Effect of Gallic Acid on the Aroma Constituents of Soymilk and Soy Protein Isolates

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ABSTRACT: Volatile compounds from soymilk were analyzed by gas chromatography/olfactometry/mass spectrometry (GCO/MS) with direct injection of various volumes of static headspaces. The most powerful odorants, determined by the minimum headspace volume required to detect by olfactometry, were (i) hexanal, (ii) acetaldehyde, (iii) methanethiol, (iv) dimethyl trisulfide (DMTS), and (v) 2-pentyl furan. Analyses of soymilk prepared with the addition of 100 ppm gallic acid revealed that the only two detectable odorants were hexanal and acetaldehyde. Sensory analyses of the soymilk treated with 100 ppm gallic acid produced a significantly lower score ($P = 0.0006$) for overall odor intensity compared with the control soymilk. Aqueous slurries of soy protein isolates (SPI) prepared with the addition of 100 ppm gallic acid also had lower odor intensities than the control SPI ($P < 0.0001$). GCO/MS analyses of headspace volatiles revealed that the gallic acid treatment had removed all detectable levels of methanethiol and DMTS while having no significant effect on the level of hexanal ($P = 0.81$).

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KEY WORDS: Acetaldehyde, dimethyl trisulfide, gallic acid, headspace volatiles, hexanal, methanethiol, olfactory analysis, soymilk, soy protein isolates.

Although soybeans provide a high-quality protein, and there are increasing reports of health benefits from consuming soy protein products (1), the demand for soybeans in human foods in the Western world has not been large. In 1971, less than 1% of the U.S. soybean crop was used as a protein source for human foods (2), and in 1999, this value was about 2% (3). This lack of utilization in the West is due largely to the undesirable flavor and odor associated with soy products (4–7). Jorge *et al.* (8) demonstrated that the introduction of as little as 2% powdered soymilk into chocolate significantly lowered sensory scores, and levels above 6% were deemed unacceptable. The components that constitute the characteristic odor of soy products have been thought to include aliphatic carbonyls, volatile FA, amines, alcohols, or furans that were derived, in part, from the action of soybean lipoxygenase (LOX) and subsequent formation of lipid oxidation products (2,9). Much work has been accomplished toward developing soybean mutants that lack the LOX (EC 1.13.11.12) isozymes (LOX-1, -2, and -3) (10,11). Using these mutants, Shen *et al.* (12,13) demonstrated that soybean oils prepared from LOX-null soybeans showed no significant improvement in oil stability. Davies *et al.* (14) reported that unblanched soymilk prepared from Century variety soybeans that lacked LOX-2 had a statistically significant reduction in "beany" odor compared to soymilk prepared from traditional Century variety soybeans (this represented a reduction in beany odor from about 4.8 to about 4 on a 10-point scale, with 10 being the strongest). Soymilk prepared from soybeans lacking LOX-1, LOX-3, LOX-1 + LOX-3, or LOX-2 + LOX-3 had higher levels of beany odor. Kobayashi *et al.* (15) analyzed solvent extracts of unblanched soybean slurries by gas chromatography/olfactometry (GCO), GC–MS, and aroma extract dilution analysis. They concluded that the main odor contributors were *trans,trans*-2,4-nonadienal, *trans,trans*-2,4-decadienal, hexanal, 2-pentyl furan, 1-octen-3-one, *trans*-2-nonenal, *trans, cis*-2,4-nonadienal, and an unidentified compound. The distillation method used by Kobayashi *et al.* (15) could not have detected methanethiol, acetaldehyde, or any other volatile compound that had a boiling point below that of ethyl ether (35°C). Also, because soymilk sold in the United States, and most of the world, is typically heated (blanched)—to deactivate the LOX, other degradative enzymes, and trypsin inhibitors—and subsequently pasteurized, research on unblanched soymilk (and soybean slurries) has limited application. Torres-Penaranda and others (16) reported that the "raw beany" aroma and flavor of blanched soymilk made from LOX-null soybeans were perceived to be lower by U.S.-born judges, higher by Chinese-born judges, and not different by Japanese-born judges when compared to soymilk prepared from traditional soybeans. A reduction of "cooked beany" aroma and flavor was reported with the use of lipoxygenase-null soybeans. The overall intensity of the soymilk aroma was not addressed. King *et al.* (17) demonstrated that there were no differences in beany flavor scores between bread, ground beef patties, and soy beverages prepared with soy flour made from normal or LOX-free soybeans.

In a previous investigation (18), GCO was used to identify major odorants from the headspace of aqueous solutions of soy protein isolates (SPI) using both static and dynamic headspace methods. Based on dynamic headspace analyses, the most powerful odorants were (i) dimethyl trisulfide (DMTS), (ii) methanethiol, (iii) hexanal, (iv) an unidentified charred sweaty feet-like odor, (v) 2-pentyl furan, (vi) 2,3-butadione, and (vii) an unknown burnt-like odor. The most powerful odorants by static headspace analyses were (i) DMTS, (ii)

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hexanal, (iii) methanethiol, and (iv) 2-pentyl furan. By using deuterium-labeled DMTS as an internal standard, DMTS was quantified at 60.1 and 45.5 ppb in the SPI on a dry basis. This corresponds to odor values of 301 and 228, respectively, in the 5% aqueous SPI slurries.

Methanethiol was quantified in slurries prepared from two different soy protein concentrates (SPC) and two different SPI using a modification of the method described by Guth and Grosch (19) with ethanethiol as an internal standard (20). Methanethiol levels were 172 and 237 ppb on a dry basis in the SPC and 237 and 167 ppb dry basis in the SPI. These values correspond to odor values of 86 and 118 in 10% SPC slurries and 80 and 56 in 6.7% SPI slurries.

This investigation was undertaken to evaluate the contribution of methanethiol and DMTS to the odor of soymilk by static headspace analyses, to find a practical and effective means to minimize the occurrence of methanethiol and/or DMTS in soy products, to determine whether such a treatment affects other primary odorants, and to determine how this treatment affects the odor intensities of soy products.

EXPERIMENTAL PROCEDURES

Chemicals. Hexanal, DMTS, methanethiol, acetaldehyde, *trans,trans*-2,4-nonadienal, *trans,trans*-2,4-decadienal, gallic acid, and (–)-epigallocatechin gallate (EGCg) were obtained from Sigma-Aldrich Chemical Co. (St. Louis MO). Bedoukian Research, Inc. (Danbury, CT) donated 2-pentyl furan.

Preparation of soymilk and SPI. Burlison variety soybeans were obtained from Purdue University USDA-ARS soybean breeding and genetics program (West Lafayette, IN) and the University of Illinois plant breeding and genetics program (Champaign-Urbana, IL). Whole soybeans were soaked in water for 10 h, drained, and rinsed several times. The hydrated soybeans were combined with water (1 part soybeans to 10 parts water). The soybean/water mixture was then ground in a Waring blender on medium speed for 1 min. The resulting slurry was immediately transferred to a glass flask and placed in a boiling water bath. The soybean slurry was stirred and brought to 85°C within 8 min. where it was held for 15 min. The soymilk was then cooled to 40°C in an ice bath, filtered through cheesecloth, bottled, and refrigerated (4°C). Gallic acid was dissolved in deionized water (0.2 g per 100 mL) and brought to pH 7.5 with the addition of 0.15 M $Ca(OH)_2$. The gallic acid premix was prepared fresh every day. Additions of gallic acid solutions replaced an equal amount of the processing water (prior to grinding the soybeans) to achieve an overall concentration of 100 ppm.

Laboratory SPI was prepared by dispersing hexane-defatted soybean flour (obtained from Protein Technologies International Inc., St. Louis, MO) in water (1 part flour to 10 parts water) at 22°C followed by additions of 1 N sodium hydroxide, as needed, until a pH of 9 was achieved and maintained for 15 min (21). After centrifugation at $1500 \times g$ for 10 min, the supernatant was adjusted to a pH of 4.5 with 1 N HCl to precipitate proteins. If gallic acid was added, the pH was reduced to 8, the gallic acid and water premix was added (100 ppm of protein slurry), and the pH was held at 8 for 10 min before further lowering to 4.5. Following centrifugation at $1500 \times g$ for 10 min, the precipitate was washed twice with water, and the protein isolate was adjusted to pH 7 with 1 N NaOH. The resulting slurry was immediately transferred to a glass flask and placed in a boiling water bath. The protein slurry was stirred and brought to 77°C within 4 min, where it was held for 15 s, cooled to 40°C in an ice bath, and freeze-dried.

Static headspace analyses. For static headspace analyses, soymilk or 5% SPI solutions (300 mL) were placed in a 1-L flask sealed with a septum and stirred. After 1 h, various volumes of the unconcentrated headspace were withdrawn using a 25-mL gas-tight syringe (preheated to 45°C) equipped with an inert gas sampling valve.

GCO/MS. GCO/MS was accomplished on a Hewlett-Packard Model 5890 Series II GC with a 5971A mass spectrometer, an ms-NoVent system (SGE International, Ringwood, Australia) and an indirect liquid-nitrogen trap (SGE International) at the beginning of the column to cryo-focus analytes. The injection sequence began by bringing the liquid-nitrogen trap to –60°C. The purge valve was closed for the first 2 min of the run. The ms-NoVent was then turned on, and the GC inlet septum purge was blocked. Up to 25 mL of the sample headspace was injected at a rate of 5 mL/min followed by a 2 min wait. The ms-NoVent was then turned off, followed by a 0.5 min wait. The cap on the septum purge was removed, the flow of nitrogen to the cryo-trap was stopped, and the GC run was begun. The column was an EC-5 capillary column (30 m × 0.53 mm i.d.) with 1.2 μm film thickness (Alltech Associates, Inc., Deerfield, IL). The helium flow rate through the columns was about 3 mL/min with 2 mL/min emerging from the sniff port (SGE International). The column temperature was held at 40°C for 2 min, then increased at 5°C/min to 165°C where it was held for 5 min, then to 220°C at 20°C/min, where it was held for 2.75 min. The electron ionization detector was set to detect in the mass range of 35 to 350 *m/z*. The injection port temperature was maintained at 130°C. All determinations were performed in duplicate. Minimum reported headspace volumes necessary to detect odorants by olfaction required confirmation by two investigators. Identification of compounds were by (i) comparison of mass spectra to a spectral database (NIST98) (ChemSW, Inc., Fairfield, CA); (ii) comparison to retention times of authentic standards; and (iii) comparison of olfactory response to authentic standards.

Quantification of adorants. Selected headspace odorants were quantified by setting the electron ionization detector to detect selected ions (SIM) in order to increase the sensitivity of the detector. Ethanethiol was used as an internal standard by first injecting 10 mL headspace from a soy product with cryofocusing followed by injecting 13.4 ng ethanethiol in 10 μL. The ethanethiol standard was prepared by flushing a 250-mL amber bottle with He for 10 min and then sealing with an inert headspace-sampling valve. A vacuum (125 mm Hg

vacuum-gauge) was pulled on the bottle using a vacuum pump attached to a 22-gauge needle. After heating the bottle to 45°C, 0.4 μL of ethanethiol was injected into the bottle, which was placed in a 45°C oven for at least 30 min prior to use. Injections of both the sample and the standard used gas-tight syringes with an inert needle valve. Methanethiol, DMTS, acetaldehyde, and hexanal were quantified by the ratio of *m/z* 47, 126, 29, and the sum of 44 and 56, respectively (at the appropriate retention times), to the *m/z* 47 and 62 for the internal standard ethanethiol. Standard curves for acetaldehyde, hexanal, and DMTS with ethanethiol as an internal standard in the appropriate range were used (Fig. 1). The ratios of the relative abundances of the selected ions were plotted against the weight ratio of ethanethiol over the sum of acetaldehyde, DMTS, or hexanal and ethanethiol. The response of methanethiol (*m/z* 47) was considered a 1:1 response with the *m/z* 47 and 62 from ethanethiol (these ions represent 31.7 and 33.8%, respectively, of the total ions produced by MS under the conditions describe). The areas of *m/z* 44 and 56 for hexanal and *m/z* 81 for 2-pentyl furan were equated on a 1:1 weight basis to estimate the concentrations of 2-pentyl furan. The injection port temperature was maintained at 130°C. Duplicate injections were performed for two separately prepared slurries for both the control and treated SPI.

Statistical evaluations of effect of treatments on the level of methanethiol, hexanal, and DMTS in the headspace above SPI slurries were done using the Statistical Analysis System (22) software package. Analyses of variance were performed by the ANOVA procedure. Least significant difference (LSD) values were computed at $P \leq 0.05$, and comparisons between means were done using the Tukey–Kramer HSD test. Odor values were calculated by converting the odorant concentrations in mg/m^3 and dividing by its published odor threshold value. For example, the odor value for methanethiol is:

$$0.23 \text{ ng}/10 \text{ mL headspace} \times 1000 \text{ mL/L} = 23 \text{ ng/L}$$

$$23 \text{ ng/L} \times 1 \text{ μg}/1000 \text{ ng} = 0.023 \text{ μg/L}$$

$$0.23 \text{ μg/L} \times 1000 \text{ L/m}^3 = 23 \text{ μg/m}^3$$

$$23 \text{ μg/m}^3 \times 1 \text{ mg}/1000 \text{ μg} = 0.023 \text{ mg/m}^3$$

$$\text{Odor Value} = 0.023 \text{ mg/m}^3 \div 0.0016 \text{ mg/m}^3 = 14.4 \quad [1]$$

Sensory analyses of soymilk and SPI. A rating approach of a difference test (23) was conducted with four replications per panelist. Eight sensory panelists were chosen and trained on the basis of their ability to identify the characteristic odor of soy protein products. Coded samples were presented in pairs to panelists in random order, and the panelists were asked to rate the overall odor intensities. Samples of either soymilk or 5% SPI slurry (50 mL) were presented to the panelists in 250-mL Teflon-capped amber jars. The panels were instructed to remove the cap, sniff the sample, and rate the odor intensity on a 15-cm scale anchored from none to intense. Marks placed on the line were converted to numbers by manually measuring the position of each mark using a ruler and converting the response to a 0 to 10 scale with 0 being none and 10 being intense. The statistical design used was the paired comparison between two samples (control and treatment). Data were tested using the ANOVA procedure of the Statisti-

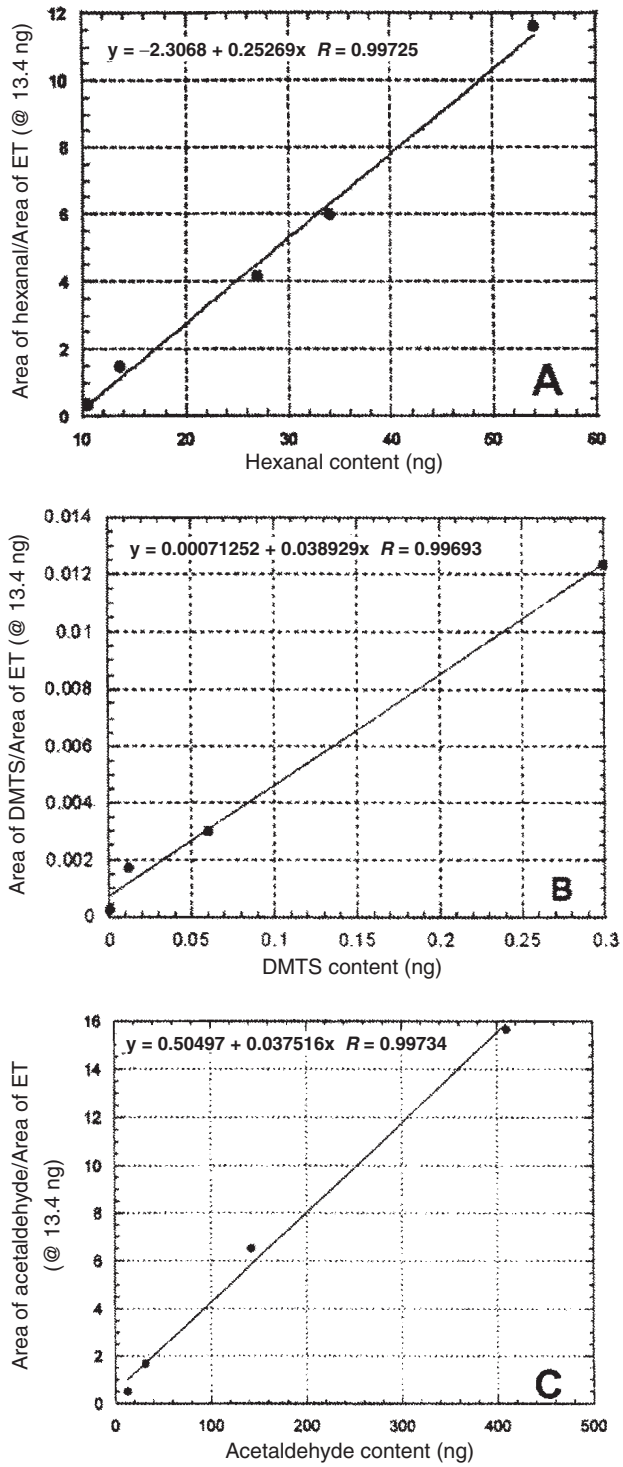

FIG. 1. GC–MS standard curves for quantifying hexanal, *m/z* 44 and 56 (A), dimethyl trisulfide (DMTS), *m/z* 126 (B), and acetaldehyde, *m/z* 29 (C) from sample headspace using ethanethiol (ET), *m/z* 47 and 62, as an internal standard.

cal Analysis System (22) software package. PROC MIXED was used to compare means and separate differences because it is better at providing covariance structures for repeated measurement scenarios than PROC GLM.

Measurement of deodorizing activity. Either gallic acid or EGCg, 0.1 g, was dispersed in 50 mL of 0.2 M phosphate buffer (pH 8), or in water from a Barnstead Nanopure 4-Module System (Fisher Scientific, Pittsburgh, PA). If phosphate buffer was not used, the pH was raised to 8.0 with either NaOH, KOH, or $Ca(OH)_2$. In a 3-mL conical vial equipped with a Teflon-lined septum, 250 µL of the gallic acid (or EGCg) premix was added to 250 µL of *ca.* 800 ppm methanethiol solution and 500 µL water (or additional phosphate buffer). For a control, the gallic acid premix was replaced with water. Samples were removed with a syringe through the septum and injected into the HPLC at various times after stirring at 25°C.

HPLC separations were accomplished on a Hewlett-Packard (Wilmington, DE) Model 1100 HPLC equipped with binary pumps, a diode-array detector, ChemStation Software, and a 20-µL injection loop. Analytes were separated on a Rainin Dynamax Microsorb 5 µm C18 column (4.6 × 250 mm) with a 5-µm C18 guard column (4.6 × 15 mm) (Ridgefield, NJ). The flow rate was 1.0 mL per min. A solvent gradient of % methanol/H_3PO_4 (999:1, vol/vol) in water/H_3PO_4 (999:1, vol/vol) was 0% at zero time to 10% at 20 min then back to 0% by 30 min. Methanethiol was monitored at 210 nm.

RESULTS AND DISCUSSION

GCO/MS analyses of the headspace volatiles from the control soymilk revealed that the most potent odorant was hexanal, which was detected at 1.25 mL of headspace (Table 1). The next most potent odorants were acetaldehyde and methanethiol, which were both detected at 2.5 mL of headspace. Next, DMTS was detected at 5 mL, and 2-pentyl furan was detected at 20 mL. These results differ from the GCO/MS data presented by Kobayashi and others (15). The most likely cause of these differences is their lack of a heat treatment in the soymilk preparation and their use of ethyl ether to recover and introduce the volatiles into the GCO/MS compared to our use of direct injections of static headspace. Not cooking the soymilk will maintain the activity of the LOX isozymes and elevate the level of volatile compounds produced by these enzymes. The lack of a heat treatment may also reduce the degradation of sulfur-containing amino acids. Extracting the soybean slurry with a solvent, concentrating the solvent, and injecting the resultant concentrated extract into the gas chromatograph will not accurately represent the volatile compounds in the headspace. The extraction process will recover some compounds at levels that are higher than would occur in the headspace because of their relatively high boiling point or because they are bound to other components in the soymilk. The majority of the most potent odorants emphasized by the solvent injection method of Kobayashi and others (15) were the higher boiling point compounds including *trans,trans*-2,4-nonadienal and *trans,trans-2*,4-decadienal. Also, by concentrating the ether extract prior to injection, compounds with boiling points below or near the boiling point (b.p.) of ethyl ether (35°C) will be lost or underrepresented.These include acetaldehyde (b.p. 21°C) and methanethiol (b.p. 6°C). Kobayashi and others (15) detected no compounds with b.p. below that of pentanal (b.p. 102°C). Because odors emanated from the GC used in this investigation at temperatures above 160°C and interfered with our ability to detect odorants, we did not attempt to analyze the higher molecular weight compounds by GCO. However, as discussed in a later section of this investigation, neither 2,4-nonadienal nor 2,4-decadienal was detected from any soy product by GC–MS-SIM analysis. This current investigation is the first to report methanethiol or DMTS as a component of soymilk and the first to demonstrate the relative importance of methanethiol, DMTS, and acetaldehyde to the odor of soymilk.

Investigation of possible methods to minimize the occurrence of methanethiol and DMTS in soy products found that gallic acid was very effective (Fig. 2). Our results on the deodorizing activity of gallic acid differ from those of Yasuda and Arakawa (24). They found that EGCg was about four times more effective than gallic acid. Differences in our assays include type of buffer, the deodorizing time (5 min vs. 1 to 151 min), and the method of monitoring the disappearance of methanethiol (GC-headspace vs. HPLC). HPLC separation of methanethiol, gallic acid, and the reaction products after 1 (A) and 51 min (B) reaction time are shown in Figure 3. Neither of these peaks representing the reaction products was observed in the controls. The methanethiol peak is almost completely gone by 51 min. Yasuda and Arakawa proposed a

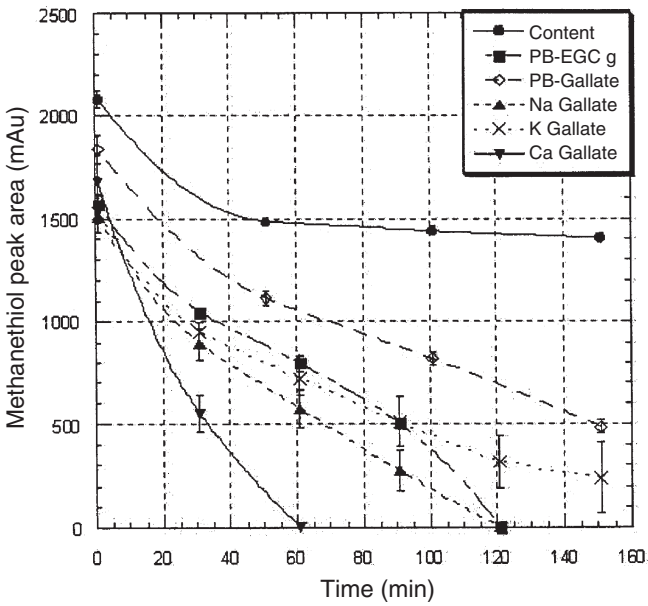

FIG. 2. Time-course disappearance of methanethiol from reaction with (–)-epigallocatechin gallate (EGCg) in phosphate buffer (PB); gallic acid in PB; calcium, potassium, and sodium gallate (all at pH 8) as measured by HPLC peak area at 210 nm. For a control, gallic acid was replaced with water and PB was used.

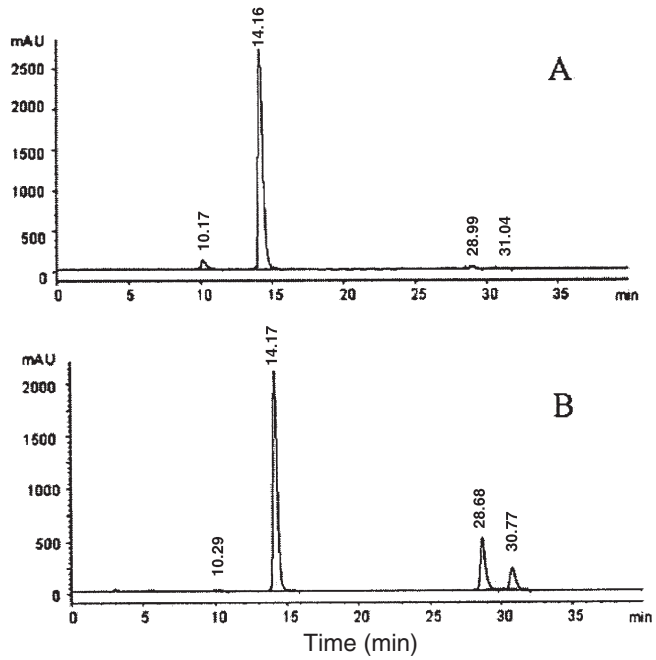

FIG. 3. HPLC chromatogram of methanethiol (10.17 min), gallic acid (14.16 min), and their reaction products (29 and 31 min) at 1 min (A) and 51 min (B). The pH was maintained at 8 with $Ca(OH)_2$, and compounds were detected at 210 nm.

mechanism for the reaction between EGCg and methanethiol involving oxidation of the triphenolic hydroxyl groups with subsequent reaction between the orthoquinone and methanethiol by either a 1,4- or a 1,6-addition. The reaction between gallic acid and methanethiol may involve a similar mechanism.

Analysis of soymilk headspace volatiles by GCO/MS demonstrated that the addition of 100 ppm of gallic acid [brought to pH 8 with $Ca(OH)_2$] at the beginning of the soymilk preparation process greatly reduced the level of both methanethiol and DMTS (Table 1). These potent odorants, detected at 2.5 and 5 mL headspace, respectively, in the control were not detected in 25 mL headspace from the treated soymilk. Soymilk samples prepared in the same manner as those represented in Table 1 were submitted to sensory analyses to evaluate their overall odor intensity (Fig. 4). On a 10-point scale (10 = strong, 0 = weak), the control soymilk was rated a 6.48 and the soymilk treated with gallic acid was rated

TABLE 1
Effect of Calcium Gallate on the Lowest Static Headspace Volume to Perceive Odorants from Soymilk[a] (the lower the volume, the more potent the odorant)

Compound	Volume from control soymilk (mL)	Volume from soymilk treated with 100 ppm Ca gallate (mL)
Acetaldehyde	2.5	5
Methanethiol	2.5	>25
Hexanal	1.25	1.25
2-Pentyl furan	20	>25
Dimethyl trisulfide	5	>25

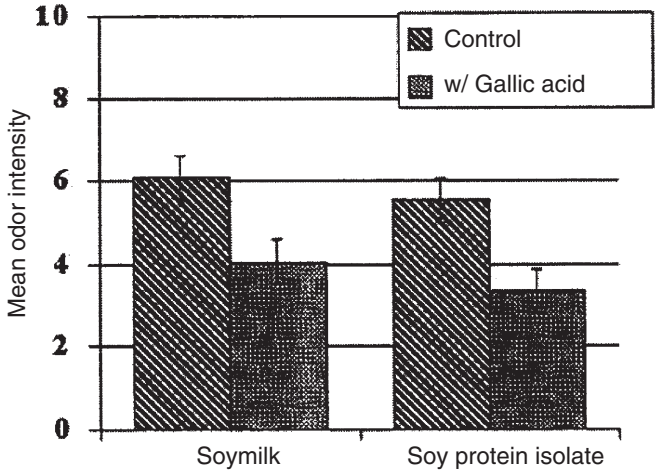

FIG. 4. Odor intensities of control soymilk and 5% aqueous soy protein isolate samples compared with corresponding samples prepared with 100 ppm gallic acid (10 = strong, 0 = weak).

a 4.05 with an SE 0.53. There was a difference (estimated at 2.43 points) between the control and treated soymilk samples ($P = 0.0006$). Sensory evaluation of SPI processed with the addition of 100 mg gallic acid to 100 g of hexane-defatted soy flour provided similar reductions in the overall odor intensities (Fig. 4). Aqueous solutions (5%) of the control SPI were rated a 5.53, whereas the SPI with gallic acid was rated a 3.33 with an SE of 0.55. There was a difference (estimated at 2.19 points) between the control and treated aqueous SPI samples ($P < 0.0001$).

GCO/MS analyses of the headspace of these aqueous SPI samples revealed that the three most potent odorants in the control SPI were methanethiol, hexanal, and DMTS (i.e., the only three compounds detected in 25-mL headspace by olfactometry). Treatment with gallic acid removed detectable levels of methanethiol and DMTS (as determined by both olfactometry and SIM analyses) but had no significant effect on the level of hexanal, $P = 0.81$ (Fig. 5). The peaks for methanethiol, hexanal, and DMTS in Figure 5A represent 0.23 (0.05), 11.4 (0.18), and 0.045 (0.006) ng, respectively, per 10 mL headspace; numbers in parentheses are SE. At first glance, hexanal would appear to be by far the predominant odorant because of its larger quantity. However, the published odor threshold in air for methanethiol is 0.0016 mg/m^3 (25) and for hexanal is 0.058 mg/m^3 (26). These are mean threshold values from several different studies. The corresponding odor values (quantity divided by threshold) for these two compounds are 14.4 and 17.9, respectively. There are two published threshold value for DMTS in air: 0.0062 mg/m^3 (27) and 0.009 mg/m^3 (28). These references are both compilations of data for multiple compounds, and because Ruth (27) does not provide the original reference, these values could both be from the same source (28). If we use the 0.0062 mg/m^3 value, the corresponding odor values for DMTS from our laboratory SPI would be 0.73. However, because we can smell DMTS from 25 mL of the headspace, the odor value is likely higher. We injected various quantities of DMTS into

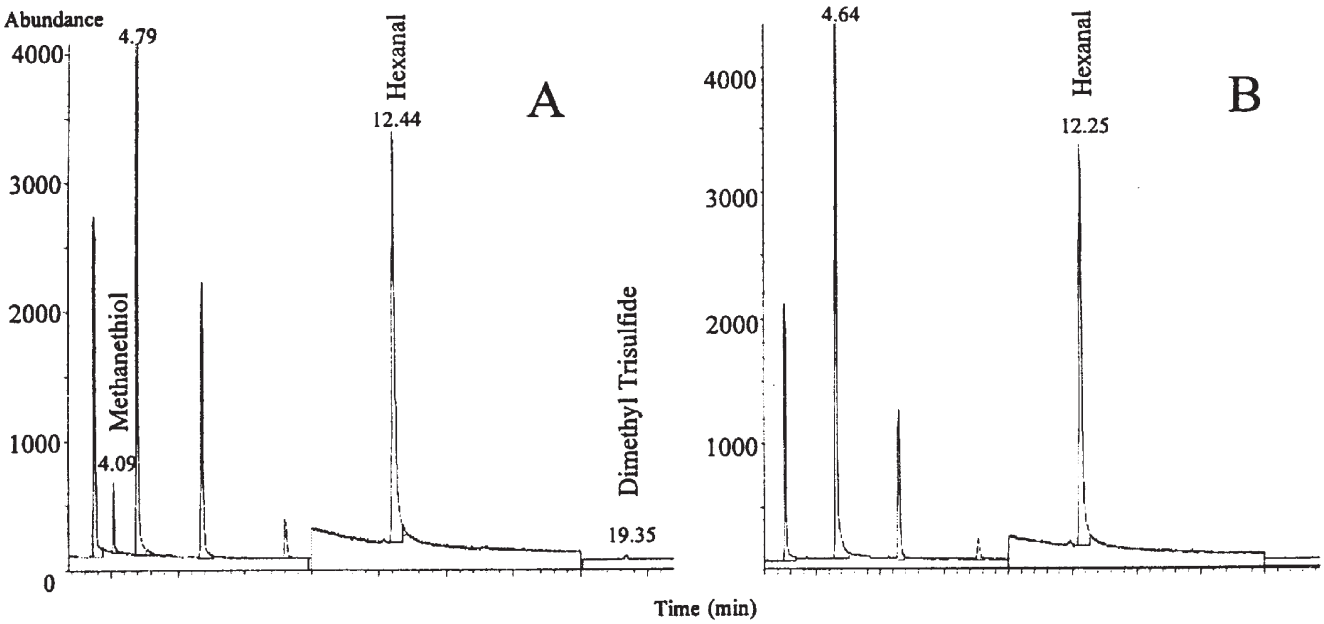

FIG. 5. GC–MS of 10 mL of headspace from (A) control soy protein isolate (SPI) and (B) SPI prepared with 100 ppm gallic acid. Detection represents only *m/z* 47 (methanethiol at 4.09 min and ethanethiol as an internal standard at 4.79 min), *m/z* 56 (hexanal at 12.44 min), and *m/z* 126 (DMTS at 19.35 min). For abbreviations see Figure 1.

the GC and were able to clearly detect 0.067 ng at the sniff port by olfactometry. One-half that amount was not clearly evident. We compared the minimum quantity of DMTS detectable to the minimum quantity of hexanal detectable using the same GCO technique. We could clearly detect 3.33 ng of hexanal at the sniff port, and 1.7 ng was not clearly detectable. This is 50 times greater than the minimum quantity necessary to detect DMTS by this method. If the odor threshold of 0.058 mg/m^3 is correct for hexanal, then the corresponding odor threshold for DMTS would be about 0.0012 mg/m^3. With 0.0012 mg/m^3 as the estimated DMTS threshold value, the estimated odor value for this DMTS in the headspace above the laboratory SPI solution would be 4. The perceived odor intensity of DMTS from this laboratory prepared SPI was lower than commercial SPI previously analyzed by GCO in our laboratory.

We also compared the minimum level of methanethiol detectable by GCO and found that we could clearly detect 0.08 ng at the sniff port and 0.04 ng was not clearly detectable. Determinations of the minimum quantity necessary to detect these compounds by olfactometry was accomplished by the same two investigators that performed the olfactometry analyses in our previous investigations (18,29). By using 0.058 mg/m^3 as the odor threshold for hexanal, the calculated odor threshold for methanethiol using this method is 0.0014 mg/m^3. This is very close to the published odor threshold value of methanethiol in air of 0.0016 mg/m^3. This method of estimating the odor threshold of a compound in air—by determining the minimum quantity that can be detected at a GC sniff port and comparing it with the minimum detectable amount of a compound with a known threshold value—is similar to the method employed by Boelens and van Gemert (30).

Quantifying odorants in the headspace and determining their corresponding odor value in air provides a more accurate representation of their impact than quantifying the odorants in solution and dividing by their odor threshold in water. By measuring headspace concentrations, interferences from the binding of odorants to soy protein or other constituents are excluded. Quantifying odorants in the headspace also provides an accurate and precise means to quantify methanethiol and DMTS in soy products treated with gallic acid. An appropriate internal standard for either methanethiol or DMTS (e.g., ethanethiol) added directly to the soy product would probably be affected by gallic acid.

Odorants in the headspace of a commercial SPI and a laboratory-prepared soymilk were also quantified using this method. The odor values for methanethiol, hexanal, DMTS, and 2-pentyl furan from a commercial SPI were 115, 26, *ca.* 9 (using 0.0012 mg/m^3), and 6.5, respectively. Odor values for acetaldehyde, methanethiol, hexanal, DMTS, and 2-pentyl furan from a laboratory soymilk were 84, 15, 103, *ca.* 11, and 7.2, respectively. This soymilk was made from a different batch of Burlison variety soybeans (from the University of Illinois) because the supply of soybeans that was used to make the soymilk that was subjected to GCO and sensory analyses (from Purdue University) was depleted. The commercial SPI was the same one used by Lei and Boatright (20) to quantify methanethiol in aqueous SPI slurries. The calculated odor value of 80 for methanethiol using the slurry method was similar to the value of 115 calculated from headspace concentrations. Neither *trans,trans*-2,4-nonadienal nor *trans,trans*-2,4-decadienal (both *m/z* 81) was detected in these soy products by GC–MS-SIM. This indicates that neither compound is a major odorant in these products. Because the

odor threshold in air for these two compounds is very low (0.00020 and 0.00022 mg/m^3, respectively) (26), we should not rule out a minor contribution. These chemical analyses support both our sensory and GCO analyses and further demonstrate that methanethiol and DMTS are major odorants from soy products.

While minimizing methanethiol and DMTS reduces the overall odor intensity of soymilk and SPI slurries, combining the gallic acid treatment with treatments to reduce hexanal, acetaldehyde, and 2-pentyl furan should further improve the flavor characteristics of SPI and soymilk. Even as these experiments demonstrate that hexanal, methanethiol, DMTS, 2-pentyl furan, and acetaldehyde are the most potent odorants from these soy products, it is likely that other odorants contribute to the overall characteristic soy odor but are not detected by GCO/MS with a 25-mL static headspace injection. These less potent contributors likely include some of the odorants identified by Boatright and Lei (29) using vacuum distillations with a liquid nitrogen trap to concentrate the odorants.

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