Technical News Feature

Effect of Maturity on Chemical Composition and Storage Stability of Soybeans

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ABSTRACT

Soybeans from different maturation stages were tested for their chemical composition and storage stability. Maturation was arrested at specified times by spraying paraquat on the plant. The same level of trypsin inhibitor activity was found regardless of maturation. However, the lipoxygenase activity and phytate content were significantly lower in immature beans. Crude oil and protein contents were similar, regardless of maturation. The crude oils from immature samples were greener in color and higher in free fatty acid content than those from mature ones. Both yield of isolated soy protein and ratio of 7S to 11S protein in immature soybeans were lower than that from mature soybeans. During storage, lipoxygenase activity decreased independently of maturation but free fatty acid content in the crude oil increased at a faster rate in immature beans than that from mature ones.

INTRODUCTION

Soybeans are used for oil extraction and meal production. Although most of the meal is consumed by the feed industry, its use by the food industry is increasing. Since the quality of raw material affects the quality of the end product, steps taken to ascertain good quality of raw soybeans will promote this use. Soybean maturity is a prime quality characteristic; a review of biochemical changes during maturation of soybeans has been published by Rackis (1) and another on food value as a function of maturity by Rackis (2). Preliminary work for this study showed that spraying of paraquat on very immature soybean plants resulted in arrest of maturation. In a recent study, Urbanski et al. (3) indicated that, when compared to undamaged soybeans, freeze-damaged soybeans showed similar oil and protein contents and trypsin inhibitor activities, but lower lipoxygenase activity and greener oil color. Also, the crude oil from freeze-damaged soybeans has less storage stability than that from undamaged soybeans, due to a more rapid increase in free fatty acid content. For soybean meal, trypsin inhibitor and lipoxygenase activity are the two major factors directly associated with its acceptance as human food (4). The former is responsible for poor protein digestibility, and the latter appears related to beany off-flavor. Collins and Sanders (5) observed that, as soybeans became more mature, trypsin inhibitor activity gradually increased. Rackis et al. (6) and Iwanicki (7) found that lipoxygenase activity also increased during soybean maturation.

Phytate in soybeans has drawn attention because it may reduce mineral bioavailability (8). The amount of phytate present varies with the variety, from 1.01 to 1.47% (9). Phytate in mature pea seeds (*Pisum salvia*, var. "Early Market") has been found to be 1.16-1.23%, but immature pea seeds contain 0.16-0.17% phytate (10). No data has been reported on phytate content in soybeans during maturation.

Whigham and Stoller (11) reported that soybean producers often use a chemical desiccant as a harvest aid and that paraquat was the most effective agent for this.

Although research described above (3) reported on the effect of frost damage on the quality of soybean oil and meal, no attempts have been made to study oil and meal quality at different maturation stages. The present objectives were: (a) to study the effect of maturity on trypsin inhibitor and lipoxygenase activity; (b) to test whether maturity affects phytate content, oil content, free fatty acid content, and oil color; (c) to study the effect of maturity on protein content, yield of isolated protein and major protein components; and (d) to determine whether ambient temperature storage of soybeans intensifies the effect of soybean maturity on oil and meal qualities.

MATERIALS AND METHODS

Sample Preparation

Soybean plants of the Williams variety were grown for this study on the South Farm of the University of Illinois at Urbana-Champaign. Three maturation stages were arrested by spraying paraquat (Chevron Chemical Company, Richmond, CA) at a concentration of 38 mL/gal water on the plants. Four to six days after the leaves became wilted, soybeans were harvested by a combine. The harvested soybeans were dried to a moisture content of 10% or below in a through-flow air drier (Proctor & Schwartz, Philadelphia, PA) at ambient temperature for 6-12 hr. The dried beans were sealed in no. 2 tin cans in air and stored at room temperature for a total of 6 months. Samples were analyzed before and after storage for phytate, trypsin inhibitor activity, lipoxygenase activity, oil content, free fatty acid content of oil, color of oil, protein content, yield of isolated protein and ratio of 7S to 11S protein. All analyses were in triplicate.

Chemical Analyses

Total solids content was determined by drying in a vacuum oven at 60 C for 24 hr. Oil content was measured by Soxhlet according to AOAC (12). Protein content was obtained as 6.25 times the micro-Kjeldahl nitrogen content (12). Phytate was determined by a method developed by Thompson and Erdman (13). This method calls for a ferric phytate precipitation of a soybean extract. For this method, soybean meal was extracted with an acidified solution of Na_2SO_4, the extract was filtered and the filtrate was treated with ferric chloride and centrifuged. The supernatant was filtered and analyzed for total P. The initial

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TABLE I.

Weight and Solids and Phytate Content of Soybean Seeds at 3 Stages of Maturity (planting date June 16, 1980)

Maturation stage	Harvest date	Fresh weight (g/100 seeds)	Solids content (%)	Phytate content[1]
1	Sept. 20	32 ± 1.2	36.95	0.866 ± 0.021[a]
2	Sept. 26	41 ± 1.8	38.65	1.080 ± 0.010[b]
3	Oct. 3	20 ± 0.5	86.21	1.260 ± 0.040[c]

[1]Means bearing different superscripts differ significantly at the 5% level.

extract was also analyzed for total P and the difference ascribed to phytate P. Free fatty acid was determined according to AOAC (12) modified by Urbanski (3); with this modification, 2.0 g of oil instead of 7.05 g were titrated with 0.01 N NaOH rather than 0.25 N NaOH. Free fatty acid was expressed as percent oleic acid. Lipoxygenase activity was determined by measuring diene formation at 234 nm (14); according to Hildebrand and Hymowitz (15), this method gives lipoxygenase 1 activity under the conditions employed.

In case of oil color, a Beckman DB-G spectrophotometer was used to measure absorbance (Method Td 2a-64 [16]). The photometric index was expressed as 100 times the sum of absorbance at 440 nm and that at 550 nm.

For assaying trypsin inhibitor activity, the Kunitz (17) Gelatin-Formal method was used. A standard curve was prepared by titrating a gelatin solution containing a known amount of trypsin before and after a 60-min incubation period. Data were plotted as log of trypsin concentration vs the increase in titer between 0 and 60 min incubation. Trypsin inhibitor activity was obtained by adding a known amount of sample extract to a solution of gelatin and trypsin and measuring the decrease in titer between 0 and 60 min incubation. The trypsin inhibitor activity was expressed as mg crystalline soybean trypsin inhibitor/g of soybean.

Isolated soy protein was prepared by the method of Horan (18). For fractionation of 7S and 11S proteins, the method developed by Thanh and Shibasaki (19) was applied. Adjusting the pH of 0.03 M tris (hydroxymethyl) aminomethane buffer extract of freeze-dried isolated soy protein to pH 6.4 with 2 N HCl caused precipitation of the 11S fraction. After centrifugation, the supernatant was adjusted to pH 4.8 and the 7S fraction was separated from the whey proteins.

Statistical Analyses

Data were analyzed using analysis of variance (20). The F-test was used to test significant differences between mature and immature soybean samples at the 5% level. If the F-test proved significant, the least-significant-difference procedure was applied to determine significant differences among treatment means (21).

RESULTS AND DISCUSSION

Total Solids Content

In this study, soybeans were harvested at three stages of maturity. Soybeans harvested on September 20, 26, and October 3 contained 36.95%, 38.65% and 86.21% solids, and were arbitrarily defined as stage 1, 2 and 3, respectively. It was observed that as seeds become more mature, the total solids content increased (Table I). This result is expected since nutrients will be accumulated as seeds grow. This is also in agreement with findings of Urbanski et al. (3). Although the change in total solids content between stage 1 and stage 2 is small, the change in fresh weight (g/100 seeds) was large. This could be due to the considerable amount of moisture accumulated in the seeds during maturation from stage 1 to stage 2. As soybeans became normally mature (stage 3), solids content increased substantially but fresh weight decreased drastically. Thus, a large amount of water was lost by each seed as it approached full maturity.

Phytate Content

Phytate content increased from 0.866% to 1.260% on dry weight basis during soybean maturation (Table I). When calculated on a per bean basis, phytate content increased from 1.0 to 2.2 mg between stage 1 and stage 3. Welch et al. (10) reported that phytic acid content of pea seeds increased from 0.16% to 1.23% during maturation. Welch and Campen (22) concluded that availability of iron from soybean seeds was not directly correlated to the phytate content of the seeds ranging from 0.61% for immature seeds to 1.71% for the mature and that immature seeds contain a factor that depresses iron availability. However, phytate content in diets has been related to mineral (particularly zinc) availability (8). Rackis (2) stated that meal from mature soybeans showed higher boron and phosphorus and lower zinc values than that from immature soybeans. Therefore, the lower phytate content of immature soybeans found here indicates a better mineral nutritional value for immature than mature soybeans.

Trypsin Inhibitor Activity

This increased from 20.89 to 21.23 mg crystalline trypsin inhibitor/g of soy solids during maturation (Table II). This is equivalent to 2.47-3.66 mg crystalline trypsin inhibitor per soybean seed. After storage for 6 months, there was no significant change in trypsin inhibitor activity (Table II). The difference in trypsin inhibitor activity between various stages of maturity was insignificant after 6 months of storage. Rackis (2) stated that TI activity in 108 soybean varieties ranged from 66 to 233 units/mg protein and that the activity generally increased during maturation.

Lipoxygenase Activity (LA)

Immature soybeans had significantly lower lipxoygenase activity (10.50 LA units/mg soy solids) than mature ones (21.39 LA units/mg soy solids) (Table II). Similar results were found in other reports (1,4,5). Hildebrand and Hymowitz (15) found that the profile of lipoxygenase-1 activity increased to maturity while lipoxygenase-2 and -3 activities became maximal between 5 and 20 days before maturity. Since the lipoxygenase assay conditions used here give lipoxygenase-1 (L-1) activity (15), L-1 activity of both mature and immature soybeans decreased remarkably

TABLE II

Effect of Soybean Maturity on Trypsin Inhibitor Activity and Lipoxygenase Activity During 6 Months Storage[1,2]

Maturation stage	Storage time (months)			
	0	6	0	6
	Trypsin inhibitor (mg SBTI/g solids)		Lipoxygenase activity (units/mg solids)	
1	20.89 ± .02[a]	20.25 ± .69[a]	10.50 ± 1.21[a]	4.90 ± .67[a]
2	21.89 ± .01[a]	20.68 ± .32[a]	16.47 ± .46[b]	5.05 ± .01[a]
3	21.23 ± .03[a]	21.13 ± .02[a]	21.39 ± .51[c]	14.58 ± 1.50[b]

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.
[2]Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

(Table II) during storage but mature beans remained higher in L-1 activity than immature ones.

Oil Content

Oil content ranged between 18.6 and 19.2% dry basis from immature to mature soybeans prior to storage (Table III). Oil content per seed was 22 mg and 33 mg (calculated) at stages 1 and 3, respectively. This result agrees with the reported data that freeze-damaged samples had slightly lower oil content than undamaged samples before storage (3). Therefore, oil is slowly accumulated during seed maturation. This is due to a simultaneous increase in both seed size and dry matter. Table III gives oil content on a dry basis. When these values are placed on a fresh weight basis by multiplying by dry matter content in Table I, we find that oil content increased from 6.9 g to 16.6 g per 100 g seeds between stage 1 and stage 3. During storage none of the samples showed a statistically significant change in oil content.

Privett et al. (23) found that in the early stages of development the lipid was virtually devoid of triglyceride but as the bean developed there was a rapid synthesis of triglyceride. They further found that saturated fatty acids decreased and unsaturated fatty acids increased rapidly as the bean developed. Roehm and Privett (24) also reported an increase in triglyceride content of the total lipid and a change in fatty acid make-up during maturation. However, Rackis (2) stated that soybeans can be consumed in the green-mature or dry-mature form without significant differences in protein and oil quality.

Free Fatty Acid Content

This decreased significantly from 1.43% to 0.23% as the soybeans become more mature (Table III). This was also reported by others (3,25,26). This result is expected since oil is constantly being synthesized from free fatty acids during maturation. Therefore, it can be concluded that, despite the same oil content in both mature and immature seeds, the oil quality of the mature seeds is superior to that of immature ones.

Six months storage of stage 1 and stage 2 soybeans resulted in an increased free fatty acid content from 1.43% to 2.18% and from 0.81% to 1.68%, respectively. At stage 3, the free fatty acid content was low and remained low during storage. The high rate of increase in free fatty acid content in immature soybeans during storage suggests more neutral oil losses during oil refining. Therefore, the least amount of neutral oil loss would be expected from mature soybeans.

Color of Crude Oil

MacMillan and Melvin (27) noted that crude oil showing a green color gave a strong absorbance at wavelengths 550 nm and 440 nm. They defined the term photometric index as 100 times the sum of absorbance at 440 and 550 nm. Photometric index of crude oil was reduced as the soybean became more mature (Table III). The same result was observed by Sanders (28) and Urbanski et al. (3). Both immature and mature samples indicated no change in photometric index during storage for up to 6 months. This indicates that the green color of immature soybeans would not disappear during storage. Therefore, the oil color of immature seeds would be inferior to that of mature seeds although both immature and mature seeds contain the same level of crude oil.

Protein Content

As shown in Table IV, protein content on dry basis in-

TABLE III

Effect of Soybean Maturity on Content and Quality of Soybean Oil During 6 Months Storage[1,2]

Maturation stage	Oil content (% dry basis)			Free fatty acid content (g oleic acid/100 g oil)			Photometric index		
	Storage time (months)								
	0	3	6	0	3	6	0	3	6
1	18.6 ± .5	18.9 ± .1	18.6 ± .3	1.43 ± .06[a]	2.01 ± .02[a]	2.18 ± .18[a]	352 ± 15[a]	337 ± 21[a]	342 ± 18[a]
2	19.0 ± .3	19.1 ± .1	19.2 ± .1	.81 ± .08[b]	1.49 ± .25[b]	1.68 ± .07[b]	276 ± 11[b]	251 ± 14[b]	254 ± 13[b]
3	19.2 ± .5	19.6 ± .2	19.4 ± .4	.23 ± .04[c]	.36 ± .01[c]	.42 ± .01[c]	182 ± 12[c]	164 ± 22[c]	180 ± 20[c]

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.
[2]Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

TABLE IV

Effect of Soybean Maturity on Protein Content, Yield of Isolated Protein and Its Composition During 6 Months Storage[1,2]

Maturation stage	Protein content (% dry basis)			Yield of isolated soy protein (% dry basis)			7S/11S			
	Storage time (months)									
	0	3	6	0	3	6	0	3	6
1	39.33 ± .06[a]	38.93 ± .42[a]	39.09 ± .09[a]	23.79 ± .97[a]	23.21 ± .37[a]	24.99 ± .16[a]	.3405 ± .0272[a]	.3460 ± .0524[a]	.3367 ± .0436[a]
2	40.04 ± .58[a]	39.30 ± .16[a]	39.83 ± .16[a]	33.07 ± .91[b]	33.23 ± .40[b]	32.27 ± 1.02[b]	.3502 ± .0136[a]	.3639 ± .0785[a]	.3551 ± .0570[a]
3	42.29 ± .33[b]	42.95 ± 1.07[b]	41.11 ± 1.00[a]	38.00 ± 1.30[c]	37.71 ± .70[c]	39.96 ± .04[c]	.6392 ± .0809[b]	.6392 ± .0504[b]	.6243 ± .0686[b]

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.
[2]Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

creased slightly during maturation from ca. 39% at stage 1 to 42% at stage 3. This result is consistent with the data reported by Urbanski et al. (3); protein content of Williams soybeans increased from 39.7% to 41.8%, dry basis, between early frost-damaged and control samples. The calculated protein content per seed increased from 47 mg at stage 1 to 73 mg at stage 3. The protein content, dry basis, remained stable in soybeans during storage, irrespective of maturation stage.

Rubel et al. (29) found that on a dry weight basis, percent protein-N showed a concomitant rise with maturation.

Yield of Isolated Soy Protein

The total yield of isolated soy protein increased significantly from 23.79% to 38.00%, dry basis, with increasing maturity (Table IV). This corresponds to an increase from 28 mg to 66 mg isolated soy protein per seed from immature stage 1 to mature stage 3. The remarkable increase in isolated soy protein during maturation shows that much more alkaline-extractable nitrogen is present in more mature soybeans, although the apparent protein contents were much the same at the three stages. This can be explained on the basis that protein was measured from total Kjeldahl nitrogen which includes nonprotein nitrogen and the immature seed could be expected to contain more nonprotein nitrogen. The yield of isolated soy protein was not affected by storage, no matter what the maturation stage. The yield of isolated soy protein in mature samples remained higher than that in immature ones during storage. These results were expected since the total protein content was also found unchanged during storage.

Ratio of 7S to 11S Protein

It has been reported that 7S and 11S proteins are the major protein components of isolated soy protein and comprise nearly 90% of the total soy protein (30). Each of them has its own unique functional properties suited for particular food products. Saio et al. (31) found the tofu-gel prepared from a soybean variety with lower 7S/11S (0.616) was harder than that from a variety with higher 7S/11S (0.983). Thus, the ratio of 7S to 11S protein is very important to functionality. Therefore, 7S/11S at different maturation stages was determined and the results are shown in Table IV. The ratio 7S/11S increased significantly between stages 1 and 3. This can be explained by the change in individual proteins; the 7S increased from 0.254 g to 0.388 g/g of isolated soy protein but 11S decreased from 0.746 g to 0.612 g. As a result, immature soybeans contain relatively more 11S protein than do mature soybeans. The current data on the ratio of 7S to 11S protein in the mature soybean is 0.634, which is fairly close to the reported value 0.673 (30). The ratio was not changed during storage, irrespective of maturation stages.

Hill and Breidenback (32) found that the 2S proteins predominated at 12 days after flowering but that 7S and 11S components were synthesized later in maturity and in larger amounts.

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