Comparison of instrumental methods for measuring seed hardness of food-grade soybean

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ABSTRACT

Seed hardness is an important factor in determining soybean suitability for natto production. There is no established methodology for testing seed texture of soybeans. The objective of this study was to develop an efficient method by examining different instruments and seed parameters that could be potentially used for testing soybean seed hardness. Five food-grade soybean genotypes with different seed sizes were used to determine seed hardness and water-absorption capacity. Water absorption capacity was expressed by swell ratios for seed weight, seed dimension, and volume of water changes before and after soaking. Seed hardness test was conducted by a one-bite method using two food-texture analyzers: a TMS-2000 equipped with shear cell (SC) and a TA-XT2i equipped with either a single blade (SB), a 2-mm probe (PB), a 75-mm cylinder (CY), or a 16-probe pea rigs (PR). The results showed that hardness testing by CY with ten seeds (CV=0.14), SB with 5 seeds (CV=0.11), and SC with 30 g steamed seeds (CV=0.14) produced dependable and consistent results with low coefficient of variance. However, SC may not be practical for early plant selection in a breeding program due to a relatively large sample requirement. Seed size was negatively, whereas swell ratio by weight and volume was positively, correlated with seed hardness, and therefore, can be used as indirect selection indicators for seed hardness.

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INTRODUCTION

Demand for food-grade soybean has been increasing as more consumers recognize the proven health benefits and nutritional value of processed soybean foods. Soybean isoflavones have been shown to have pharmaceutical functions in preventing cancer, cardiovascular disease, and osteoporosis (Omoni and Aluko, 2005). The proper physical characteristics of food-grade soybeans, such as round seeds with yellow cotyledons, yellow seed coats, and yellow hilium, are desired by consumers for certain soyfoods. Natto is fermented soy product with high, beneficial phytochemical activity, and the natto production utilizes specific types of soybeans including traditional small yellow-seeded, black-seeded, and large-seeded beans. Japan has a large market for food-grade soybeans, and the United States is a major supplier for traditional natto beans every year (Carter et al., 2003).

Seed hardness is an important quality attribute for natto soybean because it affects the water absorption, seed-coat permeability, and overall texture and quality. Seed hardness is affected by calcium content, water absorption, and cookability (Chen, et al. 1993). Calcium content of soybean seeds varies with cultivar (0.19% to 0.52%) but not with different environments (Chen, unpublished data). Water-absorption capacity of soybean seeds is usually measured by swell ratio, which is determined by changes in seed weight, seed dimension, or water volume before and after soaking. Seed-swell ratio is an important trait for determining soyfood quality (Mullin and Xu, 2001). Generally, small-seeded soybeans are preferred for natto manufacturing as they provide for better fermentation than large-seeded soybeans; however, small-seeded soybeans have a tendency to be hard in texture and require more processing time in natto making, which causes undesirable ammonia gas and higher cost of production. To date, very limited research has been conducted to evaluate instrumentation and methodologies for seed-hardness testing. In fact, potential cultivars or product for the natto market are usually examined by professional testers to determine suitability based upon sensory evaluations. At least 0.91 kg of soybean seeds are required in each test in which ten well-trained researchers taste natto products in a four-week period with four replications (Wei, 2004). This method is costly and time-consuming, and it requires making the natto products from soybean seeds. Other methods for evaluating seed texture by instruments include puncture, shear, and compression tests (Bourne, 1972 and 2002; Rhodes, 1972; Okabe, 1979). A puncture test was described as one of the simplest and most commonly used in instrumental measurements for food texture (Bourne, 2002), but it can give rise to large

MEET THE STUDENT-AUTHOR

Mioko Tamura

I am an international student from Saitama, Japan, and currently a junior majoring in crop management with a minor in crop biotechnology. I came to Fayetteville after graduation from technical high school in Japan. I enjoy the activities with the student organizations, such as Grogreen, organic farming club, and ICT (International Culture Team). I am a member of the College Honors Program and have been awarded Harvey A. & Jo York, Eddie Davis, and Hinkle scholarships.

I have been working with the soybean research program since my freshman year and have had opportunities to learn things outside the classroom and a chance to meet many friends. I am interested in organic farming and biotechnology. I hope to have a career in sustainable agricultural development in undeveloped communities. I would like to thank Dr. Pengyin Chen for his support and guidance and members of the research program, especially Dr. Bonnie Zhang, for advice on this project.

experimental errors when using single samples. The major disadvantages of shear test are 1) no uniform protocol, and 2) multiple variable sources involved in the sample preparation and testing processes. A compression test is rarely used because gases are easily trapped in most products (Wheeler, 1994). There is no standard method in testing soybean seed hardness for natto manufacturing.

The objectives of this research were 1) to evaluate five food-grade soybean genotypes for seed-swell ratio and hardness, 2) to determine the optimal amount of steamed seeds required to quantify seed hardness for each testing, and 3) to examine the relationship between the seed-swell ratio and hardness and compare different hardness-testing methods.

MATERIALS AND METHODS

Test sample preparation.

Five food-grade soybean genotypes, V97-6490, MFL-552, Hutcheson, MFS-591, and Camp, ranging in size from 6.8 to 38.1 g per 100 seeds were grown at the University of Arkansas Agricultural Experimental Station, Fayetteville, Ark., in 2004. All genotypes were subjected to the same growing conditions, and seeds harvested from each genotype were cleaned and sieved to a uniform size. Fifty grams of unbroken and uniform seeds from each genotype were soaked in 250 ml water in heat-resistant plastic boxes for 16 h. Then, seeds were recovered from the soaking water with a sieve and blot-dried with paper towels. Thereafter, soaked seed samples were pressure-cooked in an autoclave for 20 min at 121.1°C and 1.2 kg/cm^2. Sub-samples from each genotype were taken, as appropriate, for each type of hardness test.

Seed hardness measurement

Seed hardness of steamed samples was tested with a one-bite method using a TMS Texture System (TMS-2000, Food Technology Corp. Sterling, Va.) equipped with a 10-blade shear cell and a TA-XT2i food-texture analyzer (Texture Technologies, Scarsdale, N.Y.) equipped with either a single blade, a 2-mm probe, a 75-mm cylinder, or pea rigs with 16 2-mm probes. The maximum force to puncture, shear, or compress cooked-seeds in Newtons (N) was determined to represent seed hardness (Song et al., 2003) (Fig. 1). To determine proper sample size for each test method, 20, 30, 40, and 50 g of steamed seeds were used for shear-cell testing; one, five, and ten steamed seeds for cylinder compression testing; one and five steamed seeds for single-blade shear testing; one steamed seed for the 2-mm probe puncture testing; and 16 steamed seeds for pea-rigs puncture testing.

Swell ratio measurement.

Swell ratio was determined by taking dimensions and weight of dry and soaked soybean seeds and comparing volumes of the water absorbed. The seed dimension was measured based on the length, width, and thickness, perpendicular to the hilium, with a digital caliber. Seeds with broken seed-coat and stone seeds (i.e., stone seeds=no water absorption) were discarded from each sample before testing. Seeds were soaked in water for 16 h. Swell ratio by weight was expressed by the ratio of the soaked-seed weight of each genotype to the initial dry weight. Water-absorption capacity of seeds was determined by the absorbed water volume as a ratio to the volume of the seeds.

Statistical analysis.

Hardness test efficiency was assessed by the coefficient of variation (CV), which was calculated by average hardness divided by the standard deviation in each replicated test procedure. The least CV value indicates the least hardness variation among replications with a given testing procedure or sample size. All statistical analysis was performed using SAS (2003). The precision comparison of each hardness-testing method or sample size was evaluated by Fisher's least significance difference (lsd) test using the general linear model (GLM). The $P \leq 0.05$ probability level was used as the statistical-significance threshold when different combinations of replication and sample sizes were compared within and between testing procedures. Pearson's correlation coefficient (r) was used to determine the relationships between hardness-related traits. The coefficient of determination (R^2) of the linear regression model was calculated for each testing procedure.

RESULTS AND DISCUSSION

Swell ratios.

The swell ratio by volume, weight, and dimension was evaluated among all genotypes (Table 1). Cultivar V97-6490, with the largest seeds among all genotypes tested (38.1 g/100 seeds), had the highest swell ratio by volume (2.18), weight (2.42), and dimension (2.78), and its swell ratio by volume and weight was significantly higher than other genotypes. In a previous study, Tachanagaha, with 36.3 g/100 seeds, a Japanese miso cultivar, had a similar swell ratio by weight of 2.84 (Mullin and Xu, 2001). Hutcheson (15.4 g/100 seeds) and MFL-552 (21.8 g/100 seeds) had significantly lower swell ratios by volume (1.82) and by dimension (1.87) respectively, than any other genotypes. Cultivars MFL-552, Hutcheson, MFS-591 (8.7 g/100 seeds), and Camp (6.8 g/100 seeds) all showed lower swell ratios by weight

(2.30) than did V97-6490. In Mullin and Xu's (2001) study, OX 591, with a similar seed size (15.6 g/100 seeds) to Hutcheson, had a swell ratio by weight of 1.54 that was much lower than that of Hutcheson, because OX 591 was a hard-seeded line with 72.4% non-water-absorption seeds. The swell ratio by weight ranged from 2.36 to 2.51 among 16 Iowa small-seeded lines (Geater et al., 2000), which was higher than that of MFS-591 and Camp, two small-seeded soybeans in our study. Apparently, genotype affected the swell ratio significantly. Despite the seed-size difference, all genotypes except for MFL-552 exhibited similar seed-size dimension change before and after soaking. Therefore, the swell ratio by volume, weight, and dimension did not show the same trend on these five genotypes.

The correlation of seed size and swell ratio as well as correlation between swell ratios is shown in Table 2. Swell ratio by weight (WE) and volume (VO) had the highest correlation coefficient of 0.81 at $p < 0.0001$ level. Swell ratios determined by both volume and weight are more reliable than those determined by dimension. However, using seed-weight change to determine swell ratio is much easier than using volume of water absorbed or seed-dimension measurement. Seed size (SS) was significantly and negatively correlated with both swell ratio by weight (WE) and swell ratio by volume (VO), with negative correlation coefficients of –0.51 and –0.32, respectively. The correlation coefficient between seed size and swell ratio by weight was –0.81 in Mullin and Xu's study (2001). The possible reason for the lower correlation coefficient obtained in this study was that we tested the soybean genotypes with a wider seed-size range (6.8 to 38.1 g/100 seeds) than those in Millin and Xu's study (15.6 to 36.3 g/100 seeds). In addition, the specific genotypes and number of genotypes tested may have influenced the correlation coefficient. Swell ratio by seed dimension had insignificantly positive correlation with seed size and with swell ratio by weight and volume. Therefore, seed-dimension change before and after soaking might not serve as a good indicator for water absorption.

Seed hardness measured by five different methods.

The seed hardness determined by each testing procedure is given in Table 1. Seed hardness by single-blade, cylinder, and shear-cell probes showed a linear increase with increased sample size. For the single-blade procedure, the hardness of five seeds was about five times higher than the hardness of one seed. For the cylinder procedure, the hardness of one, five, and ten seeds did not show the exact proportional increase as the sample sizes increased, except for the hardness of five and ten MFS-591 seeds (25.3 N and 51.7 N, respectively). Increase of hardness was also linear but not exactly proportionate to sample size for the shear-cell procedure. The one-seed hardness by probe and single blade had similar ranges of 0.8 to 3.4N and 1.8 to 4.3N, respectively, whereas the one-seed hardness by cylinder ranged from 10.5 to 31.1N. These variations were mainly due to equipment design differences that resulted in differences in force and energy needed for compressing (cylinder), slicing and shearing (single-blade and shear-cell), and penetrating (probe and pea-rigs).

Testing method precision.

One of the most important characteristics of a reliable method is reproducibility or precision (Guo et al., 2004). Five seeds in SB, ten seeds in cylinder, and 30 g seeds in shear cell were selected to represent single-blade, cylinder, and shear cell due to the low CV (Table 3). Small seed samples tended to cause higher CV, whereas large seed samples reduced the CV for seed hardness measurement. For example, the mean CV of cylinder (0.42) and single blade (0.32) using one seed was significantly higher than that of five or ten seeds (0.20 and 0.15 in cylinder and 0.12 for five seeds in single blade). However, the CVs of shear-cell, using different sample sizes, were similar: 0.15 for 30 g sample; 0.21 for 20 g; 0.18 for 40 g and 50 g samples. In addition, a 20-g sample was not adequate to completely cover the bottom of the shear cell. Therefore, one seed test of cylinder, single blade, or 20-g seeds for shear cell was not proper sampling strategy for a precise hardness test. The probe was not recommended for testing hardness because it yielded one-seed test with a CV as high as 0.32. Similarly, the hardness of cooked, Japanese milled rice using the probe was poorly reproducible as compared to cylinder, shear cell, and single blade (Ohtsubo et al., 1990).

The CV for hardness generated by single blade, cylinder, probe, shear cell, and pea rigs was significantly different ($p < 0.05$). The CV for hardness using pea rigs and single blade with five seeds was the lowest (0.10 and 0.12) among all the methods using various sizes of seed samples, followed by shear cell with 30-g seeds (0.15) and cylinder with 10 seeds (0.15). However, the pea rigs test was relatively difficult to set up because it also required perfect alignment of 16 individual seeds on the test panel each time, and it required more time to clean the probe. Although shear-cell testing generated low CV, it required relatively large samples, which may not be practical for early plant selection in a breeding program. Therefore, single blade with five seeds and cylinder with ten seeds were highly recommended for testing soybean seed hardness due to the small amount of seeds required and easy setup.

Correlation coefficients (r) for seed hardness measured by the five methods are listed in Table 4. All correlation coefficients were positive except for pea rigs and probe or single blade. Hardness by cylinder was significantly correlated with hardness by probe, single blade, and shear cell; hardness by shear cell was significantly correlated with hardness by pea rigs and single blade. Hardness by single blade was also significantly correlated with hardness by cylinder and probe. Cylinder and single blade had the highest correlation coefficient of 0.81, followed by the correlation between single blade and probe, cylinder and probe, and pea rigs and shear cell. Shear cell was relatively weakly correlated with cylinder or single blade, with a correlation coefficient of 0.32. A much higher correlation coefficient (0.94) was found between a single-blade and 10-blade shear cell for the hardness of poultry breast meat (Xiong et al., 2006). The possible reason for the difference between the two studies was that the soybean has different texture as compared to poultry breast meat. In addition, soybean samples loaded in the shear cell consisted of many individual seeds. These correlation coefficients among five methods indicated that cylinder, single blade, and shear cell yielded very similar hardness rankings among soybean genotypes.

Relationship among seed size, swell ratio, and seed hardness.

The relationships among seed size, seed-swell ratio, and hardness were modeled using linear regression equations and are shown in Table 5. Seed size and swell ratio by volume, weight, and dimension predicted differently the hardness measured by single blade, cylinder, and shear cell. Seed size and swell ratio by weight were better predictors for seed hardness than swell ratio by volume and seed dimension in single-blade and cylinder procedures. Hardness by single blade was best predicted by the seed size with an R^2 of 0.70, followed by the swell ratio by weight with $R^2 = 0.62$ and swell ratio by volume with $R^2 = 0.41$. However, the swell ratio by dimension could hardly predict hardness by single blade due to a very low R^2 of 0.01. Hardness by cylinder was best predicted by swell ratio by weight ($R^2 = 0.53$), followed by seed size ($R^2 = 0.45$) and then by swell ratio by volume ($R^2 = 0.30$). Similarly, swell ratio by dimension ($R^2 = 0.01$) cannot be used to predict hardness by cylinder. Neither seed sizes nor swell ratios were good predictors for seed hardness by shear cell because of low R^2 values (0.02 to 0.25). Therefore, seed size and swell ratio by weight and volume can be used as indirect selection criteria for hardness without conducting a texture test. Based on the intercept and slope from the regression model, softer seeds tended to be larger and absorb more water, which is in agreement with the results from Taira's study in 1990.

In summary, cylinder with ten seeds, single blade with five seeds, and shear cell with 30 g steamed seeds were the most dependable procedures. Cylinder and single-blade probes were more practical for early plant selection in a breeding program than shear-cell probe. Seed size and swell ratio by weight and volume can be used as indirect selection indicators for seed hardness of soybean.

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Table 1. Seed physical traits and hardness of five food-grade soybeans.

Genotype	Seed size	Swell ratio			PB†	SB†		
	(g/100sd)	volume	weight	dimension	1 seed	1 seed	5 seeds	LSD
V97-6490	38.1	2.2A‡	2.4A	2.8A	3.4A	4.3bA	27.5a§A	1.6
MFL-552	21.8	2.0B	2.3B	1.9B	1.6B	3.5bB	15.2aB	1.0
Hutcheson	15.4	1.8C	2.3C	2.5A	0.8C	2.2bC	8.5aC	0.6
MFS-591	8.7	2.0B	2.3B	2.5A	1.4B	1.8bC	8.5aC	0.7
Camp	6.8	2.0B	2.3B	2.7A	1.1BC	2.2b	8.4aC	0.6
LSD		0.08	0.03	0.43	0.55		1.22	

	CY†				PR†	SC†				
Genotype	1 seed	5 seeds	10 seeds	LSD	16 seeds	20 g	30 g	40 g	50 g	LSD
V97-6490	31.1cA	99.1bA	152.5aA	14.5	72.6B	485c	800bA	970ab	1049a	207.0
MFL-552	15.5cB	48.3bB	69.6aB	7.5	26.8D	303d	422cC	523b	606a	69.8
Hutcheson	17.4cB	39.1bB	63.6aBC	6.0	57.8B	391c	649bB	760b	1189a	156.2
MFS-591	10.5cB	25.3bC	51.7aC	5.8	34.1C	348d	483cC	534b	703a	45.0
Camp	19.2c	40.0b	61.6aBC	4.5	54.2A	491c	670bB	1089a	1086a	129.5
LSD			15.18		4.74		113.28			

†PB, a 2-mm probe; SB, a TA-XT2i equipped with a single blade; CY, a 75-mm cylinder; PR, 16-probe pea rigs; SC, TMS-2000 equipped with a 10-blade shear cell

‡Means with the same lower case letter within a row were not significantly different at $p < 0.05$.

§Means with the same capital letter within a column were not significantly different at $p < 0.05$.

Table 2. Correlation among seed swell ratios and seed size of five food-grade soybeans as measured by different methods.

Traits	VO	WE	DI
SS	-0.32*	-0.51**	0.11
VO		0.81***	0.16
WE			0.14

*, **, ***: Significant at $P \leq 0.05$, 0.01, and 0.0001 probability levels, respectively.

SS, seed size; VO, swell ratio by volume; WE, swell ratio by weight; DI, swell ratio by dimension.

Table 3. Coefficient of variation for seed hardness using different testing methods and sample size of five food-grade soybeans.

No. of	CY[†]			PB[†]	SB[†]		PR[†]	SC[†]			
replication	1 seed	5 seeds	10 seeds	1 seed	1 seed	5 seeds	16 seeds	20g	30g	40g	50g
5	0.37Ac[‡]	0.20bA	0.14a[§]A	0.35A	0.30bA	0.11aA	0.09A	0.21aA	0.14aA	018aA	0.19aA
10	0.44Ac	0.20Ab	0.15Aa	0.32A	0.34Ab	0.13Aa	0.10A	0.21Aa	0.16Aa	0.18Aa	0.17Aa
15	0.44Ac	0.20Ab	0.15Aa	0.31A	0.31Ab	0.13Aa	0.11A	—	—	—	—
Mean	0.42	0.20	0.15	0.33	0.32	0.12	0.10	0.21	0.15	0.18	0.18

[†]CY, a 75-mm cylinder; PB, a 2-mm probe; SB, a TA-ZT2i equipped with a single blade; PR, 16-probe pea rigs; SC, TMS-2000 equipped with a 10-blade shear cell.

‡Means with the same lower case were not significantly different within a row ($p < 0.05$)

[§]Means with the same capital letter were not significantly different within a column ($p < 0.05$)

–, Data not available

Table 4. Correlation among seed hardness of five food-grade soybeans measured by different testing methods.

Methods[‡]	CY	PB	PR	SB	SC
CY		0.54***	0.09	0.82***	0.32*
PB			-0.09	0.67***	0.15
PR				-0.13	0.53***
SB					0.32*

[‡]CY, a 75-mm cylinder for 10 seeds data; PB, a 2-mm probe for 5 seeds data ; PR, 16-probe pea rigs;
SB, a TA-ZT2i equipped with a single blade; SC, TMS-2000 equipped with a multiple blade shear cell for 30 g seeds data.

*, **, ***: Significant at $p \leq 0.05$, 0.01, and 0.0001 levels, respectively.

Table 5. Regression model statistics for predicting seed hardness from seed physical traits.

Traits[‡]	SB[§]			CY[§]			SC[§]		
	R^2	Intercept	Slope	R^2	Intercept	Slope	R^2	Intercept	Slope
SS	0.70	27.14	-4.51	0.45	136.35	-19.14	0.02	663.92	-19.76
VO	0.41	-53	33.16	0.30	-252.93	166.11	0.02	281.76	161.28
WT	0.62	-192.96	89.32	0.53	-1029.43	480.11	0.06	-913.59	657.24
DI	0.01	10.94	0.98	0.01	55.75	9.67	0.25	202.77	162.7

[‡]SS, seed size; VO, swell ratio by volume; WE, swell ratio by weight; DI, swell ratio by dimension.

[§]SB, a TA-ZT2i equipped with a single blade for 5 seeds data; CY, a 75-mm cylinder for 10 seeds data;
SC, TMS-2000 equipped with a multiple blade shear cell for 30 g seeds data.

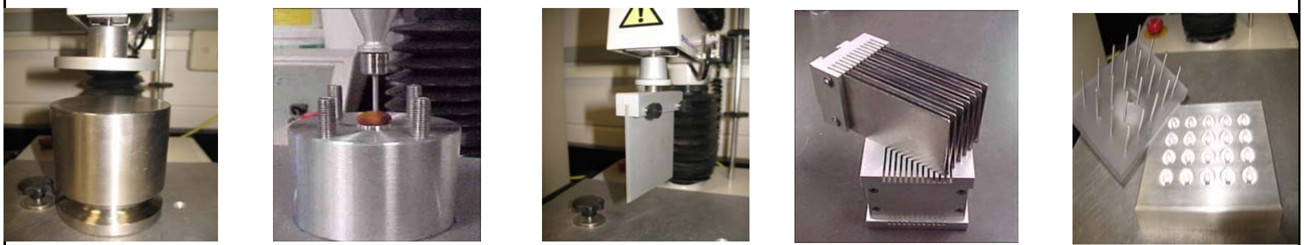

Fig. 1. Five probes used in testing seed hardness of cooked soybean.