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

Yoko Yoshikawa [a], Pengyin Chen [a,*], Bo Zhang [b], Andrew Scaboo [c], Moldir Orazaly [a]

[a] Department of Crop, Soil, and Environmental Sciences, 115 Plant Science Building, University of Arkansas, Fayetteville, AR 72701, United States
[b] Crop, Soil and Environmental Sciences, 509 Latham Hall (0404), Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, United States
[c] Division of Plant Sciences College of Agriculture, Food, and Natural Resources, 1-31 Agriculture Building, University of Missouri, Columbia, MO 65211, United States

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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 × 3 μm in size and can be found everywhere in nature, especially in soil and crop residue (Watanabe, 2006). During the fermentation by *B. subtilis* (*natto*),

* Corresponding author. Tel.: +1 (479) 575 7564; fax: +1 (479) 575 7465.
E-mail address: pchen@uark.edu (P. Chen).

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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, & Otobe, 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 Infratec 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 (×*g*) for 15 min, followed by 10 min. of centrifuging at 16,000*g*. 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 16,000*g* 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