



Evaluation of seed chemical quality traits and sensory properties of natto soybean



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ABSTRACT

Natto is a popular soyfood in Japan, and the U.S. is the largest supplier of natto soybeans. However, information on natto seed chemical and sensory properties is very limited. The objectives of this study were to evaluate differences of seed chemical and sensory properties among natto types and determine heritability and correlation. A total of 15 small-seeded natto genotypes (three superior, nine moderate and three inferior) were evaluated for protein, oil, calcium, manganese, boron and sugar content and processed into a natto product to evaluate appearance, stickiness, flavor, texture and shelf-life. The superior natto group had a higher sugar content but lower protein plus oil, calcium, manganese and boron content than other two groups. Most seed quality traits exhibited high heritability. The natto sensory preference was positively correlated with sucrose and oil content, but negatively correlated with seed hardness, protein, protein plus oil, calcium, manganese, and boron contents. Selecting soybean lines with low protein, protein plus oil, calcium, manganese, and boron content while with high sucrose will be an effective approach for soybean breeding for natto production.

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1. Introduction

Natto is a fermented soyfood mainly consumed in Japan (Taira et al., 1982). Natto is made by fermenting seeds with a bacterium, *Bacillus subtilis* (natto), which provides a unique flavour and stickiness (Watanabe, 2006). The natto market is relatively small compared to other soyfoods, such as tofu and soymilk. Also, the unfamiliar aroma, flavour, and texture of natto have kept it from becoming a popular international soyfood (Hosoi & Kiuchi, 2003; Zhang et al., 2008b). However, natto is very popular soyfood in Asia, especially Japan, which has a stable market that provides the US soybean growers very profitable opportunities.

Seed quality evaluation is essential to facilitate soybean breeding for natto production in the United States, where we have very limited information on seed chemical quality traits and sensory properties. Measuring protein and oil content is simple and easy for indirect natto selection (Geater, Fehr, Wilson, & Robyt, 2001). One of the important traits for the natto soybean is sugar content, but sugar analysis is time consuming and costly. Since protein and oil content have a strong negative correlation with total sugar ($r = -0.81$), the selection of genotypes with lower protein and oil content should produce sweeter natto (Geater & Fehr, 2000).

Total sugar and sucrose content advances the taste of soyfood. During the fermentation of natto, high total sugar and sucrose content help to produce natto with better flavour (Taira, 1990).

Negative correlations have been reported between glucose and sucrose, fructose and sucrose, and fructose and stachyose content (Hou, Chen, Gray, Giannoccaro, & Wang, 2006). On the other hand, total sugar content was positively correlated with sucrose, raffinose, and stachyose content. There was also a positive correlation between raffinose and stachyose and between glucose and fructose contents (Cicek, Chen, Saghai Maroof, & Buss, 2006). In addition, Geater and Fehr (2000) found that natto hardness was negatively correlated with the total sugar content.

Few studies have specifically reported an association of minerals, such as calcium, manganese, and boron with natto quality. However, it is important to examine if there is a correlation between these minerals, other soybean chemical quality traits, and natto quality. For example, calcium performs structural roles in the cell walls and membranes (White & Broadley, 2003); manganese is important to photosynthetic oxygen evolution in chloroplasts within most plants (Kuwabara & Murata, 1983); and boron maintains the integrity of cell walls. However, there is no information regarding the effect of these minerals on soyfood quality.

Fermentation by *B. subtilis* (natto) is a crucial step in natto production. *B. subtilis* (natto) is about $0.8 \times 3 \mu\text{m}$ in size and can be found everywhere in nature, especially in soil and crop residue (Watanabe, 2006). During the fermentation by *B. subtilis* (natto),

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amylase and protease help to produce natto with a unique flavour, soft texture, and stickiness. The stringiness and stickiness of natto is caused by poly-glutamic acid and fructan during fermentation (Yoshioka, Sekine, & Otake, 2007).

In addition, natto quality is affected by its shelf life, which is determined by changes in appearance, stickiness, flavour, and texture over time. As natto ages, the taste of natto will drastically deteriorate and white crystals, consisting of tyrosine and struvite, or ammonium magnesium phosphate (MgNH_4PO_4), will form on the surface, which is not accepted by consumers. Tyrosine is one of the components of the umami taste, but it does not adversely affect natto taste. Struvite, however, worsens the flavour of natto and dramatically reduces natto's shelf life (Muramatsu, Yasui, Suzuki, & Kiuchi, 2000).

Although natto breeding has been conducted at several universities and food processing companies, there is still very little research on natto seed quality and sensory analysis. Sensory evaluation is necessary for food products due to its psychological factors rather than field or laboratory factors (Fuller, 2005). Although it is difficult and expensive to measure sensory properties among samples and use sensory panel, it is too vital to be omitted from crop breeding and food product development (Fuller, 2005).

The specific objectives of this research were: (1) to evaluate natto seed chemical quality traits including seed protein, oil, protein plus oil content, minerals (calcium, manganese, and boron), sugars (glucose, fructose, sucrose, raffinose, stachyose, and total sugar), and sensory traits of 15 natto soybean lines across locations and year, and (2) to determine the heritability and correlation of seed chemical quality traits and natto sensory properties.

2. Materials and methods

2.1. Plant materials

The natto soybean genotypes evaluated included three superior natto varieties used in commercial natto production (ARK1, ARK2, and ARK3), nine moderate natto varieties with intermediate marketing value (MO8109, MO8750, R02-1983, R05-1953, R05-1989, R05-2206, R05-2629, R05-2734, and SS 516), and three unacceptable natto varieties determined by Japanese natto marketers (R04-245, R05-1298, and R05-1679). ARK1, ARK2, ARK3, and SS 516 are commercial natto cultivars. ARK1 was developed by Kaneko Seed Company, Gunma, Japan; and ARK2 and ARK3 were developed by Takano Foods Corporation, Ibaraki, Japan. SS 516 was released by the Southern States Seed Company, Richmond, VA. MO8109 and MO8750 natto lines were developed by Dr. J.G. Shannon at the University of Missouri Delta Research Center, Portageville, MO, and the other R-lines were developed by Dr. P. Chen at the University of Arkansas, Fayetteville, AR. Relative maturities of these lines ranged from 4.8 to 5.7, with either determinate or semi-determinate growth habit. The seed size and plant height ranged from 68 to 137 mg seed⁻¹ and 49 to 88 cm, respectively.

2.2. Field experiment

The 15 natto varieties were grown in a randomized complete block design (RCBD) with three replications at Keiser, Rohwer, and Stuttgart, Arkansas in 2008 and 2009. Keiser is in northeastern Arkansas, Rohwer is in the southeastern corner of the state, and Stuttgart is in the east-central part of the state. The soil types of these locations were Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) in Keiser and Rohwer, and Dewitt silt loam (fine, smectitic, thermic Typic Albaqualfs) in Stuttgart.

Each genotype was grown in a four-row plot with 6.1 m plot length and 0.81 m row width. On average, 175 seeds row⁻¹ were planted, and the center two rows of each plot were harvested.

The planting dates in 2008 were June 12th at Keiser, AR and May 23rd at Rohwer and Stuttgart, AR. The harvest dates in 2008 were November 7th at Keiser, October 6th at Rohwer, and October 24th at Stuttgart. The planting dates in 2009 were May 21th at Keiser and Stuttgart and May 22nd at Rohwer. The harvesting dates in 2009 were November 5th at Keiser, November 9th at Rohwer, and November 4th at Stuttgart.

The fields were prepared with a chisel plow and disk. Fertilization was applied based on soil tests conducted by the University of Arkansas Cooperative Extension Service. Pre-emergence weed control was achieved using Scepter70DG (BASF Corporation, Florham Park, NC) and Dual/Magnum (Syngenta, Greensboro, NC). Post-emergence weeds were managed with Surfactant and Firstrate herbicide (Dow Agro Sciences LLC, Indianapolis, IN), and general weed control was done using Surfactant and Flexstar (Syngenta, Greensboro, NC). All field experiments were conducted under irrigated conditions according to the irrigation schedule of Arkansas Research and Extension Center affiliated with the University of Arkansas.

2.3. Seed chemical quality traits

A twenty-five gram sample of cleaned soybean seeds was used in the analysis for seed protein and oil content (g/kg) with a Foss Infracac Grain Analyzer (NCP Soybean Project Laboratory, NCAUR-ARS-USDA, Peoria, IL). Three mineral components (calcium, manganese, and boron) were measured by inductively coupled plasma spectrophotometry (ICP) using a CIROS model ICP (Spectro Analytical Instruments, Inc., Mahwah, NJ) at the Agricultural Diagnostic Laboratory of the University of Arkansas, Fayetteville, AR. Ten grammes of seed sample from each plot was ground with a regular bean grinder (Applica Consumer Products Inc., Miramar, FL) and then screened through a 250 µm standard testing sieve (VWR International, Bridgeport, NJ). A sample of 0.25 g of dried and ground seeds was digested by 2.5 ml concentrated HNO_3 (Mallinckrodt Baker, Inc., Phillipsburg, NJ) and put into a digestion tube (Environmental Express, Mt. Pleasant, SC). The tubes were allowed to predigest overnight and were placed on a heating block (Environmental Express, Mt. Pleasant, SC) to heat slowly to 60 °C in 30 min. One ml of 30% H_2O_2 (Mallinckrodt Baker, Inc., Phillipsburg, NJ) were added and heated to 110 °C for about 1 h or until the volume was reduced to about 2 ml. The tubes were then cooled and brought to 25 ml total volume with deionized water and shaken. The samples were then settled and analyzed in a CIROS model ICP. The sugar extraction procedure was used as described by Hou, Chen, Shi, Zhang, and Wang (2009). Glucose, fructose, sucrose, raffinose, and stachyose were measured by high performance liquid chromatography (HPLC). For each plot, a 10 g seed sample was ground and screened as described above. For soluble sugar extraction, 0.15 g of soybean powder was placed in a 2 ml Eppendorf microcentrifuge tube and 1.5 ml of distilled deionized water was added. The tubes were incubated at room temperature on a horizontal shaker at 229 times gravity ($\times g$) for 15 min, followed by 10 min. of centrifuging at 16,000g. A total of 500 µl supernatants were transferred to a new tube and mixed with 700 µl acetonitrile by inversion and the mixtures were kept at room temperature for 30 min. The sample was then centrifuged at 16,000g for 10 min and 500 µl supernatant was filtered through a 0.2 µm membrane. A total of 24 µl aliquot of the extract was dissolved with 576 µl distilled water for injection to the HPLC system (Hou et al., 2006).

Glucose, fructose, sucrose, raffinose, and stachyose were separated and quantified by HPLC. A Dionex DX500 high performance anion exchange chromatograph with pulsed amperometric detection (HPAEC-PAD), equipped with a GS50 pump, an ED40 pulsed amperometric detector, an AS40 automated sampler with a 25 µl

injection loop, and a Chromeleon Chromatography Management System (Dionex, Sunnyvale, CA) were used. An analytical CarboPac PA10 pellicular anion-exchange resin column (250 × 4 mm) preceded by a CarboPac PA10 guard column (50 × 4 mm) and an AminoTrap column (30 × 3 mm), were used for sugar separation. A 90 mM NaOH solution was used at a flow rate of 1.0 ml min⁻¹ under isocratic conditions for eluting sugars. The mobile phase of 90 mM NaOH was prepared by diluting carbonate-free HPLC grade 50% (w/w) filtered through a 0.45 μm membrane and degassing with compressed nitrogen gas for 25 min (Hou et al., 2009).

Glucose, fructose, sucrose, raffinose, and stachyose were identified by comparing their retention times to those of pure standards (Sigma Chemical Co., St. Louis, MO). A series of solutions with concentrations of 10, 20, 40, 60, and 80 μg 0.6 ml⁻¹ were prepared to obtain regression curves for quantifying the five soluble sugars. A standard curve was established by using a series of sugar standards with known amount and it was used in quantification of all five soluble sugars in the testing samples.

2.4. Hardness test

Thirty grammes of unbroken uniform seeds from each progeny line were weighed and soaked in heat resistant plastic boxes containing 150 ml water at room temperature for 16 h. Seeds were recovered from soaking water with a sieve and blot dried on paper towels. Stone seeds that did not absorb water during soaking process were removed. Soaked seed samples were pressure cooked in heat resistant plastic boxes at 121.1 °C and 1.2 kg cm⁻² for 20 min. Hardness testing of 30 g cooked seeds from each sample was conducted in two replications using TMS Texture System (TMS-2000, Food Technology Corp., Sterling, VA) equipped with a 16-blade shear cell. The maximum force to compress cooked seeds in newtons (N) was determined as seed hardness (Song et al., 2003).

2.5. Natto processing

A total of 200 g of unbroken seeds from two replicated plots of each variety at each location were soaked for 16 h at room temperature. After being drained with a colander, seeds were cooked at 123 °C with 1.2 kg cm⁻¹ for 13 min in a portable autoclave ES-315 (Tomy Tech USA, Inc., Fremont, CA). Tools that were used for natto processing, such as bowls, spoons, and tips were sterilized at 121 °C with 1.05 kg cm⁻¹ for 15 min. Forty-five grammes of cooked seeds were inoculated with 4 ml of *Bacillus subtilis* (natto) starter culture, provided by Miyagino Company, Miyagi, Japan. Polystyrene paper package (PSP, 100 × 100 × 27 mm), typical for packaging natto product, was provided by Plastics Co., Ltd., Osaka, Japan. Six packages of natto, with 55 g natto product in each, were made from each sample: two packages for the tests of sensory attributes and shelf life at day 3; one package each for shelf life at days 5, 7, and 10; and one for temperature measurement during fermentation. Natto packages were covered with a perforated polyethylene film (Plastics Co., Ltd., Osaka, Japan) and the lid of each package was closed with a stapler. After the temperature of these natto packages dropped to 37 °C, they were incubated in a fermentation chamber (Temperature cycling chamber: Lunaire Limited Tenney Environmental Division, Parsippany, NJ) for 18 h. The fermentation chamber was maintained at 37 °C and 90% relative humidity (RH) for 7 h, then temperature was increased to 39 °C for 3 h followed by 41 °C for 7 h. Subsequently, the temperature was gradually decreased to 4 °C at 70% RH, all sample packages were moved into a refrigerator, and stored at 4 °C for 72 h.

2.6. Sensory properties

Appearance, stickiness, flavour and texture are the main natto sensory properties. A well-trained natto research specialist, T. Kawashima, working at Blue Horizon Inc., Cabot, AR and a natto researcher, Y. Yoshikawa, a M.S. student at the University of Arkansas, Fayetteville, AR tasted and evaluated all natto samples. Darkness and seed uniformity were used to evaluate appearance. Stickiness was measured by how well natto beans clung to chopsticks. Flavour was determined jointly by smell and taste. Good natto should have a plain or slightly sour smell and taste, whereas an ammonium odour and a dead leaf odour are undesirable. Natto texture should be neither too firm nor too soft. For all sensory traits, rating scores of 1–5 were used, where 5 = excellent, 4 = good, 3 = moderate, 2 = poor, and 1 = inferior (Meilgaard, Civille, & Carr, 2007; Mitsuboshi et al., 2006; Yoshioka, Sekine, Suzuki, & Otake, 2009).

Shelf life was evaluated at days 3, 5, 7, and 10 after natto was made. Shelf life was rated based on the density of struvite that tends to increase with time. A rating score of 1–5 was used for assessing natto shelf life, where 5 = stable (excellent) with no struvite crystals; 4 = good with a few struvite crystals; 3 = moderate with a moderate amount of struvite; 2 = poor, with some large sectors of struvite formation; and 1 = inferior with abundant struvite formation.

Finally, each sensory property rating was multiplied by a factor of 4 to obtain an index score out of a total of 100 points. A greater index score indicated better natto quality.

2.7. Statistical analysis

Seed chemical quality traits (seed protein, oil, protein plus oil, minerals, and soluble sugars) and sensory traits were analyzed using the General Linear Model procedure (PROC GLM) of SAS Version 9.2 for Windows (SAS Institute, Cary, NC, 2009). The Least Significant Difference (LSD) at $\alpha = 0.05$ was used for mean separation to detect significant differences among genotypes. Variance components were estimated by JMP 8.0 (SAS Institute, 2009) with the Restricted Maximum Likelihood (REML) method for heritability calculations.

Overall broad-sense heritability (H^2) for main variables was estimated using the following formula (Greene, Kenworthy, Quesenberry, Unruh, & Sartain, 2008):

$$H^2 = \sigma^2g / [(\sigma^2g + (\sigma^2gy/y) + (\sigma^2gl/l) + (\sigma^2gyl/yl) + (\sigma^2e/ryl)]$$

where σ^2g is the variance of genotypes, σ^2gy is the genotype × year variance, σ^2gl is the genotype × location variance, σ^2gyl is the genotype × year × location variance, σ^2e is the experimental error variance, y is the number of years, r is the number of replications, and l is the number of locations. The correlation coefficients for seed chemical quality traits and natto sensory properties were calculated based on genotypic means across the years and locations using the correlation procedure (PROC CORR) of SAS 9.2 (SAS Institute, 2009).

3. Results and discussion

3.1. Genetic and environmental effect on seed chemical quality traits

Analysis of variance showed the significance on genotype × year × location on protein, oil, and protein plus oil content (Table 1). In a similar study, Geater, Fehr, and Wilson (2000) indicated that genotypic effects on seed protein, oil, and protein plus oil content were significant, while non-significant effects were observed in year, location, and genotype × location interactions at three Iowa locations during two years, which are in agreement

Table 1

Analysis of variance for seed chemical quality traits of 15 natto soybean lines grown at three Arkansas locations over two years.

| Trait ^a | P | O | P + O | Ca | Mn | B | SUC | STA | TS |
|--------------------|--------------------------|-------------|-------------|-------------|-------------|-------------|-------------|----------|-----------|
| Year (Y) | NS ^b | NS | NS | NS | NS | NS | NS | NS | NS |
| Location (L) | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Y × L | *** ^c | *** | ** | ** | *** (14.6%) | *** | NS | NS | NS |
| Rep (Y, L) | ** | NS | NS | * | *** | *** | *** | *** | *** |
| Genotype (G) | *** (68.1%) ^d | *** (49.8%) | *** (47.0%) | *** (36.4%) | *** | *** (42.6%) | *** (27.0%) | NS | * (10.0%) |
| G × Y | NS | * | NS | * | NS | NS | NS | * (4.0%) | NS |
| G × L | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| G × Y × L | *** | *** | * | ** | ** | NS | NS | NS | NS |

^a P, protein; O, oil; P + O, protein + oil; Ca, calcium; Mn, manganese; B, boron; SUC, sucrose; STA, stachyose; TS, total sugar.^b NS, nonsignificant at the 0.05 probability level.^c *, **, and *** refers to significance at the probability levels 0.05, 0.01, and 0.001, respectively.^d % variation based on analysis of variance.

with the results of the current study. Genotype contributed 68.1% and 49.8% of variation on protein and oil content, respectively, suggesting that the protein and oil content are variety specific and stable, more genetically controlled, and less influenced by the environmental conditions. Yan, Lauer, Borges, and Leon (2010) also reported that there were significant differences in protein and oil content due to genotype and genotype × year × location interaction. However, such genotype × year × location interaction may be associated with changes in magnitude, but not the variety ranking by protein and oil means.

Analysis of variance for minerals showed significant differences on genotype × year × location for calcium, manganese, while no significant difference for boron content (Table 1). Significant differences were observed among genotypes for sucrose and total sugar content contributing 27% and 10% of the variation, respectively (Table 1). The differences among the genotypes and replication (Y × L) were observed for total sugar content due to the lack of significant interactions of year × location, genotype × location, and genotype × year × location. The differences on the replication (Y × L) and genotype × year interaction were observed for stachyose content. Geater et al. (2000) also reported non-significant effects of year, location, and genotype × year × location interactions on sucrose, stachyose, and total sugar content. Moreover, Geater et al. (2000) identified non-significant effects of year × location and genotype × location interactions for stachyose and total sugar.

3.2. Seed protein and oil

Seeds with low protein and oil content are desirable for high quality natto (Geater et al., 2000). Comparing different natto quality types showed that the superior and moderate natto lines had lower protein and higher average oil content than the inferior qual-

ity natto types (Table 2). In the combination of protein and oil content, superior natto lines had the lowest protein plus oil content, while inferior natto quality lines had the highest. These results indicated that selecting lines with lower protein plus oil content is desirable for good quality natto and should be considered in breeding. However, due to the strong negative correlation between protein and oil content (Chung et al., 2003; Geater et al., 2000; Panthee, Pantalone, West, Saxton, & Sams, 2005), it is difficult to breed lines with both low protein and low oil content (Panthee et al., 2005). Since high protein and oil content are desirable traits for commodity soybeans, natto requires specialty soybean lines with low protein plus oil content. Therefore, most elite germplasm would have undesirable seed profiles for natto.

3.3. Minerals

Identifying the association between minerals and natto quality would allow us to manipulate the breeding process by selecting for minerals for natto soybeans. To obtain soft natto low calcium content is preferred (Zhang et al., 2008a). When comparing the three natto groups, inferior quality lines had the highest average calcium content followed by the moderate and superior lines, confirming that low seed calcium is preferred for good-quality natto (Table 2).

The association between natto quality and manganese has not been thoroughly studied previously. Comparison of three natto quality lines showed large differences in the manganese means among the three groups (Table 2). Superior natto lines had significantly lower manganese content than the moderate or inferior natto quality lines. However, Mitsuboshi et al. (2006) reported that adding Mn²⁺ helped *B. subtilis* (natto) form more spores during fermentation but current research results differ. The functionality of Mn²⁺ in fermentation and natto quality needs to be further investigated.

Table 2

Seed chemical quality traits and sensory properties of three quality natto types grown at three Arkansas locations over two years.

| | Seed chemical quality trait ^a | | | | | | | | | Sensory property ^a | | | | | |
|---------------------------|------------------------------------------|-----------|-----------|------------------------------|-------------|-------------|---------------------------|---------|-----------|-------------------------------|-------|-----|-------|-------|---------|
| | P g kg ⁻¹ | O | P + O | Ca g 100 kg ⁻¹ | Mn | B | SUC g kg ⁻¹ | STA | TS | APP | STI | FLA | TEX | SHL | INDEX |
| Superior (3) ^b | 399 b | 200 a | 599 c | 240 c | 24.7 c | 27.8 c | 52 b | 50 a | 117 b | 4 a | 4 a | 4 a | 3 a | 3 a | 81 a |
| Range | (396–404) | (199–203) | (596–603) | (229–251) | (22.0–26.3) | (27.1–28.8) | (49–55) | (49–53) | (113–119) | 4 | 4 | 4 | (3–4) | 3 | (80–81) |
| Moderate (9) ^b | 400 b | 203 a | 603 b | 266 b | 32.5 b | 30.4 b | 58 a | 50 a | 122 a | 3 b | 4 a | 3 b | 3 b | 2 a | 68 b |
| Range | (386–410) | (196–213) | (592–612) | (219–286) | (27.9–35.3) | (26.5–34.2) | (55–60) | (47–52) | (117–128) | 3 | (2–5) | 3 | 3 | (1–3) | (59–74) |
| Inferior (3) ^b | 438 a | 183 b | 622 a | 312 a | 37.9 a | 36.7 a | 49 b | 50 a | 113 b | 2 c | 3 b | 2 c | 2 c | 1 b | 51 c |
| Range | (438–440) | (173–192) | (613–631) | (280–352) | (36.6–39.5) | (33.6–39.0) | (47–52) | (49–52) | (111–116) | (2–3) | (2–4) | 2 | (1–2) | 1 | (44–56) |

Different letters in the same column indicate the significant difference.

^a P, protein; O, oil; P + O, protein + oil; Ca, calcium; Mn, manganese; B, boron; SUC, sucrose; STA, stachyose; TS, total sugar; APP, appearance; STI, stickiness; FLA, flavour; TEX, texture; SHL, Shelf-life; INDEX, quality index.^b Superior (3): Mean of three superior natto types; moderate (9): Mean of nine moderate natto types; inferior (3): Mean of three inferior natto types.

Seed boron content decreased in a sequence of inferior, moderate, and superior natto lines (Table 2). Since more than 90% of boron is in the plant cell walls (Yoshioka et al., 2009), there is a possible correlation of boron with seed hardness. It was also stated that seeds with lower boron content might produce softer natto (Ishii & Matsunaga, 2004; Yoshioka et al., 2009). Current research supports this statement showing that superior natto types had lower boron content and seed hardness, while inferior natto types had high boron content and seed hardness.

3.4. Soluble sugars

Comparing the three natto quality types showed small differences in sucrose content, but superior and moderate natto types tended to have higher average sucrose than the inferior natto types (Table 2). All three natto groups had the same average stachyose content with very narrow range from 47 to 53 g kg⁻¹ (Table 2). Since *B. subtilis* (natto) bacteria requires sugar for fermentation process, higher sugar content is desired for better natto quality (Taira, 1990). There was no significant difference on total sugar content among natto genotypes in this study; however, there was a trend that superior and moderate natto lines had higher average total sugar content than the inferior quality natto types (Table 2). Thus, high sugar content appears to be associated with good natto quality.

3.5. Sensory properties

Sensory properties were evaluated in three natto quality types. The average total quality index ranged from 44 to 81 points across locations and years (Table 2). Stickiness and shelf life were the least significant variables among the five natto quality traits, while appearance, flavour, and texture showed significant differences between three natto quality types. In the overall comparison of the three natto groups, the superior natto lines exhibited the highest overall quality attributes (80–81 points for total quality index), while the inferior natto lines showed the lowest quality attributes (44–56 points for total quality index).

Wei and Chang (2004) studied natto sensory properties in total of five lines, four experimental lines (Dannato, Minnatto, Natto King, and MN91-468) and a commercial line (Itobiki natto), and reported significant differences in natto color, stickiness, aroma, bitterness, hardness, and chewiness among the genotypes. They also reported no significant differences in ammonia flavour and sweetness/sourness between experimental lines and the commercial line. Geater et al. (2000) studied 16 Iowa natto lines and reported significant differences in natto darkness and hardness among genotypes. Nevertheless, the two studies did not compare the lines with different natto quality, thus this is the first study on the differences in the sensory traits among different natto quality types.

Although the lines with the highest natto quality were suitable for natto manufacture, the three commercial natto varieties had lower seed yield than the other natto lines. Therefore, improving natto quality with competitive seed yield is critical to help natto soybean growers.

3.6. Heritabilities

Trait heritability is an important criterion in the breeding process (Walker & Schmitthenner, 1984). High heritability indicates that traits can be effectively modified using selection based on phenotype and such selection can be performed during early stages of breeding. In the current study, heritability of each trait was calculated for each location across two years. Protein, oil, protein plus oil, calcium, manganese, boron, sucrose, and total sugar content exhibited high heritability, ranging from 0.72 to 0.97, while stachyose showed low heritability (0.45) due to the lack of genetic variability among genotypes. These results were consistent with the non-significant genotype × location and genotype × year effects on most seed chemical quality traits (Table 1). Therefore, natto varieties could be tested at fewer locations and/or years to save time and labor cost.

3.7. Correlation coefficients

Seed protein and oil content exhibited a strong negative correlation (−0.74), while protein was positively correlated with protein plus oil content (Table 3). Seed protein was also positively correlated with calcium and boron content, but negatively correlated with sucrose and total sugar content. All these correlations indicated that lines with low protein, calcium, and boron and high sucrose content should be objectives for natto breeding. There was a negative correlation between seed oil and both calcium and boron content, which was consistent with the negative correlation between protein and oil content. Seed protein plus oil content was positively correlated with manganese and boron content and negatively correlated with total sugar content, showing another beneficial correlation for breeders where natto lines can be selected for low levels of protein plus oil and manganese content. Calcium was positively correlated with boron and stachyose content, but negatively correlated with total sugar content; sucrose content was positively correlated with both stachyose and total sugar content and was negatively correlated with boron content (Table 3). The positive correlations among sucrose, stachyose, and total sugar content were consistent with the report by Taira, Tanaka, and Saito (1989). Many of these seed chemical quality traits were significantly inter-correlated; some were beneficial and others were detrimental. Thus, breeders could take advantage of the beneficial correlations and use a simple trait as an indirect criterion to select other related traits. For instance, analyzing protein and oil content is relatively easy and fast, and therefore could be used as the major

Table 3
Correlation coefficients for seed chemical quality traits of 15 natto soybean lines grown at three Arkansas locations over two years.

| | O | P + O | Ca | Mn | B | SUC | STA | TS ^a |
|-----------------------|-------|-----------------|-------|------|-------|-------|------|-----------------|
| Protein (P) | −0.74 | 0.77 | 0.40 | NS | 0.53 | −0.24 | NS | −0.25 |
| Oil (O) | | NS ^b | −0.63 | 0.25 | −0.46 | 0.33 | NS | NS |
| Protein + Oil (P + O) | | | NS | 0.22 | 0.34 | NS | NS | −0.24 |
| Calcium (Ca) | | | | NS | 0.48 | −0.30 | 0.24 | NS |
| Manganese (Mn) | | | | | NS | NS | NS | NS |
| Boron (B) | | | | | | −0.21 | NS | NS |
| Sucrose (SUC) | | | | | | | 0.21 | 0.63 |
| Stachyose (STA) | | | | | | | | 0.79 |

^a TS, total sugar.

^b NS, nonsignificant at the 0.05 probability level.

Table 4

Correlation coefficients for seed chemical quality traits and sensory properties of 15 natto soybean lines grown at three Arkansas locations over two years.

| Trait ^a | P | O | P + O | Ca | Mn | B | SUC | STA | TS |
|--------------------|-----------------|------|-------|-------|-------|-------|------|-----|----|
| Appearance | −0.47 | 0.33 | −0.40 | −0.48 | −0.45 | −0.52 | NS | NS | NS |
| Stickiness | NS ^b | NS | NS | −0.30 | NS | −0.30 | 0.36 | NS | NS |
| Flavour | −0.51 | 0.38 | −0.39 | −0.48 | −0.38 | −0.57 | 0.28 | NS | NS |
| Texture | −0.42 | NS | −0.45 | −0.24 | −0.31 | −0.49 | NS | NS | NS |
| Shelf-life | −0.28 | 0.25 | NS | −0.29 | −0.54 | −0.29 | 0.25 | NS | NS |
| Quality index | −0.47 | 0.34 | −0.38 | −0.46 | −0.49 | −0.60 | 0.27 | NS | NS |

^a P, protein; O, oil; P + O, protein + oil; Ca, calcium; Mn, manganese; B, boron; SUC, sucrose; STA, stachyose; TS, total sugar.^b NS, nonsignificant at the 0.01 probability level.

chemical quality trait in evaluating natto lines since low protein plus oil lines would likely have low calcium, manganese, and boron content, and high sucrose content.

Natto appearance was negatively correlated (−0.40 to −0.52) with seed protein, protein plus oil, calcium, manganese, and boron content; but positively correlated (0.33) with oil content across locations and years (Table 4). Natto stickiness was negatively correlated with calcium and boron content, but positively correlated with sucrose content. Since *B. subtilis* (natto) bacteria requires sugar for fermentation (Taira, 1990), high sucrose content facilitates the growth and replication of *B. subtilis* (natto) that results in better stickiness of natto. There were negative correlations between natto flavour and protein, protein plus oil, calcium, manganese, and boron content, but positive correlations with oil and sucrose content, indicating that high sugar, but low protein and minerals contribute favorably to natto sensory quality. Taira (1990) and Zhang et al. (2008a) also stated that high sugar and low calcium content are preferred for acceptable natto flavour and texture. Natto texture was negatively correlated with protein, protein plus oil, calcium, manganese, and boron content. Geater et al. (2000) indicated that natto texture was positively correlated with seed size, protein, and protein plus oil content, but negatively correlated with oil, sucrose, and total sugar content. These correlations were also found in the current study indicating that chemical quality traits could be used as indirect criteria for natto quality selection without performing sensory analysis. Ishii and Matsunaga (2004) and Yoshioka et al. (2009) stated that seeds with lower boron content produced natto with softer texture. Such correlation was confirmed in present study with a correlation coefficient of −0.49. There was a negative correlation between natto shelf life and protein, calcium, manganese, and boron content and a positive correlation with oil and sucrose content. Thus, low protein, low minerals, and high sucrose content will improve the natto shelf life. Natto shelf life has not been extensively studied previously and the current study showed a strong correlation of several seed quality traits and shelf life, which can be used for selecting superior natto with a long shelf life. For overall natto quality, low protein, protein plus oil, calcium, manganese, and boron and high sucrose content are desirable and could be used as indirect selection criteria in natto breeding.

4. Conclusions

Seed chemical quality traits are essential to determine suitable natto varieties to produce high quality natto. Combined data analysis showed no significant year or location effect on variation in seed quality traits, but a significant genotype × year × location interaction effect on protein, protein plus oil, calcium, and manganese content. Results of the comparison of three natto quality types showed that superior natto types had lower protein, protein plus oil, calcium, manganese, and boron content, and higher sugar content than inferior natto types. All of the seed quality traits exhibited high heritability, with the exception of stachyose content, indicating that genetic variance was largely responsible for the phenotypic difference observed across the environments. There

was a strong positive correlation between the following: protein and both protein plus oil and boron, calcium and boron, and sucrose and both stachyose and total sugar content. In addition, there was a strong negative correlation between protein and oil, seed oil and calcium, and seed oil and boron content. Variations were observed among natto qualities in appearance, stickiness, flavour, texture, and shelf life, which effectively separated the superior natto type from the moderate and inferior natto types. In the current study, five sensory properties, appearance, stickiness, flavour, texture, and shelf life, were significantly correlated with protein, oil, calcium, manganese, boron, and sucrose content; thus the chemical quality traits can be used as indirect criteria for natto quality selection in a practical breeding program.

Overall, high yield breeding lines with low protein plus oil, calcium, manganese, and boron content, but high sucrose content will have the best chance to be accepted by natto manufactures.

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