useful discussions. We are grateful to Dr. Tamotsu Ogawa, Hokuriku Agriculture Research Station, Ministry of Agriculture, Forestry and Fisheries, for helpful advise for carrying out sensory evaluation analysis.

References

Aishima, T. and Matsushita, K. (1984). Jozo-shokuhin no jukusei to takaku-NMR supekutoru (Aging of fermented foods and multinuclear NMR). Presented at the Annual Meeting of the Agricultural Chemical Society of Japan in 1984, Tokyo, April 1-4, p. 459 (in Japanese).

Ebine, H. (1958). Estimation method of ripening of miso by pH meter. *Report of National Food Research Institute*, No. 13, 89-91 (in Japanese).

Ebine, H. (1989). Industrialization of Japanese miso fermentation. In "Industrialization of Indigenous Fermented Foods," ed. by K.H. Steinkraus, Marcel Dekker, Inc., New York, pp. 89-126.

Institute of Miso Technologists (1968). "Official Methods of Miso Analysis." Institute of Miso Technologists, Tokyo (in Japanese).

Ishida, N., Kobayashi, T., Kano, H., Nagai, S. and Ogawa, H. (1991). ^{23}Na-NMR imaging of foods. *Agric. Biol. Chem.*, **55**, 2195-2200.

Itoga, K., Yajima, Y., Yasuhira, H. and Mochizuki, T. (1976). Miso to shoyu-moromi no suibunkassei (Water activities of misos and soy sauce mashes). *Rep. Shinshu-Miso Res. Inst.*, No. 17, 32-39 (in Japanese).

Matsui, C., Suzuki, S., Aida, K., Smino, T. and Yamada, K. (1994). Study on water activity, sodium content, potassium content and composition of free amino acids of miso produced in Iwase, Fukushima. *Jpn. J. Nutr.*, **52**, 251-257 (in Japanese).

Matsumoto, I., Akimoto, T. and Imai, S. (1992). Relation between rice to soybean ratio, salt concentration, moisture and water activity of miso. *Miso Sci. Technol.*, **40**, 356-360 (in Japanese).

Matsushita, K (1990). NMR-ho de shouyu wo miru (NMR spectra of soy sauce). *J. Jpn. Soy Sauce Res. Inst.*, **16**, 251-258.

Mochizuki, T. (1978). Changes of several components and role of microbes during miso maturation—a monograph. *J. Ferment. Technol.*, **56**, 630-644 (in Japanese).

Mochizuki, T. and Imai, S. (1982). Jukusei (Fermentation and aging). In "Miso no Jozo-Gijutsu (Technology of miso manufacture)," ed. by M. Nakano, The Brewing Society of Japan, Tokyo, pp. 87-110 (in Japanese).

Osawa, Y., Hondo, S. and Yasuhira, H. (1983). Effect of moisture and salt concentration in water phase on fermentation of miso. *Rep. Shinshu-Miso Res. Inst.*, No. 24, 30-39 (in Japanese).

Shibasaki, K. and Hesseltine, C.W. (1962). Miso fermentation. *Econ. Bot.*, **16**, 180-195.

Troller, J.A. and Christian, J.H.B. (1978). "Water Activity and Food." Academic Press, Inc., New York.

Tsutsumi, C., Koizumi, H. and Ebine, H. (1968). Determination of salt in miso and soy sauce by Argentometry. *Nippon Shokuhin Kogyo Gakkaishi*, **15**, 461-465 (in Japanese).

Yamaguchi, S. and Takahashi, C. (1984). Interactions of monosodium glutamate and sodium chloride on saltiness and palatability of a clear soup. *J. Food Sci.*, **49**, 82-85.

Yoshii, H. (1975). Shokuhin no suibun-kassei (Water activity and food). *Miso Sci. Technol.*, No. 260, 5-13 (in Japanese).