Compounds Contributing to the "Beany" Odor of Aqueous Solutions of Soy Protein Isolates

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ABSTRACT

Using gas chromatography/olfactometry (GCO), major odors from the headspace of aqueous solutions of soy protein isolates were evaluated. Many corresponding odorants were identified by correlating GCO with GC/mass spectrometry (MS) on two separate stationary phases followed by comparing retention times, mass spectra, odor descriptions and odor intensities with authentic standards. Based on aroma extract dilution analyses, the most powerful odorants (strongest and most volatile first) were (1) dimethyl trisulfide, (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 is the first reported occurrence of dimethyl trisulfide in soy protein isolates.

Key Words: soybean protein isolate, headspace volatiles, olfactory analysis, dimethyl trisulfide, mass spectrometry

INTRODUCTION

THE FLAVOR OF SOY PROTEIN PRODUCTS (often described as having a beany odor and a throat-catching and bitter taste) has long limited the expanded use of soy proteins in human foods (Kinsella, 1979; McLeod and Ames, 1988; Wilson et al, 1990). The beany odor components of soy products have been thought to include aliphatic carbonyls, volatile fatty acids, amines, alcohols and furans derived, in part, from the action of soybean lipoxygenase and subsequent formation of lipid oxidation products (Wolf and Cowan, 1975; Sessa and Rackis 1977). Takahashi et al. (1979) and Maheshwari et al. (1997) treated aqueous extracts of soy flour with aldehyde oxidase, reducing selected aldehydes and the beany odor of these extracts. The contribution of individual aldehydes to the overall beany odor was not demonstrated.

Until Kobayashi et al. (1995) analyzed solvent extracts of soy milk by gas chromatography/olfactometry (GCO), GC/mass spectrometry (MS) and aroma extract dilution analysis, there was no published evidence of the relative contribution of individual odorants comprising the overall beany odor of a soy product. They concluded that the main contributors to soy milk beany odor (in order with the strongest first) 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, 1963) of 1561 on DB-Wax, and *trans,cis*-2,4-nonadienal.

Boatright and Crum (1997) analyzed the lipid extracts from "dry" commercial soy protein isolates (SPI) by GCO and GC/MS. The most potent odorants detected by GCO were butyric acid, 2-methyl butyric acid methyl ester, 2-pentyl pyridine and hexanal. Subsequent sensory analyses demonstrated that 2-pentyl pyridine had a taste threshold in water (0.000012 ppm), approximately 50 times lower than its published odor threshold, contributed a throat-catching taste and grassy-like odor and had the greatest reported flavor value of any compound reported from SPI. Our objective was to determine the major odors and corresponding odorants that contribute to the "beany" odor of aqueous solutions of SPI.

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MATERIAL & METHODS

Protein products

SPI samples were designated as Pro Fam 970 (from the Archer Daniels Midland Co., Decatur, IL) and Supro 500E (from Protein Technologies International, St. Louis, MO).

Chemicals

Butyric acid, hexanal, pentanal, 2-heptanone, dimethyl disulfide, dimethyl trisulfide, 2,3-butadione, 2,3-pentadione, benzaldehyde, acetophenone, *trans, trans*-2,4-nonadienal, and *trans,trans*-2,4-decadienal were obtained from Sigma Chemical Co. (St. Louis MO). Lancaster Synthesis Inc. (Windham NH) provided 2-pentyl pyridine. Bedoukian Research, Inc. (Danbury, CT) donated 2-pentyl furan and 1-octen-3-one was synthesized by the method of Corey and Schmidt (1979).

Concentration of headspace compounds

Volatile compounds from the headspace of aqueous solutions of SPI were concentrated by the method of Forss et al. (1967). SPI (25g) and 500 mL of water from a Barnstead Nanopure 4-Module System (Fisher Scientific, Pittsburgh, PA) were placed in a 2 L flask and distilled for 2.5 h while stirring at 24°C under 711 mm Hg vacuum. Headspace volatiles were also concentrated by inserting a stream of compressed air into a side arm of the 2 L flask and bubbling compressed air into the SPI slurry at 55 mL per min. The stream of air diverted the headspace volatiles into the liquid nitrogen-cooled trap.

Volatile compounds were collected from the liquid nitrogen trap by two methods. First, a glass splitter was attached to the outlet of the trap prior to removing it from liquid nitrogen. The two splitter exit ports were fitted with GC injector liners packed with Tenax® absorbent (Alltech Associates, Deerfield, IL). These included a 2 mm liner packed with 26 mg absorbent for GCO and, for GC/MS a 4 mm liner was packed with 106 mg absorbent. The trap was then removed from the liquid nitrogen and the distilled mixture of volatile compounds was allowed to melt. The volatiles were then stripped from the distillate with a stream of ultra-high purity helium for 15 min at 75 mL/min and trapped in the packed injection liners. Each distillate was used only once. The second method collected the volatile compounds by extracting the aqueous distillate (~7 mL) twice with 2 mL of Spectranalyzed® chloroform (Fisher Scientific, Pittsburg, PA). The combined chloroform phases were dried over anhydrous Na_2SO_4 and concentrated to 100 μL under a flow of dry nitrogen. Samples were stored in a freezer at −15°C.

Gas chromatography, olfactometry and mass spectroscopy

Identification of compounds that contribute undesirable odors to SPI was accomplished using a Perkin-Elmer Autosystem gas chromatograph with integrated autosampler equipped with a hydrogen flame-ionization detector (FID) and model 970 Intelligent Interface for data analysis. For olfaction, the chloroform recovered headspace volatiles (4 μL) were loaded onto the injector liner (maintained at 210°C) and held splitless for 1 min.

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The outlet from a EC-Wax capillary column (Alltech Assoc., Inc., Deerfield, IL) (30 m × 0.25 mm i.d.) with 0.25 μm film thickness or a DB-225 capillary column (J&W Scientific, Folsom, CA) (30 m × 0.32 mm i.d.) with 0.25 μm film thickness was divided with an O.S.-2 outlet splitter system (SGE Intl., Ringwood, Australia). One-third of the splitter outlet was directed to the FID detector (maintained at 250°C) and the other two-thirds to a sniffing port (SGE Intl., Ringwood, Australia). Make-up gas was supplied to the splitter-tee at 4 mL/min. Reported odor intensities are the mean values from both investigators. The Kovats indices (Van Den Dool, 1963) of the detected odor compounds from the solvent injections were determined using FID response to C_{10} and $C_7 - C_{25}$ odd carbon-numbered paraffins. There was no measurable delay between detected odor and FID response.

The trapped volatiles were desorbed from the absorbent packed injector liner on the Perkin-Elmer Autosystem gas chromatograph by placing the packed liner in the inlet at 30°C and increasing the temperature to 210°C at 45°C/min (4 min desorption time). Desorption on the GC/MS occurred by placing the packed liner in the 30°C injector and increasing the temperature to 210°C at 26°C/min, followed by holding at 210°C for 1 min during tuning of the mass spectrometer (8 min total desorption time). At all times during desorption the injector valves were closed and the first loop of the column was submerged in a dry ice-acetone bath. Desorbed volatiles were transferred in helium at 1.0 mL/min onto the appropriate capillary column and condensed in the first loop of the column cooled in a dry ice-acetone bath. Following the described desorption times, the dry ice-acetone bath was removed and the temperature program was begun (same on both gas chromatographs). After the described desorption times, the injector remained splitless for an additional 2 min.

GC/MS was done on a Hewlett Packard Model G1800A GCD System (Wilmington, DE) equipped with an electron ionization detector (EID) , Model G1030A Chemstation controller and capillary columns described above. The column temperature on both GC systems 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. High-purity helium was the carrier gas at 1.0 mL per min. The EID was set to detect in the mass range of 25 to 250 *m/z*. All determinations were performed in duplicate. Identification of lipid compounds was by comparison of mass spectra to a spectral database (NIST98) (ChemSW, Inc., Fairfield, CA), retention times of authentic standards and olfactory response. Solutions of each standard were prepared at concentrations similar to those found in the recovered SPI volatiles. A positive identification required a comparable odor description and odor intensity by GCO.

Aroma extract dilution analyses (AEDA; Grosch, 1993) were accomplished by diluting the chloroform recovered headspace volatiles from the aqueous solutions of SPI stepwise with chloroform in a ratio of $1{:}10^n$, where *n* was selected from 1, 2, or 3 as the dilution factor. Aliquots of 4 μL of a diluted sample were analyzed by GCO as previously described.

Additional GCO/MS was accomplished on a Hewlett Packard Model 5890 Series II GC with an O.S.-2 outlet splitter system (SGE Intl., Ringwood, Australia) that sent 1/3 of the column effluent to a 5971A MS detector (mass range of 25 to 250 *m/z)* and 2/3 to a sniffing port (SGE Intl., Ringwood, Australia). The column used was an SPB-1 capillary column (15 m × 0.53 mm i.d.) with 0.25 μm film thickness (Supelco, Bellefonte, PA). The helium flow rate through the columns was about 3 mL/min. The column temperature was held at 40°C for 5 min, then increased at 5°C/min to 165°C and held for 5 min. Sample injection was accomplished with a Tekmar LSC 2000 (Cincinnati, OH) purge and trap concentrator with a Tenax® packed trap. The transfer line was attached to a purged-packed injection port maintained at 210°C. The nickel transfer line was held at 175°C. The aqueous solution of SPI was placed into glass purge tubes connected in tandem so that foaming of the SPI solution did not contaminate the trap. The purge time was 15 min, purge flow was 40 mL/min, dry purge time was 1 min, desorb preheat temperature was 180°C, desorb time was 4 min, and desorb temperature was 180°C.

RESULTS & DISCUSSION

FOUR RESEARCHERS FAMILIAR WITH SOY products were further trained to recognize the characteristic "beany" odor of SPI. All four agreed that the aqueous solutions studied had the typical odor characteristic of SPI.

GCO descriptions and mean intensities of the odorants concentrated by both absorbent and solvent methods from the two SPIs on two stationary phases were listed and compared (Table 1, 2). Average relative intensities, and Kovat's indices (KI) (Van Den Dool, 1963) for the identified odorants, as well as for the major unidentified odorants, were compared (Table 3) on each stationary phase. Identification was by comparison of mass spectra, retention times, odor descriptions and odor intensities with authentic standards. The GCO analyses of the concentrated headspace volatiles revealed odor characteristics of the common lipid oxidation by-products along with several sulfurous odors and some charred or burnt-like odors. The sulfur-containing compounds likely resulted from the degradation of sulfur containing amino acids and burnt-like odor compounds often result from Maillard reactions. Pyrazines often contribute to burnt odors, but we were unable to identify the responsible odorants.

Concentration was very useful for identifying odorants with extremely low odor thresholds which may not have been present in sufficient quantity to provide a good mass spectrum. An example was 1-octen-3-one, the compound responsible for the strong mushroom-like odor. In our previous investigation (Boatright and Crum, 1997), we reported this mushroom odor from the lipid extracts of "dry" SPI, but were not able to identify the odorant. The absorbent method revealed the lower boiling point odorants which had been obscured by the solvent peak. The two most notable of these odorants as determined by GCO were 2,3-butadione and pentanal.

Performing AEDA with the chloroform recovered headspace volatiles provided an indication of the most powerful odorants comprising the "beany" odor of SPI (Fig. 1). From these results the strong sulfur odor, two oxidized fat odors, a burnt soy sauce and a penetrating grassy odor were the strongest contributors. Seven of the nine most powerful odorants were identified. The unidentified compound that contributed the burnt soy sauce odor may be due to co-eluting compounds and/or artifact formation in the injection port. On EC-Wax, it was not detected by the method of gradually heating the absorbent. This does not expose most compounds to the higher temperatures encountered when the samples are directly injected into the GC liner at 210°C. Also, stationary phases of different polarities usually provide less than a 200 KI shift, with the higher KI occurring on the more polar EC-Wax. On the EC-Wax column, a burnt soy sauce odor was detected at a KI of 1468. On the less polar DB-225 column, a burnt soy sauce-like odor was detected at a KI of 1811 using both isolation methods. These similar odors appeared to result from different compounds.

The AEDA results provided some contradictions to the perceived intensities of these odorants before dilution. For example, hexanal was not perceived above a dilution factor of 100 by AEDA, but was perceived as the strongest odorant in the concentrated samples. By diluting the concentrated extracts, AEDA provided a more realistic representation of the potency of these compounds in the headspace above an aqueous solution of SPI (Grosch, 1993). While 2-pentyl pyridine was detected at a dilution factor of 1000 for Supro 500E and 100 for Profam 970, it was not perceived by the evaluators as one of the most powerful odorants from the concentrated headspace volatiles. At a dilution factor of 10, hexanal was perceived as being more powerful, and at a dilution factor of 100 hexanal and 2-pentyl pyridine were rated as having similar degrees of impact. A similar relationship was found between hexanal and other odorants, such as *trans,trans*-2,4-decadienal and *trans,trans*-2,4-nonadienal. Note that since the AEDA assays used the chloroform extracts the impact of 2,3-butadione and pentanal were not evaluated due to solvent interference. While

Table 1—Comparison of clearly distinguishable odors and their intensities by GCO of concentrated headspace volatiles aqueous solutions of SPI on DB-225

Odor description	Kovats indices[a]	Headspace by $CHCl_3$[bc]	Headspace by $CHCl_3$[bd]	Headspace by absorbent[bc]	Headspace by absorbent[bd]
Buttery	—	—	—	+++	+++
Oxidized/nutty	—	—	—	+++	+++
Sulfur	—	—	—	+++	++
Buttery	1003	++	++	++	n.d.
Sulfur	1014	++	++	n.d.	n.d.
Oxidized/nutty	1040	++++	+++++	+++++	+++++
Burnt-sweaty feet	1104	++	++	++	+++
Sulfur	1109	++	n.d.	n.d.	n.d.
Flowery/grassy	1146	+++	++	++++	++++
Charred-sweaty feet	1220	++++	++++	++	+
Sulfurous/green onion	1234	+++	+++	++++	+++
Mushroom	1235	++	++	++	++
Fruity	1261	++	++	++	+++
Burnt-sweaty feet	1286	+++	n.d.	+++	n.d.
Charred melon	1330	+++	+++	+++	+++
Almonds	1338	+++	++++	++++	+++
Fatty	1372	++	+++	+++	++
Green/burnt	1387	n.d.	++++	n.d.	+
Grassy/minty	—	n.d.	n.d.	+++	+++
Burnt Grassy	1387	++	+++	++	++
Sulfur	—	n.d.	n.d.	++	n.d.
Penetrating grassy	1463	++	+++	++	++
Penetrating green	1482	+++	++	+++	+++
Green vegetable	—	n.d.	n.d.	+++	n.d.
Burnt paper	1543	++++	++++	+++	n.d.
Oxidixed/fatty	1595	+++	+++	+++	++++
Green burnt	1562	n.d.	+++	n.d.	n.d.
Oxidixed/fatty	1697	++	+++	+++	+++
Citrus/fruity	1740	++	++	++	+
Burnt soy sauce	1809	++	n.d.	++	++

[a] = Kovats calculated from the $CHCl_3$ extract of the distillate.
[b] + = mild, ++ = medium, +++ = strong, ++++=very strong, +++++ = very strong and sustained (mean of both evaluators).
[c] Profam 970
[d] Supro 500E

Table 2—Comparison of clearly distinguishable odors and their intensities by GCO of concentrated headspace volatiles aqueous solutions of SPI on EC-Wax

Odor description	Kovats indices[a]	Headspace by $CHCl_3$[bc]	Headspace by $CHCl_3$[bd]	Headspace by absorbent[bc]	Headspace by absorbent[bd]
Sweaty feet	—	—	—	++	+
Grassy/sweaty feet	—	—	—	++	+
Burnt alcohol	—	—	—	++	+
Buttery	—	—	—	++	++
Oxidized/nutty	—	—	—	++	++
Unpleasant	—	—	—	++	++
Smoky-rotten fruit	—	—	—	++	+
Buttery	1089	++	+	+	+
Sulfur	—	n.d.	n.d.	++	+
Oxidized/nutty	1121	+++++	+++++	+++++	+++++
Sulfur	1129	n.d.	+	++	++
Caramel-ketone	1172	++	n.d.	++	n.d.
Smoky-putrid	—	n.d.	n.d.	++	+++
Flowery	1194	+++	++	++++	+++
Grassy	1238	++	+	++++	++
Fruity	1245	+	++	++	++
Melon	1289	+	++	+++	++
Mushroom	1306	++	++	+++	+++
Burnt-nutty	1330	n.d.	+	++	+
Charred-sweaty feet	1342	+++	++++	+++	+++
Sulfurous/green onion	1387	+++	+++	++++	++++
Melon	—	n.d.	n.d.	++	++
Burnt-nutty	1427	++	+++	++	+++
Grassy/minty	1441	++	+++	+++	+++
Earthy/burnt	1450	+	++	++	+++
Burnt soy sauce	1468	+++	+++	n.d.	n.d.
Burnt caramel	1493	+	+++	n.d.	++
Almonds	1526	+++	+++	++	+++
Fatty-melon	1544	n.d.	n.d.	++	n.d.
Green-nutty	1557	++	n.d.	++	n.d.
Burnt celery	1569	++	+	n.d.	n.d.
Penetrating grassy	1578	++	++	n.d.	+
Harsh Burnt	—	n.d.	n.d.	+++	n.d.
Penetration Green	1654	+++	++++	+++	+
Fatty	1684	n.d.	+++	n.d.	+++
Oxidixed/fatty	1703	+++	+++	+++	+++
Oxidixed/fatty	1823	+++	+++	++	+
Citrus/fruity/burnt	1840	++	+	+++	n.d.

[a] Kovats calculated from the $CHCl_3$ extract of the distillate.
[b] + = mild, ++ = medium, +++ = strong, ++++=very strong, +++++ = very strong and sustained (mean of both evaluators).
[c] ProFam 970
[d] Supro 500E

the source of burnt soy sauce-like odor appeared to be an artifact, it was not confirmed.

While not previously identified as specific contributors to the odor of soy products, most of the compounds listed (Table 3) have been reported in headspace analyses of soy products. For example, 2,3-butadione, pentanal, 2,3-pentadione, dimethyl disulfide, hexanal, 2-heptanone, 2-pentyl furan, benzaldehyde and aceotphenone were identified by Qvist and von Sydow (1977) and/or del Rosario et al (1984). Kobayashi et al (1995) used GCO to identify most of the major odorants from soy milk. These odorants included *trans,trans*-2,4-nonadienal, *trans,trans*-2,4-decadienal, hexanal, 2-pentyl furan and 1-octen-3-one as primary odorants. The Kovat's indices (Van Den Dool, 1963) of 2-pentyl pyridine on EC-Wax (1578) is similar to the Kovat's indices of one of their unknowns on DB-Wax (1561). The odor description was not provided, however. Our investigation is the first to report dimethyl trisulfide (DMTS) as a component of SPI. From the aroma extract dilution analyses, DMTS and *trans,trans*-2,4-decadienal were the two most powerful odorants.

To investigate the possibility that DMTS could be an artifact from the distillation procedures, a purge and trap concentrator was used to inject volatile compounds from aqueous solutions of the same SPIs into a GCO/MS system. DMTS was again found to be a major odorant from aqueous solutions of SPI although this system caused over half of the standard DMTS (5 μg/5 mL water) to be degraded (as determined by MS response) into dimethyl disulfide and a lesser amount of dimethyl tetrasulfide. No such degradation occurred with our previously reported methods. This problem of standard degradation prevented us from using the purge and trap method more extensively; however, it did not indicate that DMTS was an artifact formed during distillation.

DMTS, methanethiol and β-ionone have been reported to be major contributors to the offensive odors formed when broccoli florets were stored under reduced-oxygen conditions (Hansen et al., 1992). Chin and Lindsay (1994a) demonstrated that DMTS could be formed from methanethiol in the presence of transition metals and ascorbate. Another proposed mechanism for DMTS formation in *Brassica* vegetables (including broccoli) involves the conversion of S-methylcysteine sufoxide to sulfenic acid (by the action of cystine lyase) with subsequent dimerization to methyl methanethiosufinate (Marks et al., 1992). The sulfinate could then react rapidly with hydrogen sulfide to form DMTS (Chin and Lindsay, 1994b). The reaction of methyl methanethiosulfinate and methyl methanethiosulfonate with hydrogen sulfide to form DMTS in model systems has been confirmed by Chin and Lindsay (1994) and was proposed to be the prominent mechanism for the

Table 3—Identified odor compounds with corresponding odor description and Kovat's Indicies on EC-Wax and DB-225

Odor description	Mean Intensity-headspace analyses[b]	Compound	DB-225 Kovat's indices Found	DB-225 Kovat's indices Std	EC-Wax Kovat's indices[a] Found	EC-Wax Kovat's indices[a] Std
Buttery	+++	2,3-butadione	N/A	N/A	N/A	N/A
Oxidized/nutty	+++	pentanal	N/A	N/A	N/A	N/A
Buttery	+	2,3-pentadione	1003	991	N/A	1102
Sulfur	+	dimethyl disulfide	N/A	N/A	N/A	1127
Oxidized/nutty	+++++	hexanal	1040	1045	1121	1135
Flowery	+++[c]	2-heptanone	1146	1143	1194	1206
Grassy	++[c]	2-pentyl furan	1146	1140	1238	1246
Charred-sweaty feet	+++	?	1220	?	1342	?
Sulfurous/green onion	+++	dimethyl trisulfide	1234	1233	1387	1390
Mushroom	++	1-octen-3-one	1235	1233	1306	1306
Almonds	+++	benzaldehyde	1338	1337	1526	1529
Penetrating grassy	++	2-pentyl pyridine	1463	1461	1578	1578
Penetrating green	+++	acetophenone	1482	1487	1654	1655
Oxidixed/fatty	+++	*trans*-2,4-nonadienal	1595	1601	1703	1700
Oxidixed/fatty	+++	*trans*-2,4-decadienal	1697	1708	1823	1825
Burnt soy sauce	N/A	?	1809	?	1468	?

[a] Kovat's Indices calculated from the $CHCl_3$ extract of the distillate, N/A=not available
[b] + = mild, ++ = medium, +++ = strong, ++++=very strong +++++ = very strong and sustained (mean of both evaluators for all headspace analyses)
[c] Due to overlap on DB-225, mean from Table 2 only.

formation of methanethiol and DMTS in *Brassica* vegetables**.** To determine whether the reduced oxygen conditions of our vacuum distillation had a notable effect of DMTS from aqueous SPI, we replaced the vacuum with a stream of air to drive the volatiles into the liquid nitrogen cooled trap. GCO and GC/MS analyses revealed comparable levels of DMTS (data not shown).

It is unlikely that the enzymatic conversion of S-methylcysteine sulfoxide by cysteine lyase to methyl methanethiosulfinate occurs in soy protein products because of the apparent absence of key reactants. A more likely reaction mechanism would be similar to that proposed by Nedjma and Hoffmann (1996) comprising a redox reaction involving methanethiol, H_2S and copper. Methanethiol and hydrogen sulfide have been detected in the headspace of heated SPI (Qvist and von-Sydow, 1977; del Rosario et al., 1984). Another possible mechanism could be similar to that proposed by Jung et al., (1998) for the formation of DMDS except involving H_2S to form DMTS. In this mechanism, the singlet oxygen-initiated oxidation of methionine would result in the formation of the methyl sulfanyl radical, which acts similarly to the methanethiol mechanism proposed by Chin and Lindsay (1994b) in DMDS formation. The presence of hydrogen sulfide (Chin and Lindsay, 1994b) could facilitate the synthesis of DMTS.

This investigation is the first to use GCO/MS to identify major odorants that contribute to the characteristic "beany" odor of aqueous solutions of SPI. Several compounds that have been shown to contribute to the odor of soy milk (Kobayashi, 1995), such as 1-octen-3-ol, 1-pentanol, hexanol, were not detected by GCO from SPI, even in the concentrated headspace volatiles.

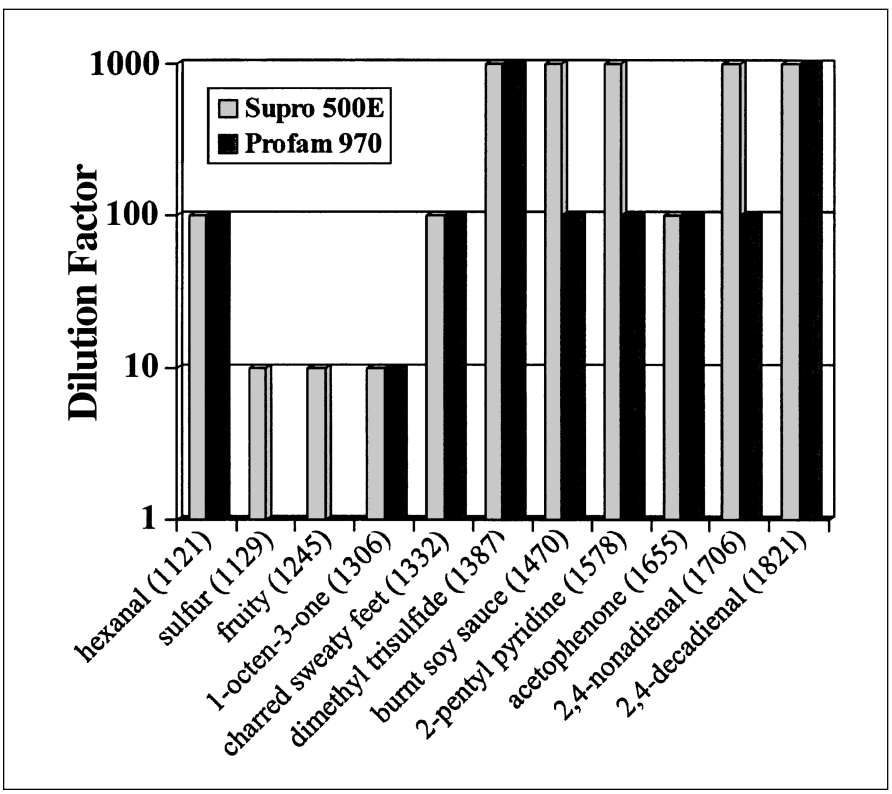

Fig. 1—Aroma extract dilution analysis of the predominate odorants or odors (with Kovat's Indices) from chloroform headspace volatiles of soy protein isolates on EC-Wax.

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