Development of a New Methanethiol Quantification Method Using Ethanethiol as an Internal Standard

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Development of a new method to quantify methanethiol in which ethanethiol was employed as an internal standard is reported. Recovery yields for methanethiol from an aqueous model system and a soy protein concentrate (SPC) aqueous slurry determined with this method ranged from 97 to 107% and from 103 to 121%, respectively. The methanethiol content of two commercial SPCs and two commercial soy protein isolate (SPI) samples, on a dry basis, ranged from 835 to 1190 times greater than the odor threshold for methanethiol. Relative standard deviations for quantifying methanethiol with the method from these samples were <5%, indicating its good reproducibility in quantifying methanethiol from soy protein products. Also investigated in the current study was the feasibility of using 5′,5-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) to determine the concentration of methanethiol in the aqueous solutions used to prepare the standard curve for quantifying methanethiol from soy protein products. The concentrations of methanethiol obtained from Ellman's reagent method were comparable with those from a weighing method and theoretical calculation.

Keywords: *Methanethiol quantification; soy protein concentrate; soy protein isolate; ethanethiol; Ellman's reagent*

INTRODUCTION

Methanethiol has been identified as one of the major odorants for the characteristic odor of cheese and associated with desirable Cheddar-type sulfur notes in good-quality Cheddar cheese (*1*). In addition, it was also reported to be one of the potent odorants for the typical offensive odor of broccoli and cabbage, especially when these vegetables had been stored under oxygen-reduced condition (*2*, *3*). The occurrence of methanethiol was first reported by Qvist and von Sydow (*4*) from soy protein isolate (SPI) heated to 121 °C for 37−41 min. However, no further investigation about its importance to the characteristic odor of SPI was conducted until recently; during our studies about the most potent volatiles contributing to the typical "beany"odor associated with soy products based upon gas chromatography olfactometry−mass spectrometry (GCO-MS) analysis of the headspace of an SPI aqueous slurry, methanethiol was identified as one of the primary odorants for SPI (*5*, *15*). Further investigation revealed that methanethiol is also a key aroma-impact compound for soy protein concentrate (SPC) (*6*) and soy milk (Boatright and Lei, unpublished data). In addition to its direct contribution to the odor of soy products, methanethiol is also likely to be related to the formation of dimethyl trisulfide, another powerful odorant for soy products (Boatright and Lei, unpublished data), which has the second highest odor activity value (ratio of concentration to its threshold) among all of the odorants identified from soy products. To fully evaluate its importance to the odor of soy products, it is required to quantify methanethiol from these products and then to calculate its odor activity value.

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Quantifying methanethiol from food resources can be accomplished either by stable isotope dilution assay (SIDA) based on GC-MS analysis (*7*, *8*) or by analyzing the headspace sample using GC equipped with a flame photometric detector (*3*). While studying the character-impact odorants of stewed beef juice, Guth and Grosch (*8*) established a methanethiol quantification method using [^{2}H]methanethiol as an internal standard and first converting the original methanethiol from the samples and the added isotope labeled internal standard to their derivatives with 4-vinylpyridine prior to GC-MS analysis. Later, Semmelroch and Grosch (*7*), when investigating the primary odorants of coffee brew, reported another method to quantify methanethiol, by which headspace was analyzed by GC-MS directly without deriving methanethiol to [β-(4-pyridyl)ethyl] thiomethyl ether with 4-vinylpyridine, still using [^{2}H]-methanethiol as an internal standard. Analyzing a headspace sample with GC equipped with a flame photometric detector is the most commonly utilized technique quantifying methanethiol from vegetables including cabbage (*3*, *9*) and broccoli (*10*−*13*). Such a technique was also employed to quantify methanethiol from the headspace of dairy products (*14*).

No matter which method is employed for methanethiol quantification, a series of standard methanethiol samples with known concentration should be made to prepare a calibration curve. Due to its low boiling point (6 °C under atmospheric pressure), the preparation of such a standard methanethiol sample is not as easy as that for liquid compounds. Guth and Grosch (*8*) described a procedure to prepare such samples by measuring the volume of methanethiol gas and then converting it to the weight of methanethiol based on its density of 1.09 μg/μL. This parameter is influenced by both temperature and atmospheric pressure; hence, utilization of these data in preparing

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methanethiol standard samples should be with prudence. Another method reported by Bettie and Torrey (*15*) was to liquify methanethiol prior to measuring the volume of liquified methanethiol with a gastight syringe and then to obtain the weight of methanethiol based upon the density of methanethiol liquid. Neither of the two methods was very convenientt; therefore, reported in this paper was the procedure to determine the concentration of methanethiol aqueous solutions with Ellman's reagent. Also reported in the paper is the development of a new methanethiol quantification procedure with ethanethiol as an internal standard and its application in quantifying methanethiol from commercial soy protein products.

EXPERIMENTAL PROCEDURES

Soy Protein Samples. SPCs Soyarich B and Procon 2000 were obtained from Central Soya Co., Inc. (Fort Wayne, IN). Archer Daniels Midland Co. (Decatur, IL) provided SPI Pro Fam 970, and Protein Technology International, Inc. (St. Louis, MO) donated SPI Supro 500E.

Chemicals. Methanethiol, ethanethiol, 4-vinylpyridine, thiourea, and iodomethane-d_3 were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). DTNB′ (Ellman's reagent} was obtained from Sigma Chemical Co. (St. Louis, MO). Ethyl ether was from Fisher Scientific (Fair Lawn, NJ).

Methods for Measuring Concentrations of Methanethiol Standard Solution. *Ellman's Reagent Method.* The procedure described by Ellman (*16*) was generally employed with modification. To a 15 mL test tube containing 3 mL of deionized water was added Ellman's reagent (200 μL, 39.6 mg of DTNB dissolved in 10 mL of 0.1 mol/L, pH 7.0, phosphate buffer). After the tube was sealed with Parafilm to reduce the loss of methanethiol, a known volume of standard methanethiol solution was introduced into the tube with a syringe. The reaction solution was mixed well by vortex after the tube was resealed with Parafilm. The absorbance measured at 412 nm was utilized to calculate the concentration of methanethiol standard solutions as follows

$$\text{concentration (ppm)} = \text{Abs}/\epsilon \times D \times \text{MW} \times 1000$$

where Abs = the absorbance measured at 412 nm, ϵ = extinction coefficient = 13600/M/cm, D = dilution factor, MW = molecular weight for methanethiol (48.11) or for [^{2}H]-methanethiol (51.11), and 1000 = conversion factor.

Weighing Method. A 25 mL flask sealed with a septum, containing ~18 mL of deionized water, was subjected to vacuum treatment for 1 h by connecting to a pump to pull out the air inside. After the weight of the flask including the water inside had been accurately weighed with a balance, methanethiol gas was introduced into the headspace of the flask from the storage tank through the septum. Then, the total weight of the flask with water and methanethiol was weighed. The amount of methanethiol added into the flask was obtained by subtracting the weight before methanethiol was added from the weight after methanethiol was introduced. More water was then introduced into the flask to the 25 mL mark with a syringe. Therefore, the concentration of methanethiol solution thus prepared could be calculated on the basis of the amount of methanethiol added and the total volume of the solution.

Method Based on Theoretical Calculation. This method was applied for the solution of [^{2}H]methanethiol, which was synthesized according to the procedure described by Guth and Grosch (*8*) with modification. To achieve complete conversion of [^{2}H]iodomethane to [^{2}H]methanethiol, an excessive amount of thiourea (1140 mg, 15 mmol) was mixed with [^{2}H]-iodomethane (1.34 g, 9.5 mmol), and then ethanol/water (1:1 v/v, 10 mL) was added into the mixture. After 15 min of stirring, the solution was refluxed for 6 h at 85 °C. The total volume of the reaction mixture was measured accurately after it was cooled to room temperature. To a sealed 25 mL volumetric flask pretreated under vacuum to pull out the air inside, the reaction mixture (100 μL) and aqueous NaOH solution (200 μL, 2 mol/L) were introduced subsequently with a syringe. After 15 min, deionized water was introduced into the flask to the 25 mL mark with a 50 mL syringe. One mole of [^{2}H]methanethiol is converted from 1 mol of [^{2}H]iodomethane, so the total amount of labeled methanethiol synthesized could be calculated. Therefore, the concentration of the above [^{2}H]-methanethiol solution was obtained on the basis of theoretical calculation. Thus, prepared [^{2}H]methanethiol solution was diluted further and measured with Ellman's reagent.

Synthesis of [β-(4-Pyridyl)ethyl] Thiomethyl Ether and [β-(4-Pyridyl)ethyl] Thioethyl Ether. [β-(4-Pyridyl)-ethyl] thiomethyl ether and [β-(4-pyridyl)ethyl] thioethyl ether, derivatives formed from methanethiol and ethanethiol with 4-vinylpyridine, were synthesized in an aqueous model system according to a procedure modified from the method described by Guth and Grosch (*8*), by which ethyl ether was employed as the reaction medium. To a 100 mL round-bottom flask containing 100 mL of deionized water was added methanethiol aqueous solution (50 μL, 0.79 μg/μL) or ethanethiol methanol solution (50 μL, 1.69 μg/μL). The above solution was stirred for 15 min, and then 4-vinylpyridine (200 μL) was added. After the reaction mixture was stirred overnight at room temperature while the flask was kept sealed, the resulting derivatives were extracted with ethyl ether (80 mL). The ether extract, after being dried over anhydrous sodium sulfate (5.0 g), was concentrated to 2−3 mL with a rotary evaporator at 50 °C followed by further concentrating to ~200 μL with a dry nitrogen stream. The resulting ether extract was subjected to GC-MS analysis.

Procedure of Quantifying Methanethiol from Soy Protein Products Using Ethanethiol as an Internal Standard. To a slurry of SPC (10.0 g/100 mL of deionized water) or SPI (10.0 g/150 mL of deionized water) in a 250 mL flask sealed with a glass stopper was added ethanethiol methanol solution (50 μL, 0.79 μg/μL). After the SPC slurry was stirred for 15 min, 4-vinylpyridine (200 μL) was introduced into the flask. The mixture was stirred overnight at 24 °C while the flask was kept sealed with a glass stopper. Then, aqueous cysteine solution (250 mg cysteine in 6 mL of deionized water) was added, and the mixture was further stirred for another 2 h. The supernatant from the SPC slurry, after being centrifuged at 5000 rpm for 15 min at 24 °C, was extracted twice with ether (80 mL × 1, 60 mL × 1). The ether extracts were combined and treated in the same way as for the extract for the derivative described above.

GC-MS. GC-MS analysis was performed on a Hewlett-Packard model G 1800 A GCD system (Wilmington, DE) equipped with an electron ionization detector (EID) maintained at 240 °C and a model G 1030A Chemstation controller. Separation was conducted on a DB-225 capillary column (30 m × 0.25 mm i.d.) with 0.25 μm film thickness (J&W Scientific, Folsom, CA), with a 1 min splitless injection. The injector liner, maintained at 210 °C, was packed with silanized glass wool. The column temperature was held at 40 °C for 5 min and then raised at 3 °C/min to 165 °C followed by another rise to 220 °C at 20 °C/min. High-purity helium at 1.0 mL/min was used as carrier gas. The EID was set to detect in the mass range of 25−250.

RESULTS AND DISCUSSION

Determining the Concentration of Methanethiol in Standard Solutions Using Ellman's Reagent. *Stability of the Complex Formed from Methanethiol and DTNB.* Because preparation of methanethiol standard solutions and the calibration curve is a crucial step for methanethiol quantification, an accurate and convenient method suitable for measuring concentrations of methanethiol standard samples was desired. In current studies, Ellman's reagent, initially invented and used to measure total sulfhydryl group contents from urine

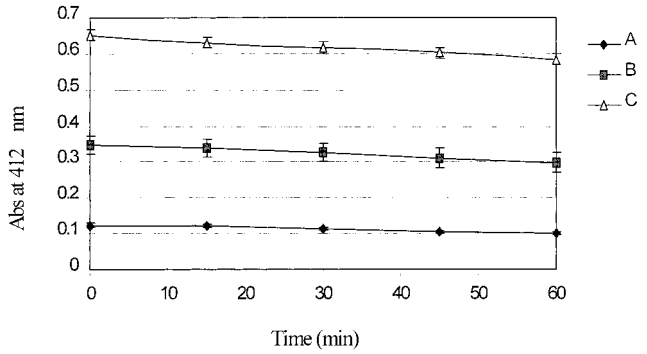

Figure 1. Changes of absorbance of the color complex from methanethiol and Ellman's reagent with time measured at 412 nm at 24 °C: (A) 0.44 ppm; (B) 1.23 ppm; (C) 2.31 ppm.

and blood tissues (*16*), was utilized to measure the concentrations of methanethiol standard solutions.

With the addition of methanethiol solution, the reaction mixture already containing deionized water and DTNB solution instantly turned a bright yellow as described by Ellman (*16*). The absorbance for the color complex at 412 nm was utilized to calculate the concentration of methanethiol in standard solutions, so the stability of the color complex formed between DNTB and methanethiol in three concentrations, 0.44, 1.23, and 2.31 ppm, was investigated by monitoring the changes in absorbance at 412 nm over 60 min at 24 °C. The results showed that the absorbance, from methanehtiol with either higher or lower concentration, decreased with time under the test condition, suggesting the complex gradually degraded or converted to another compound (Figure 1). However, only <5% loss of original absorbance was observed within 15 min after the addition of methanethiol. Therefore, when the Ellman's reagent method was used to determine the concentration of methanethiol standard solutions during this study, the absorbance at 412 nm was usually read within 15 min after methanethiol was introduced into the DTNB solution.

Feasibility of Using Ellman's Reagent To Determine the Concentration of Methanethiol. The primary concern about employing Ellman's reagent to measure the concentration of methanethiol in standard solutions was the molar extinction coefficient for the yellow complex formed from methanethiol and DTNB. There are no published data available, so the feasibility of the method in determining methanethiol concentration in standard solutions was evaluated first by the weighing method. During their effort to improve the performance of analyzing low molecular weight thiols by GC, Nedjma and Maujean (*17*) emphasized that the headspace of the thiol solution should be as small as possible to reduce the amount of thiols existing in the headspace. Therefore, with the weighing method, vacuum treatment was employed to pull out the air inside the sealed flask, which facilitated preparing methanethiol aqueous solution with minimum headspace after methanethiol and water were added. The concentrations of methanethiol solutions determined by using the Ellman's reagent method were compared with those based on the weighing method (Table 1). It was observed that, from 0.59 to 4.39 ppm, the concentrations of methanethiol in standard solutions measured using Ellman's reagent method were close to those determined by the weighing method, with the ratio (concentration from the Ellman's reagent method relative to that from the weighing method for the same sample) ranged from 0.91 to 0.94. For all of the concentrations measured, the results from the Ellman's reagent method were always slightly lower than those from the weighing method, which was explained by loss of methanethiol during the solution preparing procedure and the amount of methanethiol present in the headspace of the solutions. Both were accounted for in the weighing method but not detected by the Ellman's reagent method.

Table 1. Comparison of Concentration of Methanethiol Aqueous Solution As Determined by the Weighing Method and Ellman's Reagent Method

methanethiol concn					
weighing method (ppm)	0.59	1.47	2.34	2.94	4.39
Ellman's reagent method (ppm)	0.55	1.37	2.13	2.66	4.10
SD[a]	0.08	0.09	0.07	0.04	0.18
n[b]	5	6	6	5	5
ratio[c]	0.93	0.94	0.91	0.91	0.93

[a] Standard deviation. [b] Number of measurements. [c] Ratio = methanethiol concentration determined by Ellman's reagent method/ methanethiol concentration calculated from the weighing method.

Table 2. Comparison of Concentration of Synthesized Methanethiol-d_3 in Aqueous Solution As Determined from Theoretical Calculation and by Ellman's Reagent Method

methanethiol concn[a]					
theor calculation (ppm)	0.53	1.03	1.54	2.11	2.62
Ellman's reagent method (ppm)	0.49	0.95	1.39	2.04	2.45
SD[a]	0.01	0.01	0.01	0.02	0.04
n[b]	8	7	7	7	6
ratio[c]	0.92	0.92	0.91	0.97	0.93

[a] Standard deviation. [b] Number of measurements. [c] Ratio = methanethiol concentration determined by Ellman's reagent method/ methanethiol concentration from theoretical calculation.

Utilization of Ellman's reagent in measuring the methanethiol concentration in standard solutions was further investigated by comparing the results obtained from the method with those from the theoretical calculation for the solutions of synthetic [^{2}H]methanethiol (Table 2). Again, very close results were obtained from these two methods with the ratio (concentration from the the Ellman's reagent method relative to that from the theoretical calculation for the same sample) ranged from 0.91 to 0.97. Due to the incomplete conversion of [^{2}H]iodomethane to [^{2}H]methanethiol, loss of part of the methanethiol in solution preparation, and the amount of methanethiol in the solution headspace, the results from the Ellman's reagent method were also slightly lower than those obtained from theoretical calculation. However, the differences were usually <10% as observed from comparison of the results from the Ellman's reagent method and the weighing method. Therefore, both the weighing method and the theoretical calculation method proved that Ellman's reagent method was a reliable method suitable for measuring the concentration of methanethiol in aqueous solutions. Compared with the method described by Guth and Grosch (*8*) or the method reported by Bettie and Torrey (*15*), the Ellman's reagent method was more convenient and, at the same time, satisfactorily accurate. The detection limit for the method in measuring the methanethiol concentration was ~0.1 ppm (final concentration in the reaction mixture). It was also observed that the DTNB solution can be stored at 5 °C for 1 month without influencing the measurement of the methanethiol concentration (data not shown).

Reactivity of Methanethiol and Ethanethiol with 4-Vinylpyridine in Aqueous Media. As meth-

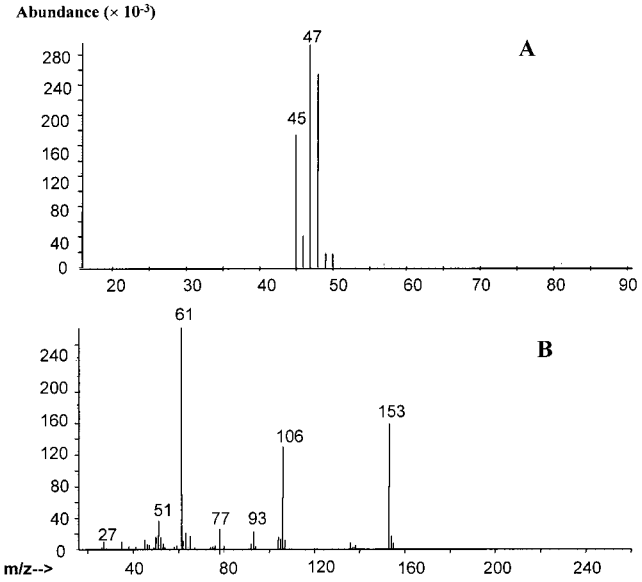

Figure 2. Comparison of the mass spectra for methanethiol (A) and [β-(4-pyridyl)ethyl] thiomethyl ether (B).

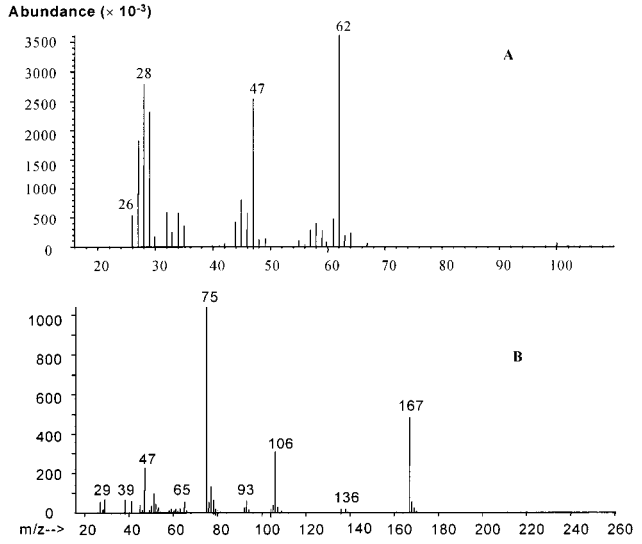

Figure 3. Comparison of the mass spectra for ethanethiol (A) and [β-(4-pyridyl)ethyl] thioethyl ether (B).

anethiol was quantified from an aqueous slurry of SPC with ethanethiol as an internal standard and with added 4-vinylpyridine to convert both methanethiol and ethanethiol to their derivatives, the reactivity of these two thiols with 4-vinylpyridine in an aqueous system was studied by adding methanethiol or ethanethiol into the aqueous solution containing 4-vinylpyridine. The mass spectra for the resulting derivatives are compared in Figures 2 and 3, respectively. The mass spectrum for [β-(4-pyridyl)ethyl] thiomethyl ether (Figure 2B) was exactly the same as reported by Guth and Grosch (*8*), indicating that this derivative can be formed as readily from methanethiol and 4-vinylpyridine in the aqueous system at room temperature as in ethyl ether. Figure 3B shows the mass spectrum of [β-(4-pyridyl)ethyl] thioethyl ether, a derivative from ethanethiol and 4-vinylpyridine. The molecular weight of the derivative is 167, which is 105 mass units higher than that for ethanethiol (MW 62). The molecular weight of 4-vinylpyridine is 105, so the above result suggested that the derivative is also formed readily from an additive

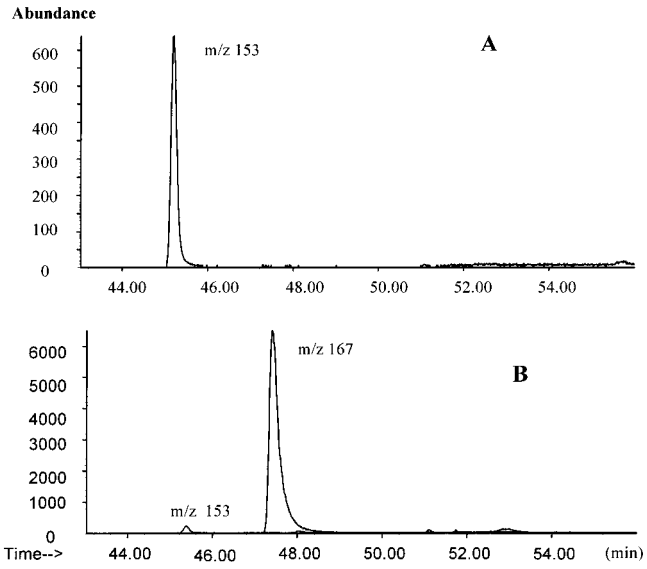

Figure 4. Mass chromatography of the derivatives from an SPC (Soyarich B) aqueous slurry with (A) only 4-vinylpyridine added and (B) both ethanethiol and 4-vinylpyridine added.

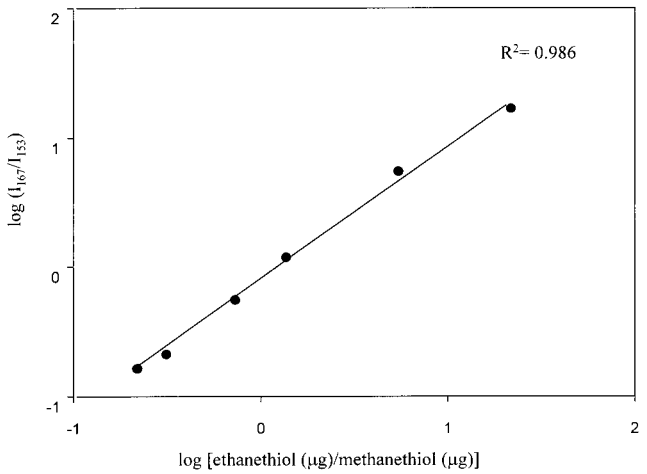

Figure 5. Calibration curve for methanethiol quantification using ethanethiol as an internal standard.

reaction between ethanethiol and 4-vinylpyridine in aqueous media.

Quantifying Methanethiol Using Ethanethiol as an Internal Standard. *Calibration Curve.* The derivatives from 4-vinylpyridine (200 μL) reacting with known amounts of methanethiol and ethanethiol in the range of 0.84−111.00 μg were extracted with ethyl ether and analyzed by GC-MS. The log values for the ratio of relative abundance of *m*/*z* 167 to *m*/*z* 153 were plotted against the log values for the weight ratio of ethanethiol to methanethiol as shown in Figure 5. A good linear relationship ($R^2 = 0.986$) was obtained over the range of weight ratio studied, which covered the amount of methanethiol existing in SPC and SPI.

Ethanethiol as an Internal Standard for Methanethiol Quantification. During our attempt to quantify methanethiol from soy protein products, [^{2}H]methanethiol was first employed as an internal standard; however, the preliminary analysis of its aqueous solution indicated that this labeled compound was not very stable, even when stored at 5 °C. Similar results were observed from the analysis of the headspace sample from the [^{2}H]-methanethiol aqueous solution with GC-MS, which revealed that >50% of the injected methanethiol was

converted into dimethyl disulfide (DMDS-d_6). However, <1% of the injected ethanethiol was oxidized into diethyl disulfide (DEDS) analyzed under same condition. Therefore, ethanethiol, an analogue to methanethiol, was chosen to replace [^{2}H]methanethiol as an internal standard. An ideal internal standard should have physical and chemical properties similar to those of the compound to be quantified and, at the same time, there no such indigenous compound should exist in the samples studied. Occurrence of ethanethiol was documented by Qvist and von Sydow (*4*) from heated SPI, but no ethanethiol was found from the unheated sample. There were no published research results indicating its existence in SPC. To investigate whether there was indigenous ethanethiol in SPC, 4-vinylpyridine (200 μL) was introduced into the SPC aqueous slurry (10.0 g of Soyarich B/100 mL of deionized water) without the addition of ethanethiol, and the resulting derivative was extracted and analyzed in the same way as for quantifying methanethiol from SPC. The corresponding mass chromatogram is shown in Figure 4A along with the mass chromatogram from the ether extract when both ethanethiol and 4-vinylpyridine were added (Figure 4B). No [β-(4-pyridyl)ethyl] thioethyl ether was observed from the ether extract obtained from SPC when only 4-vinylpyridine was added, suggesting either no indigenous ethanethiol exists in SPC or its concentration was too low to be detected by GC-MS. However, [β-(4-pyridyl)ethyl] thiomethyl ether formed from indigenous methanethiol in SPC and 4-vinylpyridine was still observed. Furthermore, as an analogue to methanethiol with only one more methylene group, ethanethiol has chemical and physical properties similar to thos of methanethiol. Therefore, it was reasonable that ethanethiol was employed as an internal standard in the quantification of methanethiol.

Recovery Yield of Methanethiol Quantified with Ethanethiol as an Internal Standard. The feasibility and accuracy of the new methanethiol quantification method was further evaluated on the basis of the recovery yields for methanethiol quantified with this method from a model system. To a 250 mL flask containing 100 mL of deionized water was added a known amount of methanethiol. Then an ethanethiol methanol solution (50 μL, 1.68 μg/μL) and 4-vinylpyridine (200 μL) were introduced into the flask, respectively. After stirring overnight at 24 °C, the reaction mixture was extracted with ether and the ether extract was analyzed by GC-MS. On the basis of the linear regression equation for the calibration curve, the amounts of methanethiol were quantified, and the results are summarized in Table 3. It was observed that, from the model system, the methanethiol quantification yields ranged from 97 to 107%, implying good recovery yields were obtained for methanethiol quantified from such a system with this method. Considering the simple composition of the model system compared with that for SPC slurries, the recovery yields for methanethiol quantified from SPC slurries were also obtained by adding known amounts of methanethiol into the SPC slurries and then quantifying the methanethiol as described under Materials and Methods. Due to the occurrence of indigenous methanethiol in SPC, the quantification of added methanethiol was accomplished by subtracting the amount of indigenous methanethiol from the total amount quantified from SPC. Table 4 shows the results for recovery yields for methanethiol quantification from SPC slurries. Compared with the model system, the recovery yield obtained from the SPC slurry was slightly higher, ranging from 103 to 121%. Such difference was likely due to the interactions of methanethiol and ethanethiol with soy proteins and other components existing in SPC and SPI. The recovery yields from both model system and SPC slurries further confirmed that quantifying methanethiol with ethanethiol as an internal standard is feasible and accurate.

Table 3. Recovery Yield of Methanethiol (MT) Quantified from a Model System Using Ethanethiol as an Internal Standard[a]

vol of MT soln added[b] (μL)	10	50	100	200	300
MT added (μg)	7.86	39.31	78.63	157.26	235.89
MT determined (μg)	8.42	41.79	76.43	156.72	240.63
SD[c]	0.68	1.25	4.96	5.37	3.14
n[d]	4	3	3	3	3
recovery yield[e] (%)	107	106	97	100	102

[a] The reaction mixture of the model system contained deionized water (100 mL), ethanethiol internal standard (50 μL, 1.68 μg/μL in methanol), 4-vinylpyridine (200 μL), and a known amount of MT aqueous solution as shown in the table. The mixture was stirred overnight at 24 °C in a sealed 250 mL flask, and the following treatment was the same as that for quantifying MT from SPC. [b] The concentration of MT solution was 0.79 μg/μL determined by Ellman's reagent method as described above. [c] Standard deviation. [d] Number of measurements. [e] Recovery yield = (amount of MT quantified ÷ amount of MT added) × 100.

Table 4. Recovery Yield of Methanethiol (MT) Quantified from SPC Using Ethanethiol as an Internal Standard[a]

vol of MT soln added[b] (μL)	5	50	100	200
MT added (μg)	3.66	36.56	73.31	146.26
MT determined (μg)[c]	3.91	37.63	83.15	177.14
SD[d]	0.42	1.70	0.09	8.35
n[e]	2	2	2	3
recovery yield[f] (%)	107	103	113	121

[a] Various volumes of MT aqueous solution (as shown in the table) were added into each Soyarich B SPC slurry (10.0 g suspended in 100 mL of deionized water). Quantification of MT was performed in the same way as for quantifying MT from SPC without adding methanethiol. [b] The concentration of MT solution used was 0.73 μg/μL determined by Ellman's reagent method as described above. [c] Data shown = amount of MT quantified from SPC with addition of exogenous MT - amount of indigenous MT determined from SPC. [d] Standard deviation. [e] Number of duplicate measurements. [f] Recovery yield = (amount of MT detected ÷ amount of MT added) × 100.

Table 5. Concentration of Methanethiol Quantified from Soy Protein Products Using Ethanethiol as an Internal Standard and Its Corresponding Odor Activity Values

soy protein product	methanethiol concn[a] (ppb)	odor activity value[b]
soy protein concentrates		
Arcon S	172.47± 2.18	860
Soyarich B	236.73 ± 9.72	1180
soy protein isolates		
Supro 500E	237.29 ± 8.59	1190
Pro Fam 970	167.07 ± 3.45	835

[a] Mean value on dry basis with standard deviation in parentheses from four measurements. [b] Odor activity value = concentration of methanethiol/threshold of methanethiol, based on the threshold of 0.2 ppb for methanethiol (*19*).

Quantifying Methanethiol from Soy Protein Products with Ethanethiol as an Internal Standard. Using the new methanethiol quantification procedure, the concentrations of methanethiol from two commercial SPC samples and two commercial SPI samples were quantified, and the results are summarized in Table 5. For all of the soy protein samples tested, methanethiol was quantified with a relative

standard deviation of <5%, implying a good reproducibility of the method in methanethiol quantification. Compared with the data reported by Qvist and von Sydow (*4*) from the SPI samples heated at 121 °C for 37−41 min, the methanethiol concentration quantified by using the new method from unheated SPI was ∼5 times higher. On the basis of its threshold in water (0.2 ppb; *19*), the odor activity values for methanethiol from the soy protein products studied ranged from 835 to 1186 (dry basis). Such results also confirmed our previous findings (*5*, *6*) from olfactory analysis of SPC and SPI headspaces, which revealed methanethiol to be one of the potent aroma-impact highly volatile compounds.

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