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Selected Odor Compounds in Soymilk As Affected by Chemical Composition and Lipoxygenases in Five Soybean Materials

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Protein, fat content, and beany odor affect consumers' choice of soy foods. Our objective was to characterize protein, lipid, and lipoxygenase activities and fatty acid compositions in food soybeans and to determine how chemical composition and enzyme activities related to the generation of beany odor components, including hexanal, hexanol, *trans*-2-nonenal, 1-octen-3-ol, and *trans*-2,*trans*-4-decadienal in soymilk. Protein, lipid, and fatty acid compositions and lipoxygenase activities of five soybean materials, including Proto, IA2032, IA 2064, IA3017, and L-Star varieties, were analyzed. Soymilk was prepared by a traditional method involving soaking, grinding, and cooking processes. Selected odor compounds in raw and cooked soymilk were analyzed by solid-phase microextraction and gas chromatography. Results showed that soybeans differed in crude protein and lipid content, lipoxygenase activities, and fatty acid compositions. L-Star had the highest and Proto the lowest lipid content among the five soybean cultivars. Protein content, lipoxygenases, and linoleic acid were positively correlated with beany odor content in soymilk made from the selected soybean materials. After boiling for 20 min, the soymilk made from L-Star and IA2032 retained the lowest odor profiles among the soymilk products made from the five selected materials.

KEYWORDS: Soymilk flavor; lipid; fatty acid; protein; lipoxygenases

INTRODUCTION

Soymilk made by the traditional cooking method has been used in East Asia for a long time. Worldwide soy food consumption is increasing rapidly due to their potential health benefits in preventing heart disease (*1*−*3*), postmenopausal syndromes (*4*), cancers, aging, and osteoporosis (*5*). Despite the health benefits, many Western consumers shy away from soy foods, primarily due to the characteristic soy odor, commonly referred to as the beany off-flavor (*6*−*8*). Soymilk is used as a beverage and also an intermediate product for tofu making. The protein content is important not only for preventing heart disease but also for making quality tofu products (*9*, *10*).

The odor components in soymilk are primarily the oxidation products of unsaturated lipids catalyzed by lipoxygenases and hydroperoxide lyase. The denaturation temperature of lipoxygenases is approximately 80 °C (*11*). Hydroperoxide lyase can be completely inactivated at 70 °C, which is lower than that for lipoxygenases (*12*). The actual sources of beany off-flavor in soymilk have not been definitely characterized. Instead, there are a host of compounds, which have been identified, that may contribute to the soy odor (*7*, *13*−*16*). Hexanal is the most commonly studied since it gives a sensory beany, grassy flavor in soymilk (*13*, *17*) and has a low detection threshold (*18*). Other important soy odor compounds include hexanal, 1-hexanol, *trans*-2-nonenal, 1-octen-3-ol, *trans*-2,*trans*-4-decadienal, and dimethyl trisulfide (*18*). Our preliminary study shows that dimethyl trisulfide does not exist in soymilk products made in our laboratories (*19*).

Soybean materials differing in lipoxygenases may lead to different degrees of the oxidation of fatty acids in soymilk during processing. Lipoxygenase-deficient soybean varieties produce soymilk with a lower off-flavor pattern than that of the normal soybean varieties (*14*, *20*−*23*). Kobayashi et al. (*14*) compared the aroma compositions in soymilk made from the Suzuyutaka (normal lipoxygenases variety), Yumeyutaka (lacking lipoxygenases 2 and 3), and Kyushu No. 111 (lacking lipoxygenases 1, 2, and 3). They found soymilk without lipoxygenases 2 and 3 or without lipoxygenases 1, 2, and 3 produced less overall volatile components than the normal-lipoxygenase Suzuyutaka soybean. Hexanal and hexanol were low but were significant in the two lipxoxygenase-deficient varieties. In addition, the concentrations of 1-octen-3-ol, *trans*-2-nonenal, and *trans*-2,*trans*-4-decandienal were very substantial in soymilk made from the two lipoxygenase-lacking varieties. Five normal-lipoxygenase soybean cultivars (HS903456, HS903515, HS903518, HS903608, and Conrad) grown in two locations have been reported to affect volatile profiles of cooked soymilk (*16*). The total contents of hexanal and hexanol in soymilk have been significantly correlated with the lipoxygenase contents in heated ground soy products (*24*).

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Fat levels in processed foods can affect the consumers' choice when body weight control is a concern. Soybean materials, which can produce soymilk products high in protein and low in lipids, are preferred by the soymilk industry. Recently, two low-linolenic acid soybean varieties, IA 3017 and IA 2064, were developed at the Iowa State University. IA 2032 and L-Star are two new lipoxygenase-null varieties. Proto is a high protein, normal-lipoxygenase variety that is grown very well in North Dakota. Soymilk made from these varieties has not been studied. Our objective was to determine protein and lipid contents, fatty acids, and lipoxygenase activities in these five selected materials and their relationships to the soy odor components in raw and soymilk prepared by a traditional method.

MATERIALS AND METHODS

Soybean Materials. Soybean materials included IA 3017, IA 2064 (harvested in 2003), and IA 2032 (2002) (Ames, IA), Proto (2003, Sinner Brothers and Breshnana, Casselton, ND), and dehulled L-Star (2004, WhiteWave Soyfood Co., Boulder, CO). The moisture content ranged from 5 to 6%. The dehulled L-Star was placed in a freezer (−20 °C), and the other four varieties were stored in an air-conditioned room (21−22 °C) until use.

Soymilk Preparation Method. Whole soybeans were soaked in tap water at room temperature for 15 h, whereas dehulled soybeans were soaked for 3.5 h to allow the beans to uptake water one time of its initial weight. The soaked beans were drained, rinsed, and ground with tap water using a bean:water ratio of 1:9 (w/w). The soybeans were ground for 3 min at high-speed using a Hamilton Beach blender (model: 585-1, Peabody, MA). The soy slurry was filtered through a muslin cloth to separate the insoluble residues from the soymilk. The raw soymilk (1 L) in a small pot was heated within a larger pot, which contained boiling water on a stove, which was set at the highest heat level, to approximately 90 °C, and then, the small soymilk pot was switched to the hot stove surface to heat to a boiling temperature of 100 °C and held at this temperature with stirring to prevent foaming for up to 20 min. Soymilk (approximately 10−15 mL) was sampled at 0, 3, 6, 9, 12, and 20 min after boiling. Immediately after sampling at each time interval, the soymilk in a small beaker was cooled in an ice bath. Immediately after cooling, a 1 mL portion was placed in a 4 mL vial sealed with a lid containing a Teflon-lined rubber septum. A portion of the same internal standard consistent with the concentration for each soy odor standard curve establishment was injected into each sample vial using a microsyringe. The sample containing internal standard was shaken for 1 min to equilibrate the internal standard in the soymilk samples. Flavor compounds analysis using gas chromatography (GC) was started immediately after cooking and analyses of all samples were completed in the same day.

Soybean Moisture, Crude Protein, and Crude Lipid Determination. Soybean materials were ground to pass a 60 mesh size. The moisture content of the bean powder was determined by the air-oven method (AOAC Method #945.15, *25*). The crude protein content was determined by the Dumas method (AOAC Method #992.23, *25*) using model FP2000 Leco instrument (Leco Corp., St. Joseph, MI) using 6.25 as the protein conversion factor. Soxhlet extraction with pet ether (AOAC Method #920.39C, *25*) was used for crude lipid content.

Fatty Acid Composition. The lipids used for fatty acid composition were extracted using a combination of hexane−isopropanol (3:2, v/v) (*26*). Fatty acids in lipids were converted to fatty acid methyl esters using sodium methoxide method and analyzed by GC (*27*) using HP5890A equipped with an autoinjector (Hewlett-Packard Product, Avondale, PA). Fatty acid methyl esters standards (GLC-21A) and an internal standard hepadecanoic acid methyl ester were obtained from NuChek Prep, Inc. (Elysian, MN).

GC of Fatty Acid Methyl Esters. The GC conditions include an injector inlet at 230 °C; column type DB-23 (30 m × 0.25 mm, 0.25 μm film, J&W Scientific, Inc., Folsom, CA); detector, flame ionization detector and temperature 300 °C; oven temperature gradient, 190 °C for 5 min, then to 220 °C for 10 °C/min and hold for 1 min, then to 240 °C at 20 °C/min and hold for 1.5 min; and carrier gas, helium at approximately 1.5 mL per min.

Lipoxygenase Activity. Hydroperoxides formed by the lipoxygenases-catalyzed oxidation of linoleic acid were analyzed by the two-step mixing colorimetric method at pH 6 as described by Anthon and Barrett (*28*). For the normal-lipoxygenase varieties, soymilk was diluted to a series of 20−200-fold dilutions and 10 μL was used for analysis. Immediately after grinding and separation of insoluble residues by filtration through a piece of muslin cloth, the soymilk was determined for lipoxygenase activity. Soymilk from the lipoxygeases-null varieties was analyzed directly without dilution. Absorbance at 598 nm vs μg of soybean materials was graphed. The lipoxygenase activity was calculated from the linear range of absorbance vs soybean mass of the series of dilution. One lipoxygenase activity unit was defined as one absorbance unit at 598 nm.

GC of Soy Odor Components in Soymilk. Commercial compounds of hexanal, hexanol, *trans*-2-nonenal, 1-octen-3-ol, and *trans*-2,*trans*-4-decadienal and an internal standard 2-methyl 3-heptanone (*18*) obtained from Sigma-Aldrich (St. Louis, MO) were used for identification and quantification. Standard curves of each odor compound containing the internal standard were established using 2% fat cow's milk as a matrix. The reason for using 2% cow's milk as a matrix is that cow's milk resembles soymilk in terms of being an emulsion beverage and containing approximately 2% lipids and 3−5% proteins but has no soy odor. Cow's milk has an additional advantage as an autoxidation-stable matrix since it contains little linoleic and no linolenic acids. Samples of raw soymilk, the soymilk just boiled (0 min), and the soymilk boiled for 3, 6, 9, 12, and 20 min were analyzed.

SPME fiber (50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane StableFlex, Supelco, Bellefonte, PA) was used for flavor extraction. Before analyzing the sample, the SPME fiber was conditioned in the injector port at 270 °C for 1 h. The sample vials and Teflon-lined silicon septum were baked (105 °C for >12 h) to remove any volatiles associated with these materials to avoid potential contamination. Samples in the vials were heated at 37 °C for 4 min in a water bath before inserting the fiber needle into the vials which were placed on an electric heater preset at 60 °C for 6 min before injecting to GC.

A HP 5890 series II gas chromatograph (Hewlett-Packard Product, Avondale, PA) equipped with a capillary column with a polar resin of DB-Wax (carbowax, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) was used for odor compound analysis. The following conditions were used for GC: oven temperature, 35 (2 min hold) to 225 °C (5 min hold) at 10 °C/min increase rate in between; detector, flame ionization detector at 235 °C; carrier gas, helium, and flow rate of approximately 2 mL/min); and inlet temperature, 235 °C. Results were expressed as mg/L soymilk.

Statistical Analysis. All experiments, including soymilk preparation and chemical analyses, were conducted in duplicate. The data were subjected to analysis of variance using the SAS 9.1 package (*29*). Significant differences among treatments were analyzed using Duncan's multiple range test with a probability level of 0.05 or less. A simple Pearson correlation coefficient method was used to analyze the potential correlations among various variables of protein, lipoxygenase activity, fatty acid compositions, and odor profiles in raw and cooked soymilk prepared from the five soybean materials/varieties.

RESULTS AND DISCUSSION

Lipid and Protein Contents. Table 1 shows that the lipid content ranged from approximately 17 to 23% with Proto having the lowest lipid content and L-Star the highest. Cai and others (*9*) reported lipid contents of 13 food soybeans varieties ranging from 17 to 21%. The fat content of Proto soybeans was 25% lower than that in L-Star. The protein content ranged from approximately 39 to 45% with Proto and L-Star being the highest. The protein contents of the five selected soybeans were consistent with those of the food soybeans as reported by Cai and others (*9*), Min and others (*16*), and the U.S. Department of Agriculture Nutrient Database (*30*).

Table 1. Moisture, Lipid, and Protein Content of Soybeans[a]

soybean	moisture (%)	lipid (%)	protein (%)
L-Star	6.54 A (0.01)	23.38 A (0.19)	45.48 A (0.00)
IA2064	4.91 E (0.04)	22.86 A (0.25)	43.09 C (0.16)
IA3017	5.26 D (0.06)	20.88 B (0.06)	38.86 D (0.08)
IA2032	5.54 C (0.02)	19.98 C (0.83)	43.72 B (0.27)
Proto	5.77 B (0.01)	17.22 D (0.25)	45.43 A (0.07)

[a] Means followed by different capital letters A, B, C, etc. in the same column indicate significant differences among different varieties at $p < 0.05$. The number in parentheses is the respective standard deviation of the mean.

The hull fraction of soybeans was approximately 8% (*31*) and had a high fiber and low protein and lipid content. Therefore, the actual lipid and protein contents of the whole L-Star soybean were slightly lower than that of the dehulled sample. Whole L-Star soybeans were not commercially available. Therefore, dehulled L-Star was used in this study. This was the reason why we compared L-Star with the other four soybeans as a material effect, not exactly a variety effect. Because consumers and the food industry prefer low-fat and high-protein foods, Proto had an advantage in this regard and was used for making soymilk with desirable claims of high-protein and reduced-fat products.

Fatty Acid Composition of Soybean Materials. Table 2 shows that significant differences existed among the fatty acid compositions of the five selected food soybean materials. Soybean fatty acids were characterized by a high content of linoleic acid (51−58%), which was higher than that (45%) reported by the U.S. Department of Agriculture (*30*). Oleic acid, palmitic acid, linolenic acid, and stearic acid were also present in significant amounts. The other four fatty acids with higher carbon numbers were in trace amounts. As expected, IA2664 and IA3017 contained low linolenic acid. Between the two lipoxygenase-null varieties, IA2032 had a significantly lower linoleic acid content (51%) than L-Star.

Lipoxygenase Activities. Lipxoygenases catalyzed hydroperoxides formation of the unsaturated fatty acids, the linoleic and linolenic acids. The hydroperoxides formed were converted to secondary oxidation products of aldehydes, alcohols, or acids by the hydroperoxide lyases and other enzymes (*32*, *33*). Among the most significant oxidation products formed from linoleic acid, hexanal and hexanol carry the green and grassy beany odor (*7*, *13*, *12*, *18*). **Table 3** shows that lipoxygenase activities of the selected soybean materials were quite different with Proto having the highest activity and the two lipoxygenase-null materials having the lowest activity. There were significantly substantial differences in the lipoxygenase activity of the three normal materials with IA2064 having only a half of the activity of Proto (**Figure 1**). Lipoxygenases in normal soybeans as affected by cultivars, year of production (*34*), and location (*35*) had been reported. The effect of the year of production of the same cultivars was greater than that among cultivars produced in the same year (*35*). This indicates that weather played an important role in the generation of lipoxygenases. Although lipoxygenase activities in normal soybean varieties may differ, however, how the differences affect odor formation in soymilk had not been investigated.

Although L-Star and IA2032 were reported to be lipoxygenases-free, there were still trace amounts of hydroperoxides detected in the soymilk, indicating that some autoxidation had occurred during soymilk making. The activities detected in the normal soybeans in our study contained higher lipoxygenase activities than the activities in the pea and green bean materials as reported by Anthon and Barrett (*28*).

Soy Odor Compositions of Soymilk. Using the flame ionization detector (FID) detector with our GC system, the detection limit was approximately 1 μg/L for hexanal, 2.5 μg/L for 1-hexanol, 5 μg for 1-octen-3-ol, 10 ppb for *trans*-2-nonenal, and 25 μg/L for *trans*-2,*trans*-4-decadienal. Part A of **Table 4** shows the statistical differences of hexanal and other off-flavor compounds in raw soymilk made from the soybeans. Hexanal and hexanol were the major odor compounds among the five odors. There was a significant material effect with L-Star having the lowest odor profile in the raw soymilk. The low-beany odor profile of L-Star soymilk was consistent with the findings of other researchers (*14*, *20*−*23*) that lipoxygenase-deficient soybeans produced less beany odor than normal soybean materials. L-Star produced lower odor components in raw soymilk than the other lipoxygenase-null soybean IA2032 even though the linoleic acid was higher in L-Star (13.0%) than that in IA2032 (10.3% of the bean mass). We do not know why hexanal in raw soymilk of IA2032 was so high, approaching the normal soybean materials. Proto and the two low linolenic acid materials (IA2064 and IA3017) had high hexanal contents, and this may be due to their high linoleic acid contents and the presence of high lipoxygenase activities.

Parts B and C of **Table 4** present the statistical differences of the odor compositions in 0 min boiled and 20 min boiled soymilk, respectively, made from each selected soybean material. In comparing the odor compositions of raw soymilk to that of 0 min boiled soymilk (first part vs second part, **Table 4**), we observed substantial reduction of odor components, particularly hexanal, hexanol, 1-octen-3-ol, and total odor compounds in this time period of cooking from raw to 0 min of boiling (just boiled). During the time of temperature increase from the room temperatures (21−22 °C) to boiling (100 °C), lipoxygenases might be active before reaching 80 °C. Above 80 °C, lipoxygenases and hydroperoxide lyase were inactivated (*6*). Differences among soybean materials were statistically significant in the raw and 0 min boiled soymilk samples. The statistical analyses of the results from soymilk cooked for 3, 6, 9, and 12 min were not presented here since the odor compositions further decreased with heating time and the differences among different materials became smaller and less significant. The final soymilk product cooked for 20 min is presented here to reflect the heat treatment effect. After 20 min of boiling, hexanal was still the major component among the five odors. Hexanol was completely eliminated in all except Proto soymilk. On the other hand, *trans*-2,*trans*-4-decadienal in the soymilk of the three normal-lipoxygenase materials/varieties increased gradually when the raw soymilk was cooked to boiling (0 min boiled) and held at boiling for 20 min. At 20 min of boiling, the concentration of *trans*-2,*trans*-4-decadienal in Proto soymilk was higher than that of hexanal. The results indicated that the formation of *trans*-2,*trans*-4-decadienal might be due to more autoxidation than that induced by lipoxygenases since lipoxygenases were all destroyed when the temperature was increased to boiling temperature, which is higher than 80 °C, the reported temperature for lipoxygenase inactivation (*6*). Approximately 92−98% of the original hexanal and 99−100% of hexanol, 95−99% of the total odor contents in raw soymilk made from the five materials, were eliminated after boiling for 20 min.

The odor compositions after boiling for 20 min did not differ widely among the five soybean materials (third part, **Table 4**). Therefore, cooking for 20 min reduced the advantages of L-Star

Table 2. Fatty Acids Composition (%) of Oil Extracted from Soybeans[a]

variety	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
IA 2032	10.7 C (0.06)	4.5 C (0.01)	24.5 B (0.08)	51.3 D (0.07)	8.1 B (0.01)	0.3 C (0.00)	0.2 A (0.00)	0.4 C (0.04)	0.1 AB (0.01)
IA 2064	10.9 B (0.07)	5.5 B (0.01)	23.5 C (0.03)	57.6 A (0.12)	1.3 D (0.01)	0.4 B (0.00)	0.2 A (0.00)	0.4 B (0.01)	0.2 A (0.00)
IA 3017	10.5 D (0.01)	6.0 A (0.01)	25.1 A (0.01)	56.0 C (0.00)	1.2 E (0.00)	0.5 A (0.00)	0.2 A (0.00)	0.5 A (0.00)	0.2 A (0.00)
L-Star	11.2 A (0.05)	3.4 E (0.00)	20.8 D (0.05)	55.8 C (0.04)	7.8 C (0.01)	0.3 E (0.00)	0.2 A (0.01)	0.4 E (0.02)	0.1 B (0.03)
Proto	10.6 C (0.01)	4.1 D (0.01)	19.2 E (0.03)	56.2 B (0.05)	8.9 A (0.01)	0.3 D (0.00)	0.2 A (0.00)	0.4 D (0.03)	0.1 B (0.03)

[a] Means with different capital letters in the same column indicate significant differences among different varieties at $p < 0.05$.

Table 3. Lipoxygenase Activity of Raw Soymilk[a]

variety	lipoxygenase activity (unit /mg soybean)
Proto	30 A (0.6)
IA3017	20 B (0.6)
IA2064	15 C (1.7)
IA2032	0.053 D (0.001)
L-Star	0.063 D (0.002)

[a] The lipoxygenase assay was conducted at pH 6 and 22 °C. One lipoxygenase activity unit is defined as one absorbance unit at 598 nm and calculated from the linear range of absorbance vs soybean mass in a series of dilution. Means followed by different letters indicate significant differences at $p < 0.05$. The number in parentheses is the standard deviation of the mean.

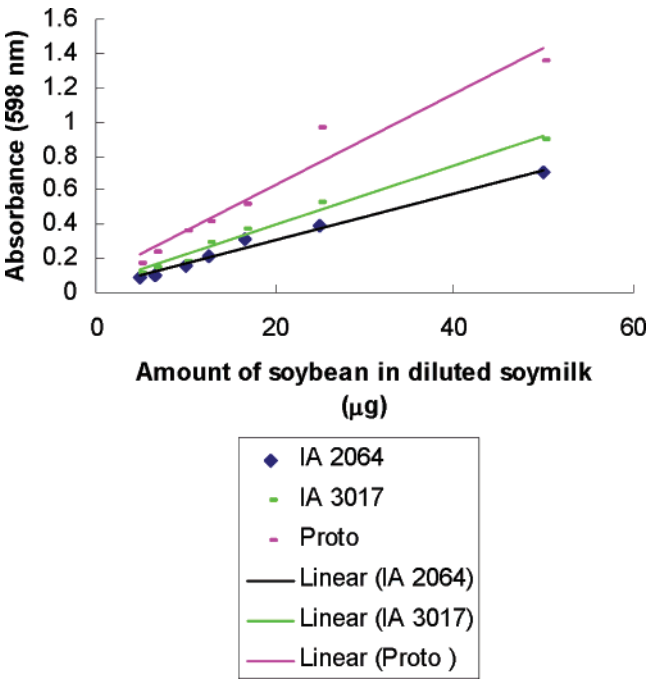

Figure 1. Lipoxygenase activity determination of the three normal soybean varieties.

and IA2032 as lipoxygenase-null materials when compared to the other normal lipoxygenases varieties. After 20 min of boiling, IA2064 had a higher ($p < 0.05$) hexanal content than that made from the other four materials, whereas Proto soymilk had significantly ($p < 0.05$) higher contents of hexanol, 1-octen-3-ol, *trans*-2-nonenal, and *trans*-2,*trans*-4-decadienal than that made from the other soybean materials. L-Star and IA2032 soymilk after 20 min of cooking still contained some hexanal but was not significantly different in total odor content, and both had the lowest beany odor among the five materials studied. The practical significance of the low odor content in the 20 min cooked L-Star and IA2032 is not known since the detection threshold values of the soy odors are lower than that retained in the soymilk (*18*, 36). The detection threshold is 4.5 μg/L in water for hexanal, 0.08 μg/L in water for *trans*-2-nonenal, and 180 μg/kg in sunflower oil for *trans*-2,*trans*-4-decadienal (*36*). Soymilk is an emulsion, which contains lipids and protein that may bind odor compounds. No research has published the threshold values of odor compounds in soymilk.

Significant Correlations among the Variables of Protein, Lipid, Fatty Acid Composition, Lipoxygenase Activity, and Soy Odor Content in Soymilk. Table 5 shows only the correlations among the above selected variables that were statistically significant at $p < 0.05$. The formation of green-grassy hexanal in raw soymilk was positively correlated with the linoleic acid content ($r = 0.93$) and lipoxygenase activity ($r = 0.63$). Lipoxygenase activity also positively correlated with hexanal in 0 min boiled soymilk either among the five materials ($r = 0.94$) or among the three normal lipoxygenase varieties. Wang and others (*24*) showed that when a soybean material was processed into various intermediate products before making soymilk, the residual lipoxygenase activity in these products was correlated with the hexanal and hexanol contents in the soymilk. However, our study is the first to show that beany odors of soymilk were associated with soybean materials of different varieties that contained different levels of linoleic acid and lipoxygenase activities. Linoleic acid was a substrate for lipoxygenases to produce oxidized flavor compounds. Efforts may be made by breeding or genetic manipulation to reduce linoleic acid and lipoxygenase activity in normal soybean varieties for soymilk making.

Table 5 also shows that protein content was correlated with the formation of green-grassy hexanal in raw soymilk, with total hexanal contents of various cooking times and with total odor compounds in raw soymilk. Min and others (*16*) showed that protein content in soymilk made from five soybean varieties was positively correlated with the total volatiles in the cooked soymilk. They also reported that several volatile compounds, including pentane, propane, pentanal, hexanal, and 2-hexenal, increased as the soybean protein content was increased. Our results showed positive correlations between soybean protein content and three other odor variables (hexanal in raw soymilk, total hexanal of all cooking times, and total odor compounds in the raw soymilk). We do not know the biochemical reason why a high-protein variety tends to have a high LOX activity. Our study is the first confirmation of this finding by Min et al. (*16*). Protein content is important, although the actual mechanism as related to odor production is not known to the soymilk industry since a high protein material is preferred because of the FDA's approval of a health claim of soy protein in the prevention of heart disease.

It should be noted that even though the statistical analyses showed positive correlations between odor content and lipoxygenase activity, cooking not only denatured the lipoxygenases but also vaporized the beany odor compounds with time. This is partially responsible for the reason why after 20 min of boiling, the odor compounds did not correlate with either linoleic or lipoxygenase activity in the three normal soybean materials.

Table 4. Soy Odor Composition in Raw, 0 min Boiled, and 20 min Boiled Soymilk (mg/L Soymilk)[a]

variety	hexanal	hexanol	1-octen-3-ol	*trans*-2-nonenal	*trans*-2,*trans*-4-decadienal	total odor compounds
			raw soymilk			
Proto	5.93 ABa (0.18)	6.07 Aa (0.05)	0.71 Ab (0.14)	0.045 Ac (0.00)	0.075 Ac (0.06)	12.19 A (0.53)
IA3017	4.61 Ca (0.29)	3.04 Cb (0.17)	0.53 BAc (0.04)	0.048 Ac (0.01)	ND Ac (0.00)	8.23 B (0.41)
IA2064	6.33 Aa (0.35)	3.8 BCb (0.06)	0.38 BCc (0.06)	0.070 Ac (0.03)	ND Ac (0.00)	10.58 AB (0.75)
IA2032	4.87 BCa (0.31)	4.28 Bb (0.12)	0.57 BAc (0.03)	0.045 Ad (0.01)	ND Ad (0.00)	9.77 B (0.47)
L-Star	2.58 Da (0.12)	0.10 Db (0.04)	0.10 Cb (0.00)	0.028 Ab (0.02)	ND Ab (0.00)	2.81 C (0.13)
			0 min boiled soymilk			
Proto	3.99 Aa (0.22)	1.90 Ab (0.26)	0.21 Ac (0.01)	0.045 Ac (0.003)	0.14 Ac (0.04)	6.29 A (0.42)
IA3017	3.41 Ba (0.05)	0.95 Bb (0.01)	0.18 ABc (0.01)	0.009 Bd (0.000)	0.070 Adc (0.01)	4.62 B (0.08)
IA2064	2.44 BCa (0.23)	1.02 Bb (0.05)	0.15 BAc (0.05)	0.028 Ac (0.001)	0.084 Ac (0.006)	3.72 BC (0.07)
IA2032	1.77 Ca (0.22)	0.59 BCb (0.10)	0.22 Ab (0.03)	0.022 Ab (0.003)	ND Ac (0.00)	2.6 C (0.25)
L-Star	0.69 Da (0.12)	0.064 Cbc (0.02)	0.10 Bb (0.02)	0.016 Bc (0.008)	ND Ac (0.00)	0.87 D (0.09)
			20 min boiled soymilk			
Proto	0.19 Bb (0.04)	0.051 Ac (0.03)	0.037 Ac (0.01)	0.032 Ac (0.001)	0.30 Aa (0.01)	0.61 A (0.04)
IA3017	0.18 Ba (0.01)	ND Bb (0.00)	ND Bb (0.00)	ND Bb (0.00)	0.19 Ba (0.08)	0.37 B (0.09)
IA2064	0.51 Aa (0.03)	ND Ba (0.00)	ND Ba (0.00)	ND Ba (0.00)	0.032 Ca (0.05)	0.54 AB (0.08)
IA2032	0.11 Ba (0.00)	ND Bb (0.00)	0.016 ABb (0.002)	ND Bb (0.00)	ND Cb (0.00)	0.13 C (0.02)
L-Star	0.089 Ba (0.01)	ND Bb (0.00)	0.012 ABb (0.002)	ND Bb (0.00)	ND Cb (0.00)	0.10 C (0.01)

[a] Means followed by different capital letters in the same column (soy materials) indicate significant differences at $p < 0.05$. Means followed by different lower case letters in the same row (odor compounds) indicated significant differences at $p < 0.05$. The number in parentheses is the standard deviation of the mean. ND denotes not detected by our GC-FID detector system; therefore, for the purpose of statistical analysis, the value 0 is used.

Table 5. Significant Correlations ($p \leq 0.05$) among Protein, Fatty Acids, Lipoxygenase Activity, and Soymilk Odor Composition

correlation variables	*r*	soybeans
linoleic acid vs hexanal in raw soymilk	0.93	three normal varieties
LOX activity vs hexanal in boiled soymilk	0.94	five varieties
LOX activity vs hexanal in raw soymilk	0.63	five varieties
LOX activity vs hexanal in 0 min boiled soymilk	0.92	three normal varieties
protein content vs hexanal in raw soymilk	0.81	three normal varieties
protein content vs total hexanal during cooking[a]	0.85	three normal varieties
protein content vs total off-flavor in raw soymilk[b]	0.93	three normal varieties

[a] The total hexanal content was the sum of all hexanal in raw and 0, 3, 6, 9, 12, and 20 min boiled samples in the three normal varieties. [b] Total off-flavor was the sum of all five selected beany odor compounds in raw soymilk samples.

The reason why both lipoxygenase-null materials, the L-Star and IA2032, had the lowest total soy odor content after boiling for 20 min among all materials might be partially due to their low total odor contents in the 0 min cooked soymilk.

The odor content differences observed in 20 min heated soymilk made from different materials need to be confirmed by the sensory evaluation methods for practical applications in the food industry (*37*−*40*). The Oriental consumers had a higher preference for flavor-rich soy foods than the Western consumers (*37*−*38*, *41*). The normal soybean varieties reported in this report may be more suitable for making soymilk by the traditional preparation method for Oriental population than for the Western population.

In summary, this study showed that soymilk cooked by the traditional method was significantly correlated with lipoxygenase activity and linoleic acid content as well as protein content of different soybean materials/varieties. Cooking significantly reduced the beany odor compositions. L-Star and IA2032 produced the lowest odor compounds after cooking. The low lipid and high protein Proto variety may be used as a soybean material for manufacturing fat-reduced soymilk to satisfy the needs of some consumers who are concerned about dietary fat intake.

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