JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Off-Flavor Related Volatiles in Soymilk As Affected by Soybean Variety, Grinding, and Heat-Processing Methods

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ABSTRACT: Off-flavor of soymilk is a barrier to the acceptance of consumers. The objectionable soy odor can be reduced through inhibition of their formation or through removal after being formed. In this study, soymilk was prepared by three grinding methods (ambient, cold, and hot grinding) from two varieties (yellow Prosoy and a black soybean) before undergoing three heating processes: stove cooking, one-phase UHT (ultrahigh temperature), and two-phase UHT process using a Microthermics direct injection processor, which was equipped with a vacuuming step to remove injected water and volatiles. Eight typical soy odor compounds, generated from lipid oxidation, were extracted by a solid-phase microextraction method and analyzed by gas chromatography. The results showed that hot grinding and cold grinding significantly reduced off-flavor as compared with ambient grinding, and hot grinding achieved the best result. The UHT methods, especially the two-phase UHT method, were effective to reduce soy odor. Different odor compounds showed distinct concentration patterns because of different formation mechanisms. The two varieties behaved differently in odor formation during the soymilk-making process. Most odor compounds could be reduced to below the detection limit through a combination of hot grinding and two-phase UHT processing. However, hot grinding gave lower solid and protein recoveries in soymilk.

KEYWORDS: *grinding, UHT, lipoxygenases, oxidation, inactivation, GC*

INTRODUCTION

In recent years, with the FDA-approved claim of health benefits for soy protein,[1] soymilk has become more popular in the United States. However, still many Western consumers dislike it because of the grassy-beany flavor.[2] Off-flavors from soymilk are represented by a mixture of many odor compounds,[3−5] among which hexanal has been studied the most in soy foods. The formation of odor compounds in soymilk processing is closely related to the composition of soybeans. Yuan and Chang[6] revealed that the hexanal content in soymilk was positively correlated with protein content, lipoxygenase activity, and linoleic acid content of soybeans. Min et al.[7] also found a high correlation between soybean protein and volatile compounds. To date, no report is available regarding soymilk flavor prepared from black soybean. Heating methods have been proven to affect the content and composition of odor compounds.[8] In addition to lipoxygenase-induced oxidation of polyunsaturated fatty acid, off-flavors could also be generated through nonenzymatic mechanisms.[9,10]

For improving soymilk quality, several treatments have been used to reduce odor content through the inactivation or inhibition of lipoxygenases.[11−14] Since the discovery of the capability of hot grinding at 80 °C to minimize soy odor in a nonquantitative report in 1967,[15] there have not been any reports on the effect of hot grinding on soymilk flavor until recent years.[5,12,13,16] However, it is difficult to make comparisons among literature because of a lack of detailed characterization of grinding and heating devices and processing conditions that lead to a wide variation of odor products.[5,16] In recent years, ultrahigh temperature (UHT) processing has been adopted by large commercial production; however, only one report is available on comparing the flavor profiles of soymilk processed by traditional methods with selected UHT methods,[4] in which the hexanal content in the UHT cooked soymilk was shown to be similar to the traditionally cooked soymilk.

Comparing how different temperatures from cold (3 °C) to hot (80 °C) affect both odor and protein recovery in soymilk has only been reported once in the literature[13] by Mizutani and Hashimoto using coarsely ground soy powder. However, this study shows grindings at both 3 and 80 °C followed by a 93−94 °C heating step are ineffective to reduce soy odor since the finished soymilk still contained 158 ppm hexanal, which is much higher than the sensory threshold value of 4.5 ppb.

A direct steam-injection UHT processor equipped with a vacuum chamber has been recently used by the soymilk industry. However, no report is available about the effectiveness of a vacuum chamber associated with UHT in the reduction of off-flavors. In this study, eight typical odor compounds were selected based on their significant contributions to the characteristic off-flavor of soymilk as reported in literature.[5,8,16,17] The objective of this study was to investigate the effect of different grinding temperatures and heating methods, including UHT-vacuum processing, on the composition of the eight odor compounds and protein and solid recoveries of soymilk using well-characterized grinding and heating systems.

Received: December 29, 2011
Revised: June 26, 2012
Accepted: July 2, 2012
Published: July 2, 2012

dx.doi.org/10.1021/jf3016199 | *J. Agric. Food Chem.* 2012, 60, 7457−7462

Table 1. Effect of Grinding Methods, Heating Methods, and Variety on Selected Volatile Compounds in Soymilk (ppm)[a]

odor compd	soybean material	grinding method	raw[b]	stove cooking	one-phase UHT	two-phase UHT
hexanal	Prosoy	cold grinding	6.60 A1 (0.16)	0.27 Ba2 (0.04)	0.25 Ba1 (0.04)	0.14 Ab1 (0.01)
		ambient grinding	3.23 B2 (0.79)	0.54 Aa2 (0.20)	0.34 Aa2 (0.01)	0.00 Bb2 (0.00)[c]
		hot grinding	0.051 C2 (0.004)	0.006 Ca2 (0.002)	0.005 Ca1 (0.003)	0.00 Bb2 (0.00)
	black soybean	cold grinding	7.12 A1 (0.47)	0.63 Ba1 (0.06)	0.26 Bb1 (0.04)	0.17 Ac1 (0.01)
		ambient grinding	7.16 A1 (0.89)	1.19 Aa1 (0.34)	0.52 Ab1 (0.03)	0.048 Bc1 (0.0005)
		hot grinding	0.16 B1 (0.03)	0.027 Ca1 (0.004)	0.012 Cb1 (0.005)	0.006 Cb1 (0.0003)
hexanol	Prosoy	cold grinding	0.34 B1 (0.03)	0.00 Aa1 (0.00)	0.00 Ba1 (0.00)	0.00 Aa1 (0.00)
		ambient grinding	2.46 A1 (0.34)	0.00 Ab1 (0.00)	0.21 Aa1 (0.07)	0.00 Ab1 (0.00)
		hot grinding	0.00 B2 (0.00)	0.00 Aa1 (0.00)	0.00 Ba1 (0.00)	0.00 Aa1 (0.00)
	black soybean	cold grinding	0.16 B2 (0.02)	0.00 Aa1 (0.00)	0.00 Ba1 (0.00)	0.00 Aa1 (0.00)
		ambient grinding	1.26 A2 (0.26)	0.011 Ab1 (0.015)	0.10 Aa1 (0.01)	0.00 Ab1 (0.00)
		hot grinding	0.038 B1 (0.015)	0.00 Aa1 (0.00)	0.00 Ba1 (0.00)	0.00 Aa1 (0.00)
2-pentylfuran	Prosoy	cold grinding	0.060 B1 (0.001)	0.24 Ba1 (0.01)	0.062 Bb1 (0.001)	0.064 Bb1 (0.001)
		ambient grinding	0.064 A2 (0.001)	0.37 Aa1 (0.02)	0.074 Ab1 (0.004)	0.081 Ab1 (0.004)
		hot grinding	0.00 C1 (0.00)	0.061 Ca1 (0.002)	0.00 Cb1 (0.00)	0.00 Cb1 (0.00)
	black soybean	cold grinding	0.061 B1 (0.001)	0.16 Aa1 (0.07)	0.058 Bb2 (0.001)	0.058 Bb1 (0.004)
		ambient grinding	0.069 A1 (0.001)	0.23 Aa2 (0.05)	0.071 Ab1 (0.005)	0.080 Ab1 (0.004)
		hot grinding	0.00 C1 (0.00)	0.00 Ba2 (0.00)	0.00 Ca1 (0.00)	0.00 Ca (0.00)
1-octen-3-one	Prosoy	cold grinding	0.39 A1 (0.02)	0.13 Ba2 (0.01)	0.13 Ba2 (0.01)	0.11 Bb2 (0.004)
		ambient grinding	0.39 A2 (0.06)	0.18 Aa1 (0.03)	0.14 Ab2 (0.004)	0.12 Ab1 (0.003)
		hot grinding	0.00 B2 (0.00)	0.00 Ca2 (0.00)	0.00 Ca2 (0.00)	0.00 Ca2 (0.00)
	black soybean	cold grinding	0.40 B1 (0.02)	0.18 Aa1 (0.02)	0.17 Bab1 (0.01)	0.14 Ab1 (0.02)
		ambient grinding	0.57 A1 (0.02)	0.22 Aa1 (0.03)	0.21 Aa1 (0.02)	0.084 Bb2 (0.004)
		hot grinding	0.40 B1 (0.01)	0.19 Aa1 (0.02)	0.082 Cb1 (0.001)	0.15 Aa1 (0.04)
1-octen-3-ol	Prosoy	cold grinding	0.47 A1 (0.001)	0.032 Aa2 (0.006)	0.037 Ba1 (0.003)	0.019 Ab1 (0.002)
		ambient grinding	0.46 A1 (0.09)	0.032 Ab2 (0.002)	0.048 Aa1 (0.007)	0.012 Bc1 (0.001)
		hot grinding	0.039 B2 (0.005)	0.012 Ba2 (0.001)	0.013 Ca2 (0.001)	0.00 Cb2 (0.00)
	black soybean	cold grinding	0.22 AB2 (0.01)	0.047 Aa1 (0.004)	0.034 Ab1 (0.001)	0.017 Ac1 (0.002)
		ambient grinding	0.25 A2 (0.04)	0.032 Ba1 (0.006)	0.031 Aa2 (0.001)	0.01 Bb2 (0.00)
		hot grinding	0.18 B1 (0.01)	0.035 Ba1 (0.005)	0.018 Bb1 (0.001)	0.017 Ab1 (0.005)
(*E*)-2-nonenal	Prosoy	cold grinding	0.032 B1 (0.002)	0.010 Ca2 (0.002)	0.00 Ab1 (0.00)	0.00 Ab1 (0.00)
		ambient grinding	0.038 A1 (0.000)	0.030 Ba1 (0.004)	0.00 Ab2 (0.00)	0.00 Ab1 (0.00)
		hot grinding	0.00 C2 (0.00)	0.039 Aa2 (0.005)	0.00 Ab1 (0.00)	0.00 Ab1 (0.00)
	black soybean	cold grinding	0.037 A1 (0.004)	0.037 Ba1 (0.005)	0.002 Bb1 (0.001)	0.00 Ab1 (0.00)
		ambient grinding	0.044 A1 (0.010)	0.035 Ba1 (0.000)	0.007 Ab1 (0.002)	0.00 Ac1 (0.00)
		hot grinding	0.00 5B1 (0.002)	0.051 Aa1 (0.005)	0.00 Bb1 (0.00)	0.00 Ab1 (0.00)
(*E*,*E*)-2,4-nonadienal	Prosoy	cold grinding	0.11 B1 (0.002)	0.090 Ba2 (0.004)	0.078 Bb1 (0.002)	0.070 Bc2 (0.002)
		ambient grinding	0.13 A1 (0.01)	0.13 Aa1 (0.02)	0.089 Ab2 (0.001)	0.077 Ab1 (0.003)
		hot grinding	0.00 C1 (0.00)	0.00 Ca1 (0.00)	0.00 Ca1 (0.00)	0.00 Ca1 (0.00)
	black soybean	cold grinding	0.11 B1 (0.003)	0.10 Ba1 (0.002)	0.081 Bb1 (0.002)	0.074 Bc1 (0.001)
		ambient grinding	0.13 A1 (0.001)	0.12 Aa1 (0.003)	0.10 Ab1 (0.004)	0.080 Ac (0.001)
		hot grinding	0.00 C1 (0.00)	0.00 Ca1 (0.00)	0.00 Ca1 (0.00)	0.00 Ca1 (0.00)
(*E*,*E*)-2,4-decadienal	Prosoy	cold grinding	0.06 1A2 (0.017)	0.41 Ba2 (0.11)	0.30 Bab2 (0.03)	0.25 Ab2 (0.01)
		ambient grinding	0.06 3A2 (0.011)	1.08 Aa1 (0.18)	0.35 Ab2 (0.01)	0.20 Ab2 (0.04)
		hot grinding	0.00 B1 (0.00)	0.00 Ca1 (0.00)	0.00 Ca1 (0.00)	0.00 Ba1 (0.00)
	black soybean	cold grinding	0.15 B1 (0.04)	0.78 Ba1 (0.02)	0.47 Bb1 (0.04)	0.56 Ab1 (0.07)
		ambient grinding	0.25 A1 (0.06)	1.17 Aa1 (0.04)	1.05 Aa1 (0.13)	0.61 Ab1 (0.02)
		hot grinding	0.00 C1 (0.00)	0.00 Ca1 (0.00)	0.00 Ca1 (0.00)	0.00 Ba1 (0.00)

[a] Means with different capital letters in the same column are significantly different among different grinding methods for the same heating methods and same variety ($p < 0.05$). Means with different lowercase letters in the same row are significantly different among different heating methods for the same grinding methods and same variety ($p < 0.05$). Means with different numbers in the same column are significantly different between two varieties for the same grinding and heating methods ($p < 0.05$). Values in parentheses are SD ($n = 6$). [b] Raw soymilk is defined as the soymilk obtained after grinding and filtration processes. [c] Zero was used for statistical analysis where the compounds were not detectable.

■ MATERIALS AND METHODS

Materials. Two varieties of soybeans (*Glycine max*) were used in this study: Prosoy (harvested in 2009) and black soybean (harvested in 2006) were obtained from Sinner Brothers and Bresnahan Co. grown in Casselton, North Dakota. The black soybean contained green-colored cotyledons, presumably due to chlorophyll. The soybeans were stored in a cool and dry air-conditioned room until use. All processing methods were replicated three times.

Soymilk Preparation. To prepare each batch of soymilk, 300 g of soybeans was soaked five times (1500 mL) in cold water (4 °C for cold grinding) or room temperature water (20 °C for ambient and hot grinding) for 16 h. The hydrated beans were drained and ground with cold water (4 °C), room temperature water (20 °C), and hot water (80.5 °C) with a bean-to-water ratio of 1:10 (w/w). All soaked beans were ground at 10000 rpm (set at high speed and 50% of maximum voltage output of a Staco transformer) with a New Hartford 1 gallon blender (model CB-2-10, CT). In order for the grinding temperature to be accurately maintained at desired temperature, the blender was covered with insulating foam (1 in. thickness). For cold grinding, ice (−2.5 °C) and precooled (4 °C) water with a proper ratio (1:3.3) were used, which resulted in an initial grinding temperature about 2 °C. For hot grinding, prewarming was done by rinsing the blender twice with boiling water, and immediately, soaked soybeans and boiling water were added. The initial grinding temperatures were recorded as the temperatures at 10 s after grinding, and in fact, the final temperatures after 3 min of grinding were about 2 °C higher than the target temperatures. All of these temperatures were determined in preliminary tests. After grinding, the soymilk was manually filtered through a piece of muslin cloth. The pressing step was done by the same person until no soymilk was pressed out to maintain the consistency.

Traditional Stove Heating of Soymilk. The method reported by Yuan and Chang[18] was used. In the current study, soymilk was heated for 20 min after boiling.

UHT Thermal Processing of Soymilk. In this study, a Microthermics Direct/Indirect Steam Injection Processor (DIP, Microthermics, Inc., Raleigh, NC) was used as reported by Yuan and Chang.[18] In this study, two sets of heating temperature and time combinations were designed as follows: 140 °C/5 s (one-phase UHT, $F_0 = 6.62$); 120 °C/80 s + 140 °C/4 s (two-phase UHT, $F_0 = 6.35$). These two UHT methods had a similar sterilization power F_0 calculated on the basis of $Z = 10$ for bacterial spore sterilization. In the heating tube, the heating medium (steam) was in direct contact with soymilk. The Microthermics Processor was equipped with a vacuum chamber (50 kPa), which was originally designed with a purpose to cool the heated soymilk and to remove the condensed water from the injected steam. However, during the vacuum evaporation process, some volatile compounds were also removed. Hence, the one-phase UHT processed soymilk was vacuum-treated once, and the two-phase UHT processed soymilk was vacuum-treated twice.

Volatile Extraction and Gas Chromatography. Our previously reported method of Yuan and Chang[8] was used for quantification of the selected volatile compounds. The standard curve was established by plotting response factors against the concentration of standard volatile compounds with 2% cow's milk as a matrix. The response factor is the ratio of peak area of volatile compounds to that of internal standard (2-methyl-3-heptanone). For all soymilk samples and cow's milk, the same amount of internal standard was added. For each sample, the amount of each volatile compound was calculated on the basis of response factors according to standard curve. Using the flame ionization detector (FID) detector with our GC system, the detection limit was approximately 1 ppb for hexanal, 2.5 ppb for 1-hexanol, 5 ppb for 1-octen-3-ol, 10 ppb for (*E*)-2-nonenal, and 25 ppb for (*E*,*E*)-2,4-decadienal. Most of them are near or below sensory thresholds.

Lipoxygenase Activity Analysis. The method reported by Anthon and Barrett[19] was adopted, and 10 μL sample dilutions were used for analysis. For soymilk from hot grinding, a 10-fold dilution was made. For soymilk from the other two grinding methods, 20-, 40-, and 60-fold dilutions were made. One absorbance unit at 598 nm was defined as one lipoxygenase unit, and the lipoxygenase activity was expressed as unit/mg dry soymilk.

Solid and Protein Analysis. The solid was determined by the air-oven method (AOAC Method 945.15, 2005). Crude protein was determined by the Kjeldahl method (AOAC Method 955.04, 2005).

Statistical Analysis. Soymilk processing was conducted in triplicate, and the following GC analyses were completed in duplicate for each soymilk. The data were subjected to analysis of variance (ANOVA) with SAS 9.1 package (SAS 2005). Significant differences among variables were determined by Duncan's multiple range test ($\alpha = 0.05$). Data were expressed as means ± SDs ($n = 6$).

■ RESULTS AND DISCUSSION

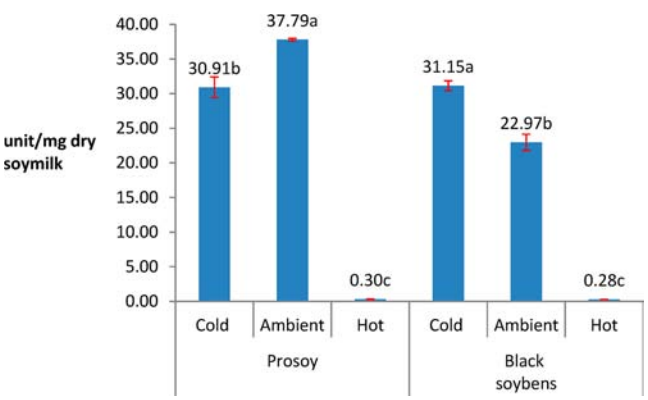

Figure 1. Lipoxygenase activity of raw soymilk. Means with different letters are significantly different among different grinding methods within the same variety ($p < 0.05$).

Effect of Grinding Methods, Heating Methods, and Variety on Hexanal in Soymilk. Table 1 shows that significant differences existed in hexanal levels among different grinding methods, heating methods, and the two varieties. Hot grinding resulted in significantly ($p < 0.05$) lower hexanal as compared with the other two grinding methods. Figure 1 shows that the lipoxygenase activity of raw soymilk after hot grinding was much lower than that from other two grinding methods. In fact, hot grinding inactivated approximately 99% of the lipoxygenase activities as compared to that in the cold ground soymilk. It is obvious that the decrease in hexanal by hot grinding was mainly due to the inactivation of the oxidative enzymes at 80 °C. Our study is the first to report the effect of grinding at various temperatures on soy odor in raw soymilk. All other reported studies had reported the odor composition in the finished soymilk products.

As compared with the raw soymilk, all further heating methods greatly reduced the hexanal content with the order of the ability to reduce hexanal content from high to low: two-phase UHT > (one phase UHT > or = stove cooking). For the soymilk after hot grinding and the two-phase UHT process, the hexanal content was reduced to 6 ppb and an undetectable level for black soymilk and Prosoy soymilk, respectively, which were very close or lower than the sensory detection threshold (4.5 ppb).[20] Therefore, the two-phase UHT heating method with the equipped vacuum chamber was very effective to reduce soy odor. As compared to the literature, Sun et al.[5] found hot temperature grinding, followed by a 95 °C and 10 min of heating, gave a similar hexanal content (242 ppb) as compared to that (264 ppb) ground at 25 °C. Lv et al.[16] were able to reduce the hexanal level to 20−70 ppb after hot grinding at

80−100 °C and followed by heating at 93−95 °C for 5 min. The study of Lozano et al.[4] using one-phase UHT resulted in hexanal with 150−250 ppb. The above literature reports showed the ineffectiveness of hot grinding or one-phase UHT process to reduce hexanal to a level lower than the sensory threshold value (4.5 ppb). The discrepancies between our results and others may be attributed to the accurate control of the temperature in grinding using the insulated device or differences in analytical methods and soybean varieties.

Under almost all heating methods, black soybean tended to produce significantly ($p < 0.05$) higher hexanal than the yellow Prosoy soybean in most cases. This differential effect may be due to their different chemical compositions and oxidative enzyme systems.[6] Black soybeans contain high phenolic contents and chlorophyll that are all different from yellow soybeans.[21]

Effect of Grinding Methods, Heating Methods, and Variety on Hexanol in Soymilk. Hexanol is not a contributor to the green, beany flavor due to its much higher sensory detection threshold (2.5 ppm) than hexanal.[20] However, it is a good indicator volatile. Hexanol is derived from 13-hydroperoxide of linoleic acid. Table 1 shows in raw soymilk, and ambient grinding gave significantly ($p < 0.05$) higher hexanol level than the other two grinding methods. After heating, in most cases, the hexanol level was reduced to below the detection limit.

Effect of Grinding Methods, Heating Methods, and Variety on 2-Pentylfuran in Soymilk. 2-Pentylfuran is a very unique odor compound. It has a beany odor note as reported by Belitz et al.[20] and Smouse and Chang.[22] As shown in Table 1, in contrast to other odor compounds, it increased with heating. According to Bradley and Min,[23] singlet oxygen could be formed by riboflavin present in soymilk, and 2-pentylfuran was generated by singlet oxygen action on linoleic acid via a specific oxidation mechanism.[7,10] Lee et al.[10] also found only soy flour stored under light could generate 2-pentylfuran. Bradley and Min[23] mainly contributed it to the formation of singlet oxygen induced by chlorophyll, which can promote the reaction in a similar manner as riboflavin.

In most cases, ambient grinding resulted in significantly ($p < 0.05$) higher 2-pentylfuran as compared with the other two grinding methods, between which hot grinding caused much lower 2-pentylfuran formation than cold grinding. These values suggest that heat inactivation of lipoxygenases could partly restrict the generation of 2-pentylfuran to some extent. Soymilk from traditional stove cooking contained several times higher 2-pentylfuran than that from the UHT methods. In stove cooking, continuous stirring and longtime exposure to light and air could lead to the extensive formation of singlet oxygen. Among our processing methods, the hot grinding and the UHT processing were particularly effective in reducing 2-pentylfuran to that below sensory threshold values.

Effect of Grinding Methods, Heating Methods, and Variety on 1-Octen-3-one in Soymilk. 1-Octen-3-one has a mushroom odor note and an extremely low threshold of 0.005 ppb in water as reported by Buttery et al.[24] In general, the contents of the 1-octen-3-one from three grinding methods followed the same trend: ambient grinding > cold grinding > hot grinding. All heating methods greatly reduced 1-octen-3-one levels, but UHT methods were more effective, and the two-phase UHT method was the most effective in the reduction of 1-octen-3-one. The effect of hot grinding and heat processing on 1-octen-3-one has not been reported by others in the soymilk in the literature.

In most cases, black soybean-made soymilk possessed more 1-octen-3-one than yellow soymilk. What is striking is that hot grinding could make 1-octen-3-one in soymilk made from yellow Prosoy soybean below the detection limit but not from black soybean. We do not know why the two varieties in our study exhibited such a large disparity under hot grinding.

Effect of Grinding Methods, Heating Methods, and Variety on 1-Octen-3-ol in Soymilk. 1-Octen-3-ol also has a mushroom odor note with an extremely low threshold of 0.005 ppb.[25] Yuan and Chang[8] and Kobayashi et al.[17] reported that lipoxygenase-deficient varieties had no advantages over normal varieties in terms of 1-octen-3-ol content. It is very likely that some 1-octen-3-ol is formed during the soaking phase via biologically (enzyme)-controlled mechanism during soaking.[26] Kobayashi et al.[17] suggested that 10-hydroperoxide was formed by other forms of hydroperoxidation other than lipoxygenase-activated oxidation, while Frankel et al.[9] found that 1-octen-3-ol was derived from 10-hydroperoxide, which was formed by photosensitized oxidation from linoleic acid. Lee et al.[10] detected higher levels of 1-octen-3-ol in soy flour stored under light as compared with soy flour stored in the dark. Our results (Table 1) showed that hot grinding reduced 1-octen-3-ol to less than 10% of that in cold and ambient ground raw yellow Prosoy soymilk. Cold grinding is not effective in reducing this odor compound when compared to ambient grinding. These results at least demonstrated that inactivating lipoxygenases by hot grinding at 80 °C for 3 min could partially inhibit the formation of 1-octen-3-ol.

In general, the two-phase UHT gave significantly ($p < 0.05$) lower odor contents in comparison with the other two heating methods, and hot grinding plus two-phase UHT was effective to make it below the detection limit for Prosoy soymilk. Without using hot grinding, Lozano et al.[4] reported high levels (dilution factors ranged from 27 to 729) of 1-octen-3-ol in traditionally cooked and one-phase UHT processed soymilk, whereas using hot grinding at 80−100 °C and a traditional cooking method, Lv et al.[16] reported 40−10 ppb of 1-octen-3-ol in soymilk.

In sharp contrast to Prosoy, hot grinding of black soybean resulted only in a slightly lower level of 1-octen-3-ol than ambient ground soymilk. This may be due to the unique oxidation mechanism of 1-octen-3-ol and the differences in the lipid composition of the two varieties.

Effect of Grinding Methods, Heating Methods, and Variety on (*E*)-2-Nonenal in Soymilk. (*E*)-2-Nonenal has a cooked carrot odor note with a low threshold of 0.08 ppb in water.[20,25] For both varieties, in raw soymilk, hot grinding produced significantly ($p < 0.05$) lower levels of (*E*)-2-nonenal than the other two grinding methods (Table 1). Yuan and Chang[8] and Kobayashi et al.[17] also reported that lipoxygenase-null varieties produced much lower (*E*)-2-nonenal in comparison with the normal varieties. These results implied that lipoxygenases were involved in the formation of this compound.

Stove cooking after hot grinding could greatly increase (*E*)-2-nonenal but not after the other two grinding methods. Yuan and Chang[8] found a similar trend in comparing the effect of stove cooking on soymilk made from normal and lipoxygenase-null varieties. In addition, Lv et al.[16] found that after hot grinding at 80−100 °C for 2 min and heating for 5 min at 93−95 °C, hot grinding did not show much advantage over ambient grinding. The results from stove heating following hot grinding suggested that (*E*)-2-nonenal might also be formed non-enzymatically during heating processes. According to Frankel et al.,[9] (*E*)-2-nonenal could be derived from 9-/10 -OOH via autoxidation and photosensitized oxidation of linoleic acid. As shown in Table 1, it was very likely that some polyunsaturated fatty acids were oxidized in stove cooking in the presence of enough light and oxygen even if the lipoxygenase had been inactivated during hot grinding.

Effect of Grinding Methods, Heating Methods, and Variety on (*E,E*)-2,4-Nonadienal in Soymilk. (*E*,*E*)-2,4-Nonadienal has a beany note and a low threshold value of 0.09 ppb.[5] Table 1 clearly shows that the order of (*E*,*E*)-2,4-nonadienal concentrations from low to high is hot grinding < cold grinding < ambient grinding. In particular, hot grinding could make it below the detection limit, which implied that lipoxygenases played a vital role in the oxidation of lipid to form this compound. Kobayashi et al.[17] also found that in raw soymilk from a lipoxygenase-null variety, no (*E*,*E*)-2,4-nonadienal was detected. Following hot grinding, all three cooking treatments kept this odor compound undetectable. For soymilk from cold grinding and ambient grinding, two-phase UHT produced the lowest level of (*E*,*E*)-2,4-nonadienal, followed by one-phase UHT and stove cooking. Different from other odor compounds, all cooking methods following the cold and ambient grinding only reduced (*E*,*E*)-2,4-nonadienal slightly, even after two-phase UHT process. This may be due to strong association of this odor compound with other components, such as proteins in the soymilk.[27]

Effect of Grinding Methods, Heating Methods, and Variety on (*E,E*)-2,4-Decadienal in Soymilk. Table 1 shows significant ($p < 0.05$) differences existed among the three grinding methods with hot grinding producing the lowest (*E*,*E*)-2,4-decadienal, followed by cold grinding and ambient grinding. (*E*,*E*)-2,4-Decadienal has a fried fatty sensory note and a detection threshold of 180 ppb in water or 0.07 ppb in palm oil as reported by Belitz et al.[20] and Buttery et al.,[24] respectively. In view of such a low threshold, hot grinding was a very efficient way to reduce it to undetectable level. According to Frankel et al.,[9] (*E*,*E*)-2,4-decadienal could be formed from linoleate through autoxidation or photosensitized oxidation. However, Kobayashi et al.[19] found much lower levels (*E*,*E*)-2,4-decadienal from a lipoxygenase-null variety as compared with the lipoxygenase-normal variety, and Yuan and Chang[8] detected it only in one normal variety. In view of their reports and our results, we can conclude that lipoxygenases contribute greatly to the presence of (*E*,*E*)-2,4-decadienal. After heating, this odor compound increased dramatically for cold grinding and ambient grinding but not for hot grinding. Yuan and Chang[8] also observed the same phenomenon. We do not know why this happened, and we postulate that may be due to thermal decomposition. (*E*,*E*)-2,4-Decadienal is derived from 9-hydroxyl linoleic acid,[9] and hydroperoxide lyase from soybean only specifically catalyzes 13-OOH.[28] It is very likely that hydroperoxides from cold and ambient grinding decomposed during heating. However, if we compared the effects of three heating methods for the cold and ambient grinding methods, the order of (*E*,*E*)-2,4-decadienal content from high to low concentration is stove cooking > one-phase UHT > two-phase UHT.

Effect of Grinding Methods on Solid and Protein Recovery. Protein extraction from soybean is a mass transfer process. How protein leaches into water is affected by both particle size and diffusion rate, which are affected by the original raw material size, temperature, and shearing during grinding. Although hot grinding may reduce odor in soymilk, it may denature soy protein to form insoluble protein aggregates before diffusion occurs to lose protein and total solid recovery. Cold grinding may reduce protein yield due to slower diffusion during mass transfer process. As shown in Figure 2, ambient grinding gave the highest protein extraction for both soybean varieties. Solid and protein recoveries were also affected by filtration equipment, varieties, and bean-to-water ratio.[15,29] With a bean-to-water ratio of 1:6, Endo et al.[12] found that the protein and solid recoveries after hot grinding were 40.4 and 38%, respectively. In the current study, these protein and solid recovery values were 51.6 and 57.8%, respectively. Using the same bean-to-water ratio (1:10) and press separation, Wilkens et al.[15] achieved a higher solid recovery (about 62%) than that of our study. This higher recovery might be attributed to the heating of slurry before filtration, which has been reported to increase protein and solid recoveries.[13,30,31] This means heating of slurry might be used to overcome the effect of hot grinding to some extent while maintaining lower off-flavor profile. Cold grinding resulted in the lowest solid and protein recoveries for the two varieties.

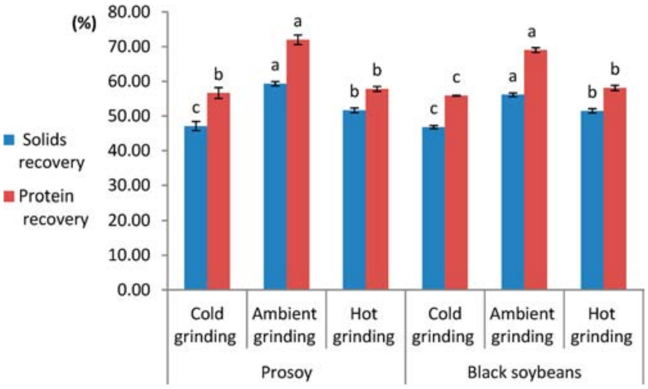

Figure 2. Solids and protein recoveries as affected by grinding methods. Means with different letters are significantly different among different grinding methods within the same variety ($p < 0.05$).

In summary, the hot grinding process with accurate temperature control used in our laboratory was effective in reducing soy odor. Cold grinding also exhibited an advantage over ambient grinding. In addition, the two-phase UHT with vacuum evaporation could effectively remove the selected volatiles to a large extent due to the use of vacuum twice that helped remove not only water but also volatile compounds in the product. As expected, proper combinations of grinding and heating methods are desirable to tackle the soy odor problem. It should be noted that in the meantime, cold and hot grinding could reduce the protein recovery and solid yield. In addition, other properties of soymilk might be affected by various treatments, such as isoflavone profile, trypsin inhibitor activity, and antioxidant capacity. Comprehensive assessment of these treatments is conducive to processing optimization. In most cases, it was more difficult to reduce soy odor in black soymilk than in yellow soymilk. Even if the combination of hot grinding and two-phase UHT treatments was used, it was not possible to make some odor compounds below their extremely low sensory threshold level. Autooxidation of polyunsaturated fatty acids is a significant factor for consideration in the design of processes to reduce soy odor in soymilk. Future studies should be done to assess the functional (sensory and other) properties of soymilk as affected by different methods of processing, particularly for improving the quality of black soymilk.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) FDA. Food labeling: Health claim; soy protein and coronary heart disease. Final Rule. *Fed. Regist.* **1999**, *64* (206), 57700−57733.

(2) Macleod, G.; Ames, J. Soy flavor and its improvement. *CRC Crit. Rev. Food Sci. Nutr.* **1988**, *27* (4), 219−400.

(3) Yuan, S. H.; Chang, S. K. C. Selected odor compounds in cooked soymilk as affected by soybean materials and direct steam injection. *J. Food Sci.* **2007**, *7*, 481−486.

(4) Lozano, p. R.; Drake, M.; Benitez, D.; Cadwallader, K. R. Instrumental and sensory characterization of heat-induced odorants in aseptically packaged soy milk. *J. Agric. Food Chem.* **2007**, *55*, 3018−3026.

(5) Sun, C. X.; Cadwallader, K. R.; Kim, H. Comparison of key aroma components between soymilks prepared by cold and hot grinding methods. In *Chemistry, Texture and Flavor of Soy*; Cadwallader, K., Chang, S. K. C., Eds.; ACS Symposium Series 1059; American Chemical Society: Washington, DC, 2010; Chapter 22, pp 361−373.

(6) Yuan, S. H.; Chang, S. K. C. Selected odor compounds in soymilk as affected by chemical composition and lipoxigenases in five soybean materials. *J. Agric. Food Chem.* **2007**, *55*, 426−431.

(7) Min, S.; Yu, Y.; Yoo, S.; St. Martin, S. Effect of soybean varieties and growing locations on the flavor of soymilk. *J. Food Sci.* **2005**, *70* (c), 1−7.

(8) Yuan, S. H.; Chang, S. K. C. Selected odor compounds in cooked soymilk as affected by soybean materials and direct steam injection. *J. Food Sci.* **2007**, *72*, 481−486.

(9) Frankel, E. N.; Neff, W. E.; Selke, E. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VII. Volatile thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate and linolenate. *Lipids* **1981**, *16*, 279−285.

(10) Lee, J. Y.; Min, S.; Lee, E. O.; Min, D. B. Volatile compounds formation in soy flour during light storage by singlet oxygen oxidation. *J. Food Sci.* **2003**, *68*, 1933−1937.

(11) Nelson, A. L.; Steinberg, M. P.; Wei, L. S. Illinois process for preparation so soymilk. *J. Food Sci.* **1976**, *41*, 57−61.

(12) Endo, H.; Ohno, M.; Tanji, K.; Shimada, S.; Kaneko, K. Effect of heat treatment on the lipid peroxide content and Aokusami (beany flavor) of soymilk. *Food Sci. Technol. Res.* **2004**, *10*, 328−333.

(13) Mizutani, T.; Hashimoto, H. Effect of grinding temperature on hydroperoxide and off-flavor contents during soymilk manufacturing process. *J. Food Sci.* **2004**, *69*, 112−116.

(14) Boatright, W. L. Effect of gallic acid on the aroma constiturents of soymilk and soy protein isolate. *J. Am. Oil Chem. Soc.* **2002**, *79*, 317−323.

(15) Wilkens, W. F.; Mattick, L. R.; Hand, D. B. Effect of processing method on oxidative off-flavors of soybean milk. *Food Technol.* **1967**, *21*, 1630−1633.

(16) Lv, Y. C.; Song, H. L.; Li, X.; Wu, L.; Guo, S. T. Influence of blanching and grinding process with hot water on beany and non-beany flavor in soymilk. *J. Food Sci.* **2011**, *76* (s), 20−25.

(17) Kobayashi, A.; Tsuda, Y.; Hirata, N.; Kubota, K.; Kitamura, K. Aroma constituents of soybean milk lacking lipoxygenase isozymes. *J. Agric. Food Chem.* **1995**, *43*, 2448−2452.

(18) Yuan, S. H.; Chang, S. K. C.; Liu, Z. S.; Xu, B. J. Elimination of trypsin inhibitor activity and beany flavor in soy milk by consecutive blanching and ultrahigh-temperature (UHT) processing. *J. Agric. Food Chem.* **2008**, *56*, 7957−7963.

(19) Anthon, G. E.; Barrett, D. M. Colorimetric method for the determination of lipoxygenase activity. *J. Agric. Food Chem.* **2001**, *49*, 32−37.

(20) Belitz, H. D.; Grosch, W.; Schieberle, P. Aroma compound. In *Food Chemistry*, 3rd English ed.; Springer Publication: New York, NY, 2004; Chapter 5, pp 342−408.

(21) Xu, B. J.; Chang, S. K. C. Comparative analysis of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *J. Agric. Food Chem.* **2007**, *72* (2), 167−177.

(22) Smouse, T. H.; Chang, S. S. A systematic characterization of the reversion flavor of soybean oil. *J. Am. Oil Chem. Soc.* **1967**, *44*, 509−514.

(23) Bradley, D.; Min, D. B. Singlet oxygen detection in skin milk by electron spin resonance spectroscopy. *J. Food Sci.* **2003**, *68*, 491−494.

(24) Buttery, R. G.; Guadangi, D. G.; Ling, L. C. Volatile aroma components of cooked artichoke. *J. Agric. Food Chem.* **1978**, *26*, 791−793.

(25) Buttery, R. G. Importance of lipid derived volatiles to vegetable and fruit flavor. In *Flavor Chemistry of Lipid Foods*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1989; Chapter 8, pp 156−164.

(26) Badenhop, A. F.; Wilkens, W. F. The formation of 1-octen-3-ol in soybeans during soaking. *J. Am. Oil Chem. Soc.* **1968**, *46*, 179−182.

(27) Suppavorasatit, I.; Cadwallader, K. R. Flavor-soy protein interactions. In *Chemistry, Texture and Flavor of Soy*; Cadwallader, K., Chang, S. K. C., Eds.; ACS Symposium Series 1059; American Chemical Society: Washington, DC, 2010; Chapter 21, pp 339−359.

(28) Gardner, H. W. How the lipoxygenase pathway affects the organoleptic properties of fresh fruit and vegetables. In *Flavor Chemistry of Lipid Foods*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1989; Chapter 8, pp 98−112.

(29) Johnson, K. W.; Snyder, H. E. Soymilk: A comparison of processing methods on yield and composition. *J. Food Sci.* **1978**, *44*, 249−353.

(30) Ono, T.; Choi, M. R. Changes in the composition and size distribution of soymilk protein particles by heating. *Agric. Biol. Chem.* **1991**, *55*, 2291−2297.

(31) Beddows, C. G.; Wong, J. Optimization of yield and properties of silken tofu from soybeans. II. Heating processing. *Int. J. Food Sci. Technol.* **1987**, *22*, 23−27.