Processing of Lipoxygenase-Free Soybeans and Evaluation in Foods

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ABSTRACT: Lipoxygenase-free soybeans were processed into flour, concentrate, and isolate and compared to normal soybeans in bread, meat patties, and a beverage, respectively. Bread made with 20% normal or lipoxygenase-free soy flour had greater ($P <$ 0.05) beany flavor than control yeast bread. There were no differences in beany flavor scores between soy flour types, normal and lipoxygenase-free. Ground beef patties made with 5% acid-washed or ethanol-washed soy protein concentrate had greater ($P < 0.05$) beany flavor than control ground beef patties. Ground beef patties made with ethanol-washed concentrate were scored lower in beany flavor than those made with acid-washed concentrate from normal soybeans. There were no differences in beany flavor between normal and lipoxygenase-free soy isolate in 2%-fat or no-fat beverages. Comminuted meat products made with lipoxygenase-free soy proteins, especially ethanol-washed concentrate, have potential for making soy foods with less beany flavor than foods made with normal soy.

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Three lipoxygenase (LOX) isozymes, LOX-1, LOX-2, and LOX-3, are found in soybeans (1). LOX isozymes require substrates containing *cis*, *cis*-1,4-pentadiene systems such as linoleic acid (18:2) and linolenic acid (18:3) in oxidation reactions. The activity of LOX isozymes has a role in the development of off-flavors characterized as beany, grassy, green, painty, astringent, and bitter in soybean products through oxidation of polyunsaturated fatty acids (2). The beany off-flavor has resulted in soy foods being unacceptable to some consumers who prefer bland flavor (3).

The effects of genetic removal of one or more of the LOX isozymes on the flavor of soybean homogenates, soybean oil, and soy food are being studied by several researchers. LOX-2 may be the main isozyme responsible for the formation of hexanal in aqueous soybean homogenates (4), but the other two isozymes are also involved (5–7). Homogenates from soybeans lacking both LOX-1 and LOX-3 had hexanal levels of 0.6 nmol/mg protein after storage at 25°C for 60 min. Homogenates lacking only LOX-2 had lower hexanal levels of 0.1 nmol/mg protein (4). Another study showed that samples lacking LOX-2 and LOX-3 had lower levels of hexanal than homogenates and flours from soybeans lacking only LOX-2 (5). The presence of LOX-3 decreased the production of hexanal, presumably by converting 13-hydroperoxy-9,11-octadecadienoic acid into forms that could not be broken down into hexanal (6). After storage for 60 min at 4°C, hexanal levels were lowest in flour from soybeans lacking the three LOX isozymes, but highest in flour from soybeans with only LOX-2 present (7).

Soymilk made from soybeans lacking LOX-2 had lower beany, rancid, and oily flavors than soymilk from soybeans lacking LOX-1, LOX-3, both LOX-2 and LOX-3, or both LOX-1 and LOX-3 (8). The concentrations of several volatiles in the headspace of soymilk from soybeans lacking the three LOX enzymes were lower than those found in soymilk from normal soybeans or those lacking both LOX-2 and LOX-3 (9). Soymilk and tofu made with LOX-free soybeans had less cooked beany flavor than soymilk and tofu made with normal soybeans (10). There were no significant differences in flavor scores between oils from normal soybeans and soybeans lacking LOX-1 after storage at 60°C for 8 d (11). Sensory differences could not be distinguished between oil from normal soybeans and oil from soybeans lacking LOX-2, LOX-2 and LOX-3 (12), or LOX-1, LOX-2, and LOX-3 (13).

Hexanal levels were greater in bread dough made with normal soy flour or soy flour lacking LOX-1 or LOX-3 than in dough made with soy flour lacking LOX-2 before and after proofing (14). Defatted soy flour in bread is used as a bleaching agent (0.5% level) and as a lower-cost replacement or extender of egg and milk proteins (15,16). At levels greater than 10%, soy addition will improve protein content and essential amino acids profile, increase loaf volume, and improve quick bread dough rheology (17). Soy concentrates, usually extruded or texturized, are used in comminuted meats to reduce fat content, increase moisture- and fat-binding, increase yield and reduce cost, and improve cohesiveness (18). Soy concentrates are allowed at 3.5% in sausage, up to 8% in chili, up to 12% in meatballs, and up to 30% in hamburger patties (15). Isolates (greater than 90% protein) are utilized in infant formula and high-protein sport beverages containing sweeteners and flavorings.

The flavor of foods made with flour, protein concentrate, and protein isolate from LOX-free soybeans has not yet been

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evaluated. The primary objective of this study was to compare the flavor of bread, meat patties, and a beverage made with products from LOX-free soybeans with the same foods made from soybeans normal in LOX content. Other measurements evaluated were color and texture.

MATERIALS AND METHODS

Materials. LOX-free and normal soy protein flours, isolates, and acid- and ethanol-washed protein concentrates (Center for Crops Utilization Research, Iowa State University, Ames, IA) were studied. LOX-free soybean genotype, produced *via* γ-ray irradiation at the National Agriculture Research Center (Yatabe, Tsubuka, Japan), was obtained from K. Kitamura (19). The obtained genotype was used as the donor parent to backcross the null alleles in developing cultivar IA 2027 that lacks LOX isozymes. Soybean seeds of both 3-alleles controlling absence of the LOX and 3-alleles controlling normal LOX lines (cultivar IA 2020) produced from the first backcross ($BC_1F_{2:4}$ lines) were cultivated and grown at the Agricultural Engineering & Agronomy Research Center of Iowa State University (Ames, IA) in 1996. A study comparing LOX-free and normal soybean lines as adapted to the United States for agronomic and seed traits of economic importance was documented by Narvel *et al.* (20).

LOX-free and normal soybean lines were harvested separately, and 25 random seeds from each soybean line were tested for LOX isozymes activity using a colorimetric assay described by Suda *et al.* (21). Seeds that showed zero activity for LOX-1, LOX-2, and LOX-3 enzymes were defined as LOX-free. Seeds of seven LOX-free lines were combined, and seeds of seven normal lines were combined to generate a sufficient amount of soybeans of each type for use in the entire study.

Soy white flakes processing. Samples (400 g) of the two soybean types were evaluated for moisture, protein, oil, and fiber by using near-infrared spectroscopy (22). The two types were similar to each other, having an average of 10.6% moisture, 38.9% protein, 17.6% oil, and 4.9% fiber. Soybeans of each type (LOX-free and normal) were processed into white flakes at the pilot plant of the Center for Crops Utilization Research, Iowa State University (Ames, IA) in 1997. Cracked and dehulled (through aspiration) whole soybeans were conditioned by heating the cotyledons up to 60°C immediately before flaking to 0.25–0.31 mm thickness with a pilot-plant scale flaking roll machine (2 rolls, 30.5 cm i.d. × 45.7 cm wide; Roskamp, Waterloo, IA). The flakes were then extracted using commercial-grade hexane (1.75:1, hexane/cotyledons) at 65.5°C in a batch-advance percolation-type solvent extractor (French Oil Mill Machinery Co., Piqua, OH). The flakes were extracted in five stages. Each extraction stage was done in 10-min intervals, with 4 min of draining between stages and 1 h final draining. To further reduce hexane content, the solvent-laden defatted white flakes were unloaded at a slow rate into a nonheated, three-deck desolventizer/toaster (Three-Deck DT, French Oil Machinery Co.) under vacuum at ambient temperature. Soy white flakes were further dried (air-drying) for 24 h at ambient temperature to remove the remaining hexane in order to produce flakes with high protein solubility [92–94 protein dispersibility index (PDI)]. After drying, a portion of the defatted flakes was milled into flour (Fitzmill, model D; Fitzpatrick Co., Elmhurst, IL) at a mill speed of 4,000 rpm, feed auger of 30 rpm, and 200 mesh (U.S. standard) screen. The remaining defatted soy white flakes were double-bagged in polyethylene bags, sealed, and stored at 2.8°C until they were further processed into concentrates and isolates. Composition of the LOX-free flakes was 62.1% protein, 7.4%, ash, and 30.5% carbohydrate by difference (dry wt basis). Normal soybean flakes contained 62.0% protein, 6.0% ash, and 32% carbohydrate.

Acid-washed soy concentrate extraction. Acid-washed soy concentrate extraction was done by mixing the soy flakes in a flakes-to-water ratio of 20 to 1 at pH 4.5 and 63°C for 5 min. The mixture was allowed to sit for 55 min under the same conditions. Hydrated flakes were centrifuged in a continuous horizontal decanter (Sharples, model P660; Pennwalt Corp., Warminister, PA) with a bowl speed of 5,700 rpm (2,768 × *g*) and backdrive at 4690 rpm (10.6 conveyor differential). The extracted protein was ground into fine powder with a 0.05-mm cutting ring (Stephen Microcut, model MC 15; Stephen Machinery Corp., Columbus, OH) to decrease fiber and then neutralized to pH 6.8. The ground protein solids were spray-dried (Compact Anhydro Spray Dryer, APV Crepaco, Inc., Attleboro Falls, MA) at inlet and outlet temperatures of 160 and 85°C, respectively. Collected concentrates were kept in double-wrapped polyethylene bags and stored at 4°C until used. Composition of acid-washed concentrate from normal soybean was 72.1% protein, 6.0% ash, and 21.9% carbohydrate. LOX-free soybean acid-washed concentrate composition was 71.8% protein, 7.4% ash, and 20.8% carbohydrate.

Ethanol-washed soy concentrates extraction. Ethanol-washed concentrates were produced by extracting the prepared white soy flakes with 70% (vol/vol) ethanol. Ethanol, preheated to 70°C, was added to the soy flakes in an ethanol-to-flake ratio of 4 to 1. The ethanol/flakes mixture was mixed (100 rpm) and stirred in a 65°C water bath shaker (Versa-Bath®S, model 224; Fisher Scientific, Itasca, IL) for 15 min and allowed to stand for 10 min in the same water bath. The slurry was vacuum-filtered to remove the ethanol, and the flakes were re-extracted twice with fresh 70% (vol/vol) ethanol (preheated to 70°C) at 65°C in the same water bath shaker. After the final filtration, the extracted soy flakes were dried in a rotary evaporator (RE 121, Buchi 011, Flawit, Switzerland) to remove excess ethanol. The remaining ethanol residue was removed by air-drying for 24 h at ambient temperature. Dried ethanol-washed protein concentrates were double-wrapped in polyethylene bags and stored at 2.8°C. Ethanol-washed concentrate from normal soybean contained 72.3% protein, and the LOX-free soybean concentrate contained 70.6% protein.

Soy isolate extraction. Defatted soy flakes were twice extracted with water at 63°C and pH 8.6 in solvent-to-flakes ratios of 10 to 1 and 5 to 1, respectively. The flake/water mixture was centrifuged using a horizontal decanter after each extraction step. The combined supernatant was acidified to pH 4.5, stirred for 5 min, and allowed to sit for 1 h. The solution was centrifuged in a continuous disk-type desludging centrifuge (Alpha-Laval, model BPTX 205; Alfa Laval Separations Inc., Oak Brook, IL) at 9,545 rpm (12,000 × *g*) with 1 min discharging interval. The collected solids were neutralized to pH 6.8 and spray-dried (Compact Anhydro Spray Dryer) at 160 and 85°C inlet and outlet temperatures, respectively. Protein isolates were double-wrapped with polyethylene bags and stored at 4°C until used. Isolate composition for the normal soybean was 92.7% protein, 5.6% ash, and 1.7% carbohydrate. LOX-free isolate contained 93.4% protein, 5.7% ash, and 0.9% carbohydrate.

Soy bread preparation. Control yeast bread was prepared by mixing bread flour (920 g), table sugar (36.8 g), salt (16.5 g), and dry yeast (14 g) in a KitchenAid mixing bowl (23). Treatments containing soy flour replaced 20% of bread flour (184 g). Shortening (23.4 g) was heated for 30 s on high power in a microwave oven (Amana Refrigeration, Inc., Amana, IA), added to the flour mixture, and stirred for 30 s with the bread hook attachment. Tap water (495 mL) at 51°C was gradually added over 5 min during stirring on low speed. The dough was kneaded for 10 min on low speed, then placed in a glass bowl sprayed with nonstick corn oil for proofing at 27°C and 88% relative humidity for 30 min.

The dough was punched down and split by weight into equal halves. Each half was rolled into a 46 × 23-cm rectangle, folded in thirds lengthwise, and rolled into a 23 × 23-cm square. The dough was rolled into a cylinder, formed into a loaf, and placed in a 21 × 11 × 6.5-cm pan sprayed with nostick corn oil for proofing for 20 min at 27°C and 88% relative humidity in a fermentation cabinet (National Manufacturing Co., Lincoln, NE). The loaves were baked in a conventional oven (Amana Refrigeration Inc.) for 45 min at 190°C. The loaves were removed from the pans and cooled on a wire rack. After cooling, loaves were wrapped in foil and plastic bags and stored at room temperature until sensory evaluation the following day.

Two loaves of each treatment were prepared for each of three replications. Both loaves were measured for height, weight, and volume. Height was measured at the center of the loaf and at both ends with a skewer and the results were averaged. Volume was measured by a displacement method utilizing rapeseed (24). One loaf of each treatment replication was analyzed for texture [Instron Universal Testing Machine (UTM), model 4500, Instron; Canton, MA] and color (Hunter Labscan II 0/45; Hunter Spectrocolorimeter, Reston, VA, with Universal software version 3.1.2). Texture was determined using two compressions (50%) each of 15 crust-free cubes (2 × 2 × 2 cm) per loaf taken from center slices of the loaves (24). The diameter of the aluminum compression disk was 35 mm. The speed of the probe was 100 mm/min. The colorimeter was standardized (white, X = 80.46, Y = 85.25, Z = 90.90) with the 44.5-mm port covered with plastic under F illuminant and 10° standard observer. Hue angle was calculated from $\tan^{-1}$ (b/a). Color was determined from the measurement of both inside halves of a freshly cut loaf and from crust of a 10-cm portion. The second loaf was used for sensory evaluation.

Soy meat patty preparation. Refrigerated ground beef (85% lean) was combined in a mixing bowl with 5% soy concentrate based on the total weight of meat and soy. Water was added at 3× the weight of the soy concentrate. Each batch was mixed until homogeneous on speed 2 with a KitchenAid mixer. Twenty-four uniform 100-g patties were made with a plastic patty-former for both acid-washed and ethanol-washed (12 for normal and 12 for LOX-free) concentrates. Control patties were made with 90% lean ground beef. All patties were wrapped in waxed paper and placed in plastic bags and stored at 4°C for 1 d until cooking.

Patties were broiled for 4.5 min, turned, and cooked until the internal temperature reached 77°C (thermocouple thermometer (Model 115KC; Omega Engineering, Inc., Stamford, CT). Patties were weighed before and after cooking to determine weight loss. Three patties of each treatment were used for color analysis by Hunter Spectrocolorimeter as described for bread. Color was determined from the measurement of both sides of the exterior and both inside halves of a freshly cooked patty sliced lengthwise. All color samples were covered with clear food-grade plastic wrap during analysis. Three patties were used for texture analysis compression by Instron UTM. Texture was determined using two compressions (50%) of 1 in. i.d. cores per patty. The diameter of the aluminum compression disk was 35 mm. The speed of the probe was 100 mm/min. The remaining six patties were used for sensory evaluation.

Soy beverage preparation. Soy isolate is used in flavored high-protein mixes designed for body builders. These mixes may be prepared with or without milk. Our intent was to mimic these sports beverages, but without sweeteners or flavorings so that potential beany flavor would not be masked. Samples with and without fat were prepared from normal and LOX-free soy isolates on the day of sensory evaluation. For samples without fat, water (464.8 g) was heated to 60°C in a microwave oven and placed in a blender (Oster Corporation, Milwaukee, WI). Xanthan gum (0.2 g) was added to stabilize and slightly thicken the beverage, and the solution was blended for 1 min. Soy protein isolate (35 g) was added and the mixture was blended for 2 min. For samples containing 2% fat, 1.0 g lecithin (as emulsifier), 0.15 g xanthan gum, and 10.0 g of partially hydrogenated soybean plastic fat (Crisco, Procter & Gamble, Cincinnati, OH) shortening were weighed into a 15-mL beaker, and the mixture was heated on high in a microwave oven for 3.5 min. The oil mixture was swirled to ensure complete dissolution of lecithin. The oil mixture was added to 454 mL of water and 35 g soy isolate and blended for 1.5 min.

Color of 75-mL samples was measured in a petri dish with a spectrocolorimeter as described for bread. Viscosity was measured with a Brookfield viscometer (Model DV-I, Brookfield Engineering Labs, Inc., Stoughton, MA) (spindle #1, speed = 100 rpm, 400 mL in 600 mL beaker) and by the time required for 10 mL to flow from a capillary tube (24). Samples of soy isolate beverage with 2% fat and without fat were prepared for sensory evaluation.

Sensory evaluation. The sensory evaluation panel for all products consisted of a total of 14 male and female students and staff of Iowa State University. Training and evaluation were conducted according to procedures described by Meilgaard *et al.* (25). Sessions were conducted in the Center for Designing Foods to Improve Nutrition, Iowa State University (Ames, IA). Panelists were in individual booths with fluorescent lighting and controlled temperature. A 15-cm intensity line scale anchored from none to strong was used. There were no attribute standards given during the sample testing, but standards were used for training purposes. During preliminary training the researchers suggested descriptor terms for flavor and textural attributes of the samples. The descriptor terms discussed and agreed upon by the panelists were used in the study. Panelists also decided the rank of each standard's attribute on the scale unless otherwise noted. Panelists were trained with the agreed-upon descriptors and standard ranks in two 1-h sessions for each food product. The panelists were trained by comparing products with and without soy (control). Panelists were trained to easily identify the control samples, which were very different from the soy-containing products in color, texture, and flavor. For yeast bread and beef patties, the control samples were served as part of the randomly coded samples. There was no control for the beverages. Panelists were trained for yeasty flavor with standard white Wonder™ bread (Interstate Brands Corporation, Springfield, MO). Wonder™ bread was also assigned a yellow color of 0 cm on the line scale for training purposes for color evaluation of breads. There was no assigned yellow standard for 15 cm. Cooked pasta was used as the training standard for wheat flavor. Raw and cooked ground soybeans were used as standards for strong beany flavor for all food samples, and for strong astringency and strong tan color for bread and beverage samples. Strong mealiness was defined as the coarse texture found in control ground beef patties. The beef flavor of control ground beef patties was the training standard for strong beefy flavor. A cooked 50:50 mixture of corn meal and Cream of Wheat® (Nabisco Inc., East Hanover, NJ) was used as the training standard for cereal flavor for meat patties. Strong creaminess was defined as heavy cream. Heavy cream was also assigned a tan color score of 0 cm on the line scale for training purposes, since its color was white.

For each bread treatment, four slices were cut from the center of each loaf, the crusts were removed and the slices were cut into 2-cm cubes. Three cubes of each treatment were presented to each panelist on paper plates and evaluated for yellowness, wheat flavor, yeast flavor, beany flavor, and astringency. Six meat patties of each treatment were cut into eight equal triangular pieces for sensory evaluation. Panelists evaluated cereal flavor, beany flavor, beefy flavor, and mealiness of each meat patty treatment. Three pieces per treatment were presented on paper plates. Panelists evaluated tan color, beany flavor, astringency, and creaminess of the soy isolate beverages. Samples (15 mL) of each treatment were presented in plastic cups.

Triplicate treatments of each food were evaluated by each panelist on three separate days over a period of 8 wk with training before each new food item. All samples were assigned three-digit random numbers and presented in random order. Bread and beverage samples were presented at room temperature. Meat patties were presented at 77°C. Panelists sampled the beverages with 2% fat before sampling the no-fat beverages in order to minimize sample carryover and were given the option of eating crackers between samples to clean their palates. Panelists were instructed to rinse their mouths with water thoroughly between samples.

Statistical analysis. A randomized complete block design was used for the soy food evaluation. The general linear models procedure of SAS (26) was used for the analysis of variance. Each soy food was evaluated independently with three replications of each treatment. Three separate batches of each flour, concentrate, and isolate were processed for replication. Least significant differences (LSD) were calculated for attributes having significant F values ($P \leq 0.05$).

RESULTS AND DISCUSSION

Sensory characteristics of soy breads. Bread made with 20% soy flour processed from normal and LOX-free soybeans had greater ($P < 0.05$) yellow color, beany flavor, and astringency than the control yeast bread (Table 1). However, there was no difference in yellow color, wheat flavor, beany flavor, and astringency between soy flour types. Bread made with LOX-free soy flour did not have different wheat flavor from the control bread. Bread made with 20% normal soy flour had the lowest ($P < 0.05$) sensory score for yeast flavor.

Since the soy flour (PDI > 90) had a natural yellow color and was added at 10 times the level normally added for bleaching action, it was not surprising that the soy bread was more yellow in color. Soy flour can have a drying effect in the mouth (16), and this was seen by the greater ($P < 0.05$) astringency scores for the bread containing either soy type than the control bread. The LOX-free soy bread was expected to have less beany flavor than the normal soy bread due to the fact that LOX has been shown to cause the formation of beany flavor compounds, but this was not so. LOX-2 has been implicated as the main isozyme responsible for the formation of hexanal in aqueous soybean homogenates (4). Defatted soy flours have been shown to have reduced LOX-2 activity compared to full-fat soy flours, but off-flavors such as hexanal were still produced in bread dough made from the defatted flour and in bread dough made from flour without LOX-2 (14). In our study beany off-flavor compounds, which bind reversibly to soy protein (27), may have formed through autoxidation, during the processing of soybeans into flakes and then have been released during mastication of the food. Oil from LOX-free soybeans had greater initial rates of oxidation and shorter induction periods than oil from normal soybeans, most likely owing to greater ($P < 0.05$) linoleic acid

TABLE 1
Effect of Soy Content on Sensory Characteristic of Food Products[a]

Bread	Yellow[b]	Wheat	Yeast	Beany	Astringency
Control[c]	$3.6^b \pm 2.3$	$7.0^b \pm 3.5$	$7.2^c \pm 3.7$	$2.7^b \pm 2.7$	$3.4^b \pm 2.8$
Normal	$8.1^a \pm 2.1$	$5.4^a \pm 3.0$	$5.4^a \pm 3.1$	$7.5^a \pm 3.1$	$5.4^a \pm 3.6$
LOX-free	$8.8^a \pm 2.5$	$6.1^{a,b} \pm 2.9$	$6.2^b \pm 3.8$	$7.4^a \pm 3.7$	$6.5^a \pm 3.9$
Meat	Mealy	Cereal	Beef	Beany	
Control[c]	$10.3^a \pm 3.3$	$3.6^b \pm 3.4$	$10.6^a \pm 3.4$	$1.8^b \pm 2.6$	
Normal	$6.1^b \pm 2.7$	$5.9^a \pm 3.6$	$5.4^b \pm 3.2$	$6.6^a \pm 4.6$	
LOX-free	$6.4^b \pm 2.9$	$5.5^a \pm 3.7$	$6.1^b \pm 3.9$	$5.4^a \pm 4.3$	
Beverage	Tan color	Beany	Astringency	Creaminess	
Normal[c]	$9.1^a \pm 3.9$	$8.2^a \pm 4.6$	$7.8^a \pm 3.4$	$7.2^a \pm 3.3$	
LOX-free	$6.5^b \pm 3.5$	$7.3^a \pm 4.0$	$6.9^a \pm 3.7$	$8.2^a \pm 3.8$	
			NS		

[a]Values are means of three replications. Values in columns with different roman superscript letters are significantly different ($P <$ 0.05).
[b]Scores from 15-cm line scale: 0 = none, 15 = strong.
[c]Controls do not contain soy; other bread is with soy flour, other meat is with soy concentrate, and beverage is with soy isolate. LOX, lipoxygenase; NS, not significant.

levels (13). Therefore, autoxidation during processing of the soybeans may have caused an off-flavor in the LOX-free soy bread that was similar to the level produced in the normal soy bread.

Sensory characteristics of soy extended meat patties. Main effect means for beany flavor of meat patties are reported in Tables 1 and 2. Ground beef patties (90% lean) made with 5% acid-washed or ethanol-washed soy concentrate had less ($P <$ 0.05) mealiness and beefy flavor but greater ($P < 0.05$) beany flavor and cereal flavor than the control ground beef patties (Table 1). Patties with normal and LOX-free soy concentrate were not different in any characteristic evaluated. Patties made with ethanol-washed soy concentrate had lower ($P <$ 0.05) beany flavor scores and greater ($P < 0.05$) beefy flavor scores than patties made with acid-washed concentrate (Table 2). Both concentrate types had greater ($P < 0.05$) beany flavor than the patties made without soy concentrate. Although there was no difference in beany flavor between normal and LOX-free beef patties, the interaction means ($P = 0.06$) indicate that the flavor difference between normal and LOX-free soybeans may be more noticeable in the acid-washed concentrate (scores for beany flavor were normal 8.7, LOX-free 6.3) than in the ethanol-washed concentrate (normal 4.6, LOX-free 4.4).

As previously stated, beany off-flavor compounds, which could have been formed during the initial soybean processing steps owing to autoxidation, can easily and reversibly bind to soy proteins owing to their hydrophobicity and functional groups as well as changes in conformation (27). Soy protein products with less beany flavor can be produced with ethanol soaking. This reduces LOX activity and denatures proteins, exposing hydrophobic regions and causing an increased binding efficiency for the off-flavors, probably irreversibly (28–31). Also, hexane-defatted soy flours can contain residual lipids and off-flavors, whereas ethanol is effective in removing residual lipids and off-flavors from soy protein (29). Therefore, the ethanol-washed soy protein concentrate samples in our study may have had less beany flavor as a result of the following three factors: (i) the removal of protein-bound flavor compounds by the ethanol-wash, (ii) loss of LOX activity in the ethanol-washed normal soy concentrate and lack of activity in the ethanol-washed LOX-free soy concentrate,

TABLE 2
Effect of Treatments on Sensory Characteristics of Food Products[a]

Meat type	Mealy[b]	Cereal	Beefy	Beany
Control[c]	$10.3^a \pm 3.3$	$3.6^b \pm 3.4$	$10.6^a \pm 3.4$	$1.8^c \pm 2.6$
Acid-washed	$5.5^c \pm 2.6$	5.9a ± 4.1	$4.9^c \pm 3.1$	$7.5^a \pm 4.7$
Ethanol-washed	$7.0^b \pm 3.0$	$5.4^a \pm 3.3$	$6.5^b \pm 4.0$	$4.5^b \pm 4.2$
Beverage	Tan color	Beany	Astringency	Creaminess
2% Fat	$4.7^b \pm 3.6$	$8.1^a \pm 3.2$	$6.4^b \pm 3.2$	$9.3^a \pm 3.0$
No-fat	$10.9^a \pm 3.7$	$7.3^a \pm 4.4$	$8.3^a \pm 4.0$	$6.2^b \pm 3.2$
		NS		

[a]Values are means of three replications. Values in columns with different roman superscript letters are significantly different ($P \leq 0.05$).
[b]Scores from 15-cm line scale: 0 = none, 15 = strong.
[c]Control does not contain soy, other meat is with soy concentrate, and beverage is with soy isolate.

and (iii) stronger irreversible binding of the volatiles to the unfolded, denatured soy concentrate, so that they can no longer be released during mastication of the food.

The decrease in beefy flavor observed for our soy meat patties had been seen previously in beef patties with higher soy concentrate levels. Brewer *et al.* (32) found lower beef flavor scores as well as higher off-flavor scores in patties made with 20% commercial soy protein concentrate than in control beef patties. Berry (33) also found that ground beef patties made without soy were rated higher in ground beef flavor than patties made with 20% commercial soy protein concentrate. In another study, Berry *et al.* (34) observed no differences between control beef patties and 19% normal soy concentrate patties in ground beef flavor after 4 mon of frozen storage. The authors stated that this may have been due to rancid flavors detected in the control beef patties that masked the beef flavor.

Since panelists were trained to recognize soy-containing products by color, texture, and flavor, it was not surprising that the evaluation of the control bread and control beef patties were different from the soy-containing samples. The primary objective of this study was to determine differences between normal and LOX-free soy products, and the control samples served as a comparison for both types of soy. It was expected that bread and beef patties containing LOX-free soy would have less intense beany flavor than products with normal soy. Torres *et al.* (10) noted that soymilk and tofu made with LOX-free beans had less cooked beany flavor than soymilk and tofu made with normal beans. Soymilk and tofu are rather bland in flavor, and the difference in beany flavor may be too subtle to detect in products such as yeast bread and beef patties, which have more intense and complex flavors.

Sensory characteristics of soy beverages. Soy beverages made with and without 2% fat were not different from each other in beany flavor (Table 1). The tan color and astringency were greater ($P < 0.05$) in the no-fat soy beverage than in the 2% fat soy beverage (Table 2). The no-fat soy beverage was less creamy ($P < 0.05$) than the 2% fat beverage (Table 1), which is most likely due to the lack of fat. There was no difference between the normal soy beverage and the LOX-free soy beverage in creaminess, astringency, and beany flavor, but the LOX-free soy beverage had lower ($P < 0.05$) scores for tan color.

Soy protein isolate is expected to have a blander flavor, because carbohydrate and lipid fractions are more completely removed during processing than for flour or concentrate (35). However, commercial soy isolates were found to have beany odor and an off-flavor as determined by sensory evaluation of dry isolate and 33% slurries (36). Several volatiles were found in the commercial soy isolates including butanal, pentanal, hexanal, and their ketone and alcohol forms. Maheshwari *et al.* (36) stated that the presence of water caused a greater release of odors and flavors in the soy isolate slurries than in the dry form. This may be why our samples scored high in beany flavor, as they were served as an aqueous beverage. Beany flavors were most likely present in the soy isolates because they were formed during the initial processing steps and were reversibly bound by the protein (27).

Textural characteristics of soy foods. There were no differences in texture among the control and soy breads made from LOX-free and normal soy flour, but the soy bread weighed approximately 20 g less than the control bread. This may be due to the stickiness of the soy bread dough compared to the control dough, and this resulted in a loss of dough that stuck to the bowl and utensils during preparation. The normal soy bread had greater height than the control bread. There were no differences between soy breads in weight, height, or volume (data not shown). Raidl and Klein (17) found that the volumes of quick-bread loaves made with 5 and 15% defatted soy flour in place of wheat flour were greater than a control loaf, which they thought may have been due to improved retention of leavening gases by the increased batter viscosity in soy breads. The authors (17) stated that gluten formation is not as important in quick breads as it is in yeast breads. Other investigators found significantly lower loaf volumes in yeast-leavened bread made with 20% defatted soy flour (35). No differences in interior color were noted (Table 3). The LOX-free bread crust was darker ($P < 0.05$) and less yellow ($P < 0.05$) than the normal bread crust, which may be due to Maillard browning (Table 3) (17).

There were no textural differences between cooked ground beef (100%) patties and patties made with 5% LOX-free soy concentrate nor among normal soy concentrate, acid-washed concentrate, and ethanol-washed concentrate (Tables 3 and 4). Both types of 5% soy ground beef patties had lighter interior (‘L’) color and more yellow color (‘b’) inside than the control patties (Table 3). Patties made with LOX-free and normal soy concentrate had the same exterior ‘L’ color values, and LOX-free patties were lighter ($P < 0.05$) (‘L’) than the control patties (Table 3). The interior ‘L’ values for patties made with acid-washed concentrate and ethanol-washed concentrate were greater ($P < 0.05$) than the interior ‘L’ value of control patties (Table 4). Interior ‘a’ and ‘b’ values were not different. The outside color of the ground beef patties made from ethanol-washed soy concentrate was lighter ($P < 0.05$) than the control patties (Table 3). There were no other differences in color among the soy ground beef patties and the control.

The viscosity of beverages made with soy isolate was not different between normal and LOX-free treatments and between 2%-fat and no-fat treatments (Tables 3 and 4). Soy beverage made from LOX-free soy isolate had a higher ‘a’ value than beverage made from normal isolate; there were no differences in ‘L’ and ‘b’ values (Table 3). Soy beverage made with 2% fat had lower ‘L’, higher ‘a’, and higher ‘b’ values than no-fat soy beverage due to the presence of oil (Table 4).

The use of LOX-free soybean proteins in foods did not seem to improve the flavor of soy foods in this study, but it did in soy milk and tofu (10). Ethanol-washed concentrate produced soy meat patties with the least beany flavor. The beany flavor of the meat patties made with acid-washed, LOX-free soy concentrate was noticeably lower than that of meat patties made with acid-washed, normal soy concentrate. Therefore, a product made with acid-washed LOX-free soy concentrate or a product made with ethanol-washed soy concentrate may have more acceptable flavor, to consumers who

TABLE 3
Effect of Soy Content on Objective Measurements of Food Products[a]

	Hardness		Interior color				Exterior color			
Bread	N[b]	J[c]	L	a	b	Hue angle	L	a	b	Hue angle
Control[d]	4.5 ± 1.3	0.02 ± 0.01	76.2 ± 1.5	0.9 ± 0.3	15.2 ± 1.4	86.7	45.5^{c} ± 2.6	10.0 ± 2.1	16.3^{c} ± 0.6	58.7^{b}
Normal	4.0 ± 0.2	0.02 ± 0.002	76.2 ± 1.0	1.5 ± 0.4	17.6 ± 0.7	85.0	27.3^{a} ± 0.8	8.5 ± 2.4	8.1^{a} ± 1.4	44.2^{a}
LOX-free	5.9 ± 1.6	0.03 ± 0.008	77.4 ± 1.4	1.3 ± 0.3	17.4 ± 1.3	85.8	23.6^{b} ± 0.9	5.6 ± 0.8	4.8^{b} ± 0.2	40.8^{a}
	NS	NS	NS	NS	NS	NS		NS		

	Hardness		Interior color				Exterior color			
Meat patties	N[b]	J[c]	L	a	b	Hue angle	L	a	b	Hue angle
Control	208.0 ± 6.2	0.71 ± 0.08	46.1^{b} ± 1.8	3.88 ± 0.4	10.0^{b} ± 0.1	68.7^{b}	31.2^{b} ± 0.9	3.79 ± 0.4	6.10 ± 1.2	57.9
Normal	190.9 ± 36.7	0.81 ± 0.24	53.8^{a} ± 2.1	3.90 ± 0.2	10.9^{a} ± 0.7	70.2^{a}	$37.0^{a,b}$ ± 2.8	3.74 ± 0.4	6.28 ± 0.7	59.2
LOX-free	187.6 ± 40.4	0.78 ± 0.26	55.4^{a} ± 1.8	3.70 ± 0.1	10.9^{a} ± 0.6	71.3^{a}	40.2^{a} ± 6.1	3.65 ± 0.3	7.13 ± 2.5	61.6
	NS	NS		NS				NS	NS	NS

	Viscosity		Color			
Beverage	cPs[e]	mL/s[f]	L	a	b	Hue angle
Normal	32.6 ± 1.1	33 ± 2.2	80.4 ± 5.6	1.91^{b} ± 1.0	18.2 ± 1.8	84.2^{a}
LOX-free	45.8 ± 10.1	37 ± 3.1	78.2 ± 6.1	2.53^{a} ± 1.0	18.5 ± 2.0	82.4^{b}
	NS	NS	NS		NS	

[a]Values are means of three replications. Values in columns with different superscript letters are significantly different ($P < 0.05$).
[b]Newtons, peak force of compression (50%), Instron Universal Testing Machine.
[c]Joules, total force of compression (50%), Instron Universal Testing Machine
[d]Control does not contain soy; other bread is with soy flour, other meat is with soy concentrate, and beverage is with soy isolate.
[e]Centipoise, Brookfield viscometer.
[f]Time to flow through 10-mL pipette.

prefer bland flavor, than products made with normal soybean proteins. More work needs to be done to determine the flavor attributes of texturized soy protein from LOX-free soybeans, as most comminuted meat products are made this way.

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TABLE 4
Effect of Treatments on Objective Measurements of Food Products[a]

			Interior color				Exterior color			
Meat patties	N[b]	J[c]	L	a	b	Hue angle	L	a	b	Hue angle
Control[d]	208.0 ± 6.2	0.71 ± 0.08	46.1^{b} ± 1.8	3.88 ± 0.37	9.97^{b} ± 0.14	68.7^{b}	31.2^{b} ± 0.9	3.79 ± 0.44	6.10 ± 1.2	57.9
Acid-washed	179.5 ± 38.4	0.77 ± 0.25	54.8^{a} ± 2.4	3.79 ± 0.18	10.8^{a} ± 0.42	70.7^{a}	$36.8^{a,b}$ ± 2.8	3.68 ± 0.36	5.94 ± 0.58	58.2
Ethanol-washed	199.0 ± 35.8	0.82 ± 0.25	54.5^{a} ± 1.7	3.81 ± 0.23	11.0^{a} ± 0.6	70.9^{a}	40.5^{a} ± 6.1	3.71 ± 0.42	7.46 ± 2.3	62.6
	NS	NS		NS				NS	NS	NS

	Viscosity		Color			
Beverage	cPs[e]	mL/s[f]	L	a	b	Hue angle
2% Fat	38.1 ± 6.3	35 ± 3.8	74.6^{b} ± 4.2	3.08^{a} ± 0.54	19.9^{a} ± 1.1	85.4^{a}
No fat	42.9 ± 13.4	36 ± 3.3	83.9^{a} ± 1.2	1.37^{b} ± 0.52	16.8^{b} ± 0.8	81.2^{b}
	NS	NS				

[a]Values are means of three replications. Values in columns with different superscript letters are significantly different ($P \leq 0.05$).
[b]Newtons, peak force of compression (50%), Instron Universal Testing Machine.
[c]Joules, total force of compression (50%), Instron Universal Testing Machine
[d]Control does not contain soy; other meat is with soy concentrate, and beverage is with soy isolate.
[e]Centipoise, Brookfield viscometer.
[f]Time to flow through 10-mL pipette.

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