Effects of Soaking Soybeans in Dilute Acids on Biologically Active Components

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Soaking soybeans in dilute acids affected activities of lipoxygenase, trypsin inhibitor and urease. Effects of soaking time, acid concentration and soaking temperature were investigated. Lipoxygenase activity was completely eliminated by soaking in 0.3 M HCl at either 23°C or 40°C for 8 hr. Less than 50% trypsin inhibitor remained and urease was inactivated to an acceptable level (0.04 Δ pH).

KEY WORDS: Acid soaking, lipoxygenase inactivation, soybeans, trypsin inhibitors, urease inactivation.

Dry soybeans are traditionally soaked in cold water prior to the preparation of soymilk in the Orient. Overnight soaking of whole or dehulled soybeans hydrates and tenderizes the beans prior to grinding for water extraction. The resultant soymilk, however, has a highly oxidized-painty and beany flavor that is generally unacceptable to non-Asian populations. Numerous modifications to reduce off-flavor and off-odor include hot water extraction (1), acid grinding with heating (2,3), soaking in alkalis and salts (4,5) and soaking in 0.5% $NaHCO_3$ solution and blanching prior to cellular disruption (6). Some modifications have resulted in flavor improvements.

No study has been reported on dilute acid solution as a soaking medium in the preparation of soymilk or soy flour. Our earlier investigation showed that direct acid grinding at pH 3.0 irreversibly inactivated lipoxygenase (7), which is involved in catalyzing the oxidation of polyunsaturated fatty acids and the production of off-flavor (8). Sensory studies also showed that off-flavor was effectively controlled by pH adjustment (9). The objective of the present study was to investigate the effect of soaking dry soybeans in dilute hydrochloric acid (HCl) on the activities of lipoxygenase, trypsin inhibitor and urease.

MATERIALS AND METHODS

Sample preparation. Williams 82 variety soybeans *(Glycine max)* were obtained from the USDA Soybean Germplasm Collection Center, Agronomy South Farm, University of Illinois, Urbana, IL.

Whole beans were heated at 99°F for 15 min in a Proctor Standard variable circulation dryer (Proctor-Schwartz Inc., Philadelphia, PA) with a downward flow of hot air. Immediately after heating, the beans were passed through a drum dehuller (10). The distance between the stationary concave surface and the revolving corrugated drum was 0.11 inch, which is less than the diameter of the soybeans. Loose hulls and fines were removed by aspiration. Dehulled soybeans were stored at 0°C.

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Acid-treated, full-fat soy flour (FFSF) was prepared from dehulled soybeans that were previously soaked in 0.15 M and 0.3 M HCl. At each soaking time, 40 g soybeans were treated in ten-fold volume of dilute acids for 0–24 hr at either 4°C or 23°C. After soaking, the soybeans were drained, thoroughly washed and resoaked in distilled water for the same duration as the initial soaking. Four changes of distilled water ensured adequate removal of residual acid. Soybeans soaked in distilled water at room temperature for the same duration were the control. The beans were then drained, blotted dry, freeze-dried without heat for 72 hr, ground and stored at 0°C.

Chemical analyses. Lipoxygenase activity (LA) of defatted samples was assayed at 23°C by the spectrophotometric method of Surrey (11) with linoleic acid in borate buffer (pH 9.2). Trypsin inhibitor activity (TIA) was assayed by AOCS Official Method Ba12-75 (12) with a modification by Hamerstrand *et al.* (13). Urease activity (UA) was determined by AOCS Official Method Ba 9-58 (12). Activity was expressed as the difference of pH (Δ pH) between the test and blank samples.

RESULTS AND DISCUSSION

Control (soaked in distilled water) and lipoxygenase activity (LA). The effect of soaking dehulled soybeans in distilled water on LA, TIA and UA of FFSF is shown in Table 1. LA increased from 13.90 unit/mg solid to 23.56 unit/mg solid or almost 170% after soaking in distilled water for 4 hr and remained constant for another 4 hr. LA gradually decreased with longer soaking, but remained higher than the original level. The increase in LA was probably due to the decrease in solid content of the sample as a result of leaching solubles during the first 8 hr of soaking. However, after 8 hr of soaking, the gradual decrease in LA could be due to enzyme inactivation as a result of prolonged exposure to water. LA could also be detected in soaking water in the range between 0.27–0.35 unit/mg solid. Data shown in Table 1 indicate that most

TABLE 1

Effect of Soaking Dehulled Soybeans in Water at 23°C (Control) on LA, TIA and UA of FFSF

Soaking time (hr)	LA Unit/mg solid[a]	TIA Activity, mg/g[a]	UA Activity,[a] Δ pH
0	13.90 ± 0.41	26.62 ± 1.92	2.09 ± 0.02
4	23.56 ± 0.23	22.50 ± 0.98	2.17 ± 0.08
8	23.29 ± 0.57	16.21 ± 0.33	1.97 ± 0.04
12	19.43 ± 0.27	15.09 ± 0.86	1.99 ± 0.02
16	17.88 ± 0.30	12.50 ± 0.50	2.07 ± 0.03
20	16.07 ± 0.32	11.30 ± 0.79	1.97 ± 0.08
24	15.95 ± 0.11	9.50 ± 1.16	1.98 ± 0.03

[a]Means of three replications ± S.D.

of the leaching took place during the initial 4 hr of soaking. The control study simulated the Oriental pretreatment of soybeans for the preparation of soymilk. High LA in soaked beans results in typically strong off-flavors in traditional soymilk.

Trypsin inhibitor activity (TIA). TIA gradually decreased in soybeans from the initial level of 26.62 mg/g as the time of soaking increased in distilled water. After 8 hr the remaining TIA was 61% while after 24 hr, it was 36%. Leaching was believed to be the main factor involved in reducing TIA.

Urease activity (UA). UA of the full-fat soy flour increased slightly after soaking in water for 4 hr. With further soaking from 8–24 hr, UA remained fairly constant at a level of about Δ pH = 2.00. Urease was not inactivated or leached during soaking in water.

Acid study: Lipoxygenase activity (LA). The effect of soaking in dilute acids on LA of acid-treated FFSF is shown Figure 1. Rapid reduction of LA was observed in all acid-soaking conditions during the first 4 hr. No LA was detected after 8 hr of soaking in 0.15 M HCl at 23°C, or 0.30M HCl at 4°C and 23°C. However, the beans soaked for 8 hr in 0.15 M HCl at 4°C still contained residual LA of 0.33 unit/mg solid or 2.5% of the original. Under the same condition of soaking, however, sensory data showed that the slurry was rendered essentially free from off-flavor typical for the enzyme-active slurry (9). The optimum soaking time for complete elimination of LA, therefore, seemed to lie between 8–12 hr when 0.15 M HCl was used at 4°C. No LA was detected after 12 hr of soaking in either 0.15 M or 0.30 M HCl at 4°C or 23°C.

Trypsin inhibitor activity (TIA). The effect of soaking dehulled soybeans in dilute acid on TIA of acid-treated FFSF is shown in Figure 1. TIA was greatly influenced by the acid concentration and soaking temperature. TIA increased to 107% in the first 4 hr of soaking in 0.15 M HCl at 4°C. This was probably due to a greater rate of leaching of other solubles, such as carbohydrates, during the initial soaking at low temperature. No significant change from the soaked sample was noted in case of soaking in 0.3 M HCl at 4°C. There was little difference between soaking in 0.15 M and 0.3 M HCl at 4°C for longer time. In the first 4 hr of soaking at 23°C, TIA decreased 11% and 16% in 0.15 M and 0.3 M HCl, respectively. No difference in TIA was noted between water-soaked samples and samples soaked in 0.15 M HCl at 23°C. As soaking time increased to 8 hr, TIA decreased only 5% and 11% in samples soaked in 0.15 M and 0.3 M HCl at 4°C, respectively. However, when soaked at 23°C, more than 40% and 50% reduction of TIA was observed for 0.15 M and 0.3M HCl, respectively. This trend continued with the time of soaking (Fig. 1).

Since acid did not influence the TIA, the observed decrease with increased time of soaking was attributed mainly to leaching of the protease from the intact cotyledons into the soaking medium. Leaching of trypsin inhibitor by soaking was also reported by Collins and Sanders (14).

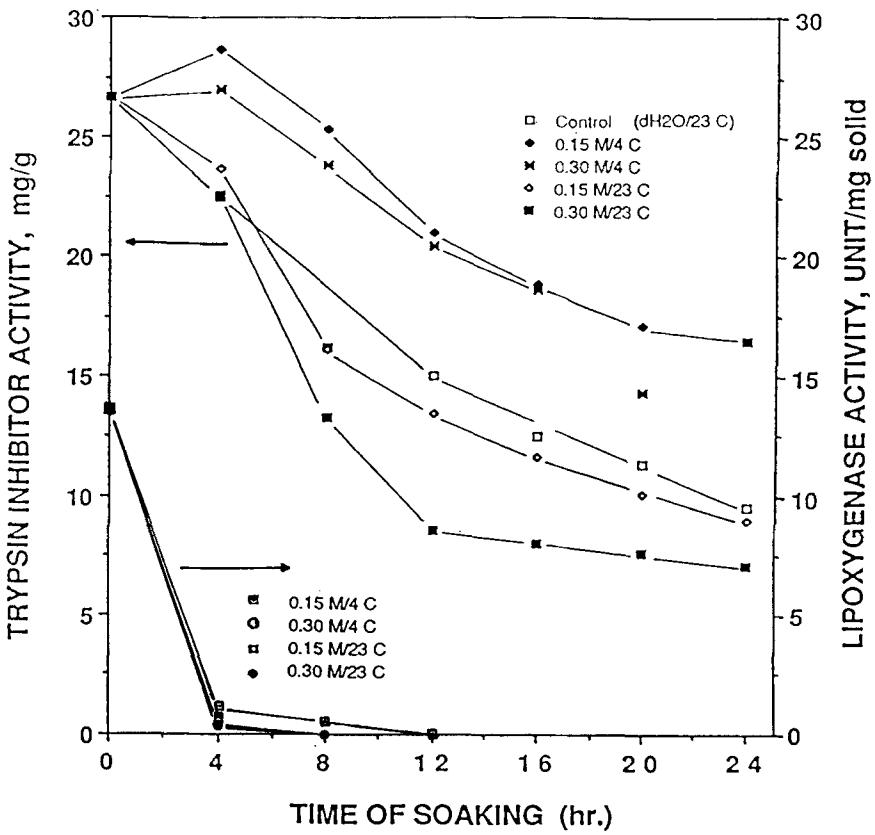

FIG. 1. LA and TIA of acid-treated FFSF after soaking in dilute HCl for various times at 4°C and 23°C.

TABLE 2

Effect of Soaking Dehulled Soybeans in Dilute Acids on UA of Acid-Treated FFSF

Soaking time (hr)	Urease index, Δ pH			
	4°C		23°C	
	0.15 M	0.3 M	0.15 M	0.3 M
0	2.09 ± 0.02	2.09 ± 0.02	2.09 ± 0.02	2.09 ± 0.02
4	1.28 ± 0.10	0.47 ± 0.02	1.07 ± 0.06	0.04 ± 0.00
8	0.06 ± 0.01	N.D.	0.07 ± 0.00	0.04 ± 0.00
12	0.05 ± 0.00	N.D.	0.02 ± 0.00	N.D.
16	N.D.[a]	N.D.	N.D.	N.D.

[a]N.D., no detectable activity.

Trypsin inhibitor is very stable in acidic conditions (15–17) and acid grinding does not result in a significant change in TIA for a range of acid pH (7).

Urease activity (UA). The effect of acid soaking dehulled soybeans on UA of acid-treated FFSF is shown in Table 2. Urease index (Δ pH) was 1.28 and 0.47 after soaking for 4 hr at 4°C in 0.15 M and 0.3 M HCl, respectively. As soaking temperature increased to 23°C, urease index decreased to 1.07 and 0.04, respectively, in 4 hr. After 8 hr of soaking, samples for all conditions were essentially free from UA.

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