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## Instrumental and Sensory Characterization of Heat-Induced Odorants in Aseptically Packaged Soy Milk

Patricio R. Lozano,<sup>†</sup> Maryanne Drake,<sup>§</sup> Daniel Benitez,<sup>#</sup> and Keith R. Cadwallader<sup>\*,†</sup>

Department of Food Science and Human Nutrition, University of Illinois, 1302 West Pennsylvania Avenue, Urbana, Illinois 61801; Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695; and Cargill, Inc., 2525 Ponce de Leon Boulevard, Suite 800, Coral Gables, Florida 33134

Predominant heat-induced odorants generated in soy milk by ultrahigh-temperature (UHT) processing were evaluated by sensory and instrumental techniques. Soy milks processed by UHT (143 °C/14 s, 143 °C/59 s, 154 °C/29 s) were compared to a control soy milk (90 °C/10 min) after 0, 1, and 7 days of storage (4.4 ± 1 °C). Dynamic headspace dilution analysis (DHDA) and solvent-assisted flavor evaporation (SAFE) in conjunction with GC-olfactometry (GCO)/aroma extract dilution techniques and GC-MS were used to identify and quantify major aroma-active compounds. Sensory results revealed that intensities of overall aroma and sulfur and sweet aromatic flavors were affected by the processing conditions. Odorants mainly responsible for the changes in sulfur perception were methional, methanethiol, and dimethyl sulfide. Increases in 2-acetyl-1-pyrroline, 2-acetyl-thiazole, and 2-acetyl-2-thiazoline intensities were associated with roasted aromas. A marginal increase in intensity of sweet aromatic flavor could be explained by increases in 2,3-butanedione, 3-hydroxy-2-butanone,  $\beta$ -damascenone, and 2- and 3-methylbutanal. Predominant lipid-derived odorants, including (*E*,*E*)-2,4-nonadienal, (E,E)-2,4-decadienal, (E,Z)-2,4-decadienal, (E)-2-nonenal, (E)-2-octenal, 1-octen-3one, 1-octen-3-ol, and (E,Z)-2,6-nonadienal, were affected by processing conditions. Intensities of overall aroma and sulfur notes in soy milk decreased during storage, whereas other sensory attributes did not change. Color changes, evaluated by using a Chroma-meter, indicated all UHT heating conditions used in this study generated a more yellow and saturated color in soy milk in comparison to the control soy milk.

KEYWORDS: Thermal processing; cooked off-flavors; soy milk; UHT; storage effect; color

#### INTRODUCTION

Soy milk, a water extract of soybeans, is an excellent source of protein and essential fatty acids. It is cholesterol-free and relatively cheap in comparison with other sources of protein. Despite the beneficial attributes of soy milk, its consumption in the Western world has been limited due to its unacceptable beany flavor (1). A considerable number of studies have been conducted to determine the volatile compounds responsible for the beany off-flavor in soy milk. As a result, in the past decade novel processing technologies have been developed to reduce beany off-flavors, obtain better yields, eliminate antinutritional factors, and extend the shelf life of soy milk. High temperature short time (HTST) and ultrahigh-temperature (UHT) processing methods have been a crucial part of this development (1, 2). The use of high-temperature processing combined with aseptic packaging has opened new markets and avenues for the distribution of soy milk (2). Unfortunately, color changes, loss of nutrients, and creation of "cooked off-flavors" have been encountered in soy milks produced under these conditions. A viable solution to this problem has been difficult to address because little information exists about the chemical reactions that occur at these temperatures and also due to the uncertainty of the effect of storage on these heat-induced aroma compounds.

The aim of this project was to characterize the major aromaactive components generated under UHT conditions, to assess their impact on the overall soy milk aroma and flavor, and to determine the effect of short-term storage on key heat-induced aroma compounds.

#### MATERIALS AND METHODS

**Chemicals.** Analytical grade authentic compounds were obtained from Aldrich Chemical Co. (St. Louis, MO) except for 2-acetyl-1pyrroline, which was a gift from Dr. R. Buttery (USDA, ARS, WRRC, Albany, CA);  $\beta$ -damascenone, which was provided by Firmenich Co. (Princeton, NJ); and  $\delta$ -octalactone and  $\gamma$ -nonalactone, which were

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<sup>\*</sup> Corresponding author [telephone (217) 333-5803; fax (217) 333-1875; e-mail cadwlldr@uuc.edu].

<sup>&</sup>lt;sup>†</sup> University of Illinois.

<sup>&</sup>lt;sup>§</sup> North Carolina State University.

<sup>#</sup> Cargill, Inc.

Table 1. Soy Milk Flavor Lexicon Employed in Descriptive Analysis

| descriptor                                                                                         | definition                                                                                                                                                                                                                                                                | reference                                                                                                                                                                         |
|----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| overall aroma intensity                                                                            | Aroma (Evaluated Orthonasally)<br>overall aroma intensity of the product                                                                                                                                                                                                  |                                                                                                                                                                                   |
| sweet aromatic<br>sulfur                                                                           | Flavors (Evaluated When Product Is Placed in the Mouth)<br>sweet aromatic associated with cooked oatmeal (retronasally)<br>sharp aromatic associated with cooked eggs and cooked                                                                                          | Quaker oatmeal, 50 g in 500 mL of water hard-boiled mashed egg, steamed or boiled                                                                                                 |
| cereal/grain<br>flour paste<br>metallic<br>astringent <sup>a</sup>                                 | cruciferous vegetable<br>aromatics associated with grain<br>aromatics associated with flour paste<br>aromatics associated with metal or rare beef juices<br>mouthfeel sensation of drving, drawing, or puckering of                                                       | broccoli or cabbage<br>Cheerios, 50 g in 500 mL of water<br>flour, 60 g in 500 mL of water<br>1-octen-3-one, 20 ppm in ethanol<br>1% alum in water or black tea (soak six bags in |
| sweet <sup>a</sup><br>sour <sup>a</sup><br>bitter <sup>a</sup><br>viscosity <sup>a</sup><br>chalky | the tongue or oral cavity<br>basic taste associated with sucrose<br>basic taste associated with acid<br>basic taste associated with bitter compounds<br>force required to move a spoon back and forth in the sample<br>degree to which the sample has very fine particles | 500 mL of water for 10 min)<br>5% sucrose in water<br>0.08% citric acid in water<br>0.08% caffeine in water<br>water = 1, cream = 3<br>chew raw potato cube                       |

<sup>a</sup> Reference taken from Meilgaard et al. (14).

purchased from Bedoukian Research (Danbury, CT). (E,E)-3,5-Octadien-2-one was synthesized on the basis of a previously published procedure (3). (Z)-2-Nonenal was synthesized from (Z)-2-nonen-1-ol (Bedoukian Research Inc.) by oxidation with Dess-Martin periodinane (0.3 M in dichloromethane; Aldrich Chemical Co.) following the procedure described by Meyer and Schreiber (4). Odorless distilled water was prepared by boiling glass-distilled water in an open flask until its volume was reduced by one-third of the original volume.

**Soy Milk Production.** The soybean variety selected for this experiment was VINTON 81 because of its high protein yield (5). Fresh whole beans from the 2004 harvest were obtained from the Illinois Center for Soy Foods at the University of Illinois (Urbana, IL).

A standardized soy milk procedure was adopted to consistently provide a homogeneous product for volatile analysis by eliminating possible sources of variation during its manufacturing. This procedure was a combination of two previously published methods (6, 7). It consisted of a predrying step at 93.3 °C for 10 min applied to the beans solely to weaken the soybean hulls and minimize the damage of the rotating drum when the hulls were separated from the cotyledons. After dehulling, the beans were soaked with odorless distilled water in a proportion of 1:5 w/w (water to wet soybeans), and the mixture was held at 4  $\pm$  1 °C for 14 h. The soaked beans were washed with cold distilled water for 1 min, and the water was discarded. A double "hotgrinding" step using a 1:7 w/w (odorless distilled water to dry beans) ratio followed, using a bean disintegrator BMI 300 (Beam Machines Inc., San Francisco, CA) equipped with a stainless steel mesh screen no. 40. This step was to create an emulsion and limit lipoxygenase activity by keeping the temperature at about 80 °C (8). The obtained slurry was later filtered using a vibration screen separator KM-1-SS (Kason, Montreal, Canada) with a no. 200 stainless steel mesh screen to separate the soy milk from the okara.

Heat Treatment. Three heat combinations in the UHT range (143 °C/14 s, 143 °C/59 s, and 154 °C/29 s) and one control in the HTST range (90 °C/10 min) were selected for this experiment. All treatments were replicated twice using two different batches of fresh (same day) soy milk each time. The heating conditions chosen for control (90 °C/ 10 min) followed the procedure described by Feng and Acree (9) to reduce solely the microbial contamination of aerobically processed soy milk and make it safe for the sensory panelists. The mildest UHT treatment was chosen to mimic the extreme conditions applied for sterilizing cow's milk (143 °C/14 s) (10), whereas the other two UHT treatments were selected on the basis of experimental (11) and mathematical modeling data (12) that provide the appropriate holding time and processing temperature combinations at the UHT range (121-154 °C) to destroy 90% of the soy milk trypsin inhibitor (TI). This extent of heat inactivation has been reported to provide adequate TI destruction where the maximum nutritive value or protein efficiency in soy milk is acquired (13).

*Equipment.* A miniature (0.8–1.4 L/min) HTST/UHT processing system (Microthermics, Raleigh, NC) using a direct steam injection

system, a laminar flow high-efficiency particulate air (HEPA) hood, and an automatic fill control to simulate aseptic filling was used to process the soy milk. The system has two stages of heating; an initial preheating stage to increase the temperature of the soy milk from 4 to 121 °C and a second stage to bring the soy milk to the UHT temperatures described above. The system was cleaned and sterilized with an odorless detergent and steam at 125 °C for 20 min, respectively. One liter glass Pyrex bottles, equipped with Teflon-lined lids and previously autoclaved (125 °C/20 min) and tested for residual odors, were used to store the soy milk under aseptic conditions. Samples were stored at refrigeration temperature (4.4 ± 1 °C) after being processed. Soy milk samples were analyzed at 0, 1, and 7 days after processing by sensory and analytical techniques.

**Sensory Evaluation of Soy Milk.** Eight panelists (females, 45–55 years of age) trained in the Spectrum method of descriptive analysis for the generation of qualitative and quantitative data evaluated the products (*14*). Each panelist had greater than 500 h of previous experience in the sensory evaluation of food products including previous experience with fluid milk, soy milk, and soy protein concentrates and isolates. Panelists received an additional 6 h of training (three 2-h sessions) focused on soy milk. During the focused training, panelists evaluated and discussed an array of commercial and experimentally produced soy milks. Specific attributes, attribute definitions, and references were developed by the panelists (**Table 1**). Panelists used the 15-point universal Spectrum intensity scale (*14*). Analysis of variance of data collected from the last part of training indicated that the panel and panelists could consistently use the attributes to differentiate the products.

Samples were removed from refrigeration approximately 1 h prior to evaluation and shaken well to distribute any sediment. Approximately 40 mL was dispensed into 2-oz portion cups labeled with three-digit random codes and covered. Panelists independently evaluated the orthonasal aroma and then the flavor (retronasal aromatics and basic tastes) and viscosity of the samples in a randomized balanced order. References were available for calibration during evaluation. Distilled/ deionized water and soda crackers were provided for palate cleansing. Panelists rested 3 min between individual samples followed by a 15 min rest period between each replication. Samples were analyzed in triplicate by each panelist.

*Color Changes.* Soy milk changes in coloration were registered by using a Chroma-meter (Cr 400, Konica Minolta). This instrument measured the color changes by exposing the soy milk to various wavelengths and expressing the differences in factors of *L* (lightness), *a* (redness–greenness), and *b* (yellowness and blueness). For better understanding of the color change relationship, lightness (*L*), chroma  $(\sqrt{a^2+b^2})$ , and hue  $[\tan^{-1}(b/a)]$  values were calculated.

Instrumental Evaluation of Soy Milk Aroma. Heat-induced odorants created in soy milk required more than one technique to be properly isolated. This is because highly volatile components of soy milk are not completely recovered with any single extraction or distillation technique, whereas the semivolatile compounds, important in the milk's characteristic flavor, can be well recovered by headspace techniques. In this study, two techniques, solvent-assisted flavor evaporation (SAFE) and dynamic headspace dilution analysis (DHDA), were used to cover a wider range of aroma-active volatile components.

DHDA was carried out in a 100-mL capacity purge and trap vessel (SIS Inc.) using nitrogen as purge gas. Ten milliliters of fresh soy milk and 10  $\mu$ L of an internal standard solution (containing 0.19  $\mu$ g/ $\mu$ L of 2-ethylbutyric acid, 0.18  $\mu g/\mu L$  of 2,4,6-trimethylpyrazine, and 0.19  $\mu g/\mu L$  of 2-methyl-3-heptanone in methanol) was placed in the vessel and stirred throughout extraction. A thermal desorption tube (Supelco, Bellefonte, PA) containing approximately 200 mg of Tenax TA was attached to the vessel to trap the volatiles of the sample. The vessel was maintained at 50 °C by circulating warm water through the external water jacket. The equilibration time of the sample was 5 min at 50 °C. Nitrogen was used as the purge gas at 50 mL/min, and headspace dilutions were carried out by changing the purging time in factors of 5 (i.e., 25, 5, or 1 min) as described by Cadwallader and Baek (15). After purging, the trap was removed from the vessel, dry purged with nitrogen gas for 17 min, and then desorbed for 10 min at 220 °C (TDS2, Gerstel, Germany) into a CIS 4 (Gerstel) inlet held at -150 °C (solvent vent mode, 50 mL/min helium vent flow). The CIS 4 inlet was then heated (12 °C/s from -150 to 260 °C with a final hold time of 10 min; purge value delay was 1.10 min) in the splitless mode, and the desorbed compounds were injected into the GC column of an Agilent 6890 GC (Palo Alto, CA) equipped with an OD2 olfactometry port (Gerstel). Two separate experiments using a polar (Stabilwax DA) capillary column (15 m  $\times$  0.32 mm i.d.  $\times$  0.5  $\mu$ m film) from Restek Co. (Bellefonte, PA) and a nonpolar (DB-5MS) column (15 m  $\times$  0.32 mm i.d.  $\times$  0.5  $\mu m$  film) from J&W Scientific (Folsom, CA) were carried out to obtain the retention indices (RI) for aroma active compounds on both phases. The GC oven was programmed from 35 to 225 °C at a rate of 10 °C/min with initial and final holding times of 5 and 20 min, respectively. The carrier gas was helium at a constant flow rate of 2.2 mL/min. Detector and sniffing port temperatures were 250 °C. The end of the capillary column was split 1:1 between the flame ionization detector (FID) or flame photometric detector (FPD) and sniff port. To prevent drying of the nasal mucosa, humidified air (30 mL/ min) was supplied to the sniff port. Retention data were represented as retention indices (RI) based on the Van den Dool and Kratz (16) approach. Analysis of each dynamic headspace dilution was carried out in triplicates, and sniffing was performed by two experienced assessors.

SAFE Extraction. A modified SAFE system was used to isolate the volatile compounds of soy milk. This system was selected due to its higher yields of volatile compounds from fatty matrices and reduced thermal alteration of the flavor odorants during the isolation (17). For the volatile extraction, the system consisted of two liquid nitrogencooled traps, a transfer head, and a 5-L round-bottom flask. The whole system was operated under high vacuum (10<sup>-5</sup> Torr). Two liters of soy milk, 225 g of salt, and 10  $\mu$ L of internal standard solution (containing 19.29  $\mu g/\mu L$  of 2-ethylbutyric acid, 18.95  $\mu g/\mu L$  of 2,4,6trimethylpyrazine, and 19.86 µg/µL of 2-methyl-3-heptanone in methanol) were introduced into the system at a rate of 25 mL/min to avoid foaming. The total extraction time was approximately 2.5 h per sample. After the SAFE extraction (approximately 800 mL of extract was obtained from each sample), the aqueous solution trap was removed from the system and thawed overnight with 50 mL of diethyl ether and 225 g of salt to accentuate the transfer of aroma compounds to the solvent. After filtration through no. 2 filter paper (Whatman Inc., Florham Park, NJ) to remove the remaining salt, the extract was acidified to a pH between 2.0 and 3.0 by adding 3 mL of 10% HCl. An organic acidic-neutral phase and a basic aqueous phase were obtained from this adjusted solution by extracting it (three times) with 50 mL of diethyl ether. For the basic organic fraction, the obtained basic aqueous phase was adjusted to pH >8 by adding 4.0 mL of 2 M NaOH and later extracted (three times) with 5 mL of diethyl ether. The acidic-neutral organic phase was concentrated at 40 °C using a Vigreux column (150 × 15 mm) (Ace Glass Inc., Vineland, NJ) to 20 mL and then extracted (three times) with 10 mL of 0.5 M NaHCO3 to

obtain a neutral organic fraction and an aqueous phase containing the acidic components. The aqueous fraction was adjusted to pH <2.0 with 15 mL of 10% HCl and then extracted (three times) with 5 mL of diethyl ether to obtain the acidic organic fraction. Each organic fraction was concentrated to a volume of 10 mL at 40 °C by using a Vigreux column and a stream of purified nitrogen gas. Anhydrous sodium sulfate (2 g) was used for moisture removal. Samples were finally concentrated to 200  $\mu$ L. Each of the original organic fractions was diluted sequentially with diethyl ether in a ratio of 1:3 according to the aroma extract dilution analysis technique (AEDA) (18). This method was employed to detect the potency of the odor-active compounds in each of the organic fractions. Two microliters of each dilution was injected using a cold on-column mode in the gas chromatography-olfactometery (GCO) system. The number of serial dilutions analyzed for each fraction depended on the potency of the compounds, and it was carried out until no odor was detected. A GCO system (Agilent 6890 GC) equipped with an FID, a cool on-column injector, and a sniffing port (DATU, Geneva, NY) was employed. Each extract (2 µL), original extract or dilution, was analyzed on a polar (Stabilwax DA) or nonpolar (DB-5MS) column as previously described.

**Identification of Aroma-Active Components.** Compounds were positively identified by comparison of their mass spectra, retention indices (*16*), and odor properties with those of authentic standards. A compound was considered to be tentatively identified when only two of the above criteria were met.

Dynamic Headspace Analysis/Gas Chromatography–Mass Spectrometry (DHDA/GC-MS). For GC-MS analysis of DHDA, a thermal desorption system (TDS2, Gerstel) and a CIS 4 inlet were connected to an Agilent 6890 GC-5973 mass selective detector (MSD). The conditions of desorption and injection were the same as previously described for DHDA. Separations were performed using either a Stabilwax DA (FFAP) or DB-5 MS column (30 m × 0.25 mm i.d. × 0.25  $\mu$ m film thickness; J&W Scientific). The oven was programmed from 35 to 225 °C at a rate of 10 °C/min with initial holding times of 5 and 25 min, respectively. Helium was used as carrier gas at a constant rate of 1.0 mL/min. The MSD was held at 280 °C with an ionization voltage at 70 eV and a mass range from 33 to 350 amu. For better sensitivity the electron multiplier voltage was 200 V above the autotune setting.

Gas Chromatography-Mass Spectrometry. GC-MS was performed for the organic fractions obtained by SAFE injected using a cold oncolumn method into an Agilent 6890 GC-5973 MSD system. Separations were performed using the columns described above for DHDA/ GC-MS. The initial temperature of the inlet was maintained at 35 °C during injection and thereafter was maintained at 3 °C above oven temperature (oven tracking mode). The oven and mass selective detector conditions were the same as described for DHA/GC-MS analysis.

*Quantification.* An MS response factor ( $f_i$ ) for each positively identified odorant was determined by adding known amounts of authentic standards plus internal standards into deodorized water. Sample preparation by SAFE–solvent extraction and GC-MS analysis were performed in the same way as described above for the soy milk samples. The MS response factor of a compound relative to an internal standard was used to determine its concentration and was calculated as described by Zhou et al. (19). 2-Methyl-3-heptanone, 2-ethylbutyric acid, and 2,4,6-trimethylpyrazine were used as internal standards for compounds in the *neutral*, *acidic*, and *basic* organic fractions, respectively. Average concentration (n = 2) as well as coefficient of variation was calculated for each observation (0, 1, and 7 days) on each treatment.

**Statistical Analysis.** A randomized complete block design (RCB) with repeated measures over time was employed in the sensory analysis. After the normality of the data had been determined, an analysis of variance (ANOVA) and a least significant difference (LSD) analysis was performed to determine significant differences in the perception of each of the descriptors (p < 0.05). For the analytical data, an average (n = 2) of the concentration of the major volatile compounds of interest was used to determine the approximate concentrations of these compounds as well as their standard deviations.



Figure 1. Effect of heat processing on sensory attributes of soy milk.

**Table 2.** Overall Aroma and Sulfur Intensities Determined by SensoryDescriptive Analysis (Intensity Scale of 0-15 Points)<sup>a,b</sup>

| overall                                                   | treatment                                                                                                                                |                                                                                                                                             | storage time (days                                                                                                                           | 5)                                                                                    |
|-----------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| aroma                                                     | effect                                                                                                                                   | 0                                                                                                                                           | 1                                                                                                                                            | 7                                                                                     |
| 90 °C/10 min<br>143 °C/14 s<br>143 °C/59 s<br>154 °C/29 s | $\begin{array}{c} 2.06 \pm 0.11 \text{ a} \\ 2.32 \pm 0.11 \text{ a} \\ 2.71 \pm 0.11 \text{ ab} \\ 2.89 \pm 0.11 \text{ b} \end{array}$ | $\begin{array}{c} 2.44 \pm 0.15 \text{ Aa} \\ 2.45 \pm 0.13 \text{ Aa} \\ 2.83 \pm 0.13 \text{ Aa} \\ 2.30 \pm 0.21 \text{ Ab} \end{array}$ | $\begin{array}{c} 2.08 \pm 0.14 \text{ Aa} \\ 2.29 \pm 0.14 \text{ Aa} \\ 2.82 \pm 0.14 \text{ Aab} \\ 3.23 \pm 0.14 \text{ Ab} \end{array}$ | $1.66 \pm 0.13$ Ba<br>2.22 $\pm 0.13$ Ab<br>2.50 $\pm 0.13$ Bb<br>2.44 $\pm 0.13$ Bbc |

| sulfur                                                    | treatment                                                                                                                                 | :                                                                                                                                           | storage time (day                                                                     | S)                                                                                    |
|-----------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| aroma                                                     | effect                                                                                                                                    | 0                                                                                                                                           | 1                                                                                     | 7                                                                                     |
| 90 °C/10 min<br>143 °C/14 s<br>143 °C/59 s<br>154 °C/29 s | $\begin{array}{c} 0.96 \pm 0.21 \text{ a} \\ 1.38 \pm 0.21 \text{ ab} \\ 2.09 \pm 0.21 \text{ bc} \\ 2.29 \pm 0.21 \text{ c} \end{array}$ | $\begin{array}{c} 1.62 \pm 0.25 \text{ Aa} \\ 1.67 \pm 0.23 \text{ Aa} \\ 2.40 \pm 0.23 \text{ Aa} \\ 2.51 \pm 0.23 \text{ Ab} \end{array}$ | $0.84 \pm 0.23$ Ba<br>$1.39 \pm 0.23$ Aa<br>$2.18 \pm 0.23$ Aab<br>$2.84 \pm 0.23$ Ac | $0.41 \pm 0.23$ Ba<br>$1.06 \pm 0.23$ Ba<br>$1.71 \pm 0.23$ Bab<br>$1.54 \pm 0.23$ Bb |
|                                                           |                                                                                                                                           |                                                                                                                                             |                                                                                       |                                                                                       |

<sup>*a*</sup> Within rows, different upper case letters indicate significant differences at p < 0.05. <sup>*b*</sup> Within columns, different lower case letters indicate significant differences at p < 0.05.

#### **RESULTS AND DISCUSSION**

**Sensory Analysis.** The effects of UHT processing conditions on overall aroma and sulfur, sweet aromatic, grain, flour, and metallic flavors, sweet taste, and astringent mouthfeel were evaluated (**Figure 1**). Overall aroma and sulfur flavor intensities increased (p < 0.05) as a function of an increase in processing temperature. Sweet aromatic intensity increased only marginally (p < 0.1) with higher temperature. Flavor characteristics such as metallic, cereal, and flour and viscosity were not affected by heat processing (p > 0.05). Bitter taste and chalkiness (mouthfeel) were not detected in the experimental samples, although they were identified in some of the commercial samples used for language generation.

Perceived intensity changes in sulfur flavor and overall aroma intensities as a function of an increase in processing temperature and time of storage are shown in **Table 2**. The highest overall aroma intensity was registered at  $2.89 \pm 0.11$ , and it corresponded to the highest temperature applied (154 °C/29 s). At time 0 there was no difference between the overall aroma

intensity for the control soy milk (90 °C/10 min) and soy milks produced using the other two UHT conditions (143 °C/14 s and 143 °C/59 s).

Sulfur flavor intensities also changed in relation to the processing conditions. Although sulfur intensity in the control (90 °C/10 min) did not differ from the mildest UHT treatment (143 °C/14 s), a difference (p < 0.05) in sulfur intensity was observed between the control and the highest temperature treatment (154 °C/29 s). This difference was also significant (p < 0.05) between control and 143 °C/59 s treatments. These results suggest that higher temperature (>143 °C) is more detrimental to soy milk flavor than an extended processing time at a lower temperature. Soy milks processed at 143 °C for 14 and 59 s did not differ in sulfur intensity.

Storage time affected the intensity of the overall aroma (p < 0.05), which decreased during storage time with day 7 being significantly lower than days 0 and 1. This trend was observed in all treatments including the control and suggests that some odorants created during heat processing might decrease due to reaction with the complex soy milk matrix or with other volatile compounds during storage.

Similar to the results found for the overall aroma, storage time also influenced (p < 0.05) sulfur intensity among soy milks, with day 0 having the strongest intensity and day 7 the weakest (**Table 2**). There was no difference between days 0 and 1. A similar pattern was reported by Colahan-Sederstrom and Peterson (20) in their study of fluid milk processed under UHT conditions, for which they observed that reactions during the first week of storage could be attributed to the reactivity of the heat-induced sulfur-containing compounds.

Aroma-Active Compounds. Twenty-six aroma-active compounds were detected in the headspace of soy milk by DHDA (**Table 3**). Seventeen of these odorants [dimethyl sulfide, 2-methylbutanal, 2,3-butanedione, hexanal, 2-heptanone, nonanal, 1-octen-3-one, (*E*)-2-octenal, 1-octen-3-ol, methional, (*E*)-2-nonenal, (*Z*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4nonadienal, (*E*,*Z*)-2,4-decadienal, (*E*,*E*)-2,4-decadienal, and  $\beta$ -damascenone] have been previously identified as volatile components of soy milk (1, 20, 21–26). Hexanal, reported as oromo

aamnaund

Table 3. DHDA-GCO Comparison of Aroma Components of Control (90 °C/10 min) and UHT-Processed Sov Milks

|     |                |        |         |         |         | flavor dilu | tion factor |                 |         |         |        |  |
|-----|----------------|--------|---------|---------|---------|-------------|-------------|-----------------|---------|---------|--------|--|
|     |                |        | da      | iy 0    |         |             | day 1       |                 |         | day 7   |        |  |
|     |                | 90 °C/ | 143 °C/ | 143 °C/ | 154 °C/ | 143 °C/     | 143 °C/     | 154 °C/         | 143 °C/ | 143 °C/ | 154 °C |  |
| B-5 | <b>RI FFAP</b> | 10 min | 14 s    | 59 s    | 29 s    | 14 s        | 59 s        | 29 s            | 14 s    | 59 s    | 29 s   |  |
| 00  | <700           | 5      | 5       | 25      | 25      | 25          | 5           | nd <sup>d</sup> | 1       | 1       | 1      |  |
| 00  | 777            | 25     | 25      | 1       | 25      | 1           | 5           | 25              | 25      | 1       | 25     |  |
| 61  | 942            | 25     | 5       | 5       | 25      | 25          | nd          | nd              | 5       | nd      | nd     |  |
| 00  | 1005           | 25     | 25      | 25      | 25      | 25          | 25          | 25              | 25      | 25      | 25     |  |
| 11  | 1095           | 25     | 25      | 25      | 5       | 25          | 25          | 25              | 25      | 25      | 25     |  |
| 00  | 1183           | 25     | nd      | nd      | 5       | nd          | nd          | 25              | nd      | 5       | 5      |  |

| compound                           | aiuma          | KI DB-5 | NIFFAF | 10 11111 | 14 5 | 59.5 | 295 | 14 5 | 395 | 295             | 14 5 | 395 | 295 |
|------------------------------------|----------------|---------|--------|----------|------|------|-----|------|-----|-----------------|------|-----|-----|
| methanethiol <sup>b</sup>          | sulfury        | <600    | <700   | 5        | 5    | 25   | 25  | 25   | 5   | nd <sup>d</sup> | 1    | 1   | 1   |
| dimethyl sulfide <sup>b</sup>      | corn, sulfury  | <600    | 777    | 25       | 25   | 1    | 25  | 1    | 5   | 25              | 25   | 1   | 25  |
| 2-methylbutanal <sup>a</sup>       | dark chocolate | 661     | 942    | 25       | 5    | 5    | 25  | 25   | nd  | nd              | 5    | nd  | nd  |
| 2,3-butanedione <sup>a</sup>       | buttery        | <600    | 1005   | 25       | 25   | 25   | 25  | 25   | 25  | 25              | 25   | 25  | 25  |
| hexanal <sup>a</sup>               | green          | 811     | 1095   | 25       | 25   | 25   | 5   | 25   | 25  | 25              | 25   | 25  | 25  |
| 2-heptanone <sup>a</sup>           | oxid           | 900     | 1183   | 25       | nd   | nd   | 5   | nd   | nd  | 25              | nd   | 5   | 5   |
| (Z)-4-heptenalc                    | crabby, fatty  | 853     | 1235   | 1        | 5    | 25   | 25  | 25   | 5   | 5               | 25   | nd  | 1   |
| 3-hydroxy-2-butanone <sup>a</sup>  | dark chocolate | 718     | 1296   | nd       | nd   | 5    | 25  | nd   | 1   | 1               | nd   | nd  | 1   |
| 1-octen-3-one <sup>a</sup>         | mushroom       | 988     | 1301   | 25       | 25   | 25   | 25  | 25   | 25  | 25              | 25   | 25  | 1   |
| 2-acetyl-1-pyrroline <sup>b</sup>  | popcorn        | 929     | 1331   | nd       | 5    | 5    | 25  | 25   | 25  | 25              | 25   | 25  | 5   |
| dimethyl trisulfide <sup>a,b</sup> | cabbage        | 978     | 1373   | 25       | 5    | 5    | 25  | 25   | 25  | 25              | 25   | 25  | 5   |
| nonanala                           | tallow         | 1108    | 1406   | 1        | 1    | 1    | 5   | 1    | nd  | nd              | 5    | 1   | 5   |
| (E)-2-octenal <sup>a</sup>         | fat, pungent   | 1058    | 1423   | 25       | 1    | 1    | 5   | 2    | 25  | 5               | 25   | 5   | 25  |
| 1-octen-3-ola                      | mushroom       | 992     | 1438   | 25       | 25   | 5    | 5   | 25   | 25  | 25              | 25   | 5   | 25  |
| methional <sup>b</sup>             | cooked potato  | 912     | 1495   | 5        | 5    | 5    | 25  | 25   | 5   | 5               | 5    | 5   | 5   |
| (Z)-2-nonenala                     | fatty          | 1148    | 1504   | 1        | 25   | 5    | 25  | 25   | 1   | 25              | 25   | 5   | 5   |
| (E)-2-nonenalc                     | tallow         | 1156    | 1529   | 1        | 5    | 25   | 25  | 25   | 25  | 25              | nd   | 5   | nd  |
| (E,Z)-2,6-nonadienala              | cucumber       | 1162    | 1581   | 1        | 5    | 5    | 25  | 25   | 1   | 25              | 25   | 25  | 5   |
| 2-acetylthiazole <sup>a</sup>      | roasted meat   | 1035    | 1674   | 1        | 5    | 5    | 5   | nd   | nd  | 25              | 5    | 25  | nd  |
| (E,E)-2,4-nonadienala              | bitter/hay     | 1204    | 1698   | 25       | 5    | 5    | 25  | 25   | 1   | 5               | 1    | 1   | 1   |
| 2-acetyl-2-thiazolinec             | tobacco        | 1110    | 1728   | nd       | nd   | 5    | 25  | 25   | 1   | 5               | 25   | 5   | 1   |
| (E,Z)-2,4-decadienala              | rancid, fatty  | 1291    | 1752   | 25       | 5    | 5    | 5   | 25   | 1   | 5               | 1    | 1   | 5   |
| (E,E)-2,4-decadienal <sup>a</sup>  | oil/fatty      | 1340    | 1809   | 25       | 5    | 5    | 25  | 25   | 25  | 25              | 25   | 25  | 25  |
| $\beta$ -damascenone <sup>c</sup>  | apple sauce    | 1390    | 1828   | 5        | 5    | 25   | 5   | 5    | 5   | 5               | 1    | 1   | 1   |
| guaiacola                          | smoke          | 1096    | 1862   | 1        | 5    | 5    | 25  | 25   | nd  | 1               | 25   | 1   | 1   |
| vinylguaiacola                     | spices         | 1365    | 2178   | 1        | 5    | 1    | 25  | nd   | nd  | 25              | 25   | 1   | 25  |
|                                    |                |         |        |          |      |      |     |      |     |                 |      |     |     |

<sup>a</sup> Compound positively identified (RI, odor, MS). <sup>b</sup> Compound tentatively identified (RI, odor, FPD). <sup>c</sup> Compound tentatively identified (RI, odor). <sup>d</sup> Not determined.

the main contributor to the green/cut-grass aroma of soy milk due to its low threshold (4.5 ppb in water) (27, 28) and relatively high abundance (3-17.9 ppm) (29), was present in all of the treatments. Other important aroma contributors of soy milk, (E,E)-2,4-nonadienal, (E,Z)-2,4-decadienal, and (E,E)-2,4-decadienal (9, 22, 24, 29), also showed high flavor dilution (FD) factors in all treatments. (E)-2-Nonenal and (Z)-2-nonenal, both compounds reported previously as products of the thermal decomposition of hydroperoxides from linoleic and linolenic acids (30), were identified in the headspace of control and UHT soy milks. The consistent high FD factors observed for 1-octen-3-one and 1-octen-3-ol for all treatments, including the control, suggest that these two compounds might be formed before the heating step in soy milk processing as suggested by Badenhop and Wilkens (31).

For the headspace analysis, volatile sulfur compounds, such as methanethiol, dimethyl sulfide, and dimethyl trisulfide, were detected even in the mildest of the heating conditions and, in general, their concentrations and intensities increased as a result of an increase in UHT temperature. Compounds with roasted aroma notes also increased in intensity as a function of higher processing temperature (Table 3).

The effect of UHT processing was more evident in the FD factors determined by AEDA compared to with DHDA results as shown in Table 4. The highest UHT treatment applied (154 °C/29 s) caused an increase in the number and potency of the aroma components of soy milk. Overall roasted aroma intensity was affected due to the formation of heterocyclic compounds such as 2-acetyl-1-pyrroline, 2-acetylthiazole, and 2-acetyl-2thiazoline, which increased in odor potency at the highest UHT conditions. These compounds, previously reported as main contributors to the "burnt flavor" of roasted soybeans (32) and "cooked flavor" in UHT-processed cow's milk, provide popcorn, roasted, and roasted/burnt aromas, respectively (20, 27). Other roasted odorants that showed an increase in intensity at UHT temperatures were *p*-vinylguaiacol (4-vinylguaiacol) and guaiacol (2-methoxyphenol), which have been reported to be formed by decarboxylation of ferulic acid and decarboxylation followed by an oxidation of p-vinylguiacol, respectively, at high temperatures (33, 34). In addition, it has been suggested that guaiacol can be formed by decarboxylation of vanillin acid (34).

Another positively identified compound that seemed to be greatly affected by an increase in process temperature was 2,3butanedione (diacetyl), as indicated by the five logarithmic (i.e., log<sub>3</sub>) increase in the potency of this odorant in the UHT treated soy milks. In addition, a reduction product of 2,3-butanedione, 3-hydroxy-2-butanone, was also detected in high abundance in the three UHT treatments (Table 6). Other compounds also responsible for sweet/buttery notes include 2-methylbutanal, 3-methylbutanal, and 2,3-pentanedione, which increased in UHT-treated soy milks (Table 5). Increase in the potency of diacetyl and the formation of 3-hydroxy-2-butanone might be partially responsible for the marginal increase of sweet aromatic attribute detected by sensory analysis (Figure 1).

Methional, a thermally generated product of methionine (8, 9, 23, 25), with a characteristic potato-like note, underwent the greatest increase in intensity as a function of higher UHT treatment. This change is consistent with the increase in sulfur flavor perception detected by sensory analysis. Our results agree with those of a similar study in which the aroma compounds generated in cow's milk processed under UHT conditions were investigated (20). These researchers reported that methional became the highest impact aroma-active compound with an FD factor of 128 after UHT processing,  $\delta$ -decalactone ranked second with an FD of 64, and finally 2-acetyl-1-pyrroline and 2-acetylthiazole ranked third with the same FD factors of 32. It is important to highlight that in that study the authors decided to use a storage time of 3 weeks after the milk was processed

Table 4. Major Intermediate and Semivolatile Aroma Components Determined by AEDA of Control (90 °C/10 min) and UHT-Processed Soy Milks

|                                       |                   |                 |                             |                     |                 | flavor dilut | ion factor   |              |
|---------------------------------------|-------------------|-----------------|-----------------------------|---------------------|-----------------|--------------|--------------|--------------|
| compound                              | aroma             | fr <sup>c</sup> | <b>RI</b> <sup>e</sup> FFAP | RI <sup>e</sup> DB5 | 90 °C/ 10 min   | 143 °C/ 14 s | 143 °C/ 59 s | 154 °C/ 29 s |
| dimethyl sulfide <sup>b</sup>         | eggy, corn        | NF              | 769                         | <600                | nd <sup>d</sup> | 81           | 9            | nd           |
| 2-methylbutanal <sup>b</sup>          | dark chocolate    | NF              | 908                         | *                   | 1               | 27           | 9            | 81           |
| 3-methylbutanal <sup>a</sup>          | dark chocolate    | NF              | 913                         | 655                 | 9               | 27           | 9            | 729          |
| pentanal <sup>a</sup>                 | butter            | NF              | 974                         | 736                 | 27              | 9            | 1            | 3            |
| 2.3-butanedione <sup>a</sup>          | butter            | NF              | 982                         | 600                 | 3               | 243          | 243          | 243          |
| 1-penten-3-one <sup>b</sup>           | plastic           | NF              | 1060                        | 683                 | nd              | 27           | nd           | nd           |
| 2.3-pentanedione <sup>a</sup>         | ,<br>milkv        | NF              | 1085                        | 696                 | 27              | 3            | 9            | 81           |
| hexanal <sup>a</sup>                  | leaf              | NF              | 1095                        | 807                 | 243             | 243          | 243          | 729          |
| (Z)-4-heptenal <sup>b</sup>           | tallowy, creamy   | NF              | 1247                        | 853                 | 3               | 81           | 3            | 9            |
| 2-butanone-3-hydroxy <sup>a</sup>     | milky, butter     | NF              | 1283                        | 706                 | nd              | 243          | 243          | 81           |
| 1-octen-3-one <sup>a</sup>            | mushroom          | NF              | 1296                        | 988                 | 27              | 27           | 3            | 81           |
| 2-acetyl-1-pyrroline <sup>a</sup>     | popcorn           | BF              | 1338                        | 925                 | 1               | 27           | 3            | 81           |
| dimethyl trisulfide <sup>a</sup>      | cabbage           | NF              | 1376                        | 963                 | 1               | 9            | 9            | 9            |
| nonanal <sup>a</sup>                  | tallowy, fruity   | NF              | 1385                        | 1108                | 27              | nd           | 1            | 1            |
| (E)-2-octenal <sup>a</sup>            | potato, nuts      | NF              | 1424                        | 1049                | 9               | 81           | 27           | 243          |
| 1-octen-3-ol <sup>a</sup>             | mushroom          | NF              | 1434                        | 1012                | 27              | 243          | 729          | 729          |
| methional <sup>a</sup>                | cooked potato     | NF              | 1442                        | 903                 | 3               | 243          | 243          | 2187         |
| acetic acida                          | vinegar           | AF              | 1445                        | 646                 | 27              | 9            | 9            | 9            |
| (Z)-2-nonenal <sup>b</sup>            | cucumber, potato  | NF              | 1508                        | 1148                | 1               | 9            | 3            | 1            |
| (E,E)-2.4-heptadienal <sup>a</sup>    | fatty, fried      | NF              | 1512                        | 1061                | 3               | 9            | 1            | 1            |
| (E)-2-nonenal <sup>a</sup>            | cucumber          | NF              | 1526                        | 1168                | 243             | 81           | 81           | 243          |
| unknown                               | cucumber          | NF              | 1539                        | *                   | 1               | 27           | 9            |              |
| propanoic acid <sup>a</sup>           | sweatv            | AF              | 1549                        | *                   | 3               | 9            | 9            | 9            |
| (E,E)-3.5-octadien-2-one <sup>a</sup> | milky, candy-like | NF              | 1569                        | 1098                | 9               | 3            | 3            | 3            |
| (E,Z)-2,6-nonadienal <sup>b</sup>     | cucumber          | NF              | 1589                        | 1155                | 81              | 3            | 9            | 9            |
| 2-acetylthiazole <sup>a</sup>         | burnt             | BF              | 1637                        | 1042                | 3               | 9            | 27           | 243          |
| butanoic acida                        | cheesy            | AF              | 1653                        | *                   | 3               | 27           | 27           | 81           |
| unknown                               | beany             | NF              | 1671                        | *                   | 1               | 27           | 9            | 81           |
| (E,E)-2,4-nonadienal <sup>a</sup>     | beany             | NF              | 1691                        | 1210                | 81              | 9            | 243          | 81           |
| 2-acetyl-2-thiazoline <sup>b</sup>    | burnt popcorn     | BF              | 1748                        | 1116                | nd              | 81           | 27           | nd           |
| (E,Z)-2,4-decadienal <sup>a</sup>     | fatty, oxid       | NF              | 1751                        | 1242                | 243             | 81           | 27           | 81           |
| (E,E)-2,4-decadienal <sup>a</sup>     | oven, spices      | NF              | 1812                        | 1345                | 3               | 243          | 9            | 243          |
| hexanoic acid <sup>a</sup>            | sweaty, ham       | AF              | 1824                        | *                   | 9               | 9            | 9            | 27           |
| $\beta$ -damascenone <sup>b</sup>     | apple sauce       | NF              | 1830                        | 1397                | 3               | 81           | 27           | 3            |
| ,<br>guaiacol <sup>a</sup>            | spices, oven      | NF              | 1841                        | 1097                | 1               | 81           | 81           | 243          |
| unknown                               | medicine          | NF              | 1859                        | *                   | nd              | 3            | 3            | 1            |
| $\delta$ -octalactone <sup>b</sup>    | burnt sugar       | NF              | 1974                        | 1288                | 1               | 27           | 9            | 1            |
| maltol <sup>a</sup>                   | burnt sugar       | NF              | 2019                        | 1133                | nd              | 9            | 9            | 9            |
| octanoic acid <sup>a</sup>            | sweaty            | AF              | 2028                        | *                   | 9               | 9            | 9            | nd           |
| $\gamma$ -nonalactone <sup>a</sup>    | burnt caramel     | NF              | 2044                        | 1363                | 1               | 81           | 9            | 3            |
| p-cresol <sup>b</sup>                 | medicine          | NF              | 2053                        | 1085                | 1               | 3            | 27           | 243          |
| vinylguaiacol <sup>a</sup>            | phenolics, oven   | NF              | 2193                        | 1365                | nd              | nd           | 27           | 27           |
| skatole <sup>b</sup>                  | mothball-like     | NF              | 2452                        | 1380                | nd              | 1            | 1            | 27           |
|                                       |                   |                 |                             |                     |                 |              |              |              |

<sup>a</sup> Compound positively identified (RI, odor, MS). <sup>b</sup> Compound tentatively identified (RI, odor). <sup>c</sup> Organic fraction in which compound was detected (NF, neutral fraction; AF, acidic fraction; BF, basic fraction). <sup>d</sup> Not detected. <sup>e</sup> Retention index.

under the assumption that in the first 2 weeks reactive sulfurcontaining compounds were degrading (20, 35). In the present study, the low-threshold sulfur-containing odorants methanethiol and dimethyl sulfide increased with processing temperature. Both compounds have been associated with methionine (36) and methional (37) as precursor and intermediate, respectively, and they have been previously identified in the headspace of aqueous solutions of soy protein isolates (37). Dimethyl trisulfide increased 3-fold in relation to the control (90 °C/10 min), and it followed the same trend as the other sulfurcontaining odorants. The degradation trend of methional (threshold of 0.2 ppb) (28) toward lower threshold oxidative products methanethiol (0.02 ppb) (37), dimethyl sulfide, and dimethyl trisulfide (0.01 ppb) (38) may be responsible for the increase in sulfur aroma detected in the sensory analysis, where the highest treatment applied (154 °C/29 s) produced the highest score in sulfur aromas.

Effect of Storage on Major Odorants in UHT Soy Milk. The potency of methional decreased with storage for soy milks treated for 143 °C/14 s and 154 °C/29 s (Table 5). Dimethyl sulfide was also reduced after 7 days of refrigeration. However, dimethyl trisulfide, a known degradation product of methional, increased from day 0 to day 7. It is possible that the degradation of methional toward the formation of highly volatile sulfur compounds such as dimethyl sulfide continues even at low temperatures, being significant after 7 days of refrigerated storage.

A marginal reduction was observed in the perception of compounds with sweet aromas after 7 days of storage. This reduction might be explained by the ambiguous trend found in odorants with buttery and sweet aromas. For instance, the potency of 3-hydroxy-2-butanone, 2,3-pentanedione, and 2-methylbutanal was reduced beyond two serial dilutions after 7 days of refrigeration. However, other sweet aroma volatiles such as 2,3-butanedione,  $\delta$ -octalactone, and  $\gamma$ -nonalactone remained constant or underwent only minimal change.

Other important contributors to the aroma of soy milk such as hexanal, 1-octen-3-ol, (E)-2-nonenal, (E,Z)-2,6-nonadienal, (E,E)-2,4-nonadienal, (E,Z)-2,4-decadienal, and (E,E)-2,4-decadienal did not change over 7 days of refrigerated storage.

The effect of storage on the concentration of some selected key aroma compounds in soy milk is shown in **Table 6**. In accordance with the trend described above by AEDA, diacetyl and sulfur compounds seem to be strongly affected by heat and

#### Table 5. Effect of Storage (4.4 °C) on the Major Aroma Components of UHT Soy Milk

|                                                     |                 |     |        |        |         |         | flavor dilu | tion factor |         |         |
|-----------------------------------------------------|-----------------|-----|--------|--------|---------|---------|-------------|-------------|---------|---------|
|                                                     |                 |     |        |        |         | day 0   |             |             | day 7   |         |
| compound                                            | oromo           | fre |        |        | 143 °C/ | 143 °C/ | 154 °C/     | 143 °C/     | 143 °C/ | 154 °C/ |
| compound                                            | aiuilla         | 11- | NEFFAF | KI DB3 | 14 5    | 09.5    | 295         | 14 5        | 59.5    | 295     |
| dimethyl sulfide <sup>b</sup>                       | eggy, corn      | NF  | 769    | <600   | 81      | 9       | nda         | nd          | nd      | 3       |
| 2-methylpropanal <sup>b</sup>                       | peanut          | NF  | 836    | <600   | 1       | 1       | 9           | nd          | 9       | 81      |
| 2-methylbutanal <sup>a</sup>                        | dark chocolate  | NF  | 908    | *      | 27      | 9       | 81          | 3           | 3       | 3       |
| 3-methylbutanal <sup>a</sup>                        | dark chocolate  | NF  | 913    | 655    | 27      | 9       | 729         | 1           | 27      | 243     |
| pentanal <sup>a</sup>                               | butter          | NF  | 974    | 736    | 9       | 1       | 3           | 9           | nd      | 9       |
| 2,3-butanedione <sup>a</sup>                        | butter          | NF  | 982    | 600    | 243     | 243     | 243         | 243         | 243     | 243     |
| unknown                                             | plastic         | NF  | 1060   | 683    | 27      | nd      | nd          | nd          | 1       | nd      |
| 2,3-pentanedione <sup>b</sup>                       | milky           | NF  | 1085   | 696    | 3       | 9       | 81          | 3           | 1       | 3       |
| hexanal <sup>a</sup>                                | leaf            | NF  | 1095   | 807    | 243     | 243     | 729         | 243         | 243     | 243     |
| 1-penten-3-ol <sup>a</sup>                          | pungent         | NF  | 1126   | 695    | 243     | 9       | 3           | 27          | 1       | nd      |
| 2-heptanone <sup>a</sup>                            | fatty           | NF  | 1181   | *      | 1       | 3       | 9           | 1           | 3       | nd      |
| (Z)-4-heptenal <sup>b</sup>                         | tallowy, sweet  | NF  | 1247   | 853    | 81      | 3       | 9           | 3           | 3       | 3       |
| 3-hydroxy-2-butanone <sup>a</sup>                   | milky, butter   | NF  | 1283   | 706    | 243     | 243     | 81          | 27          | 9       | 27      |
| 1-octen-3-one <sup>a</sup>                          | mushroom        | NF  | 1296   | 988    | 27      | 3       | 81          | 81          | 9       | 243     |
| 2-acetyl-1-pyrroline <sup>a</sup>                   | popcorn         | BF  | 1338   | 925    | 27      | 3       | 81          | 9           | 1       | 243     |
| dimethyl trisulfide <sup>a</sup>                    | cabbage         | NF  | 1376   | 963    | 9       | 9       | 9           | 81          | 27      | 3       |
| nonanal <sup>a</sup>                                | tallowy, fruity | NF  | 1385   | 1108   | nd      | 1       | 1           | nd          | nd      | nd      |
| (E)-2-octenal <sup>a</sup>                          | potato, nuts    | NF  | 1423   | 1049   | 81      | 27      | 243         | 3           | 81      | 243     |
| 1-octen-3-ol <sup>a</sup>                           | mushroom        | NF  | 1434   | 1012   | 243     | 729     | 729         | 81          | 243     | 243     |
| methional <sup>a</sup>                              | cooked potato   | NF  | 1442   | 903    | 243     | 243     | 2187        | 3           | 243     | 9       |
| acetic acid <sup>a</sup>                            | vinegar         | AF  | 1445   | 646    | 9       | 9       | 9           | 3           | 9       | 9       |
| (Z)-2-nonenal <sup>b</sup>                          | cucumber, hay   | NF  | 1508   | 1148   | 9       | 3       | 1           | nd          | 81      | 9       |
| (E,E)-2,4-heptadienal <sup>a</sup>                  | fatty, fried    | NF  | 1512   | 1061   | 9       | 1       | 1           | 9           | 3       | 3       |
| (E)-2-nonenal <sup>a</sup>                          | cucumber        | NF  | 1526   | 1168   | 81      | 81      | 243         | 27          | 81      | 243     |
| unknown                                             | cucumber        | NF  | 1539   | *      | 27      | 9       | 1           | 3           | 81      | nd      |
| propanoic acid <sup>a</sup>                         | sweaty          | AF  | 1549   | *      | 9       | 9       | 9           | 3           | 9       | 3       |
| (E,E)-3,5-octadien-2-one <sup>a</sup>               | milky, fruity   | NF  | 1569   | 1098   | 3       | 3       | 3           | nd          |         | 9       |
| ( <i>E</i> , <i>Z</i> )-2,6-nonadienal <sup>b</sup> | cucumber        | NF  | 1589   | 1155   | 3       | 9       | 9           | 9           | 81      | 243     |
| 2-acetylthiazole <sup>a</sup>                       | burnt           | BF  | 1637   | 1042   | 9       | 27      | 243         | 27          | 27      | 9       |
| butanoic acid <sup>a</sup>                          | chessy          | AF  | 1653   | *      | 27      | 27      | 81          | nd          | 9       | 27      |
| unknown                                             | beany           | NF  | 1671   | *      | 27      | 9       | 81          |             | nd      | nd      |
| (E,E)-2,4-nonadienal <sup>a</sup>                   | beany           | NF  | 1691   | 1210   | 9       | 243     | 81          | 27          | 243     | 243     |
| 2-acetyl-2-thiazoline <sup>b</sup>                  | burnt popcorn   | BF  | 1748   | 1116   | 81      | 27      | nd          | 3           | 1       | nd      |
| (E,Z)-2,4-decadienal <sup>a</sup>                   | fatty, oxid     | NF  | 1752   | 1242   | 81      | 27      | 81          | 81          | 243     | 243     |
| (E,E)-2,4-decadienal <sup>a</sup>                   | oven, spices    | NF  | 1812   | 1327   | 243     | 9       | 243         | 81          | 27      | 243     |
| hexanoic acid <sup>a</sup>                          | sweaty, ham     | AF  | 1824   | *      | 9       | 9       | 27          | 9           | 27      | 27      |
| $\beta$ -damascenone <sup>b</sup>                   | apple sauce     | NF  | 1830   | 1397   | 81      | 27      | 3           | 27          | 81      | 27      |
| guaiacol <sup>a</sup>                               | spices, oven    | NF  | 1841   | 1097   | 81      | 81      | 243         | 81          | 81      | 243     |
| unknown                                             | medicine        | NF  | 1859   | *      | 3       | 3       | 1           | 1           | nd      | nd      |
| $\delta$ -octalactone <sup>b</sup>                  | burnt sugar     | NF  | 1967   | 1288   | 27      | 9       | 1           | 1           | 3       | 1       |
| maltol <sup>a</sup>                                 | burnt sugar     | NF  | 2019   | 1133   | 9       | 9       | 9           | 1           | nd      | nd      |
| octanoic acid <sup>a</sup>                          | sweaty          | AF  | 2028   | *      | 9       | 9       | 1           | nd          | 9       | 3       |
| $\gamma$ -nonalactone <sup>a</sup>                  | burnt caramel   | NF  | 2044   | 1363   | 81      | 9       | 3           | 27          | 81      | 27      |
| p-cresol <sup>b</sup>                               | medicine        | NF  | 2054   | 1085   | 3       | 27      | 243         | 81          | 3       | 9       |
| vinylguaiacol <sup>a</sup>                          | burnt           | NF  | 2193   | 1365   | 1       | 27      | 27          | 9           | 81      | 243     |
| skatole <sup>b</sup>                                | mothball-like   | NF  | 2452   | 1380   | 1       | 1       | 27          | nd          | 1       | 9       |
|                                                     |                 |     |        |        |         |         |             |             |         |         |

<sup>a</sup> Compound positively identified (RI, odor, MS). <sup>b</sup> Compound tentatively identified (RI, odor). <sup>c</sup> Organic fraction in which compound was detected (NF, neutral fraction; AF, acidic fraction; BF, basic fraction). <sup>d</sup> Not detected. <sup>e</sup> Retention index.

short-time storage time. Short-time storage affected the concentration of some lipid-generated compounds and Strecker degradation compounds, but the main effect was on sulfur compounds such as methional, thus corroborating the hypothesis that methional could have decomposed to form other more reactive sulfur compounds after processing.

**Changes in Color.** UHT processing conditions for soy milk also seemed to cause color changes. Lightness, chroma, and hue values were calculated from the original L, a, and b values as shown in **Table 7**. Lightness, hue, and chroma average values indicate that heating conditions in the UHT range generated a more yellow and saturated color in soy milk in comparison to the original color obtained with temperature conditions of the control (90 °C/10 min).

Lightness, which is an indicator of the light-reflecting capacity of the milk, did not change significantly (p > 0.05) with an increase in processing temperature, contrary to the trend found by Rhim et al. (40) and Van Buren et al. (41). This behavior could be explained by the small content of heat-coagulable protein (42) in soy milk. In UHT-treated soy milks, chroma values, which describe the degree of saturation or purity of color, increased in comparison to the control. Among the UHT treatments, 143 °C/59 s had the biggest incremental change, and this indicates that a longer exposure to heat increases the degree of color saturation of soy milk even more than a higher temperature with a shorter exposure (154 °C/29 s). These results are in accordance with the results found by Kwok and others (43), who suggested that this color saturation increment is related to the time of processing and follows zero-order kinetics.

Finally, hue values, which are a representation of the color change in terms of red, orange, yellow, green, and blue, seemed to be reduced by the heat treatments in the UHT region. This reduction in seven degrees resulted in a yellower color of the

| Table 6. | Average ( | n = 2 | Concentrations | (Micrograms | per L | iter ± | Standard | Deviation | ) of Selecte | d Heat-Induced | Odorants | in UHT | Soy | Milk |
|----------|-----------|-------|----------------|-------------|-------|--------|----------|-----------|--------------|----------------|----------|--------|-----|------|
|          |           |       |                |             |       |        |          |           |              |                |          |        |     |      |

|                                            |                    |                  | da               | ay 0             |                   |                  | day 7            |                   |
|--------------------------------------------|--------------------|------------------|------------------|------------------|-------------------|------------------|------------------|-------------------|
|                                            |                    | 90 °C/           | 143 °C/          | 143 °C/          | 154 °C/           | 143 °C/          | 143 °C/          | 154 °C/           |
| compound                                   | aroma              | 10 min           | 14 s             | 59 s             | 29 s              | 14 s             | 59 s             | 29 s              |
| methanethiol <sup>a</sup>                  | cooked potato      | $nd \pm nd$      | $0.8 \pm 0.0$    | $2.2\pm0.1$      | 1.3 ± 0.1         | $0.3 \pm 0.1$    | $0.7\pm0.1$      | $0.2\pm nd$       |
| 3-methylbutanal                            | dark chocolate     | $0.3 \pm 0.2$    | $3.3\pm0.4$      | $8.6 \pm 6.1$    | 4.1 ± 1.6         | $0.7 \pm 0.2$    | $1.9 \pm 1.4$    | $1.1 \pm 0.6$     |
| pentanal                                   | buttery            | $0.9\pm0.5$      | $0.1 \pm nd$     | $3.8 \pm 2.7$    | $4.2 \pm 1.4$     | $3.1\pm nd$      | $5.6 \pm 3.9$    | $0.7\pm0.0$       |
| 2,3-butanedione                            | buttery            | $0.1 \pm 0.1$    | $9.7 \pm 3.9$    | $9.6 \pm 2.4$    | $9.5 \pm 4.6$     | $5.1 \pm 1.3$    | $7.5 \pm 0.2$    | $2.2 \pm 0.8$     |
| hexanal                                    | leaf               | $142.9 \pm 10.0$ | $146.9 \pm 52.3$ | $156.2 \pm 67.2$ | $248.7 \pm 6.8$   | $146.3 \pm 13.4$ | $172.8 \pm 5.0$  | $144.5 \pm 15.6$  |
| 1-penten-3-ol                              | pungent            | $101.7 \pm 38.4$ | $101.2 \pm 8.5$  | $64.8\pm34.4$    | $68.4\pm3.8$      | $59.6 \pm 22.9$  | $45.2 \pm 6.5$   | $37.7 \pm 25.2$   |
| 3-hydroxy-2-butanone                       | milky, butter      | $nd \pm nd$      | $536.0 \pm 15.2$ | $366.2 \pm 0.1$  | $174.4 \pm 1.5$   | $366.9 \pm 37.1$ | $221.8 \pm 7.5$  | $52.5 \pm 21.0$   |
| dimethyl trisulfide <sup>a</sup>           | cabbage            | $0.0\pm nd$      | $1.4 \pm 0.1$    | $2.9 \pm 0.1$    | $0.9 \pm 0.1$     | 1.6± nd          | $2.4 \pm 1.7$    | $0.8 \pm 0.4$     |
| 1-octen-3-ol                               | mushroom           | $343.8 \pm 29.7$ | 323.5 ±53.1      | $334.4 \pm 91.0$ | $314.9 \pm 143.4$ | $303.2\pm78.9$   | $299.2 \pm 79.4$ | $359.9 \pm 122.2$ |
| (E)-2-octenal                              | nuts, almonds      | $8.9 \pm 0.2$    | $nd \pm nd$      | $nd \pm nd$      | $nd \pm nd$       | $nd \pm nd$      | $nd \pm nd$      | $nd \pm nd$       |
| methional <sup>a</sup>                     | cooked potato      | $0.01 \pm nd$    | $0.03 \pm 0.02$  | $0.1 \pm 0.02$   | $0.2 \pm 0.1$     | $0.02 \pm 0.01$  | $0.10 \pm 0.0$   | $0.14 \pm 0.01$   |
| acetic acid                                | vinegar            | $168.7 \pm 54.5$ | $147.8 \pm 62.7$ | $327.9 \pm 16.8$ | $194.2 \pm 48.8$  | $138.3 \pm 39.4$ | $89.2\pm6.0$     | $121.8 \pm 40.3$  |
| (E,E)-2,4-heptadienal                      | fatty, fried       | $23.2 \pm 8.9$   | $18.0 \pm 8.0$   | $16.2 \pm 14.7$  | $9.7 \pm 2.9$     | $6.6 \pm 3.3$    | $6.3 \pm 1.9$    | $13.8\pm4.6$      |
| (E)-2-nonenal                              | fried, hay         | $0.4\pm nd$      | $nd \pm nd$      | $nd \pm nd$      | $nd \pm nd$       | $nd \pm nd$      | $nd \pm nd$      | $nd \pm nd$       |
| propanoic acid                             | sweaty             | $7.3\pm nd$      | $7.4 \pm nd$     | $26.6 \pm 8.7$   | $8.8 \pm 0.9$     | $9.0 \pm 4.1$    | $5.1 \pm 1.0$    | $6.2 \pm 3.3$     |
| ( <i>E</i> , <i>E</i> )-3,5-octadien-2-one | cucumber           | $28.4 \pm 6.1$   | $10.6 \pm 4.7$   | $7.7 \pm 4.7$    | $10.9 \pm 0.7$    | $5.0 \pm 0.7$    | $10.8 \pm 4.7$   | $5.5\pm0.8$       |
| 2-acetylthiazole                           | burnt              | $nd \pm nd$      | $15.9 \pm 11.3$  | $19.4 \pm 7.1$   | $7.4 \pm 3.5$     | $nd \pm nd$      | $19.3 \pm 4.0$   | $18.7\pm nd$      |
| butanoic acid                              | cheesy             | $36.9 \pm 1.9$   | $30.9 \pm 3.6$   | $59.5 \pm 5.9$   | $51.4 \pm 5.9$    | $36.6 \pm 10.5$  | $29.7 \pm 25.1$  | $79.8\pm24.6$     |
| (E,E)-2,4-nonadienal                       | beany              | $3.8 \pm 0.1$    | $7.5 \pm 0.4$    | $7.5 \pm 1.3$    | $10.9 \pm 4.3$    | 2.7±1.00         | $5.3 \pm 1.3$    | $6.8\pm0.9$       |
| (E,Z)-2,4-decadienal                       | fatty, oxid        | $3.6 \pm 2.9$    | $3.0 \pm 2.1$    | $3.5\pm0.7$      | $5.8 \pm 1.8$     | $1.1 \pm 0.6$    | $2.8\pm0.9$      | $5.6 \pm 0.1$     |
| (E,E)-2,4-decadienal                       | oven, spices       | $43.0 \pm 5.6$   | $35.0 \pm 3.4$   | $42.8\pm5.6$     | $67.4 \pm 24.2$   | $26.4\pm6.00$    | $43.5\pm3.0$     | $49.7 \pm 0.1$    |
| hexanoic acid                              | sweaty, ham        | $456.7 \pm 26.3$ | $156.1 \pm 15.6$ | 188.1±20.1       | $256.9 \pm 3.1$   | $144.5 \pm 1.6$  | $150.2 \pm 41.0$ | $159.5 \pm 15.1$  |
| guaiacol                                   | spices, oven       | $nd \pm nd$      | 3.4± 2.1         | $6.2 \pm 1.7$    | $5.5 \pm 2.0$     | $0.2 \pm 0.1$    | $0.4 \pm 0.3$    | $0.1 \pm 0.1$     |
| octanoic acid                              | sweaty, horse-like | $28.6 \pm 10.7$  | $13.5\pm nd$     | $15.7 \pm 3.5$   | $19.4 \pm 3.5$    | $11.4 \pm 1.3$   | $15.4 \pm 2.6$   | $35.3\pm33.0$     |
| $\gamma$ -nonalactone                      | burnt caramel      | $7.8\pm12.7$     | $12.2 \pm 10.7$  | $11.8 \pm 4.7$   | $12.7 \pm 0.9$    | $11.4 \pm 3.1$   | $17.0\pm6.4$     | $19.7\pm11.8$     |
| vinyl guaiacol                             | phenolics, oven    | $2.8\pm0.7$      | $1.5\pm0.5$      | $4.8\pm3.2$      | $7.6\pm1.5$       | $0.4\pm0.1$      | $8.9\pm4.9$      | $11.5\pm15.8$     |

<sup>a</sup> Compound analyzed by flame photometric detection.

Table 7. Color Changes in Soy Milk Treated at UHT Conditions<sup>a</sup>

| treatment                                                 | lightness                                                                                                    | chroma                                                                                                                              | hue                                                                                                                                   |
|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| 143 °C/14 s<br>143 °C/59 s<br>154 °C/29 s<br>90 °C/10 min | $61.8 \pm 0.2 \text{ a}$<br>$61.7 \pm 0.2 \text{ a}$<br>$61.0 \pm 0.2 \text{ a}$<br>$61.2 \pm 0.2 \text{ a}$ | $\begin{array}{c} 14.3 \pm 0.2 \text{ a} \\ 15.2 \pm 0.2 \text{ b} \\ 13.6 \pm 0.2 \text{ ac} \\ 9.7 \pm 0.2 \text{ d} \end{array}$ | $\begin{array}{c} 100.8 \pm 0.5 \text{ a} \\ 99.7 \pm 0.5 \text{ a} \\ 99.6 \pm 0.5 \text{ a} \\ 106.3 \pm 0.4 \text{ b} \end{array}$ |

<sup>a</sup> Within columns, different letters indicate significant difference at p < 0.05.

milk by the heat treatment (p < 0.05). This color change has been reported mainly as an effect of Maillard reactions occurring in soy milk following heat treatment (27).

UHT conditions commercially used to produce shelf-stable soy milk create heat-induced roasted and sulfur odorants in soy milk and increase the potency of other, mainly lipid-derived, volatiles normally found in soy milk. The pathway of the formation of sulfur compounds may be explained by an increase in the potency of methional and its posterior decomposition to yield methanethiol, which then oxidizes to dimethyl sulfide, a reactive molecule that can promote the appearance of more sulfur compounds such as dimethyl disulfide and dimethyl trisulfide. Sweet aromatic perception changed only marginally due to UHT heat treatment. This may be related to the increase in potency of 2,3-butanedione and the formation of 3-hydroxy-2-butanone, a reduction product of 2,3-butanedione. Storage time after processing affects the overall aroma and sulfur perception of soy milk. One day of storage at refrigeration temperature (4.4 °C) caused no significant changes in the perception of sulfur compounds. Seven days showed a significant decrease in the sulfur intensity in all of the treatments. Further storage studies at refrigeration and room temperatures are advised in order to assess sulfur reactivity beyond 7 days of storage. Results found in this study can benefit the industry by enabling the production of soy milk with better flavor properties and reducing costs

associated with the removal or masking of heat-induced odorants.

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