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Badenhop,A.F.; Hackler,L.R.

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Effects of Soaking Soybeans in Sodium Hydroxide Solution as Pretreatment for Soy Milk Production [1]

A. F. Badenhop and L. R. Hackler
Cornell University
New York State Agricultural Experiment Station
Geneva, New York

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RECENT RESEARCH IN the area of off-flavor formation during soy milk production has shown that the major off-flavors associated with soy milk are of lipid origin and are produced by the action of the enzyme lipoxidase on the unsaturated fatty acids, principally linoleic and linolenic acids. Wilkens et al. (1) were able to demonstrate that if the soybeans were ground with water at or near 100°C., nearly all of the rancid off-flavor formation was prevented and the resulting milk was quite bland. However, Badenhop and Wilkens (2) found that when a high-temperature grinding operation is preceded by an initial soaking operation, a significant quantity of 1-octen-3-ol is formed, the amount varying with several environmental conditions including pH. An alkaline soaking solution decreased the formation of 1-octen-3-ol. As a result of these findings, this investigation was made to determine the effects of soaking soybeans in sodium hydroxide solutions on the flavor, acceptability, and nutritional value of the resulting soy milk.

The effects of soaking maize in sodium hydroxide solution have been reported by Kodicek (3), who found that increased quantities of cyanogen bromide-reactive material, presumably niacin, were released by alkali treatments. Furthermore, such alkaline extracts were shown to improve the growth of rats fed niacin-deficient diets (4,5). Kodicek et al. (6) also demonstrated a similar effect in pigs. Harper et al. (7) reported that the beneficial effect of alkali treatment of maize could not be attributable to the correction or prevention of an amino acid imbalance but only to the release of niacin from an unavailable form.

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Materials and Methods

Soybeans of the variety Harosoy 63, grown locally, were used in this study. Reagent-grade sodium hydroxide was used for preparation of soaking solutions. Samples were prepared with four different concentrations of sodium hydroxide as shown in the experimental design (Fig. 1).

The beans were soaked for 2 hr. at 50°C. in a steam-jacketed kettle large enough to accommodate 5-kg. samples of beans and 25 kg. of soaking solution (1:5 w./w. ratio). The temperature was maintained at 50°C. by periodically turning on the steam. After soaking, the beans were drained and rinsed thoroughly with distilled water, then ground by passing them through a Rietz disintegrator (Model RA-4-K-53) having a screen with 0.023-in. perforations. The beans were ground with sufficient boiling distilled water to achieve a ratio of 1:10 (w./w. dry beans to water). The resulting slurry was next filtered through a Shriver size 12 plate-and-frame filter press.

After filtration, the resulting milk (approximately 40 kg.) was divided into two portions; one was filled into No. 10 cans which were then closed and heat-processed at 121°C. for 10 min. This portion was later subsampled for the taste-panel work. The milk, both heat-processed and "raw," was then freeze-dried, as well as a portion of the residue from the filtration step.

Amino acid analyses were determined by ion-exchange column chromatography of acid hydrolysates; a Technicon Amino Acid Autoanalyzer (Model NC-3) was used. Cystine values were determined by the method of Moore (8); tryptophan was measured by the method of Horn and Jones (9); and available lysine was determined by the method of Rexen and Christensen (10).

Protein efficiency ratios (PER's) were determined by growth studies with male weanling rats of the Holtzman strain. The soybean supplements were used as the protein source in test diets at a level of approximately 10% crude

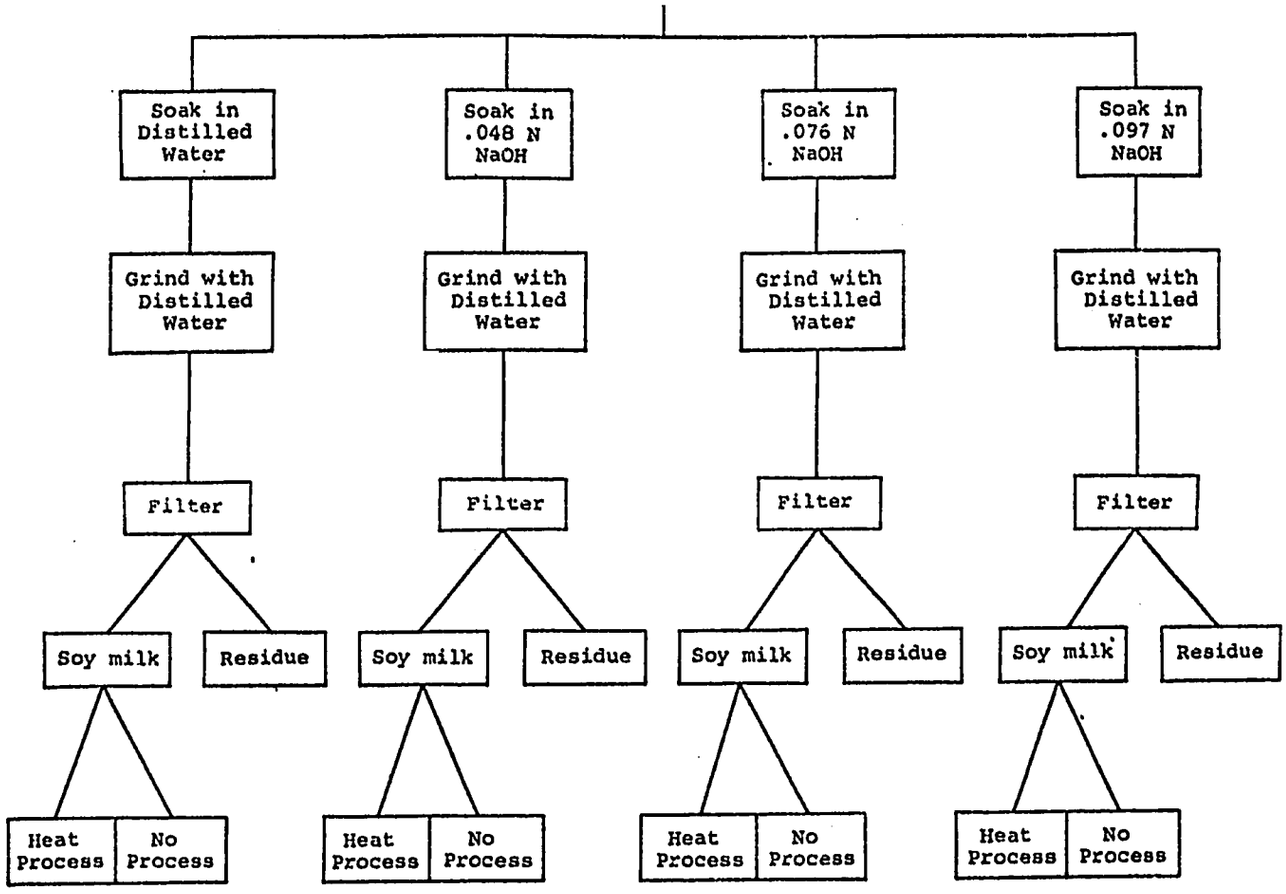

Fig. 1. Design of the experiment.

protein (Kjeldahl N X 6.25) and were fed *ad libitum* to groups of 10 rats for 28 days. Because of insufficient sample, some of the groups were limited to fewer than 10 rats and the feeding period was shortened to 25 days. Since the soybean fractions differed in protein and fat content, adjustments were made in the amounts of dextrose and corn oil added in order to maintain a relatively constant calorie-to-protein ratio. The experimental diets were compared to a casein control diet containing 10% protein. Complete details of the experimental methods used in the rat-growth studies have been reported previously (11).

Trypsin inhibitor measurements were obtained by the procedure of Learmonth (12) as modified by Van Buren et al. (13). This method compares the retardation of gelatin liquefaction by the enzyme trypsin in the presence of various dilutions of the sample. Results are expressed as percent of activity present in the nonheat-treated soybeans.

Niacin content of the samples was determined by a microbiological method (*Lactobacillus arabinosus*) (14). The amount of growth was determined by measuring the turbidity of the media at 625 mμ.

Sodium analyses were conducted on an atomic absorption spectrophotometer (Perkin Elmer Model 303) at 295 mμ.

Color measurements were obtained with a Hunter color-difference meter.

The four soy milks of different pH values were subjected to a taste panel of 14 members who were asked to rank them in order of increasing preference and rate them on a scale of 1 to 10.

Results and Discussion

Table I shows the composition of soaking solutions used in preparation of samples and amount of solution taken up by the beans during soaking. These data indicate that sodium hydroxide increases the rate of hydration of the beans. The amount of solution taken up increased from 1.15 g. of distilled water to 1.61 g. of 0.097N NaOH per g. of dry beans.

Table II shows the composition of the four resulting milk samples and the four residues or filter cakes. The solids content of the four samples indicates that at higher pH values, more solids are lost during the filtration step.

Table I. Concentrations of Soaking Solutions, Amounts of Solution Imbibed, and pH of Resulting Soy Milk

Sample No.	Sodium Hydroxide Concentration g./liter	Sodium Hydroxide Concentration moles/liter	Time of Soak min.	Amount of Soln. Imbibed g. of soln./g. of beans	pH of Resulting Milk
1	0.00	0.000	120	1.15	6.55
2	1.92	0.048	120	1.40	7.37
3	3.04	0.076	120	1.59	8.04
4	3.88	0.097	120	1.61	9.18

Table II. Composition of Soy Milks and Residues

	pH	Suspended Solids %	Percent Solids[a] Protein %	Fat[b] %	Sodium %
Soy milk	6.55	6.46	46.4	19.96	0.041
Residue	...	...	29.1	14.44	0.036
Soy milk	7.37	6.18	48.0	19.92	0.431
Residue	...	...	26.4	16.67	0.246
Soy milk	8.04	5.84	47.5	18.84	0.627
Residue	...	...	31.5	19.50	0.380
Soy milk	9.18	5.17	45.8	19.26	0.810
Residue	...	...	33.6	21.22	0.483

[a]Dry-weight basis.
[b]The term "fat" here means ether-extractable substances.

The protein content of the soy milk solids, when expressed on a dry-weight basis, indicates that an enrichment effect occurred when the pH was raised from 6.55 to 7.37, in that the protein content increased from 46.4 to 48.0%. A further increase in pH, however, decreased the protein content of the solids to 47.5% in the pH 8.04 sample and 45.8% in the pH 9.18 sample. The protein content of the residues decreased from 29.1% in the pH 6.55 sample to 26.4% in the pH 7.37 sample and then increased to 31.5% at pH 8.04 and 33.6% at pH 9.18. The ether-extractable fat in the soy milk solids was relatively unaffected by pH, as seen from the table. The fat content of the residue was observed to increase with pH. This is contrary to what would be expected and may be due in part to an enrichment effect caused by leaching of solids from the beans during soaking. The amounts of solids so leached increased from 9 g. per liter of soak water at the lowest pH value to 30 g. per liter at the highest. Since approximately 75% of the soak-water solids are substances other than protein and fat, the leaching effect may produce profound effects on the composition of milk and residue fractions. More laboratory studies are needed to evaluate properly the importance of these effects. The sodium content of the eight fractions is also included in Table II.

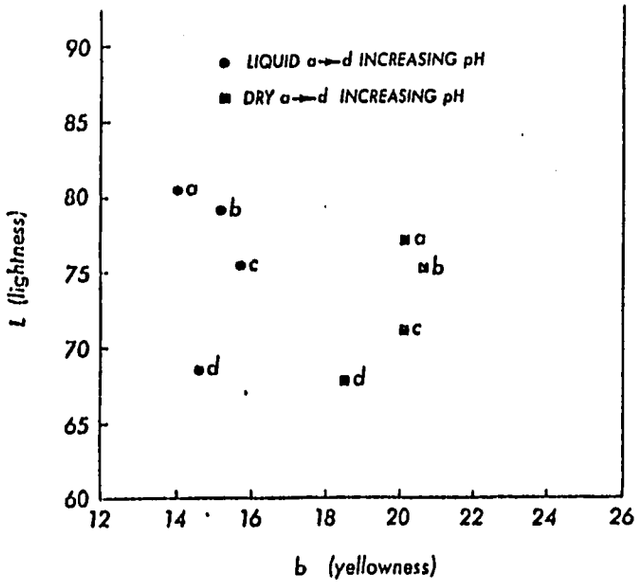

Fig. 2. Effect of pH on color of the heat-processed soy milk samples expressed in terms of Hunter color values (L vs. b).

Taste-panel results of the four different soy milk samples were analyzed with the use of Kramer's (15) revised tables for determining differences from rank sums. The sample with pH 7.37 had a significantly better flavor than the other three samples at the 5% level. The sample prepared from beans soaked in water (pH 6.55) was judged significantly poorer than the other three samples at the 1% level. The same samples were resubmitted to the panel to determine any perceptible difference in mouth-feel. This panel showed no significant differences among the four samples at the 5% level. It is of interest to note, however, that of the 14 panelists, five preferred the most alkaline sample and only two preferred the nonalkaline sample. This indicates that the alkaline treatment may have a desirable effect on mouth-feel.

Figure 2 shows a plot of Hunter color values, L vs. b, of both liquid and dried heat-processed samples of soy milk. It is evident that as the pH increases, the resulting milk becomes darker, i.e., the L value becomes smaller. The b value, which is a measure of the yellowness of the sample, shows that the three liquid samples of alkaline pH were more intensely yellow than the nonalkaline sample. The pH 8.04 sample was the most intense yellow. The dried samples showed a different pattern in terms of Hunter, b. The pH 7.37 sample was the most intense yellow and the pH 9.18 sample had a lower b value than the nonalkaline sample.

Table III shows the trypsin inhibitor (TI) activity of the 12 samples expressed as the percent of activity in whole untreated soybeans. From these data it is evident that heat-processing of the milk at 121°C. for 10 min. results in retention levels of 7.6% and lower. These values agree closely with those reported by Hackler et al. (16), that heating soy milk at 121°C. for 5 to 10 min. destroys 90 to 95% of the TI activity. Far more interesting is the apparent effect of pH on TI activity as seen from inspection of the values for the "raw" soy milks. The retention drops from 68% in the pH 6.55 sample to 17% in the pH 9.18 sample. Such an effect could be predicted in the light of findings of Kunitz (17) in his work with crystalline soybean trypsin inhibitor: he showed that 0.1N NaOH at 36°C. for 2 hr. would destroy the inhibiting activity. Considering the higher temperatures involved (50°C. during the 2-hr.

Table III. Trypsin Inhibitor Retention, Cystine Content, and PER Values of 12 Samples

Sample Description	pH	Trypsin Inhibitor Retained %	Cystine g./16 g. N	Protein Efficiency Ratio[a]
Milk, raw[b]	6.55	68.1	1.74	1.44
Milk, heat-processed	6.55	7.6	1.66	2.41
Residue	...	20.0	1.48	2.11
Milk, raw	7.37	60.0	1.72	1.60
Milk, heat-processed	7.37	5.9	1.41	2.18
Residue	...	20.0	1.37	2.13
Milk, raw	8.04	45.0	1.56	1.75
Milk, heat-processed	8.04	5.0	1.37	2.20
Residue	...	10.0	1.01	2.14
Milk, raw	9.18	17.3	1.30	1.88
Milk, heat-processed	9.18	2.7	1.15	1.70
Residue	...	6.4	1.32	2.09

[a]Adjusted to a casein standard with a PER of 2.5.
[b]The term "raw milk" means milk which was not heat-processed at 121°C.

soaking of the beans and nearly 100°C. during the grinding operation), the resulting decrease in activity is not surprising, even though the highest sodium hydroxide concentration used in the soaking solutions was approximately 0.1N.

The results of the amino acid analyses of the 12 fractions indicated that the effect of the alkaline treatment on the amino acid content of the soy milk and residue samples was minimal. The only amino acid which seems to be affected by pH is cystine (Table III). The cystine level in the "raw" soy milk decreased from 1.74 g. per 16 g. N in the pH 6.55 sample to 1.30 g. per 16 g. N in the pH 9.18 sample. When the soy milks were heat-processed for 10 min. at 121°C., the effects of heat and pH seemed to be additive. The cystine level dropped from 1.66 g. per 16 g. N in the pH 6.55 sample to 1.15 g. per 16 g. N in the pH 9.18 sample. Thermal lability of cystine at higher pH is well documented. The disulfide bond is apparently the most vulnerable position in the molecule, and one of the major products found to occur is lanthionine. Several mechanisms have been suggested for its formation, including one which involves cysteine as an intermediate product (18).

Table III shows the results of the rat weight-gain experiments. Examination of the PER's indicates that definite effects can be attributed to the alkali treatments. For the "raw" milk samples it may be noted that, as the final pH of the milk increased, the PER values also increased. This effect can be explained at least in part by examination of the trypsin inhibitor values for these samples, which range from 68.1% in the pH 6.55 sample to 17.3% in the pH 9.18 sample. The trend for the heat-processed milk samples is just reversed, in that with increasing pH the PER values decreased sharply. Apparently, part of the reason for this is the increased thermal lability of cystine, which has been discussed previously. The nutritional quality of the residue protein was relatively unaffected by pH. Since the residues were not heat-processed, some of the trypsin inhibitor activity remained in them; this partially explains their inferiority to the milk samples.

An anomalous situation exists, however, in that the trypsin inhibitor values of the higher-pH residues were quite low, whereas the PER values obtained for these samples were nearly the same as those for the lower-pH samples. This would indicate that other inhibiting substances, unaffected by alkaline pH, may play a significant role in determining the nutritive value of the unprocessed residues.

The niacin content of the soy milks and residues are shown in Table IV. In the light of earlier work done by various researchers (3,4,5,6,7) on alkaline extraction of corn, the results obtained in this study were somewhat surprising. The niacin content of alkaline-extracted soy milks did increase up to a pH of 8.04. The pH 8.04 soy milk contained approximately 25% more niacin than the pH 6.55. The niacin content of the pH 9.18 sample, however, was considerably lower than that of the pH 8.04 sample and was even lower than that of the water-extracted sample. Theories proposed by various workers for the increase in extractable niacin at alkaline pH are varied. Kodicek (3), who first observed the effects of extraction methods on niacin, suggested that alkaline conditions liberated a bound form of niacin. Snell and Wright (19) attributed the differences in amount of niacin extracted to the completeness of extraction, since they found that finely ground samples gave comparable results by both alkaline and neutral extraction methods. Melnick et al. (20) assumed that alkaline hydrolysis increased the availability of some water-soluble derivatives or precursor of niacin, since they found that alkaline hydrolysis of water extracts gave results similar to those obtained for direct alkaline extracts. Andrews et al. (21) confirmed the results of Melnick et al. (20) and also found that strong acid hydrolysis would produce the same results.

Table IV. Niacin Content of Samples

Sample Description	pH	Niacin mg./100 g. dry matter
Milk, raw[a]	6.55	3.50
Milk, heat-processed	6.55	3.96
Residue	...	0.68
Milk, raw	7.37	3.72
Milk, heat-processed	7.37	3.85
Residue	...	0.55
Milk, raw	8.04	4.82
Milk, heat-processed	8.04	4.98
Residue	...	0.43
Milk, raw	9.18	2.56
Milk, heat-processed	9.18	3.69
Residue	...	0.71

[a]The term "raw milk" means milk which was not heat-processed at 121°C.

The results obtained in this work would tend to support the original theory that bound niacin or a niacin precursor is released by alkaline extraction conditions. This would seem to be the case, because all of the samples were treated with 1N H_2SO_4 at 121°C. for 30 min. during the preparation for niacin analysis, which should acid-hydrolyze any precursor to active niacin. Therefore if the precursor is soluble in water as Melnick suggests, and equal quantities were extracted at all four pH levels, the quantity of niacin measured after sample preparation should have been approximately the same in all of the milk samples. This, of course, does not explain the decreased amount of niacin in the pH 9.18 sample. On the basis of work by Kodicek (3), one would expect to extract more niacin at this higher pH. An analogous situation exists with the niacin content of the residue where the amount of niacin remaining decreased with higher pH. Again, however, the highest pH residue contained more niacin than any of the other samples. These unexpected values may be the result of difficulties experienced with the filtration of the pH 9.18 sample which caused more of the milk to remain in the residue. This would increase the niacin content of the residue above the expected value.

Conclusion

Soaking soybeans in a sodium hydroxide solution of approximately 0.05N is a desirable pretreatment prior to a high-temperature grinding operation for the production of soy milk. This procedure produced soy milk of pH 7.37 which was judged by a panel of 14 members to have a significantly better flavor than a water-soak control treatment at the 1% level of significance.

Alkaline conditions were found to render trypsin inhibitor more heat-labile and therefore easier to destroy during heat-processing. Alkaline soaking conditions were also found to increase the niacin content of the soy milk slightly.

A disadvantage of alkaline treatment of soybeans prior to soy milk production appears to be partial destruction of the amino acid cystine, which is reflected in low PER values obtained for the processed soy milk samples of higher pH. The extent of this problem is apparently not very serious except in soy milk of pH 8.0 and higher. Since the beneficial effects of alkaline soaking treatments on the flavor of soy milk occur below pH 8.0, the use of an alkaline soaking treatment as a means of producing a more palatable soy milk would seem to pose no serious nutritional problem.

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