Natto Characteristics as Affected by Steaming Time, Bacillus Strain, and Fermentation Time

Q. Wei, C. Wolf-Hall, and K.C. Chang

ABSTRACT: Natto was made in a laboratory scale from soybean using 2 steaming times, 9 Bacillus natto strains, and 6 fermentation times. Natto characteristics including color, firmness, viscosity, ammonia content, and bacteria population were determined. The highest viscosity value of the final products was the natto inoculated with Bacillus natto "Itobiki strain"; the 2nd was natto with B. natto NRRL B-3383 strain. A higher steaming time reduced fermentation time and ammonia content in the final products. A combination of soaking at the room temperature for 20 h (weight increase ratio: 2.1 to 2.3), steaming at 121 °C for 40 min, inoculated with "Itobiki strain" or NRRL-3383 strain, and fermented at 40 to 42 °C for 18 h was able to produce good natto products in a laboratory scale.

Key words: natto quality, fermentation, Bacillus subtilis (natto), soybean, processing

Introduction

Natto is a traditional fermented soybean food, originating in the northern parts of Japan about 1000 years ago (Fukushima 1986). Similar products popular in the Southeast Asia are "shi" in China, "thua-nao" in Thailand, "chung-kook-jong" in Korea, and "tao-si" in Philippines (Ohta 1986). There are 3 types of natto in Japan: itohiki-natto, yukiwai-natto, and hama-natto. Itohiki-natto is very popular and produced in large amounts. The word "natto" usually refers to itohiki-natto (Fukushima 1986).

Natto is a low-cost, high-nutrition, and long shelf-life food. Good quality natto products should be covered with a white-colored mucous substance, have characteristic flavor, palatably soft texture, light yellow color and is able to generate silky and sticky mass when mixed/stirred with a pair of chopsticks. During the manufacturing process, nutritional value is improved through heat denaturation of soy proteins, trypsin inhibitors, and bacteria-enzyme hydrolysis of the proteins into easily digestible peptides. After sufficient fermentation, the intact soybeans are covered with a white-colored viscous substance and have a slimy appearance, softer texture, and unique flavor (Steinkraus 1983). After fermentation the natto products may be eaten without heating, and are stored in a cooler or freezer in the supermarket for purchasing. In Japanese homes, traditional natto is eaten with a mixture of thinly chopped green onion, seaweed, mustard, and a small amount of soy sauce and served as a side dish along with steamed rice (Ohta 1986). Natto also can be used as a flavoring agent for preparing meat, seafood, and vegetable dishes and as an ingredient for sauce production (Lin 1991).

Natto contains about 59.5% moisture, 16.5% protein, and 10.0% lipid (Ohta 1986). Natto manufacture generally includes steps of soaking, steaming/cooking, inoculating, and incubating (Steinkraus 1983; Ohta 1986; Maruo and Yoshkawa 1989). The quality of natto product varies greatly with the conditions of soaking, steaming, fermentation, and cultivars of soybean (Sakurai 1960; Maruo and Yoshkawa 1989). Matsumoto and others (1993) studied the effect of temperature and inoculum size on natto’s appearance, number of B. natto, temperature, hardness, and color during fermentation. The results indicated that natto’s fermentation preceded in good condition at 35 °C, with an inoculation of 10^4 to 10^6 cells/g, or at 40 °C, with 10^2 to 10^4 cells/g of the starter. From the standpoint of the appearance, color, and hardness, 18 to 20 fermentation hours were sufficient. Matsumoto and others (1995) investigated the effect of soybean steaming conditions during manufacturing on the quality of natto products. From sensory evaluation of the final natto products, they concluded that the optimum steaming time for soybeans was 30 to 40 min at 1.5 kg/cm² pressure. There is a controversy on fermentation time from the different aspects of consideration. Sakurai (1960) believed 6 h (40 to 42 °C) of fermentation to be favorable, judging from the palatability, digestibility, and yield of finished product. Ohta (1986) demonstrated 18 to 20 h (40 to 42 °C, 80 to 90% of RH) to be necessary for complete fermentation. Maruo and Yoshkawa (1989) suggested that natto product was considered to be of good quality after 16 to 18 h of fermentation (42 °C, 85 to 90% of RH). After studying the changes in molecular weight of proteins during fermentation, Ikeda and Tsuno (1985) implied that natto formation appears to occur at 14 h of fermentation of soybean.

Complex biochemical reactions occur during natto fermentation (Hayashi 1959; Ikeda and Tsuno 1984, 1985; Kanno and Takamatsu 1987; Kanno and others 1982, 1985; Kusano 1969, 1971; Maruo and Yoshkawa 1989; Ohno and others 1990; Ohta 1986; Taira and others 1983; Taguchi and others 1986). The degree and speed of the reactions, and substances produced depend on the conditions of soaking, steaming, fermentation, and bacteria strains as well. Natto has different aroma, texture, flavor, viscosity, and amount of mucus under various combinations of processing. In the literature, there are no small-scale laboratory methods available for evaluating the suitability of soybean cultivar for natto processing. The objective of this study was to determine natto product characteristics as affected by various treatments of steaming times, bacteria strain, and fermentation times. It is imperative that a lab-processing scheme could be identified for future investigation of the suitability of soybeans for natto making.

Materials and Methods

Soybean
Soybean samples SSND91-2330 (Danatto) were obtained
Natto Quality as Affected by Processing . . .

Preparation of inoculum

Several colonies from nutrient agar or 10 g of a commercial natto product were cultured in a sterilized nutrient broth (NB) (200 ml per 250-ml Erlenmeyer flask) at 40 °C for approximately 16 h on a rotary shaker (200 rpm). B. natto population in NB increased dramatically after 2 to 14 h of growth at 40 to 42 °C. The OD 660 values were measured with a spectrophotometer (Model 20D, Milton Roy Company, Rochester, N.Y., U.S.A.). The cells produced from an incubation period of 16 h with a high viable population were harvested from 200 mL of NB. After 16 h of growth, OD 660 value of the NB inoculated with the 10-gram commercial bean was about 1.5, and the bacteria populations were in the range of 10^7 to 10^8 cfu/mL. The cell pellet from 200 mL of NB centrifuged at 12,000 rpm (22100 × g) (BECKMAN J2-HS, Beckman Instruments, Inc., Palo Alto, Calif., U.S.A.) for 25 min was diluted in 20 mL of Butterfield’s phosphate buffer to prepare a suspension, which was used for inoculating the steamed soybeans.

Preparation of soybean as fermentation medium

After removing impurities, the soybean samples were washed, soaked in water at the room temperature (21 to 23 °C) for approximately 16 h until the weight increase ratio (WIR) to the original dry weight was in the range of 2.1 to 2.3, and then drained. Soaked beans were placed in a steaming shelf and steamed at 121 °C (1.2 kg/cm²) for 20, 25, 30, 35 and 40 min in a retort (Reid Boiler Works, Inc., Bellingham, Wash., U.S.A.). Pressure in the retort was built up in 15 min and released gradually in 5 min after the steaming time was ended. Steamed beans were then covered immediately with an aluminum foil and weight was measured. The beans (30 to 40 °C) were inoculated quickly with the bacteria inoculum.

Inoculation and incubation

Five milliliter inoculum preparation of B. natto was added to 120 g of steamed beans. Steamed beans were placed into sandwich Styrofoam® boxes (90 × 90 × 35 mm). The inoculum and beans were thoroughly mixed together, and covered with a piece of perforated plastic film on the top of packaged beans. Inoculated beans were fermented in a bacteriological incubator (Stabil-Therm Dry Type, Gravity Convection, BLUER, Blue Island, Ill., U.S.A.) at 40 to 42 °C for 10, 12, 14, 18 and 20 h, respectively, under 85 to 90% of RH.

Broken bean ratio of steamed beans

After soaking to achieve weight increase ratio of 2.1 to 2.3 and steaming (121 °C) for a period of time (20, 25, 30, 35, or 40 min), the weight of the sample was recorded, then the broken beans were picked out of the sample and weighed. The ration of the percentage of the weight of broken beans to the intact beans were recorded as the broken bean ratio of the steamed beans.

Moisture content of steamed beans

A 50-g portion of steamed beans was weighed out after cooling to the ambient room temperature on an aluminum film. A portion of the steamed bean was mashed by using a mortar and a pestle. The moisture content was determined after drying at 105 °C for 16 h in a hot air oven (Precision Scientific Inc., Chicago, Ill., U.S.A.), and expressed as the percentage of the weight of the sample.

Color of steamed beans and natto products

The color of the steamed beans and natto was measured with a colorimeter (Model CL-23 Lab Colorimeter, Gardner Laboratory Instrument, Bethesda, Md., U.S.A.) using a standard white tile as reference (L = 91.94, aL = 1.03, bL = +1.14).

pH value of natto products

A portion of 1.8-mL double distilled water was added to a mashed sample of 3 g of natto. The pH value was measured with a digital pH/millivolt meter (Model 61, Orion Research, Boston, Mass., U.S.A.).

Firmness of steamed beans and natto products

After steaming, the steamed beans were cooled to room temperature before testing firmness property. Natto products were stored in a refrigerator to inhibit fermentation, and were equilibrated to room temperature before testing firmness with an Instron Universal Testing Machine (Model 1011, Instron Corp., Canton, Mass., U.S.A.). A Kramer shear cell and a weight beam of 500 kg were used. The speed of the crosshead and the recording chart was set at 20 mm/min. The results were expressed as the force (kg) to shear through 100 g of the steamed beans or natto.

Viscosity of natto

Fifteen grams of the natto product was weighed and shaken vigorously in 15 mL of pH 7.4, 0.2 M sodium phosphate buffer for 2 min. The mixture was allowed to settle for at least a minute, so that the foam rose to the top. An aliquot of 500 mL of the extract was pipetted into the plate cup of a Wells-Brookfield Cone/Plate viscometer (Stoughton, Mass., U.S.A.) and measured at 3.0 rpm at 30C.

Ammonia content of natto

The ammonia content of the natto product was determined using a modified colorimetric method (AOAC Method 973.25 1995). The modified bromine solution was prepared with 10 mL of NaOH solution (50% of NaOH in carbon dioxide-free water) and 90 mL of double distilled water by adding 1.0 mL of concentrated bromine reagent. After mixing a portion of sodium hydroxide, fresh thymol, and bromine solution, the color intensity of the mixture in a 2.5% phosphotungstic acid solution (180 mL for 20-g bean sample) was related to the ammonia content. Separation of the clear color solution and the turbid portion was conducted by using n-butanol. The absorption of the color solution was measured...
with a spectrophotometer (Model 20D, Milton Roy Co., Rochester, N.Y., U.S.A.) at 580 nm. The ammonia content (mg/100g natto product) was calculated by using a standard curve of ammonia.

Population of *B. natto*

The viable populations of cells in the inoculum and finished natto products were determined by the aerobic plate count procedure using the spread plate method (FDA, Bacteriological Analytical Manual 1995).

Statistical analysis

Two completely randomized single-factor designs with five-level intervals of steaming time (20, 25, 30, 35, and 40 min) and 9-level types of bacterial species (NRRL B-3383, NRRL B-3384, NRRL B-3385, NRRL B-3386, NRRL B-14202, ATCC 15245, Yamada natto strain, Mito natto strain, and Ito-biki natto strain) were applied for determining the effect of the conditions of steaming time and bacteria strain. One randomized complete block design with factorial arrangement was used to determine the effect of fermentation time (10, 12, 14, 16, 18, and 20 h) of natto processing in which there were 4 observations per treatment of incubating time within each block of steaming time. The other factor to affect fermentation time was bacteria strains with two levels (two types of *B. natto* strains) of effect. Analyses of variance (ANOVA) and Duncan’s Multiple Range Test were conducted using the statistical analysis system (SAS Institute, Inc. 1989) for color, pH, firmness, viscosity, ammonia content, and bacteria population of steamed bean and natto product analyses, respectively.

Results and Discussion

Effect of steaming time

Statistical analysis showed that different steaming times did not significantly (p > 0.05) affect moisture content, broken bean ratio, and firmness of the steamed beans (Table 1). Steaming time longer than 25 min made the steamed beans darker gradually; however, steaming time did not affect the color of the final fermented product (Table 2). In a study of suitability of soybean varieties grown in Ibaraki prefecture (Japan) for natto production, Taira and others (1983) reported that the average of moisture content and broken bean ratio after steaming were 59.5% (56.1 to 60.6%) and 5.8% (1.5 to 15.0%), respectively. In their study, WIR of the samples of various soybean cultivars after soaking was 2.48 (2.55 to 2.41). The small seed size cultivars were steamed for 23 min at 2.0 kg/cm², the middle and large seed size cultivars were steamed for 35 min at 1.9 kg/cm². In the other study of suitability of small seed cultivars for natto processing, Taira and others (1987) reported that the moisture content and broken bean ratio after steaming were in the range of 58.2 to 61.6% and 0 to 18%, respectively. In their research, WIR of the cultivars of the small seed type after soaking was 2.19 to 2.49 and the samples were steamed for 20 to 30 min under the pressure of 1.7 to 2.0 kg/cm² (Taira and others 1987). The broken bean ratios in this study were lower (1.49 to 2.53%) than that reported by (Taira and others 1987) and the moisture contents were similar to their values. In our study, the WIR of Danatto soybean after soaking was in the range of 2.1 to 2.3 and the steam pressure used to cook Danatto was 1.2 kg/cm² (121 °C).

The firmness of steamed beans was not affected by changing the steaming time (Table 1); however, the firmness of the final fermented natto product was affected (Table 2). When steamed for 20 min, the natto product had the highest Instron reading (kg) compared with that of the beans steamed for 25, 30, 35 and 40 min. There was no significant difference (p > 0.05) in the firmness of natto products between steaming times of 30, 35 and 40 min (Table 2). In the studies of Taira and others (1983 and 1987), the firmness determination method differed from the method in this study. Although different steaming periods did not significantly (p > 0.05) influence the firmness of steamed beans, however, the longer steaming times might have made the microstructure of the beans more penetrable to bacteria, thereby, affecting the texture of the final products.

The viscosity (21.5 to 42.2 cps) and ammonia-nitrogen content (approximately 85 to 107 mg/100g), important criteria for the quality of natto products, were statistically the same (p > 0.05) in the different steaming periods (Table 2). Taira and others (1983 and 1987) did not report viscosity values of the natto products, but ammonia-nitrogen contents (215 to 360 mg/100g on a dry matter basis) were generally higher than that observed in our study.

Table 1—Effect of steaming times on selected physical properties of steamed beans

<table>
<thead>
<tr>
<th>Properties</th>
<th>Steaming times (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>60.09 ± 0.51</td>
</tr>
<tr>
<td>Broken bean ratio (%)</td>
<td>1.49 ± 0.72</td>
</tr>
<tr>
<td>Color (L value)</td>
<td>42.13 ± 0.84</td>
</tr>
<tr>
<td>Firmness (kg)</td>
<td>45.8 ± 1.79</td>
</tr>
</tbody>
</table>

*Means (SD) of at least 3 replicates, Means within the same row with different letters differed significantly (p < 0.05).*

Table 2—Effect of steaming times on selected physical and chemical properties of natto

<table>
<thead>
<tr>
<th>Properties</th>
<th>Steaming times (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Color (L value)</td>
<td>35.74 ± 0.84</td>
</tr>
<tr>
<td>pH Value</td>
<td>266.78 ± 0.18</td>
</tr>
<tr>
<td>Firmness (kg)</td>
<td>51.8 ± 2.75</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
<td>21.5 ± 6.10</td>
</tr>
<tr>
<td>Ammonia content (mg/100g)²</td>
<td>266.78 ± 25.28</td>
</tr>
<tr>
<td>Bacteria population (10⁶ cfu/g)³</td>
<td>5.5 ± 1.90</td>
</tr>
</tbody>
</table>

*Means (SD) of at least 3 replicates, Means within the same row with different letters differed significantly (p < 0.05).*

²Soybean sample soaked in water at the room temperature for 16 h and steamed at 121 °C.

³Soybean sample soaked in water at the room temperature for 16 h, steamed at 121 °C inoculated with a commercial Ito-biki natto culture (Takahashi Food Corporation of Tokyo, Japan), and fermented at 40–42 °C and 85–90% of RH for 16 h.

Data based on a dry matter basis. Moisture content of the natto samples in the range of 59.0–61.0%.

Data based on a wet matter basis.
ness decreased as the steaming time increased from 20 to 30 min, but the ammonia content did not show significant differences (p > 0.05).

A high-quality natto product is supposed to have a high viscosity, low ammonia-like flavor, and a palatably soft texture. A range of 100 to 150 mg/100g bean (on a wet matter basis) of ammonia-nitrogen is acceptable by Japanese consumers in Japan (Maruo and Yoshkawa 1989).

Effect of bacteria strains

The 9 strains of *B. natto* grew well on the PCA plates when we checked for the viability of the cultures upon receipt. Therefore, they were inoculated onto steamed (35 min) Danatto soybean and fermented at 40 to 42 °C for 16 h, and the firmness, viscosity, and ammonia content of the final products were determined.

According to the literature, natto fermentation proceeded in good condition at 35 °C, with an inoculation of $10^4$ to $10^6$ cells/g or at 40 °C, $10^2$ to $10^4$ cells/g of the starter, judging from the appearance, color, and hardness for 18 to 20 fermentation hours (Matsumoto and others 1993). From a preliminary study conducted in our lab, the initial population in the inoculated beans was in the range of $10^6$ to $10^7$ cfu/g, and the population of bacteria in the final products was between $10^8$ and $10^9$ cfu/g bean.

The strains from the commercial Yamada, Mito natto, ATCC, and some NRRL strains had high populations (2.9 to $5.0 \times 10^9$ cfu/g) after 16 h growth on the Danatto soybean. However, their natto products had much lower viscosity (4.1 to 5.2 cps) than the natto inoculated with the strain from the commercial Itobiki natto under the same incubation conditions (Table 3). Poly-glutamic acid (PGA) and polysaccharide are responsible for the viscosity of natto (Fujii and others 1975). The *B. natto* cultures, such as the NRRL B-3386, NRRL B-3385, and the strain from the commercial Mito natto, tended to produce more ammonia (262.65 to 280.50 mg/100g bean on a dry matter basis). The NRRL B-3384 was, obviously, a poor one for natto processing, because of its low population ($0.4 \times 10^9$ cfu/g), the firm texture (62.6 kg) of its natto products, and the low viscosity (5.9 cps). The pH of the steamed beans was slightly acidic (pH 6.2 to 6.8) and that natto products was slightly alkaline (pH 7.2 to 7.6) (Maruo and Yoshkawa 1989). The insufficient fermentation of the NRRL B-3384 strain was associated with a lower natto pH value (6.79). The natto made with the strain from the commercial Itobiki natto had a soft texture (48.2 kg), high viscosity (23.0 cps), and ammonia content (0.121% on a wet matter basis equivalent to 301.55 mg/100 g on a dry basis), which is in the acceptable range (0.1 to 150 mg/100g on a wet basis) of consumers as described by Maruo and Yoshkawa (1989). The natto produced with “Itobiki strain” possessed a very strong aroma of natto and a dense mucous appearance. The quality of natto made from NRRL B-3383, with a softer texture (43.9 kg), and 9.9 cps viscosity was the next best to that made from “Itobiki strain”.

Effect of fermentation time

There seems to be a disagreement in the literature about optimum duration of fermentation time for industrial natto making, which ranges from 6 h (Sakurai 1960) to 20 h (Beuchat 1983). Most of the reports preferred the fermentation time of 16 to 18 h (Ohta 1986; Maruo and Yoshkawa 1989).

The pH value of the steamed bean was 6.25. Fermentation lead to an increase in pH value (Figure 1). Using “Itobiki strain” and under 40 min steaming condition, natto products showed the quick increase in pH value during 10 h of fermentation (pH = 7.21) (Figure 1). There were no significant changes (p > 0.05) in pH from the 10 to 20 h of fermentation (pH = 7.58). The pH value of natto beans cooked for 35 min was 6.59 in 10 h of fermentation and did not had a significant change (p > 0.05) until 20 h of fermentation (pH = 7.87). Inoculated with NRRL B-3383 strain and steamed for 40 min, natto products had the pH value 7.08 until 14 h of fermenta-
Natto Quality as Affected by Processing . . .

The relationships between firmness and fermentation time differed for all 4 conditions of bacteria strain-steaming time treatments (Figure 2). Statistical results showed no significant difference in the firmness of the natto products which were produced by steaming soaked beans for 40 min and followed by inoculating with the “Itobiki strain” for all of the fermentation times (Figure 2). However, the firmness of natto products made by steaming soaked beans for 40 min and inoculating with NRRL B-3383 strain had a decreasing trend as fermentation time increased (Figure 2). There were no significant differences (p > 0.05) between the firmness values of natto inoculated with the both strains in 16 and 18 h of fermentation, and 16, 18, and 20 h of fermentation, respectively, if beans were steamed for 35 min. The firmness of natto inoculated with NRRL B-3383 strain had an irregular pattern, which was similar to “Itobiki strain” when beans were steamed for 35 min. It is not clear why the natto steamed for 35 min increased firmness when fermentation time increased from 18 to 20 h. Comparing 35 min- and 40-min steaming time, 40-min steaming yielded natto products with substantially lower firmness, which was observed in products made by both strains (Figure 2). Longer steaming might have made nutrients more accessible to bacteria. Bacteria colonies and mucilage produced by the microorganism grown on soybean substrate covered the surface of the beans of 40 min steaming earlier during fermentation than that did on the beans of 35 min steaming. The mucilage might have prevented moisture loss during the later stage of fermentation.

In Japan, when natto is served, it is stirred vigorously with

Table 3—Effect of Bacillus natto strains on selected physical and chemical properties of natto

<table>
<thead>
<tr>
<th>Properties</th>
<th>NRRL B-3383</th>
<th>NRRL B-3384</th>
<th>NRRL B-3385</th>
<th>NRRL B-3386</th>
<th>NRRL B-14202</th>
<th>ATCC 15245</th>
<th>Yamada Natto</th>
<th>Mito Natto</th>
<th>Itobiki Natto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color (L value)</td>
<td>36.87b</td>
<td>37.34b</td>
<td>34.16de</td>
<td>34.09de</td>
<td>33.15e</td>
<td>34.24de</td>
<td>35.26cd</td>
<td>36.33cd</td>
<td>35.04cd</td>
</tr>
<tr>
<td>pH Value</td>
<td>7.94a</td>
<td>6.79b</td>
<td>8.02a</td>
<td>7.90a</td>
<td>8.06a</td>
<td>8.01a</td>
<td>7.74ab</td>
<td>8.16a</td>
<td>8.10a</td>
</tr>
<tr>
<td>Firmness (kg)</td>
<td>43.3d</td>
<td>62.6a</td>
<td>53.4bc</td>
<td>51.2cd</td>
<td>61.2ab</td>
<td>57.3ab</td>
<td>62.8ab</td>
<td>53.7ab</td>
<td>48.2cd</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
<td>9.9b</td>
<td>5.9c</td>
<td>4.5c</td>
<td>5.5c</td>
<td>4.1c</td>
<td>5.2c</td>
<td>4.3c</td>
<td>4.7c</td>
<td>23.0a</td>
</tr>
<tr>
<td>Ammonia Content (mg/100g)</td>
<td>259.50ab</td>
<td>113.30c</td>
<td>279.53a</td>
<td>280.50a</td>
<td>230.98b</td>
<td>168.33c</td>
<td>124.50d</td>
<td>262.65ab</td>
<td>301.55a</td>
</tr>
<tr>
<td>Bacteria Population ×10⁹ (cfu/g)</td>
<td>(1.49)</td>
<td>(0.31)</td>
<td>(0.73)</td>
<td>(2.38)</td>
<td>(2.36)</td>
<td>(2.13)</td>
<td>(2.02)</td>
<td>(4.15)</td>
<td>(2.54)</td>
</tr>
</tbody>
</table>

* Means (SD) of at least 3 replicates. Means within same row with different letters are significantly different (p < 0.05).

*Soybean sample was soaked in water at the room temperature for 16 h (Weight increase ratio: 2.1–2.3), steamed at 121 °C for 35 min, and fermented at 40–42 °C and 85-90% of RH for 16 h.

Data based on a dry matter basis. Moisture content of the natto samples in the range of 59.0–61.0%.

Data based on a wet matter basis.
a pair of chopsticks to generate the silkiness/stickiness to form a mass of beans. Silky stickiness feeling due to a high viscosity of the mucilage is the most important criterion of a good natto. In a preliminary study we found that objective instrumental viscosity analysis was correlated with sensory viscosity (unpublished data). In general, the natto steamed for 40 min produced higher viscosity than that steamed for 35 min (Figure 4). There was a significant increase ($p < 0.05$) of natto viscosity along the fermentation times from 10 to 18 h for all 4 conditions. The viscosity continued to increase for the natto inoculated with the NRRL B-3383 strain, steamed for 35 min, and fermented from 18 to 20 h. However, the viscosity decreased significantly ($p < 0.05$) for the natto inoculated with the “Itobiki strain”, steamed for 40 min, and fermented between 18 and 20 h, during which the bacteria might have secreted enzymes to break down some of the mucilage. For the natto inoculated with the NRRL B-3383, the highest viscosity occurred at 18 h of fermentation for 40 min steaming, and at 20 h of fermentation for 35 min steaming. The “Itobiki strain” produced a significantly higher ($p < 0.05$) viscosity than did the NRRL B-3383 strain at 35 and 40 min steaming times during 12, 14, 16, 18, and 20 h of fermentation (Figure 4). The increasing trend in viscosity with fermentation time implies that a longer time might be required when using the NRRL B-3383 to produce a natto product with the same level of viscosity as that produced by the “Itobiki strain” at 18-h fermentation (Figure 4). However, a relatively longer steaming time may be used to shorten the fermentation time to obtain a viscous natto.

The ammonia content, like the viscosity, increased as the fermentation proceeded (Figure 5). There were significant differences ($p < 0.05$) in the ammonia content of the natto products during every 2-hour interval of fermentation for both steaming times and the 2 $B. natto$ strains. The “Itobiki strain” tended to produce more ammonia than the NRRL B-3383 did under both 35 and 40 min steaming times, respectively. Both bacteria strain and steaming time affected the ammonia content of the natto products. Based on the results, the “Itobiki strain” may be used to produce acceptable natto product when steamed for 40 min and incubated for various periods of times (12 to 20 h) depending upon the desired range of ammonia content and viscosity.

**Conclusion**

**A longer steaming time could shorten the fermentation time needed to obtain a good quality natto product.** In the late stage (18 to 20 h) of fermentation, the natto steamed for a longer time (40 min) contained lower contents of ammonia as compared to the natto steamed for 35 min. Under the same experimental conditions, the “Itobiki strain” produced higher viscosity and softer texture natto from the Danatto soybean than the NRRL B-3383 strain. Firmer texture, higher concentration of ammonia, and lower quantity of mucilage could result from a prolonged fermentation (20 h). Under our laboratory conditions, a steaming time of 40 min, the “Itobiki strain” or NRRL B-3383 strain, an inoculation size of $10^6$ to $10^7$ cfu/g (on a wet bean matter basis), and the fermentation time of 18 h were suitable for natto making. Therefore, these combinations of the processing conditions may be used as a laboratory method for evaluating soybean cultivar for natto making.

**References**


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