Identification of Selenomethionine in Soybean Protein

Kyoden Yasumoto,* Tetsuya Suzuki, and Munehiro Yoshida

The chemical form of selenium contained in soybean protein with a normal range of selenium levels was determined by proteolytic enzyme digestion, gel permeation column chromatography, thin-layer chromatography, and gas chromatography–mass spectrometry (GC–MS). Pepsin digestion of soybean protein isolate paralleled the liberation of dialyzable selenium. Actinase E digestion of pepsin- and pancreatin-digested soybean gave rise to selenium-containing substances located on thin-layer chromatography at the same position as selenomethionine. Analytical data obtained by GC–MS confirmed an occurrence of selenomethionine in the digest as a constituent in the soybean protein.

Selenium is an esstential trace element for animals (Schwartz and Foltz, 1957; Thompson and Scott, 1969). Its function in animals has been explained in relation to the function of glutathione peroxidase (GSH-Px) (Flohè et al., 1973; Rotruck et al., 1973). An endemic called Keshan disease in the northeastern part of mainland China was identified as related to the deficiency of selenium (Keshan Disease Research Group of the Chinese Academy of Medical Sciences, 1979a,b; Chen et al., 1980). This report led to the knowledge of the importance of selenium as an essential trace nutrient for humans.

It has been reported that the average selenium intake in Japanese adults is around 100 μg/day per capita (Sakurai and Tsuchiya, 1975; Yoshida and Yasumoto, 1987), sufficient to fulfill the RDA (recommended daily allowance) for selenium proposed by the U.S. National Academy of Science (Food and Nutrition Board, 1980). In general, selenium in food is found in the protein fraction (Franke, 1934). However, the chemical forms of selenium are not necessarily homogeneous and often are heterogeneous (Underwood, 1977; Yoshida et al., 1981a), leading to variations in the bioavailability of this element in foods (Schwartz and Foltz, 1958; Mathias et al., 1967; Cantor et al., 1975; Yoshida et al., 1981b). While there have been many reports on selenium availability in foods, the chemical form has not strictly been identified.

Recently, plant proteins have been utilized in many fields of food processing for various purposes. Effort has been made to manufacture new food materials using plant protein as the major ingredient. A typical example is effective utilization of soybean protein.

This paper describes the identification of chemical forms of selenium occurring in the soybean protein. It has already been revealed by our preliminary work using an in vitro digestion system, gel permeation chromatography, and paper partition chromatography that one of the major chemical forms of selenium in soybean protein was selenomethionine (Yasumoto et al., 1983, 1984). On the basis of previous data, the authors conducted analyses to identify the chemical form of selenium in the major component of soybean protein subfraction by using GC–MS and confirmed the occurrence of the selenium in soybean protein as selenomethionine.

MATERIALS AND METHODS

Materials. Defatted soybean flakes at low temperature, unheated soybean protein concentrate, and unheated

---

Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan (K.Y., T.S.), and Department of Public Health, Kansai Medical University, Moriguchi, Osaka 570, Japan (M.Y.).

Table I. Protein Content and Amount of Selenium in Subfractionated Soybean Protein[a]

subfraction	protein, g	Se μg	Se μg/g protein
whole buffer extract	11.3	4.5	0.40
11S globulin	1.8	0.66	0.35
7S globulin	4.3	1.51	0.36
polymerized 7S globulin	0.5	0.01	0.02
whey proteins	0.9	0.50	0.58

[a] Each value represents the mean of three measurements.

soybean isolate were provided by Fuji Oil Co. (Osaka, Japan). Materials were stored at -20 °C or in the refrigerator at 3–5 °C until analysis.

Pepsin used for primary digestion of soybean protein was purchased from Wako Pure Chemicals, Osaka. Pancreatin and leucine aminopeptidase were purchased from Sigma Chemical Co. (St. Louis, MO). Actinase E (previously called Pronase E) was purchased from Kaken Seiyaku Co. (Tokyo, Japan). Selenomethionine and selenocystine used as the standard for thin-layer chromatography (TLC) and GC–MS analyses were purchased from Sigma. All other chemicals and reagents used were guaranteed reagent grade.

Quantitative Analysis of Selenium. Selenium contents in soybean materials were determined by wet digestion and followed fluorometric measurement according to Watkinson (1966).

Subfractionation of Soybean Protein. Low-temperature defatted soybean flakes were subfractionated into 11S globulin, 7S globulin, and 2S globulin, according to Thanh and Shibasaki (1976). Purity and content of the subfractionated soybean globulin were examined and determined by polyacrylamide gel electrophoresis and the micro-Kjeldahl method (Baker, 1961) using a Kjeldhal nitrogen analyzer (Mitsubishi Chemical Industries, Inc., Tokyo, Japan; Model KN-01) and Lowry's method (1951), respectively.

Proteolytic Hydrolysis of Soybean Protein. Since the preliminary analysis showed that the absolute content of selenium is predominant in the 7S globulin fraction (Table I), the 7S globulin fraction was provided for identification of the chemical form of selenium in soybean protein.

A preliminary experiment (Yasumoto et al., 1983) also revealed that selenium in the soybean protein mostly became dialyzable by pepsin digestion at 37 °C for 24 h. Therefore, the 7S globulin was subjected to pepsin digestion in a dialysis tube. Hydrolysis of the 7S globulin by pepsin was carried out by 1% pepsin (1:10 000) in 0.1 N HCl at 37 °C for 15 h. To examine the release of selenium into the dialysate from the 7S globulin inside the

dialysis tube, an aliquot of dialysate was taken at appropriate time intervals and subjected to selenium determination. After 15-h incubation, the dialysate was neutralized with 0.1 N NaOH and lyophilized. The lyophilized pepsin digest was then suspended in 10 vol of 1% pancreatin solution. The mixture was adjusted to pH 7.5 with 0.1 N NaOH and then incubated at 37 °C for 24 h. After 24-h digestion by pancreatin, the reaction medium was immersed in boiling water for 5 min to terminate the enzyme reaction, followed by the addition of Actinase E to 1% concentration to incubate for another 24 h at pH 7.5.

The digest thus obtained was centrifuged at 8000g for 60 min at 0 °C to remove debris and then subjected to ultrafiltration on an Amicon ultrafiltration apparatus (filter YM 10) to remove molecules larger than 10000 Da.

In order to examine whether dialyzable selenium such as selenate, selenite, or selenium-containing free amino acids and/or peptides existed in the 7S globulin, the 7S globulin was preliminarily incubated with 0.1 N HCl in a dialysis tube by dialyzing against 0.1 N HCl for 24–48 h. Dialysates were provided for selenium determination after lyophilization.

Subfractionation of Digested Soybean 7S Globulin on the Gel Permeation Chromatography. The proteolytic digestion products of soybean 7S globulin by pepsin, pancreatin, and Actinase E, respectively, were chromatographed on a gel permeation column using 2.5-cm i.d. × 90-cm length packed with Toyopearl HW-40F (molecular permeation range 100–10000; Toyo Soda Co., Tokyo, Japan). The digestion products were eluted with 25 mM sodium phosphate buffer (pH 7.5) at a flow rate of 1.0 mL/min. Eluates were collected every 10 mL, lyophilized, and then provided for selenium determination and identification of chemical feature of selenium by GC–MS.

Analytical and Preparative Thin-Layer Chromatography (TLC) of the Selenium-Containing Fraction. Fractions containing selenium were collected and lyophilized. Lyophilized fractions were dissolved in a small volume of water for TLC on the precoated cellulose plate (Merck, Darmstadt, West Germany) developed with chloroform–methanol–17.5% ammonia (41:41:18, by volume) (Brenner, 1969).

Selenomethionine and selenocystine were cochromatographed as references for 10 cm in a filter paper lined saturation chamber by taking care in spotting not to overlap with the sample zone during development. The chromatographed plate was dried with cool air and scraped every 0.2 R_f value to determine the distribution of selenium on the plate. The scraped fraction containing selenium was extracted with water and centrifuged, and the supernatant was lyophilized and dried over phosphorus pentoxide. This dried extract was trimethylsilylated with N,O-bis(trimethylsilyl)acetamide (Tokyo Kasei Kogyo, Co., Tokyo, Japan) immediately before GC–MS analysis.

Identification of Selenium-Containing Amino Acids by GC–MS. The unknown component containing selenium recovered from the cellulose thin-layer plate was subjected to the GC–MS analysis by mass chromatography. Conditions for the GC–MS analysis were as follows: instrument, Shimadzu-LKB-9000 gas chromatograph–mass spectrometer equipped with digital computer data system type DGB-300; separation column, 2% silicone OV-17 on Chromosorb W (60–80 mesh, acid washed, base washed, silanized), 3 mm (i.d.) × 1 m; column temperature, raised from 150 to 270 °C at a temperature program rate of 6 °C/min; carrier gas, helium (30 mL/min); temperatures of ion source, molecular separator, and flash heater, 290,
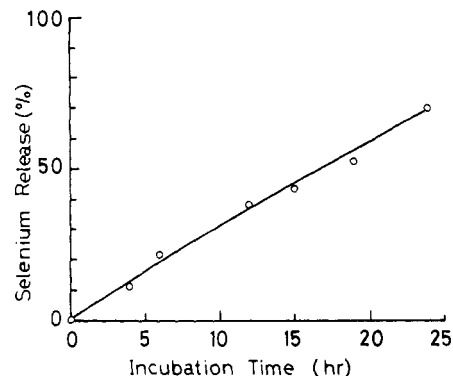

Figure 1. Release of selenium into dialysate from soybean 7S globulin during pepsin digestion.

280, and 270 °C, respectively; electron energy, 22 eV; accelerating voltage, 3.5 kV; trap current, kept at 60 μA; scanning speed, run at 8; filter, 240 Hz; slits, 0.1 mm for inlet and outlet of the analyzer. In case of running mass chromatography, mass spectra were recorded every 6 s from 1 to 10 min by scanning from m/z 30 to 550.

Estimation of Selenium Distribution in Amino Acids after Artificial Digestion. Selenium content in the protein fraction as amino acid analogues was determined by measuring selenium content in the amino acid prepared by enzymatic hydrolysis of a soybean protein isolate with pepsin, pancreatin, and Actinase E as described in the previous section. Digested soy protein isolate was centrifuged at 10000g for 1 h to remove debris and then ultrafiltrated. The filtrate (MW <5000) was further digested by leucine aminopeptidase–prolidase at 37 °C for 48 h (Matoba et al., 1982). The digest thus obtained was separated on cellulose TLC as described in the previous section, followed by subfractionation every 0.2 R_f unit for selenium determination.

RESULTS

Selenium Content in Soybean Products. The selenium content in several soybean products determined by Watkinson's method is as follows (μg/g of protein): soybean flake A, 0.52; soybean flake B, 0.57; soybean protein concentrate, 0.43; soybean protein isolate A, 0.45; soybean protein B, 0.39; soybean protein isolate C, 0.24. For the identification of chemical forms of selenium in the soybean, the soybean protein isolate containing 0.39 μg of Se/g of protein was used.

The selenium contents in the major subfactions of soybean protein prepared according to Thanh and Shibasaki (1976) are shown in Table I. The 7S globulin was the most predominant, followed by the 11S globulin and the whey fraction in which the 2S globulin is contained. The content of polymerized form of 7S globulin, formed as an artifact during the subfractionation process, was the smallest. On the other hand, the absolute amount of selenium in the subfraction was most abundant in the 7S globulin fraction. However, the selenium content in 1 g of protein was the highest in the whey protein fraction.

Release of Selenium from Soybean 7S Globulin. No selenium release from the 7S globulin was found during preliminary dialysis up to 48 h. On the other hand, selenium was released into the dialysate from the dialysis tube with the incubation time after addition of pepsin (Figure 1). After 15 h, about 44% of the total selenium in the 7S globulin was released, and about 70% of the total selenium was released after 24-h incubation. To avoid lowering the relative concentration of selenium by releasing excessive amounts of other amino acids, pepsin digestion was terminated at 15 h for further analysis.

Selenomethionine in Soybean Protein

J. Agric. Food Chem., Vol. 36, No. 3, 1988 **465**
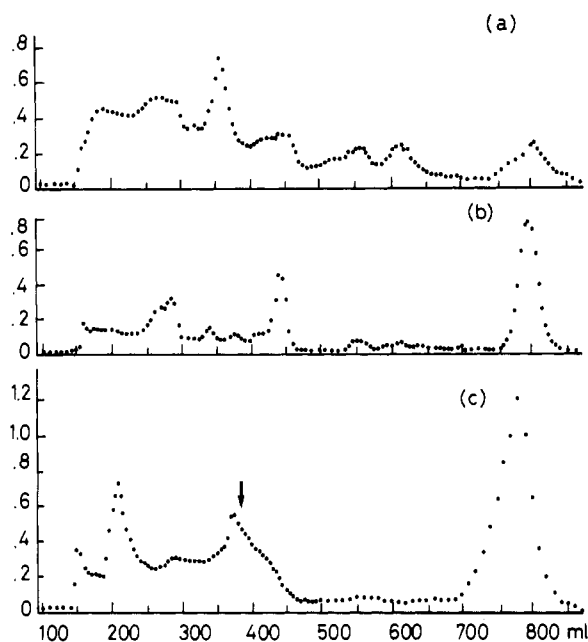
Figure 2. Elution patterns of digests of soybean 7S globulin on Toyopearl HW-40F gel permeation chromatography: (a) dialysate of soybean 7S globulin by pepsin; (b) digest of (a) by pancreatin; (c) digest of (b) by Actinase E. Eluates were monitored at 280 nm (ordinate, absorbance at 280 nm). Selenomethionine was eluted around 380 mL indicated by the arrow.

Table II. Distribution of Selenium in the Eluate of Proteolytic Digests of 7S Globulin on Toyopearl HW-40F Gel Permeation Chromatography

digest	fraction	MW	V_e, mL	Se, %
(a) dialysate of 7S globulin	1	500<	150–310	69
by pepsin digestion	2	500–170	320–400	29
	3	170–150	400–480	0
	4	150>	490–660	2
	5	150>	730–900	0
(b) digest of (a) by	1	500<	150–310	31
pancreatin	2	500–170	320–400	61
	3	170–150	410–500	4
	4	150>	520–660	4
	5	150>	740–850	0
(c) digest of (b) by	1	500<	150–310	1>
Actinase E	2	500–170	320–470	99<
	3	170–150	520–600	0
	4	150>	650–850	0

Elution Pattern and Selenium Distribution of Digests on Gel Permeation Chromatography. Elution patterns of proteolytic digests of the 7S globulin on the Toyopearl HW-40F gel permeation column are shown in Figure 2, and distribution of selenium in the eluates is shown in Table II. Since the amounts of peptides released were not determined, the absolute amounts of peptides and selenium released are not presented. The major elution peaks of digests of the soybean 7S globulin moved to the low molecular peptide fragment with proceeding proteolytic digestion. After the digestion by Actinase E, selenium was mostly recovered from the eluate of which selenomethionine was eluted. A large elution peak with 280-nm absorption was observed at around 800 mL. However, this peak contained no selenium at all. After the digestion by Actinase E, selenium was found in fraction c-2. Therefore fraction c-2 was collected, lyophilized, and subjected to TLC on the cellulose plate for further fractionation of selenium-containing compounds.

Mobility of Amino Acids on the Cellulose TLC. On cellulose TLC developed with chloroform–methanol–17.5% ammonia, authentic selenomethionine gives an R_f value of 0.74, while selenocystine moves to the position of R_f 0.31.

Table III. Distribution of Selenium in Soybean Protein Isolate on Cellulose Thin-Layer Plate after Artificial Digestion by Pepsin, Pancreatin, Actinase E, Leucine-aminopeptidase, and Prolidase

zone	R_f	Se, %	zone	R_f	Se, %
1	0.6–0.8	40	3	0.2–0.4	16
2	0.4–0.6	27	4	0–0.2	16
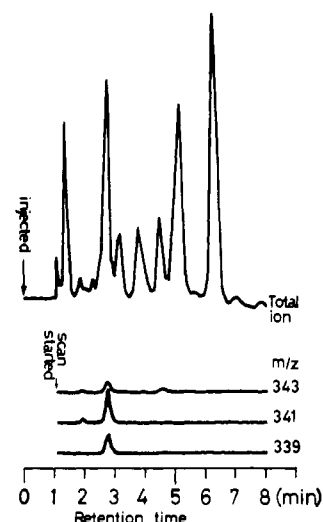
Figure 3. Mass chromatogram of the selenium-containing fraction recovered from gel permeation and subsequent cellulose thin-layer chromatography after digestion by pepsin, pancreatin, and Actinase E. Total ion, total ion chromatogram: m/z 343, M^+ peak of TMS derivative of selenomethionine from ^{82}Se; m/z 341, M^+ peak from ^{80}Se; m/z 339, M^+ peak from ^{78}Se. The authentic (trimethylsilyl)selenomethionine was eluted at 2.7 min. Detailed experimental conditions are shown in the text.

On the preparative-scale cellulose TLC plates, selenium was mainly recovered from zone at R_f 0.6–0.8, corresponding to selenomethionine, and zone at R_f 0.4–0.6, which does not correspond to either selenocystine or selenocysteine. Then, the zone corresponding to selenomethionine (R_f 0.7–0.8) was recovered preparatively from the TLC plate and subjected to analysis by GC–MS. On a separate cellulose plate, it was found that selenium was recovered most abundantly at R_f 0.6–0.8 when the digest by Actinase E was further digested by leucine aminopeptidase and prolidase at 37 °C for 48 h (Table III). Meanwhile, proteolytic enzyme treatment without soybean 7S globulin gave no selenium either at R_f 0.2–0.6 or R_f 0.6–0.8.

Identification of Selenium-Containing Compound by GC–MS. The result of TLC strongly suggested the presence of selenomethionine in the soybean protein 7S globulin. So the subsequent analysis was concentrated on the identification of selenomethionine by GC–MS. Since selenium comprises at least five stable isotopes in a constant relative ratio, i.e., ^{80}Se (100%), ^{78}Se (47%), ^{82}Se (18%), ^{76}Se (18%), and ^{77}Se (15%), it is comparatively easy to detect selenium-containing compounds by chasing mass numbers specific to selenium-containing fragments. If the unknown compound recovered from the cellulose plate is selenomethionine, ratios of the molecular ion peak and fragment peaks containing selenium must be 1:2:0.5 due to the natural abundance of ^{78}Se, ^{80}Se, and ^{82}Se. The authentic trimethylsilyl derivative of selenomethionine was eluted at 2.7 min. The trimethylsilylated fraction that was recovered from the cellulose plate also showed fragment peaks of m/z 339, 341, and 343 with peak ratio of 2:1:0.5 at 2.7 min (Figure 3). This result fulfills the requirement for the presence of selenomethionine in the fraction. The
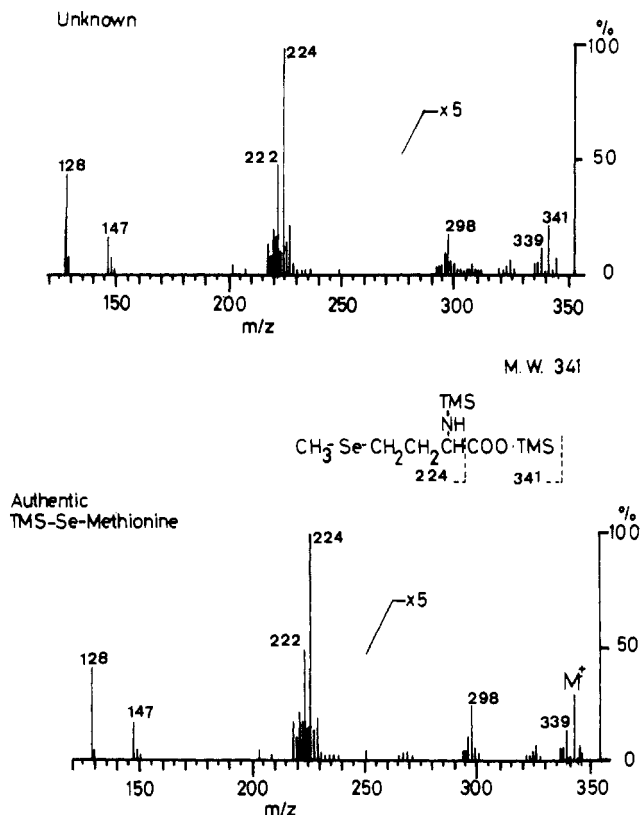
Figure 4. Mass spectra of the selenium-containing unknown compound eluted at 2.7 min and the authentic (trimethylsilyl)-selenomethionine.

whole mass spectrum of the peak eluted at 2.7 min coincided well with that of authentic selenomethionine (Figure 4).

DISCUSSION

In the present study the following facts have been revealed: (1) Selenium contained in the soybean protein or at least in the 7S globulin should be present in the form of nondialyzable compound. In other words, selenium is not present in a dialyzable form such as selenate, selenite, free amino acid, or low molecular weight peptide. (2) Selenium in the 7S globulin is mostly released into a low molecular component or free amino acid during digestion by pepsin, pancreatin, and Actinase E. (3) Selenomethionine is at least one of the major chemical forms of selenium in soybean protein.

Selenium determination of soybean protein isolate after the artificial proteolytic digestion using pepsin, pancreatin, Actinase E, leucine aminopeptidase, and prolidase and subsequent cellulose TLC fractionation revealed that about 60% of the selenium was recovered from the zone in which selenomethionine located. The remaining 40% was recovered from R_f 0.4–0.6. However, the chemical form of selenium in this zone remains unknown. Since we did not prevent oxidation of selenocysteine and selenocystine, their identification would have been almost out of question, even if they had existed in the soybean protein. Identification of selenocysteine in protein was reported by Broderick et al. (1985), using proteolytic digestion.

According to some recent reports, the predominant chemical form of selenium in animal tissues appears to be selenocysteine (Forstrum et al., 1978; Beilstein et al., 1981), obtained from animals injected with ^{75}Se selenite. In addition, identification of selenocysteine in a protein by mass spectrometry has also been done (Kraus et al., 1983). On the other hand, selenomethionine has been identified as a constituent of acetoacetyl thiolase isolated from Clos-

tridium kluyveri (Hartmanis and Stadtman, 1982; Sliwkowski and Stadtman, 1985), of selenium-rich wheat (Olson et al., 1970), or of proteins and amino acids from marine algae (Bottino et al., 1984) by means of ion-exchange chromatography or TLC. However, this is the first report identifying selenomethionine directly by GC–MS in commercial foods with a normal range of selenium level, especially soybean.

ACKNOWLEDGMENT

LITERATURE CITED

Baker, P. R. W. "The Micro-Kjeldahl Determination of Nitrogen: An Investigation of the Effects of Added Salt and Catalysts". Talanta 1961, 8, 57–71.

Beilstein, M. A.; Tripp, M. J.; Whanger, P. D. "Evidence for Selenocysteine in Ovine Tissue Organelles". J. Inorg. Biochem. 1981, 15, 339–347.

Bottino, N. R.; Banks, C. H.; Irgolin, K. J.; Micks, P.; Wheeler, A. E.; Zingaro, R. A. "Selenium Containing Amino Acids and Proteins in Marine Algae". Phytochemistry 1984, 23, 2445–2552.

Brenner, M.; Niederwieser, A.; Pataki, G. "Amino Acids and Derivatives". In Thin-Layer Chromatography: A Laboratory Handbook, 2nd Revised ed.; Stahl, E., Ed.; Springer Verlag: Berlin, Heidelberg, New York, 1969; Chapter 5.

Broderick, D. J.; Beilstein, M. A.; Whanger, P. D. "Problems Associated with Hydrolysis of Selenium Containing Proteins". Fed. Proc. 1985, 44, 1509.

Cantor, A. H.; Scott, M. L.; Noguchi, T. "Biological Availability of Selenium in Feedstuffs and Selenium Compounds for Prevention of Exudative Diathesis in Chicks". J. Nutr. 1975, 105, 96–105.

Chen, X.; Yang, G. Q.; Chen, J.; Chen, X.; Wen, Z.; Ge, K. "Studies on the Relations of Selenium and Keshan Disease". Biol. Trace Elem. Res. 1980, 2, 91–107.

Flohè, L.; Gunzler, W. A.; Shock, H. H. "Glutathione Peroxidase: A Selenoenzyme". FEBS Lett. 1973, 32, 132–133.

Food and Nutrition Board Recommended Dietary Allowances, 9th revised ed.; National Academy of Sciences: Washington, DC, 1980.

Forstrum, J. W.; Zakowski, J. J.; Tappel, A. L. "Identification of the Catalytic Site of Rat Liver Glutathione Peroxidase as Selenocysteine". Biochemistry 1978, 17, 2639–2644.

Franke, K. W. "A New Toxicant Occurring Naturally in Certain Samples of Plant Foodstuffs. II. The Occurrence of the Toxicant in the Protein Fraction". J. Nutr. 1934, 8, 609–613.

Hartmanis, M. G. N.; Stadtman, T. C. "Isolation of a Selenium-Containing Thiolase from Clostridium kluyveri: Identification of the Selenium Moiety as Selenomethionine". Proc. Natl. Acad. Sci. U.S.A. 1982, 79, 4912–4916.

Keshan Disease Research Group of the Chinese Academy of Medical Sciences "Observations on Effect of Sodium Selenite in Prevention of Keshan Disease". Ch. Med. J. 1979a, 92, 471–476.

Keshan Disease Research Group of the Chinese Academy of Medical Sciences "Epidemiologic Studies on the Etiologic Relationship of Selenium and Keshan Disease". Ch. Med. J. 1979b, 92, 477–482.

Kraus, R. J.; Foster, S. J.; Ganther, H. E. "Identification of Selenocysteine in Glutathione Peroxidase by Mass Spectroscopy". Biochemistry 1983, 22, 5853–5858.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. "Protein Measurement with the Folin Phenol Reagent". J. Biol. Chem. 1951, 193, 265–275.

Mathias, M. M.; Hogue, E. E.; Loosli, J. K. "Biological Value of Selenium in Bovine Milk for Rat and Chick". J. Nutr. 1967, 93, 14–20.

Matoba, T.; Doi, E.; Yonezawa, D.; Oste, R.; Nair, B. M. "An Approach to Assessing the Gastrointestinal Digestion of Rice and Wheat Proteins: Use of a Model System with Pepsin,

Pancreatin and Intracellular Peptidases". *Agric. Biol. Chem.* 1982, *46*, 465–472.

Olson, O. E.; Novacek, E. J.; Whitehead, E. I.; Palmer, I. S. "Investigation on Selenium in Wheat". *Phytochemistry* 1970, *9*, 1181–1188.

Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. "Selenium: Biological Role as a Component of Glutathione Peroxidase". *Science (Washington, D.C.)* 1973, *179*, 588–590.

Sakurai, H.; Tsuchiya, K. "A Tentative Recommendation for Maximum Daily Intake of Selenium". *Environ. Physiol. Biochem.* 1975, *5*, 107–118.

Schwartz, K.; Foltz, C. M. "Selenium as an Internal Part of Factor 3 against Dietary Necrotic Liver Degeneration". *J. Am. Chem. Soc.* 1957, *79*, 3292–3293.

Schwartz, K.; Foltz, C. M. "Factor 3 Activity of Selenium Compounds". *J. Biol. Chem.* 1958, *233*, 245–251.

Sliwkowski, M. X.; Stadtman, T. C. "Incorporation and Distribution of Selenium into Thiolase from *Clostridium kluyveri*". *J. Biol. Chem.* 1985, *260*, 3140–3144.

Thanh, H. V.; Shibasaki, K. "Major Proteins of Soybean Seeds. A Straightforward Fractionation and Their Characterization". *J. Agric. Food Chem.* 1976, *24*, 1117–1122.

Thompson, J. N.; Scott, M. L. "Role of Selenium in the Nutrition of the Chick". *J. Nutr.* 1969, *97*, 335–342.

Underwood, E. J. "Selenium". In *Trace Element in Human and Animal Nutrition*, 4th ed.; Academic: New York, 1977; pp 302–346.

Watkinson, J. H. "Fluorometric Determination of Selenium in Biological Material with 2,3-Diaminonaphthalene". *Anal. Chem.* 1966, *38*, 92–97.

Yasumoto, K.; Suzuki, T.; Yoshida, M. "Selenomethionine in Soybean Proteins and Its Intestinal Absorption". *Daizu Tanpakushitsu Eiyo Kenkyuukai Kaishi (Nutr. Sci. Soy Protein Jpn.)* 1984, *5*, 48–52.

Yasumoto, K.; Iwami, K.; Yoshida, M. "Nutritional Efficiency and Chemical Form of Selenium, an Essential Trace Element, Contained in Soybean Protein". *Diazu Tanpakushitu Eiyo Kenkyuukai Kaishi (Nutr. Sci. Soy Protein Jpn.)* 1983, *4*, 35–40; *Selenium-Tellurium Abstracts* 1985, *101*, 129289j.

Yoshida, M.; Yasumoto, K. "Selenium Contents of Rice Grown at Various Sites in Japan". *J. Food Com. Anal.* 1987, *1*, 71–75.

Yoshida, M.; Yasumoto, K.; Iwami, K.; Tashiro, H. "Distribution of Selenium in Bovine Milk and Selenium-Deficiency in Rats Fed Casein-Based Diets, Monitoring by Lipid Peroxide Level and Glutathione Peroxidase Activity". *Agric. Biol. Chem.* 1981a, *45*, 1681–1688.

Yoshida, M.; Iwami, K.; Yasumoto, K.; Iwai, K. "Bioavailability of Selenium Contained in Milk Casein, Soybean Proteins and Skipjack Strips". *Nippon Nogei Kagaku Kaishi (J. Jpn. Soc. Agric. Chem.)* 1981b, *55*, 689–693.

Characterization of Essential Oil of Parsley

James E. Simon* and James Quinn

A germplasm collection of parsley (*Petroselinum crispum*), consisting of 104 accessions from the USDA Plant Introduction Station including curly and flat leaf and Hamburg types, was greenhouse grown and the essential oil extracted from fresh leaves by water distillation and analyzed by GC and GC/MS for essential oil content and composition. The essential oil content ranged from 0.00 to 0.16% (v/fresh weight), and the constituents include α-pinene, β-pinene, myrcene, α-phellandrene, β-phellandrene, terpinene, terpinolene and 1-methyl-4-isopropylbenzene, 1,3,8-*p*-menthatriene, thymol, myristicin, apiol, plus three unknowns, two of molecular weight 168 and one of 268. Individual accessions varied greatly in essential oil composition. In general, the major constituent was 1,3,8-*p*-menthatriene, followed by β-phellandrene, myristicin, and myrcene. Parsley accessions high in the specific constituents (as a percent of essential oil) 1,3,8-*p*-menthatriene (68%), myristicin (60%), β-phellandrene (33%), apiol (22%), myrcene (16%), terpinolene and 1-methyl-4-isopropylbenzene (13%), and MW 268 dimer (10%) were identified. Thymol was detected in seven accessions, as 2% or less, and this is the first report of this compound in parsley leaf oil.

Parsley [*Petroselinum crispum* (Mill.) Nym. ex A.W. Hill, Apiaceae] is native to Europe and Western Asia (Bailey and Bailey, 1976) and cultivated in the United States as an annual for its aromatic and attractive leaves. The two major types of parsley are the common or curly leaf parsley (var. crispum) and the flat leaf, Italian parsley (var. neapolitanum Danert) (Simon et al., 1984). A third lesser grown parsley type is the Hamburg or turnip-rooted parsley [var. tuberosum (Bernh.) Crov.], which is cultivated to a limited extent for its enlarged edible root.

Fresh, dried, and dehydrated leaves are used as a condiment, garnish, and flavoring ingredient. A fixed oil and an essential oil can be extracted from the leaves and seeds. The constituents of the fixed oil have been reported elsewhere (Balbaa et al., 1975; Constantinescu et al., 1972).

The essential oil of parsley is used as a flavoring agent or fragrance in perfumes, soaps, and creams. The commercial essential oil of parsley is largely derived from the seed or the herb harvested at seed formation, prior to ripening (Heath, 1981). Parsley leaf oil, much more characteristic of parsley aroma, is not generally extracted and used because of the low essential oil yield.

The essential oil of parsley leaves has been previously reported (Garnero and Chretien-Bessiere, 1968; Kasting et al., 1972; Freeman et al., 1975; MacLeod et al., 1985). MacLeod (1985) identified 45 constituents including the following that constituted over 1% of the total peak area: α-pinene, β-pinene, myrcene, limonene, α-phellandrene, *p*-cymene, α-terpinolene, 1,3,8-*p*-menthatriene, 4-isopropenyl-1-methylbenzene α-terpineol, *p*-methylaceto-phenone, α-elemene, apiol, and myristicin.

Although the composition of essential oil is influenced by the plant genetic base and development and environmental conditions (Bernath, 1986), little information is

Department of Horticulture, Purdue University, West Lafayette, Indiana 47907.