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Lo,W.Yau-lai; Steinkraus,K.H.; Hand,D.B.; Hackler,L.R.; Wilkens,W.F.

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Soaking Soybeans before Extraction as it Affects Chemical Composition and Yield of Soymilk

Winston Yau-Lai Lo, K. H. Steinkraus, D. B. Hand, L. R. Hackler and W. F. Wilkens
New York State Experimental Station, Department of Food Science and Technology, Cornell University, Geneva New York 14456

SUMMARY

As the soaking time for soybeans increased, larger quantities of water-soluble solids leached into the soak water where they were lost in the usual methods of manufacturing soymilk. Analysis of the dry solids found in the soak water showed the following composition: 23.3% crude protein (of which 48.7% was non-protein nitrogen); 2.8% fat; 4.5% sucrose; 1.5% raffinose; 3.5% stachyose; and 63.7% other carbohydrates. Changes, apparently metabolic, in the soybeans during soaking caused protein to decrease from 43% in the non-soaked control to 38% for the 24-hr soak to 36% for the 72-hr soak. Non-protein nitrogen increased during the same intervals from 0.16% to 0.28% to 0.86%. Fat decreased during soaking from 24% to 19% (72-hr soak).

INTRODUCTION

The term soymilk is generally applied to the aqueous extract of soybeans, which is similar to cow's milk in appearance. However, the flavor of the two products is quite different. The association of the term "milk" in the description of this soybean extract is unfortunate. It often misleads those who are unfamiliar with it to compare soymilk unfavorably with the more familiar flavor and texture of cow's milk.

The first recorded history of the soybean plant in China was written in Sheng Nang's "Materia Medica" in 2838 B.C. (Lo, 1964). However, it was Whai Nain Tse who introduced soymilk to the people some years later (Piper *et al.*, 1923). The history of soymilk in China covers more than two millennia.

By the classical Chinese method of manufacturing soymilk, soybeans are soaked in cold water until hydrated, ground finely with added water in a stone mill to form a slurry, filtered to remove the insoluble residue, and the soymilk is boiled to improve flavor. Yield of soybean solids in the form of soymilk is estimated to be about 65% by this process.

Hand *et al.* (1964) reported a method of manufacturing soymilk that involved dehulling and fine grinding of steam-dried soybeans and slurrying the powder with water to produce a soymilk. This utilized 90% of the soybean solids.

Also recently, Mustakas *et al.* (1964), in cooperation with the Wenger Machinery Company, developed another process for manufacturing soymilk that is currently receiving considerable attention. By this procedure, dehulled soybean flakes, properly conditioned with moisture, are fed into an extruder and forced through an orifice under conditions of short-time, high-temperature, high-pressure from which the bean mixture emerges cooked, puffed, and dried. The puffed material is then finely ground and can be slurried with water to form soymilk.

In the Hand *et al.* (1964) and Mustakas *et al.* (1964) processes, no soaking or soak water losses are involved. The yield of soybean solids in the form of milk is greatly improved. However, soybeans are complex biological entities containing a variety of anti-growth factors (Liener, 1951, 1953). Ostensibly, the Chinese have used the pre-soak to make the beans grind more easily. However, the Chinese process also results in the removal of the insoluble residue. Hackler *et al.* (1963) reported that the ingredients extracted in soak water failed to support the growth of rats, and that the proteins in the insoluble residue had a better nutritional value than those in the soymilk. Very little information is available in the literature concerning the composition of soak water or the materials removed from soybeans by soaking. This study was undertaken to determine the nature of these substances and of the changes that take place in the bean during soaking. The yield of solids in soymilk in relation to the extent of soaking was also studied.

EXPERIMENTAL

The soaking studies were performed on three kg samples of Harasoy variety soybeans.

Sample 1. Non-soaked control. The whole beans were steamed at 100°C for 45 min to inactivate enzymes, and surface moisture was then removed by drying for 10 min at 105°C in a tray dryer. The beans were dehulled in a burr mill, properly spaced to yield principally cotyledons. Hulls were removed by air separation. The split beans were pulverized in a hammermill equipped with a 40-mesh screen. The bean flour was slurried with warm water to a 16%-solids mixture and was homogenized at 6,000 lb/in.^{2} The ho-

mogeneous slurry was freeze dried.

Sample 2. Soybeans soaked 24 hr at 1°C. Soybeans were soaked in three times their weight of water at 1°C for a total of 24 hr. The beans were wet dehulled in an electrically-driven vegetable peeler after soaking for 16 hr. The dehulled beans were soaked in another volume of fresh water for another 8 hr. The total soak water was saved and freeze dried before analysis. The hydrated, dehulled beans were slurried by grinding them with water in the ratio of 1:8 (beans:water) through the 0.023-inch screen of a Reitz disintegrator. The slurry was freeze dried before analysis.

Sample 3. Soybeans soaked 72 hr. The whole beans were pre-soaked for 4 hr and wet dehulled as in Sample 2. The beans were then given a total of 72 hr of soaking, including pre-soak, with water being changed four times after 16 or 18 consecutive hr of soaking. At each interval, the soak water was saved, freeze dried individually, and analyzed to determine the composition of the soak water as soaking progressed.

The soaked beans were ground as in Sample 2. The slurry was passed through a press filter and separated into a milk and a residue fraction. The fractions were homogenized and freeze dried as in other samples.

CHEMICAL ANALYSIS OF SAMPLES

Moistures. A 1-g sample was oven-dried at 95 to 100°C to constant weight. Usually a period of 4 hr was required.

Nitrogen by micro-Kjeldahl. A 100 to 150 mg sample was digested in the presence of 2 g of K_2SO_4, selenium granules (Hengar), and 3 ml of concentrated H_2SO_4. Digestion was completed in approximately 2 hr. Seven min were given for each distillation; distillate was received in 20 ml of 1% boric acid. The quantity of nitrogen was determined by titration with 0.1*N* HCl. Methylene blue-methyl red (1:2) was used as the indicator, giving a violet end point. A conversion factor of 6.25 was used for the estimation of protein.

Non-protein nitrogen. The method recommended by Becker *et al.* (1940) was used.

A 1 g sample was treated with 40 ml of 0.8*N* TCA followed by mechanically shaking for 30 min to precipitate the proteins; precipitates were separated by centrifugation before filtering. Each extraction was repeated, and the supernatants were combined and analyzed for non-protein nitrogen by the micro-Kjeldahl method.

Ether extractable fat. The method 22.033 of AOAC (1965) was used.

A pre-dried 1-g sample was extracted with anhydrous ether for 4 hr. Ether was recovered from fat through evaporation and recondensation. The extracted fat was then dried and weighed.

Chloroform-ethanol extractable fat. A pre-dried 1-g sample was extracted with 95% ethanol for 15 min and then with equal volumes of chloroform and ethanol for 20 min. The fat thus released was finally extracted with ether, dried, and weighed.

Fiber. The method 22.038 of AOAC (1965) was used.

The fat-free residue from the ether extracted sample was used. The loss on ignition of the sample after digestion with 1.25% of H_2SO_4 and 1.25% of NaOH was defined as the weight of fiber.

Sugar analysis of soak water of soybeans. Sucrose, raffinose, and stachyose were determined by the paper chromatographic method (Shallenberger *et al.*, 1961) for quantitative determination of reducing sugars and sucrose.

Freeze-dried soak water solids were dissolved in 80% hot ethanol; a concentration of 100 to 300 μg was used for spotting the paper chromatograph. Each sugar was identified by running knowns in parallel on one-dimensional descending chromatogram for 72 hr. The quantity of each identified sugar was determined by overnight digestion with invertase of corresponding unsprayed sections of the paper having

Table 1. Recovery of solids in soak water, soymilk, and residues from soybeans soaked for 0, 24, and 72 hr at 1°C.

Sample series no.	Fraction	Yield of solids (%) based on initial dry weight of soybeans	
1	Soybeans Non-soaked control	88	
2	Soybeans Soaked 24 hr	84	
2	Soak water 24 hr	5	
3	Soymilk from 72 hr soaking	50	
3	Residue	28	
3	Soak water No. 1 4 hr	0.75	
3	Water saved from wet dehulling	4.13	(4.88)[1]
3	Soak water No. 2 20 hr	2.63	(7.51)
3	Soak water No. 3 38 hr	0.75	(8.26)
3	Soak water No. 4 54 hr	1.08	(9.34)
3	Soak water No. 5 72 hr	0.38	(9.78)
3	Combined soak water 72 hr	0.72	

[1] Cumulative losses of solids in soak and dehulling water.

Table 2. Comparison of various analytical data on soaked beans, soak water solids, soymilk, and residues derived from soybeans soaked for 0, 24, and 72 hr.

Sample series no.	Fraction	Protein nitrogen %	Non-protein nitrogen %	Total fat %	Free fat %	Fiber %	Moisture %
1	Soybeans Non-soaked control	7.21	0.17	25.3	18.9	1.54	5.02
2	Soybeans soaked 24 hr	6.79	0.19	26.5	20.5	2.13	8.74
2	Soak water 24 hr	1.84	1.89	2.80	.....	0.68	.....
3	Soymilk from 72 hr soaking	7.83	0.36	23.2	18.0	0.00	3.68
3	Residue	5.85	0.19	20.6	16.9	4.48	3.63
3	Soak water 4 hr	1.44	1.40				
3	Soak water 38 hr	1.70	1.80				
3	Soak water 54 hr	1.68	0.95				
3	Soak water 72 hr	1.82	0.94				

Table 3. Comparison of yields in grams of protein, non-protein nitrogen, total fat, fiber, and carbohydrates (by difference) per 100 g of soybean on moisture-free basis.

Sample series no.	Fraction	Total protein g	Non-protein nitrogen g	Total fat g	Fiber g	Carbohydrate g
1	Soybeans Non-soaked control	42.96	0.163	24.0	1.46	22.6
2	Soybeans soaked 24 hr	37.77	0.166	23.4	1.86	21.2
2	Soak water 24 hr	0.69	0.11	0.14	0.42	3.2
	Total of 24 hr soak water and 24 hr soaked beans	38.46	0.276	23.54	2.28	24.4
3	Soymilk from beans soaked 72 hr	26.59	0.185	12.5	0.00	13.0
3	Residue	7.56	0.561	6.00	1.39	13.2
3	Soak water No. 5 72 hr	1.54	0.11	.....	.....	.....
	Total of milk residue, and soak water of 72 hr	35.69	0.856	18.5	1.29	26.2

Table 4. Comparison of soymilks prepared from soybeans soaked for about 16 and 72 hr.

Length of soak (hr)	Chemical composition of soymilk[2]					
	Crude Protein %	Free fat %	Bound fat %	Fiber %	Moisture %	Others (Carbohydrates) %
Overnight[1] (about 16 hr)	49.4	13.1	15.6	0.1	3.2	18.6
72	51.9	18.0	5.2	0.0	3.7	21.2

[1] The composition of soymilk is obtained from Hand *et al.* (1964). The Clark soybean was used, and methods of analysis were similar to the ones used in this paper.
[2] Freeze-dried milk.

the same R_f values as the knowns and calculated from equivalents of reducing sugars which were, in turn, determined with sugar reagents and colorimetry.

RESULTS AND DISCUSSION

The effect of soaking time on loss of solids from beans. It was found that the longer the soybeans were soaked before extraction the greater was the loss of solids in the soak water. On the average, beans soaked for 24 hr resulted in a 5% loss, while soaking for 72 hr resulted in a loss of 10% of solids (Table 1).

The rate of loss of solids decreased with time of soaking. This was shown from the cumulative weights of solids recovered from various fractions of soak water as soaking time increased from 4 to 72 hr (Table 1).

To keep soak water losses at a minimum, soaking should be continued only long enough to permit the soybeans to about double their initial dry weight. This permits the soybeans to be easily ground with most of the cells rupturing and releasing their constituents to the soymilk fraction.

Chemical analysis of the soak water solids after soaking beans for 24 hr showed that it had the following composition:

Crude protein	23.6%
Fat	2.8
Fiber	0.8
Sucrose	4.5
Raffinose	1.5
Stachyose	3.5
Other carbohydrates (By difference)	63.5

Of the total nitrogen, approximately one-half was non-protein nitrogen. The ratio of protein nitrogen to non-protein nitrogen remained fairly constant (1:1) in soak water solids through 38 hr of soaking (Table 2). As soaking was continued for 54 and 72 hr, the ratio of protein to non-protein nitrogen changed to about 2:1. Protein nitrogen in the soak water solids was fairly constant after 24 hr of soaking. Loss of protein nitrogen in the soak water was somewhat less when the beans were soaked for only 4 hr.

In contrast to the soak water solids, the soybeans, the residue following soymilk extraction, and the soymilk all had relatively high protein/non-protein nitrogen ratios in the range of 21 to 42 (Table 2). This was true regardless of the length of soak.

Metabolic changes in soybeans during soaking. Total protein in the interacting system, i.e., soaked beans plus soak water (Table 3) decreased from 43% (non-soaked control) to 38% (24-hr soak) to 36% (72-hr soak) in soybeans soaked at 1°C, apparently as a result of metabolic changes.

The decrease in total proteins was accompanied by an increase in non-protein nitrogen from 0.16% (non-soaked control) to 0.28% (24-hr soak) to 0.86% (72-hr soak). The increase in non-protein nitrogen materials presumably consisted of polypeptides and amino acids, the breakdown products of proteins.

There was also a decrease in fat from 24% (non-soaked control) to 19% (72-hr soak).

An increase in carbohydrates (by difference) during the soaking process was also observed. The carbohydrate content increased from 23% (control) to 24% (24-hr soak) to 26% (72-hr soak).

Although the above changes are similar to those occurring during seed germination, it is unlikely that they are identical, since soaking beans involved the complete immersion of the seed and limited the oxygen supply.

The effect of soaking on the composition of soymilk. The composition of soymilks prepared with an overnight soak (16 hr) (Hand *et al.*, 1964) and a 72-hr soak is compared in Table 4.

Considering the differences in the varieties of beans used, the only real difference between the two samples is the ratio of free to bound fat.

The implication of this difference to the quality of the soymilks is not apparent. Therefore, it can be concluded that the soaking of beans for as long as 72 hr did not result in measurable differences in the gross composition of the soymilks produced.

However, quantitative analyses of true protein and non-protein nitrogen, along with total and free amino acids, might have shown differences in the crude protein fractions with increased soaking time. Other areas worthy of detailed study are the specific sugars and higher oligo- and polysaccharides, which might show differences in soymilks prepared with different soaking times. Vitamin content may be altered during soaking (De, 1948).

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