Headspace Evaluation of Methanethiol and Dimethyl Trisulfide in Aqueous Solutions of Soy-protein Isolates

W.L. BOATRIGHT AND Q. LEI

ABSTRACT: Volatile compounds from 2 samples of aqueous soy-protein isolates (SPI) (7%) were analyzed using both static and dynamic headspace methods. Based on dynamic headspace analyses, the most powerful odorants were (1) dimethyl trisulfide, (2) methanethiol, (3) hexanal, (4) an unidentified charred, sweaty feet-like odor, (5) 2-pentyl furan, (6) 2,3-butadione, and (7) an unknown burnt-like odor. The most powerful odorants by static headspace analyses were (1) dimethyl trisulfide, (2) hexanal, (3) methanethiol, and (4) 2-pentyl furan. Using deuterium labeled DMTS as an internal standard, DMTS was quantified at 60.1 and 45.5 ppb in the SPIs. This corresponds to odor values of 6014 and 4554, respectively. Using a cool, on-column technique, direct injection of concentrated-headspace volatiles and solvent-recovered volatiles with an internal standard of d_6-DMTS detected both methanethiol and DMTS at similar levels as with the traditional injection methods.

Key Words: soybean-protein isolate, headspace volatiles, olfactory analysis, dimethyl trisulfide, methanethiol

Introduction

WHILE SOYBEANS PROVIDE A HIGH-QUALITY PROTEIN, AND there are increasing reports of health benefits from consuming soy-protein products (FDA 1999), the demand for soybeans in human foods has not been large. In 1971, less than 1% of the U.S. soybean crop was used as a protein source for human foods (Wolf and Cowan 1971), and today this value is about the same (United Soybean Board 1999). This is largely due to the undesirable flavor and odor associated with soy products (Kinsella 1979; McLeod and Ames 1988; Wilson and others 1990; Freese 1999).

Takahashi and others (1979) and Maheshwari and others (1997) treated aqueous extracts of soy flour with aldehyde oxidase, reducing selected aldehydes and the beany odor of these extracts. Kobayashi and others (1995) analyzed solvent extracts of raw-unheated soy milk by gas chromatography/olfaction (GCO), GC/mass spectrometry (MS), and aroma extract dilution analysis. They concluded that the main contributors to the odor of raw soy milk (in order with the strongest 1st) were *trans,trans*-2,4-nonadienal, *trans,trans*-2,4-decadienal, hexanal, 2-pentyl furan, 1-octen-3-one, trans-2-nonenal, an unidentified compound with a Kovat's indices (Van Den Dool and Kratz 1963) of 1561 on DB-Wax, and *trans,cis*-2,4-nonadienal.

In our previous investigation (Boatright and Lei 1999), gas chromatography/olfactometry (GCO) was used to identify major odorants from the headspace of aqueous solutions of soy-protein isolates (SPI). Volatile compounds in the headspace were concentrated under vacuum and recovered from a liquid-nitrogen trap by both solvent extraction and by using an absorbent method. From aroma extract dilution analyses, the most powerful odorants (strongest and most volatile 1st) were (1) dimethyl trisulfide (DMTS), (2) *trans,trans*-2,4-decadienal, (3) an unidentified burnt soy sauce-like odor, (4) 2-pentyl pyridine, (5) *trans,trans*-2,4-nonadienal, (6) hexanal, (7) an unidentified charred, sweaty feet-like odor, (8) acetophenone, and (9) 1-octen-3-one. This was the 1st reported occurrence of dimethyl trisulfide in soy-protein isolates. Dimethyl trisulfide was among the odorants previously identified from hydrolyzed vegetable protein (Aaslyng and others 1998). Our objective was to evaluate the primary odorants from aqueous solutions of the same 2 SPI samples using direct injection of both dynamic and static headspace volatiles.

Results and Discussion

THE ADDITION OF THE CRYOFOCUSING AND MS NO-VENT COMponents to our GCO/MS system allowed us to inject up to 25 mLs of headspace gas without contaminating our MS detector. The olfactory results from the concentrated headspace volatiles are presented in Fig. 1. Compounds that were detected at the lowest headspace volumes represent the most powerful odorants. The most powerful odorant with this method was DMTS, detected at 1-mL headspace from both samples. This correlates well with our previous findings using the absorbent and solvent methods to recover the concentrated headspace volatiles

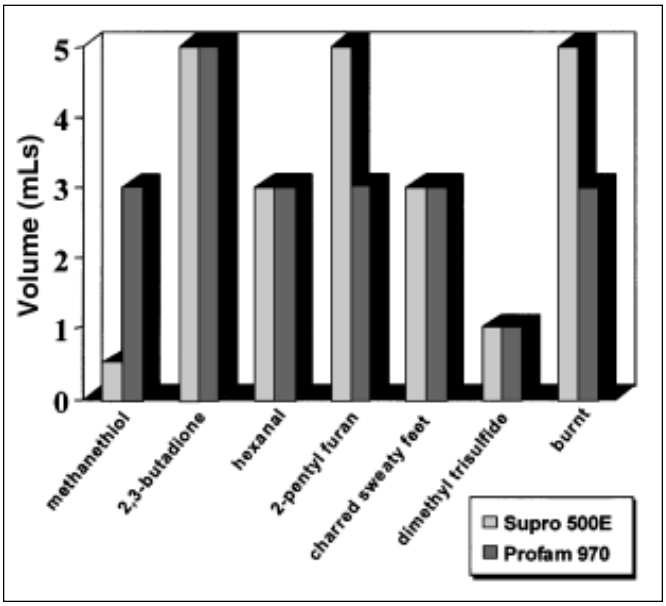

Fig. 1—Lowest headspace volume from concentrated SPI volatiles required to perceive odorants

(Boatright and Lei 1999) where DMTS was also found to be the most powerful odorant. Another powerful odorant, not detected by the 2 previous methods, was methanethiol, detected in 0.5 and 3 mLs from the 2 samples. Methanethiol was not detected with the solvent method because it is much more volatile than chloroform, and the process of concentrating the solvent resulted in the evaporation of methanethiol. It was likely not detected using the absorbent method because methanethiol is relatively polar and not absorbed onto the Tenax absorbent. The discovery of methanethiol is particularly interesting because it is a likely substrate for the synthesis of DMTS (Chin and Lindsay 1994; Nedjma and Hoffmann 1996). Qvist and von Sydow (1974) previously reported the presence of methanethiol in the concentrated headspace volatiles from aqueous slurries of SPI that had been heated at 121 °C for 37 to 41 min, but not in the unheated SPI.

Sensory results from directly injecting the concentrated headspace volatiles provided several differences from our previous aroma extract dilution analyses (AEDA) (Boatright and Lei 1999) using the same 2 SPI samples. 2,3-butadione was not detected using the AEDA because it co-eluted with the solvent. The strength of 2-pentyl furan as a potent odorant is clearly not represented by the AEDA analyses. Also, several compounds detected by AEDA (2-pentyl pyridine, acetophenone, 2,4-nonadienal, and 2,4-decadienal) are not represented in the direct headspace injection. This is due to the use of a larger-bore capillary column (0.53 mm in contrast to 0.25 mm) with a thicker stationary phase (1.2 μM in contrast to 0.25 μM). Using the same oven-temperature programming, the later eluting compound eluted too late to be detected by olfactometry.

Results from the injection of static headspace volatiles with cryofocusing are shown in Fig. 2. Injecting 25 mLs of headspace allowed only 4 compounds to be detected by olfactometry. Again, DMTS was the most powerful odorant as determined by the minimal headspace required to detect (1.25 and 5 mls) in the 2 samples. Hexanal required 2.5 and 5 mLs, methanethiol required 10 and 20 mLs, and 2-pentyl furan required 25 mLs in the 2 samples. The PTI sample required less headspace to detect DMTS and hexanal by the static headspace method, and methanethiol by both the static and dynamic methods. The ADM sample required less headspace to detect 2-pentyl furan and an unidentified burnt-like odorant by the dynamic headspace method. Both methods resulted in the same odorants being detected in each SPI sample. With the static headspace method, again 2-pentyl furan was confirmed as a major odorant. The AEDA method appears to greatly underestimate the importance of 2-pentyl furan. It is not clear why this difference occurred. Because the difference occurred between the methods of recovering the concentrated volatiles after distillation (solvent and absorbent in contrast to headspace methods), it may relate to the physical characteristics of 2-pentyl furan in water compared to the other volatile compounds, or perhaps the difference could be due to degradation of 2-pentyl furan in both the chloroform extraction and the tenax-absorbent methods. This is an excellent example of why it is prudent to use several different methods to evaluate the occurrence of compounds from complex-organic material.

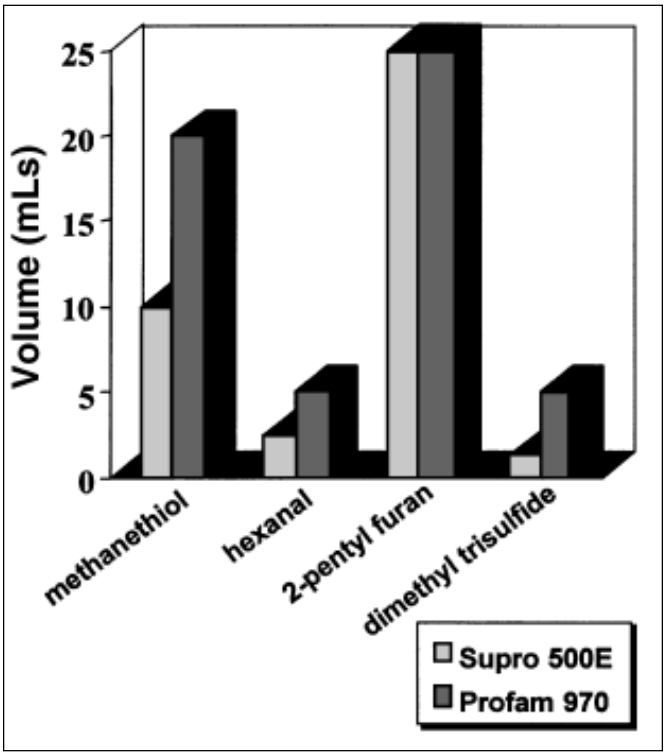

Fig. 2—Lowest static headspace volume from SPI volatiles required to perceive odorants

DMTS in the aqueous SPI was quantified by incorporating d_6-DMTS into the slurry just prior to distillation. The DMTS content of the ProFam 970 SPI was 60.14 ppb (s.e. 2.18) and 45.54 ppb (s.e. 3.38) in the Supro 500E. With an odor threshold value in water of 0.01 ppb (Whitfield and Last 1991), this corresponds to odor values (level in SPI divided by odor threshold) of 6014 and 4554, respectively. This is the highest reported odor value of any compound found in SPI. 2-pentyl pyridine's flavor value was higher (Boatright and Crum 1997), but this is because its published taste threshold is about 50 times lower than its published odor threshold. While the Supro 500E contained less DMTS than the ProFam 970, the amount of headspace required to detect DMTS by the static headspace method was less with the Supro 500E sample. This may be due to differences in the interaction between DMTS and other components in the aqueous mixture of these samples.

To further investigate the possibility that any of these compounds could be an artifact resulting from the elevated temperature of the GC injector (130 °C) used in these headsapce analyses and 210 °C used in the solvent-injection method, we installed a cool, on-column injector on our GCO/MS system. Maintaining the injector and oven at 35 °C and injecting the concentrated headspace volatiles directly onto the column provided similar olfactory results as using the higher injection temperature. Injecting solvent-recovered volatiles containing an internal standard of d_6-DMTS produced very similar ratios of m/z 132 to 126 using the cool, on-column method (1:1.79), compared to using the typical solvent-injection port (1:1.76) with a temperature of 210 °C. This is very strong evidence that none of the major odorants found in this study were chromatographic artifacts.

Conclusions

THIS IS THE 1ST INVESTIGATION TO REPORT METHANETHIOL AS A component of unheated-SPI and the 1st to demonstrate the relative importance of compounds that contribute to the odor of aqueous solutions of soy-protein products using either static or dynamic headspace analyses with GCO/MS techniques. In order to confirm the relative contribution of each odorant, we hope to combine the major odorants and re-create the characteristic odor of aqueous SPI. While accurately quantifying the major odorants will provide a useful starting point, the complex and varied interaction between odorants, soy proteins, and other compounds of SPI will require these concentrations in pure water, or aqueous solution of other proteins, to be significantly altered to obtain a comparable odor profile. Also, synergistic effects and the contributions from other minor odorants may make a significant contribution to the overall odor profile of aqueous solutions of soy-protein products.

Materials and Methods

Protein products

SPI samples were designated as Pro Fam 970 (from the Archer Daniels Midland Co. (ADM), Decatur, Ill., U.S.A.) and Supro 500E (from Protein Technologies International (PTI), St. Louis, Mo., U.S.A.).

Chemicals

Hexanal, dimethyl trisulfide, methanethiol, and 2,3-butadione, were obtained from Sigma Aldrich Chemical Co. (St. Louis, Mo., U.S.A.). Bedoukian Research, Inc. (Danbury, Conn., U.S.A.) donated 2-pentyl furan. Deuterium-labeled dimethyl trisulfide was prepared by the methods of Milligan and others (1963) using iodomethane-d_3 (Isotec Inc., Miamisburg, Ohio, U.S.A.) in place of the unlabeled iodomethane.

The concentration of d_6-dimethyl trisulfide was determined by gas chromatography/mass spectrometry (GC/MS) with the corresponding unlabeled compound used as an internal standard. The standard curve was prepared with mixtures containing known amounts of the labeled and unlabeled compound in the appropriate range. The ratio of the relative abundances of the *m/z* 126 for the unlabeled DMTS and *m/z* 132 for the labeled DMTS were plotted against the weight ratio of the labeled compounds over the sum of the labeled and unlabeled compounds as described by Guth and Grosch (1990).

Dynamic and static headspace analyses

Volatile compounds from the headspace of aqueous solutions of SPI were concentrated by the method of Forss and others (1967). SPI (25 g) and 500 mL of water from a Barnstead Nanopure 4-Module System (Fisher Scientific, Pittsburgh, Pa., U.S.A.) were placed in a 2-L flask and distilled for 2.5 h while stirring at 24 °C under 711-mm Hg vacuum. Volatile compounds were collected from the liquid-nitrogen trap by placing a septum over the trap outlet and bringing it to 25 °C. Various volumes of headspace gas were withdrawn using a syringe equipped with an inert-gas sampling valve.

For static headspace analyses, SPI (25 g) and 500 mL of water from a Barnstead Nanopure 4-Module System (Fisher Scientific) were placed in a 2-L flask sealed with a septum and stirred. After 1 h, various volumes of the unconcentrated headspace were withdrawn using a syringe equipped with an inert-gas sampling valve.

Quantifying dimethyl trisulfide in SPI

The procedure for concentrating volatiles was repeated with the incorporation of d_6-dimethyl trisulfide into the SPI slurry prior to distillation. Labeled DMTS was added at a level corresponding to 60 parts per billion (ppb) of the SPI (dry basis). The volatile compounds were extracted from the aqueous distillate (about 7 mL) twice with 2 mL of Spectranalyzed chloroform (Fisher Scientific). The combined chloroform phases were dried over anhydrous Na_2SO_4 and concentrated to 100 uL under a flow of dry nitrogen. Samples were stored in a freezer at −15 °C.

Gas chromatography, olfactometry, and mass spectroscopy

GCO/MS were accomplished on a Hewlett Packard Model 5890 Series II GC with a 5971A mass spectrometer and an MS-Novent system (SGE Intl., Ringwood, Australia). During injection of up to 25 mLs of headspace gas, the helium purge through the MS-Novent system prevented contamination of the mass spectrometer. Analytes were cryo-focused on an indirect liquid-nitrogen trap (SGE Intl.) at the beginning of the column maintained at -60 °C during the injection process. The column was a EC-Wax capillary column (30 m × 0.53 mm i.d.) with 1.2 μm film thickness (Alltech Associates, Inc., Deerfield, Ill., U.S.A.). The helium flow-rate through the columns was about 3 mL/min with 2 mL/min emerging from the sniff port (SGE Intl.). The column temperature was held at 40 °C for 5 min, then increased at 3 °C/min to 165 °C, then to 220 °C at 20 °C/min. The electron-ionization detector was set to detect in the mass range of 35 to 250 *m/z*. The injection-port temperature was maintained at 130 °C for headspace analyses and at 210 °C for solvent injections. All determinations were performed in duplicate. Minimum reported headspace volumes necessary to detected odorants by olfaction required conformation by both investigators. Identification of compounds were by (1) comparison of mass spectra to a spectral database (NIST98) (ChemSW, Inc., Fairfield, Calif., U.S.A.); (2) comparison to retention times of authentic standards; and (3) comparison of olfactory response to authentic standards. Cool, on-column injections were accomplished by installing an on-column injection sleeve on the Hewlett Packard GCO/MS system. Injections were made with a 12.5-cm needle directly onto the column. The injection port and oven were maintained at 35 °C during the injection with cryofocusing.

References

Aaslyng MD, Elmore JS, Mottram DS. 1998. Comparison of the aroma characteristics of acid-hydrolyzed and enzyme-hydrolyzed vegetable protein produced from soy. J Agri Food Chem 46:5225-5231.

FDA, 1999. Federal Register Oct 26 1999 Vol. 64 Number 206, Rules and Regulations p 57699-577733.

Boatright WL, Crum AD.1997. Odor and flavor contribution of 2-pentyl pyridine to soy protein isolates. J Am Oil Chem Soc 74:1575-1581.

Boatright WL, Lei Q. 1999. Compounds contributing to the "beany" odor of aqueous solutions of soy protein isolates. J Food Sci 64:667-670.

Chin HW, Lindsay RC. 1994. Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. Food Chem 49:387-392.

Forss DA, Jacobsen VM, Ramshaw EH. 1967. Concentration of volatile compounds from dilute aqueous solutions. J Agri Food Chem 15:1104-1107.

Freese B. 1999. Soy is healthy, but will we eat it? Successful Farming 97(7): http://www.agriculture.com/sfonline/sf/1999/soy.html. Meredith Corp.

Guth H, Grosch W. 1990. Deterioration of soya-bean oil: Quantification of primary flavor compounds using a stable isotope dilution assay. Lebensmittel-Wissenschaft und-Technologie 23:513-522.

Kinsella JE. 1979. Functional properties of soy proteins. J Food Sci 56:242-257.

Kobayashi A, Tsuda Y, Hirata N, Kubota K, Kitamura K. 1995. Aroma constituents of soybean [Glycine max (L.) Merril] milk lacking lipoxygenase isozymes. J Agri Food Chem 43:2448-2452.

Maheshwari P, Murphy PA, Nikolov ZL. 1997. Characterization and application of porcine liver aldehyde oxidase in the off-flavor reduction of soy proteins. J Agri Food Chem 45:2488-2494.

McLeod G, Ames J. 1988. Soy flavor and its improvement. Crit Rev Food Sci Nutr 27:219-402.

Milligan B, Saville B, Swan JM. 1963. Trisulphides and tetrasulphides from bunte salts. J Chem Soc. 3608-3614.

Nedjma M, Hoffmann N. 1996. Hydrogen sulfide reactivity with thiols in the presence of copper (II) in hydroalcoholic solutions or cognac brandies: Formation of symmetrical and unsymmetrical dialkyl trisufides. J Agri Food Chem 44:3935-3938.

Qvist IH, von Sydow ECF. 1974. Unconventional proteins as aroma precursors. Chemical analysis of the volatile compounds in heated soy, casein, and fish protein model system. J Agri Food Chem 22:1077-1084.

United Soybean Board. 1999. Soybeans checkoff research shows growing appetites for soy foods. SoySource: Sept. 16.

Takahashi N, Sasaki R, Chiba H. 1979. Enzymatic improvement of food flavors IV. Oxidation of aldehydes in soybean extracts by aldehyde oxidase. Agri Biol Chem 43:2557-2561.

Van Den Dool H, **Kratz PD.** 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatog 11:463-471.

Whitfield FB, Last JH. 1991. Vegetables. In: Maarse H, editor. Volatile compounds in foods and beverages. New York: Marcel Dekker, Inc. p 214, 237.

Wilson LA, Johnson LA, Hayenga ML, Love MH. 1990. Protein used for food and industrial products. In: Greiner CA, editor. Economic implications of modified soybean traits. Ames, Iowa: Iowa Agricultural and Home Economics Experiment Station. **p 11-19.**

Wolf WJ, Cowan JC. 1971. Soybeans as a food source. Boca Raton. CRC Press Inc. p 33-77.

MS 19991041 received 10/14/99; revised 2/18/00; accepted 4/10/00.

This project was funded in part by the Kentucky Soybean Promotion Board. Published with the approval of the Director of the Kentucky Agricultural Experiment Station as Journal Article #99-07-136.

Authors are with the Animal Science Department, University of Kentucky, Lexington, KY 40546-0215. Direct correspondence to William L. Boatright (E-mail: wlboat1@pop.uky.edu).