ALCOHOL TREATMENT OF SOYBEANS AND SOYBEAN PROTEIN PRODUCTS[1]

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ABSTRACT

Earlier studies suggest that some undesirable flavors of soybeans and soybean products result from enzymatic oxidation of unsaturated lipids and subsequent breakdown of the hydroperoxides formed. Such enzyme action is believed to occur as soon as the seed is crushed. To test this hypothesis, soybeans and soybean protein products were steeped or wet-milled with aqueous ethyl alcohol at 25°C to inactivate enzymes *in situ* or as soon as the cellular structure is disrupted. The treated materials were dried at reduced temperatures and pressures and then assayed for lipoxygenase, urease, and trypsin inhibitor activities. Nitrogen solubility indexes and organoleptic properties were also determined. When aqueous alcohol was used in a concentration range of 40 to 60% (v/v), enzymatic activities and nitrogen solubility indexes were reduced extensively, but trypsin inhibitor was only partially inactivated. Flavor evaluation indicated improved products, *i.e.*, the characteristic beany, bitter flavor of untreated soybeans was reduced in materials treated with aqueous alcohol. The flavor of other raw legumes, including lima beans, split peas, black-eyed peas, and peanuts, also improved after a similar aqueous alcohol treatment.

Because of their functional and nutritional properties, soy protein products find outlets in a variety of processed foods (1). Estimated U.S. production of soy flours, concentrates, isolates, and textured flours in 1976 was 625, 80, 75, and 110 million lb, respectively.[2] Industry representatives indicate that one factor limiting use of soybean protein products is flavor (2).

For several years the Northern Regional Research Center has worked on improving the flavor of soybeans and soybean products. A review in 1975 (3) details progress made on improving the flavor of soybean products. Recent studies (4) indicate that some undesirable flavors associated with soybean products result from breakdown of fatty acid hydroperoxides formed by the action of lipoxygenase (EC 1.13.1.13). Heat inactivates lipoxygenase, but such treatments insolubilize the major soybean proteins and tend to generate a cooked or toasted flavor. Therefore, an alternative method was used for inactivating lipoxygenase *in situ* or during grinding. Either soaking or wet-milling soybeans in aqueous ethyl alcohol improves their flavor. The effect of alcohol concentration on flavor, lipid-oxidizing enzymes, urease, trypsin inhibitor, and nitrogen solubility index of both whole soybeans and of various derived materials was, therefore, investigated.

MATERIALS AND METHODS

Preparation of Soybean Protein Products

Soybeans were cracked, dehulled, and flaked to yield a full-fat flake. The flakes were defatted by extracting with pentane-hexane at room temperature.

[1]Presented at the 59th Annual Meeting, Montreal, Canada, Oct. 1974.

Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

[2]Personal communication, N. R. Lockmiller.

Soybean concentrate was prepared by extracting defatted flakes with dilute acid (20:1) at pH 4.5–4.6. Isolated soybean sodium proteinate was prepared from defatted flakes by water extraction (20:1), centrifugation, reextraction with water (10:1), centrifugation, precipitation at pH 4.5 to 4.6 with dilute hydrochloric acid, washing, and freeze-drying.

Alcohol Treatment of Soybeans and Soybean Products

Certified, seed-grade soybeans (Amsoy and Hawkeye varieties) from the 1972 crop were used. After initial experiments on whole beans to optimize conditions, soybeans and soybean products were routinely soaked (with occasional stirring) in aqueous alcohol mixtures for 24 hr at room temperature. Four volumes (ml) of aqueous alcohol per gram of soybeans or soybean product was used for soaking.

The solvent and soaked beans were then transferred to a rotary evaporator, and unabsorbed solvent was removed at ~30°C. Finally, absorbed solvent was removed by freeze-drying the beans for 24 hr. This drying procedure was

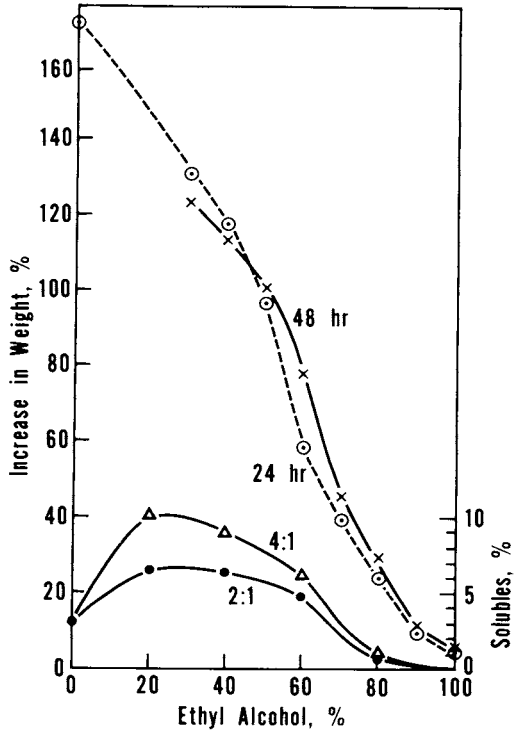

Fig. 1. Uptake of aqueous alcohol and soybean solids dissolved when soybeans are soaked at different alcohol concentrations. Solids dissolved are based on volume of drained solvent and are expressed as per cent of weight of soybeans. The per cent of soluble solids was determined on beans soaked for 24 hr at solvent:bean ratios of 2:1 and 4:1.

followed to avoid removal of nonvolatile solids from the treated soybean products. All dried products were ground in a Wiley mill equipped with a 40-mesh screen.

To improve the process by avoiding long steeping periods, soybeans were wet-milled in a Quaker City mill (Model 4E). Soybeans were dispersed in aqueous alcohol (approximately 4:1) and immediately ground to approximately 20 mesh in the mill. This slurry was passed through the mill several more times until all particles were approximately 40–60 mesh. The entire operation took only 15–20 min for 0.5–1.0 kg quantities. After grinding, the slurry was dried as described for whole soybeans to enable comparison of steeped and wet-milled samples.

Analytical Procedures

Nitrogen solubility index (NSI) of both treated soybeans and soybean products was determined according to official procedures (5). Lipoxygenase activity was measured with an oxygen electrode by the procedure of Christopher *et al.* (6) at pH 9.0 and at 20°C. Urease activity was assayed by titrating the NH_3 released from urea substrate in 5 min (7) with standard acid to an end point of pH 5.1. Soybean trypsin inhibitor (SBTI) was measured by the method of Kakade *et al.* (8).

Organoleptic evaluations were conducted on 2% aqueous dispersions as described by Kalbrener *et al.* (9). Flavors and odors were scored on a 10-point scale where 1 is strong and 10 is bland. Flavor intensity values (FIV) were calculated according to Rackis *et al.* (10) for seven flavor categories: grassy/beany, bitter, astringent, cereal/grain, musty, chalky, and bland.

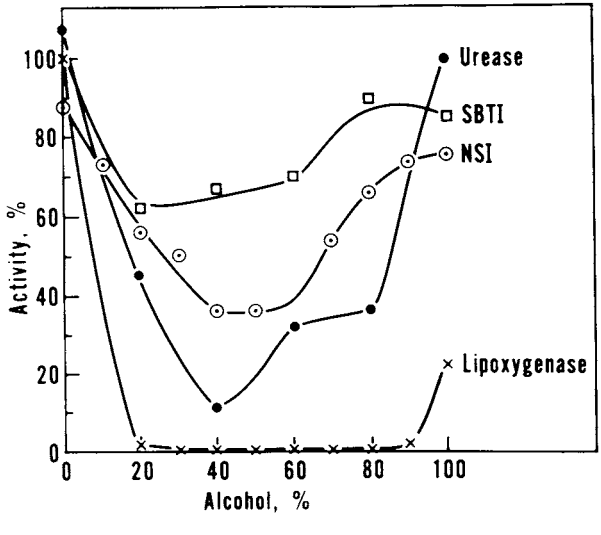

Fig. 2. Effect of soaking soybeans for 24 hr in various alcohol concentrations on nitrogen solubility index (NSI), urease, lipoxygenase, and soybean trypsin inhibitor (SBTI).

RESULTS

Preliminary studies at room temperature showed that in 24 hr soybeans and soybean products absorb nearly maximum amounts of aqueous alcohol. The increase in weight of drained soybeans was measured after soaking in different concentrations of alcohol for 24 and 48 hr (Fig. 1). Also shown in Fig. 1 is the amount of dissolved solids in the drained, unabsorbed solvent. Solvent ratios of 4:1 removed up to 10% of the solids in 24 hr, whereas a 2:1 solvent ratio dissolved a maximum of 6.5% of the whole soybeans in 24 hr.

Both NSI and several biological activities are affected by soaking Amsoy soybeans for 24 hr at various alcohol concentrations (Fig. 2). NSI of treated full-fat soybean flour is nearly what would be expected from the results of Smith *et al.* (11). The lipid-oxidizing enzyme, lipoxygenase, is greatly inactivated by the intermediate concentrations of aqueous ethyl alcohol. Less than 1% of the original activity remains after beans are soaked in 30 to 80% aqueous ethyl alcohol.

A taste panel evaluated full-fat soybean flours prepared from soybeans steeped with various concentrations of alcohol. Their flavor scores and the grassy/beany

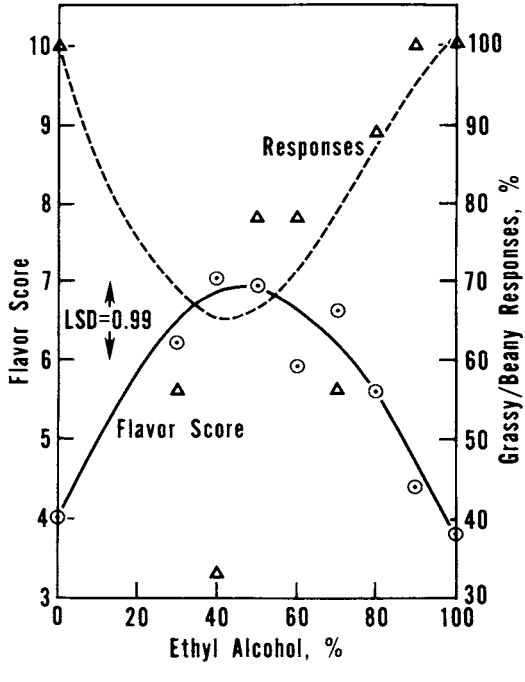

Fig. 3. Effect of alcohol concentration used for steeping on flavor of soybean flour. Grassy/beany responses are expressed as per cent of panelists giving this flavor description. Curves were fitted by the method of least squares. Least significant differences between flavor scores of samples were calculated at 0.99.

responses are plotted in Fig. 3. The maximum (best) flavor score is obtained when soybeans are treated with 40 to 60% alcohol. This maximum flavor score and minimum grassy/beany response occur where the NSI and lipoxygenase curves of Fig. 2 are at minimal values.

Organoleptic evaluations of full-fat flours from 50% alcohol-treated and untreated soybeans are compared in Fig. 4a. The bar graph shows the FIV of six flavor characteristics of soybeans (chalky, musty, cereal/grain, astringent, bitter, and grassy/beany) and overall flavor score. As can be seen, the alcohol treatment

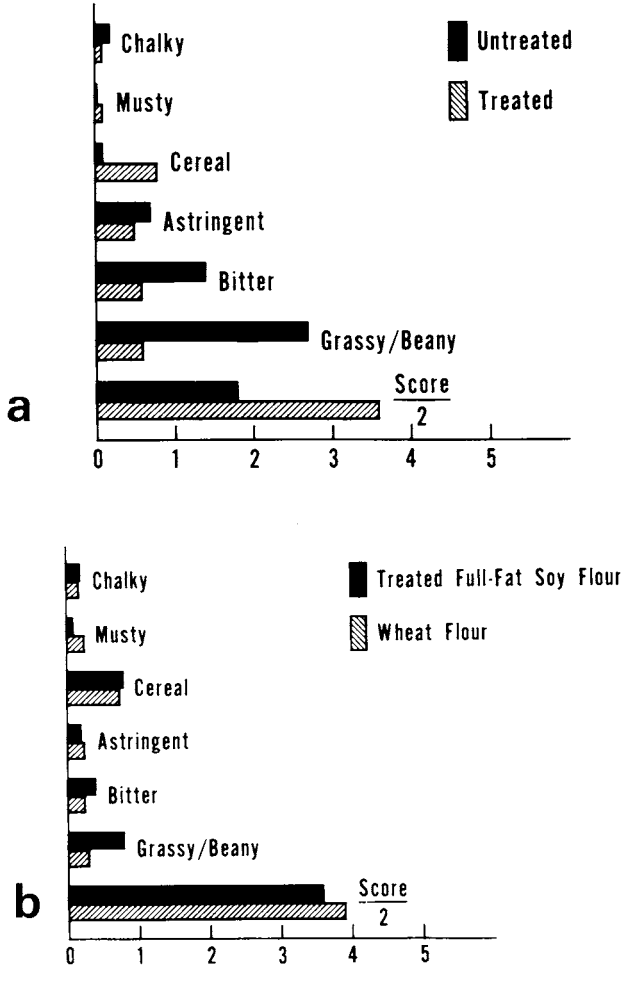

Fig. 4. Bar graphs for flavor intensity values of flavor notes and overall flavor scores of a) treated and untreated full-fat soybean flour, and b) treated full-fat soybean flour and wheat flour. Note that flavor scores are divided by 2 to facilitate graphing.

of soybeans reduces the intensity (FIV) of all flavor notes, except cereal, and also improves the overall flavor score.

A second bar graph (Fig. 4b) compares FIVs for flavor notes of a full-fat flour from 50% alcohol-steeped soybeans and an all-purpose wheat flour. The flavor responses for the treated soybeans are comparable to those for wheat flour.

These two bar graphs (Fig. 4a and 4b) also confirm the consistency of the taste panel. The flavor notes and scores of the two soybean flours are from different tastings at different times; however, the taste panel reported almost identical descriptions and overall scores.

Flavor scores of the steeped (24 hr) and wet-milled samples are compared in Fig. 5. Flavor scores of both improved in the mid range of alcohol concentration. Lower flavor scores for the wet-milled samples may have resulted from incomplete penetration of alcohol into grit particles even after 2 to 3 hr of contact during grinding and drying.

Organoleptic responses for soybeans soaked in 50% alcohol at different temperatures appear in Table I. Flavor score of the beans soaked at 25°C is significantly better than those treated either at 4° or 78°C. At room temperature or higher there is also a large decrease in the grassy/beany response. Apparently, near room temperature is optimum for the treatment of soybeans with aqueous alcohol.

Various soybean protein products prepared in the laboratory also were treated with 50% aqueous alcohol at room temperature. Free solvent was removed on a rotary evaporator and the samples were finally freeze-dried. After grinding in a Wiley mill, the samples were presented to the taste panel as pairs (*i.e.*, control and

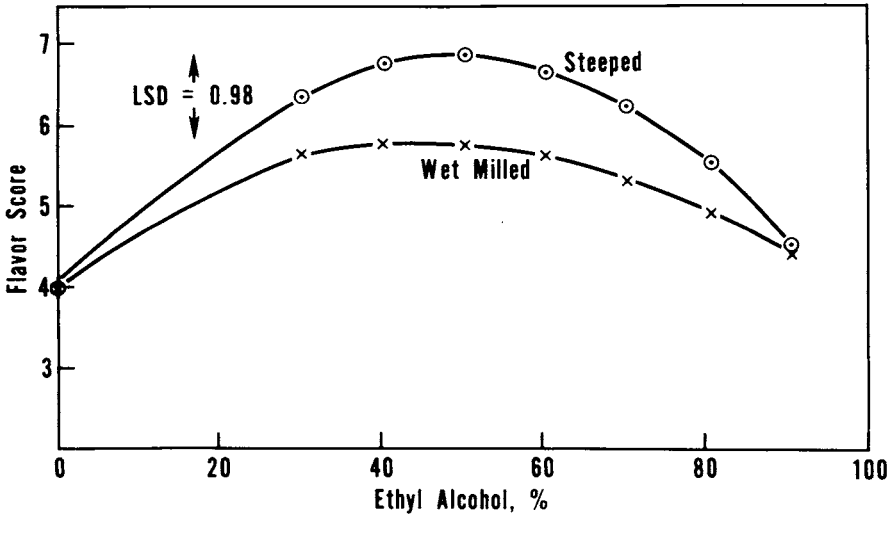

Fig. 5. Effect of alcohol concentration on flavor scores when soybeans are steeped or wet-milled. Least significant difference between samples was calculated at 0.98 and curves were fitted by method of least squares.

treated sample) in 2% dispersions. Results of the tastings and lipoxygenase activity measurements are given in Table II.

When controls and treated samples are compared, a 50% aqueous alcohol treatment always improves the flavor score. There is also a decrease in both the grassy/beany and bitter response of the taste panel members even for samples free of lipoxygenase activity prior to treatment. This apparent anomaly is discussed later.

A cursory examination was made of the effect of 50% aqueous alcohol on flavor scores of four other raw legumes in comparison with soybeans (Table III). The largest increase in flavor score occurred with soybeans, but significant or highly significant increases were also obtained for the other legumes.

TABLE I
Flavor Scores and Descriptions of Whole Soybeans after Soaking in 50% Ethyl Alcohol at Various Temperatures

Characteristic	Soaking Temperature		
	4° C	25° C	78° C
Flavor score	5.2	6.5*	5.2
FIV[a]			
Bland		0.15	
Cereal grain	0.23	0.77	0.85
Grassy/beany	1.69	0.85	0.62
Chalky	0.15	0.23	0.15
Musty	0.54	0.46	0.46
Bitter	0.62	0.31	0.54
Astringent	0.85	0.46	0.23

[a]FIV = flavor intensity value.

TABLE II
Effect of 50% Alcohol Treatment on Flavor Scores and Descriptions of Laboratory-Prepared Soybean Products

Characteristic	Full-Fat Soyflour		Defatted Soyflour		Soy Concentrate		Sodium Proteinate	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Flavor score	3.6	7.1**	4.3	7.3**	5.1	6.8*	6.1	7.7**
FIV[a]								
Cereal grain	0.09	0.82	0.58	0.83	0.44	0.78	0.40	0.80
Grassy/beany	2.73	0.64	2.42	0.83	1.56	0.67	1.00	0.40
Chalky	0.18	0.09	0.25	0.17	0.22	0.22	0.30	0.20
Musty	...	0.09	...	0.08	...	0.11	...	...
Bitter	1.36	0.55	1.42	0.42	0.78	0.22	0.90	0.30
Astringent	0.73	0.45	0.92	0.17	0.78	0.67	0.30	0.30
LU/g[b]	98	<1	59	<1	<1	<1	<1	<1

[a]FIV = flavor intensity value.
[b]Lipoxygenase units/g = μmol O_2/min/g.

DISCUSSION

Treating soybeans, soybean products, and other legumes with aqueous alcohol improves their flavor scores. Flavor notes that decrease by this treatment are grassy/beany and bitterness. In contrast, an increase in cereal-grain flavor occurs as a result of alcohol soaking.

The improvement in flavor of whole soybeans that can be accomplished by either steeping or wet-milling with alcohol at room temperature seems to parallel the inactivation of lipoxygenase, a lipid-oxidizing enzyme which has been implicated in generation of the undesirable flavors of soybeans (4, 12, 13). Results provide some support to the idea that the flavor components in soy products are generated by enzyme action when the seed structure is disrupted. By diffusing alcohol into the intact soybeans, lipoxygenase was inactivated *in situ* before disrupting the cellular structure. Because the alcohol solutions were removed by evaporation, nonvolatile compounds remained in the treated samples. Consequently, nonvolatile flavor compounds either were never formed or do not contribute to the flavor problem of soy products.

Volatile flavor components, if present in the intact seed, may have been lost during removal of the alcohol solutions by vacuum evaporation and freeze-drying. If the volatile compounds were lost by this means, the flavor scores and grassy/beany responses (Fig. 3) suggest that maximum loss was in the range of 40 to 50% alcohol and then decreased sharply as alcohol concentration increased. Volatilization of flavor compounds during removal of alcohol appears to explain the flavor improvement of soybean products listed in Table II because these products had distinct flavors such as grassy/beany before treatment as noted by the flavor responses for the control samples. All these samples had a history of lipoxygenase activity because they were all prepared from untreated soybeans by conventional cracking, flaking, and defatting; there was, therefore, opportunity

TABLE III
Flavor Evaluation after Alcohol Treatment of Various Legumes

Legume	Flavor Score		
	Untreated	Treated	△
Lima beans *Phaseolus limensis*	5.0	6.8**	+1.8
Split peas *Pisum sativum*	4.8	6.3*	+1.5
Black-eyed peas *Vigna sinensis*	4.6	6.1**	+1.5
Peanuts *Arachis hypogaea*	4.8	6.3**	+1.5
Soybeans *Glycine max*	3.8	6.7**	+2.9

for enzyme action before and during conversion into concentrate and isolates. The low activity in the concentrate and isolate before alcohol treatment can be ascribed to loss of the enzyme by washing the isoelectrically precipitated products during preparation.

Results confirm the claims of Beaber and Obey (14) that mere contacting of defatted flakes or isolated protein with alcohol is sufficient to improve their flavor. However, they used primarily 95% ethanol, which was found to be ineffective as compared to 40 to 50% ethanol in the treatment of whole soybeans. Results also confirm Daftary's (15) work in which soybeans were soaked in an alcohol-water mixture to inactivate enzymes contributing to the objectionable beany flavors.

Residual urease and SBTI activities indicate that products prepared by alcohol treatment may need additional processing to ensure maximum nutritive value. Selective stability of these proteins to aqueous alcohol as compared to instability of lipoxygenase is not surprising since 80% ethyl alcohol has been used in the crystallization of SBTI (16).

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