

study on triglycerides could lead to more complete information and more valuable results for avocado maturity determination. Such research work is worth undertaking.

ACKNOWLEDGMENT

Technical assistance in collecting fruits from the Agronomic Station of Corsica Island has been provided by M. Vogel. We express our gratitude to J. R. Llinas for his help in statistical analyses.

LITERATURE CITED

- Afnor *Recueil des Normes Françaises, Corps Gras, Graines Oléagineuses, Produits Dérivés*, 2nd ed.; Afnor: Paris, 1981.
- Ahmed, E. M. Barmore, C. R. *Tropical and Subtropical Fruits*; Nagy, S., Shaw, P. E., Eds; Avi: West Port, CT, 1980; p 121.
- Foucart, T. *Analyse Factorielle*; Masson: Paris, 1982.
- Gaydou, E. M.; Rasoarahona, J.; Bianchini, J. P. *J. Sci. Food Agric.* 1983, 34, 1130.
- Gaydou, E. M.; Iatrides, M. C.; Ramanarivo, R.; Artaud, J.; Peiffer, G. *J. Agric. Food Chem.* 1984, 32, 651.
- Gaydou, E. M.; Ralambofetra, E.; Rakotovao, L.; Llinas, J. R. *Analisis* 1985, 13, 379.

- Lebart, L.; Morineau, A.; Fénelon, J. P. *Traitements de Données Statistiques. Méthodes et Programmes*; Dunod: Paris, 1982.
- Lewis, C. E. *J. Sci. Food Agric.* 1978, 29, 857.
- Lewis, C. E.; Morris, R.; O'Brien, K. *J. Sci. Food Agric.* 1978, 29, 943.
- Lozano, Y.; Duverneuil, G.; Yamasaki, K. *Fruits* 1982, 37, 545.
- Lozano, Y.; Ratovohery, J.; Gaydou, E. M. *Rev. Fr. Corps Gras* 1985, 10, 377.
- Lozano, Y. F.; Ratovohery, J. V.; Gaydou, E. M. *Fruits* 1987, 42, 305.
- Mazliak, P. *The Biochemistry of Fruits and Their Products*; Hulme, A. C., Ed.; Academic: London, 1970; p 209.
- Metcalfe, L. D.; Wang, C. N. *J. Chromatogr. Sci.* 1981, 19, 530.
- Peyronnel, D.; Artaud, J.; Iatrides, M. C.; Rancurel, P.; Chevalier, J. L. *Lipids* 1984, 19, 643.
- Romeder, J. M. *Méthodes et Programmes d'Analyse Discriminante*; Bordas: Paris, 1973.

Received for review November 25, 1986. Accepted July 7, 1987. This work was supported in part by the Fonds d'Aide et de Coopération (France) and by a research grant from the CIES for J.V.R.

Selenium Content of Vegetables, Fruits, and Cereals in Galicia (Northwest Spain)

M. Mejuto-Martí, M. Bollain-Rodríguez, C. Herrero-Latorre, and F. Bermejo-Martínez*

The selenium content of 60 samples of vegetable foods has been determined fluorimetrically. After wet destruction of organic matter, the complex 4,5-benzopiazselenole was formed by reaction between Se(IV) and 2,3-diaminonaphthalene and extracted with cyclohexane. The results suggest that the population of Galicia (NW Spain) consumes an average 11.20 μg of selenium/person per day in food of vegetable origin.

The nutritional value of selenium has been studied in experimental animals for over 20 years (Schwarz and Foltz, 1957), and there has been growing interest in its role as an essential trace element for animals (NRC Agricultural Board, 1971) and for man (Levander, 1975). It has been found, for example, that selenium-supplemented diets effectively prevent the appearance of certain nutritional disorders in battery chickens and rats (Hartley, 1959).

According to Morris and Levander (1970), the selenium content of human food it is varied. Vegetables and fruit are generally poor in selenium (0.01 $\mu\text{g}/\text{g}$), though mushrooms (Pipponen, 1984), garlic (Olson and Palmer, 1984), and horseradish (Levander, 1976) can contain quite large amounts. The selenium content of plants is in any case strongly influenced by the quantity of biologically available selenium in the soil in which they grow (Kubota et al., 1967), and hence by their geographical origin. For example, Mondragon and Jaffe (1976) have found that various food products contain higher selenium levels when acquired in the marketplace in Caracas than when bought in the United States.

With respect to their intrinsic capacity for accumulation of Se, plants may be divided in three groups (Rosenfield and Beath, 1964): group I, plants that may contain up to 10 000 $\mu\text{g}/\text{g}$; group II, plants rarely containing more than a few hundred micrograms/gram; group III, plants rarely containing more than about 30 $\mu\text{g}/\text{g}$, among them fruit and cereals.

MATERIALS AND METHODS

Apparatus. Ordinary laboratory glassware was employed. In order to eliminate fluorescence due to detergents and samples, all glassware was washed after each use with tap water, 1:1 nitric acid, and distilled water.

Fluorimetric determinations were carried out on a Farrand Model A4 fluorimeter (Farrand Optical Co. Inc.) equipped with a mercury vapor lamp and 1-cm light path cells.

Reagents. *Selenium Standard Solution.* A vial of Merck Titrisol selenium standard solution containing 1.000 \pm 0.002 g of Se is made up to 1 L, with deionized distilled water. Working solutions are derived from this standard by successive dilutions with 0.1 M HCl.

2,3-Diaminonaphthalene (DAN) Solution. Solutions (0.1%, (w/v) of DAN in 0.1 M HCl are made up daily and extracted before use with 25 mL of cyclohexane/100 mL of DAN to eliminate fluorescence.

Departamento de Química Analítica, Facultad de Química, Universidad de Santiago de Compostela, Santiago de Compostela, Spain.

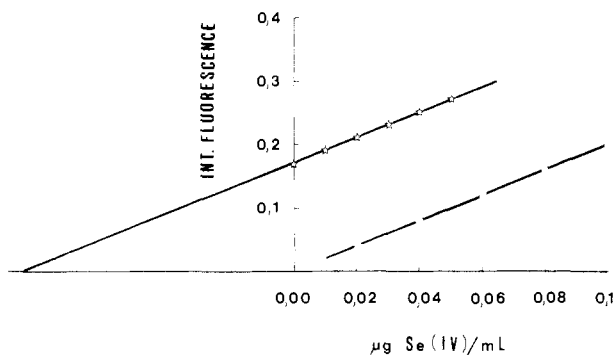


Figure 1. Accuracy of the method used for selenium determination applied for *Actinidia chinensis*, as determined by the method of additions.

EDTA-Hydroxylamine Hydrochloride Solution. EDTA- $\text{Na}_2 \cdot 2\text{H}_2\text{O}$ (0.3 g) and 25 g of hydroxylamine hydrochloride are dissolved in distilled water, and the solution is made up to 1 L.

Samples. Sixty samples of food of vegetable origin were obtained from food shops or from growers (36 of fruit, 6 of nuts, 7 of cereals or flour, 8 of mushrooms, 2 of bread, 2 of cocoa). The samples were dried at 100–105 °C for 2–4 h depending on their humidity and then ground manually to a fine powder in a mortar. Two 1-g subsamples of each were taken for analysis.

Procedure. After digestion with a 4:1 mixture of nitric and perchloric acids and a 1:1 mixture of nitric and sulfuric acids, by the method of Analytical Methods Committee, Royal Society of Chemistry (1979), samples were diluted to 50 mL with distilled water and boiled gently for at least 5 min with 5 mL of 37% HCl. After cooling, 5 mL of 50% formic acid and 10 mL of the EDTA-hydroxylamine hydrochloride solution were added to eliminate interferences and bring acidity within the range pH 1.5–2.0 with about 35 mL of a 1:1 mixture of ammonia and water. 4,5-Benzopiazselenole was formed by adding 5 mL of the 0.1% DAN solution and warming in a water bath at 50 °C for 30 min. The mixture was allowed to cool, transferred to decanting funnels, extracted with 10 mL of cyclohexane, and washed with 25 mL of 0.1 M HCl, and the fluorescence of the complex was measured.

Calibration curves must be prepared for each series of measurements employing the same procedure, but with organic matter being destroyed with just 1 mL of perchloric acid.

Reproducibility and Accuracy. The reproducibility of the Se measurements in the present study was determined by using the procedure described above to measure the Se content of each of a series of random food samples 10 times. The accuracy of the method was estimated by addition: for each of a series of random food samples, the concentration of Se was determined both without addition of Se standard and after various quantities of standard has been added.

RESULTS AND DISCUSSION

The use of fluorimetry for the determination of selenium has been recommended in recent years by a variety of authorities including the Analytical Methods Committee of the Royal Society of Chemistry (1979) and the IUPAC (1984). Though the need for temperature control, the slow digestion process, and the extraction of the complex from samples and standards by shaking for 1 min with cyclohexane make this technique lengthier than some other methods referred to by Inhat (1976), 2,3-DAN has proved to be a sensitive linear reagent for the determination of selenium in food when interferences from Al, Fe, Cu, etc.,

Table I. Reproducibility of the Method

sample	std dev of 10 determinations, $\mu\text{g/mL}$	% error
fruit (<i>Punica granatum</i>)	1.35×10^{-3}	1.77
mushroom (<i>Lactarius piperatus</i>)	1.49×10^{-2}	2.19
cereal (wheat flour)	4.65×10^{-4}	25.43

Table II. Accuracy of the Method

sample	Se ⁴⁺ added, ppm	Se ⁴⁺ found, ppm	recovery, %
<i>Actinidia chinensis</i>		0.085	
	0.01	0.095	100.00
	0.02	0.104	99.05
	0.03	0.115	100.00
	0.04	0.128	102.40
<i>Lactarius piperatus</i>		0.500	
	0.10	0.600	100.00
	0.20	0.720	102.86
	0.30	0.820	102.50
	0.40	0.925	102.78
wheat flour		1.000	
	0.50	0.020	
	0.10	0.120	100.00
	0.20	0.235	102.27
	0.30	0.320	100.00
	0.40	0.425	101.19
	0.50	0.525	100.96

Table III. Selenium Content of Vegetable Food in Galicia

sample	no. of samples	range ^a	mean ^a
fruit	36	0.01–0.08	0.015
mushroom ^b	8	0.20–1.80	0.690
cereals and flour	7	0.015–0.09	0.034
nuts	6	0.013–0.135	0.034
bread	2	0.025–0.030	0.020
cocoa	1		0.029
vegetables	44	0.01–0.8	0.047

^a Micrograms of Se/gram of dry weight. ^b Not counting the data for *Boletus edulis* [17.34 μg of Se/g, similar to the results of Piepponen et al. (1983)], which would distort the mean.

Table IV. Contribution of Food of Vegetable Origin to Mean Selenium Intake by the Galician Population^a

sample	mean, ppm	g consumed/person per day		Se intake, in $\mu\text{g/person per day}$	
		Randoin	OERGA	Randoin	OERGA
fruit	0.0019	135	142.2	0.26	0.27
mushrooms	0.051	10	10	0.51	0.51
cereals	0.022	35	52.1	0.77	1.15
bread	0.017	400	298.8	6.8	5.08
cocoa	0.028	25	6.6	0.7	0.18
fresh green veg	0.0078	300	147.3	2.34	1.15
dry legumes	0.011	25	19	0.28	0.21
potatoes	0.0044	300	602	1.32	2.65
total				12.98	11.20

^a Data obtained from fresh weights.

are eliminated by complexation with EDTA.

Table I lists the results of the reproducibility study and Table II those of the accuracy study (the results of the accuracy experiments using *Lactarius piperatus* are also shown in Figure 1). Both reproducibility and accuracy are within accepted limits (5% relative error, 95–105% accuracy), and other experiments carried out in our laboratory have shown the complex formed, 4,5-benzopiazselenole, to be stable for at least 2 h.

Table III lists the means and ranges of the selenium contents of the various groups of food studied. Of the 60 samples analyzed, the mushrooms had by far the highest selenium levels, followed by cocoa. The lowest selenium

contents were those of fruit.

On the basis of the data of Table III, together with similar data obtained for vegetables by Herrero-Latorre et al. (1986), the contributions of these kinds of food to the mean intake of selenium in Galicia/person per day have been estimated by multiplying the mean Se content of each group by the mean consumption of that kind of food in Galicia/person per day (OERGA, 1980). The results are shown in Table IV together with those of similar calculations carried out using the mean consumptions recommended in the balanced diet tables of Randoïn et al. (1969). It may be noted that in spite of their high Se levels, mushrooms make a relatively modest contribution to total Se intake because their consumption is not widespread in Galicia. The total Se intake in food of vegetable origin, 11.20 μg /person per day, is very similar to the figure of 13.01 μg /person per day obtained using the tables of Randoïn et al. (1969). The fact that both figures are considerably lower than those for other parts of the world [in Canada, for example, cereals alone provide 62-112 μg of Se/person per day (Thompson et al., 1975)] may be attributed to the low selenium content of soils.

Registry No. Se, 7782-49-2.

LITERATURE CITED

- Analytical Methods Committee, Royal Society of Chemistry, *Analyst* 1979, 104, 778.
Hartley, W. J. *N.Z. J. Agric.* 1959, 100, 259.

- Herrero-Latorre, C.; Mejuto-Martí, M. C.; Bollain-Rodríguez, M. H.; Bermejo-Martinez, F. *Anales Bromatologia* 1987, 39(1), 133.
Ihnat, M. *J. Assoc. Off. Anal. Chem.* 1976, 59, 911.
Instituto Nacional de Estadística, Ministerio de Economía, Observatorio Estadístico Regional de Galicia (OERGA) *La Alimentación en Galicia*, Santiago de Compostela, 1980.
IUPAC *Pure Appl. Chem.* 1984, 56, 645.
Kubota, J.; Allaway, W. H.; Carter, D. L.; Cary, E. E.; Lazar, V. A. *J. Agric. Food Chem.* 1967, 15, 448.
Levander, O. A. *J. Am. Diet. Assoc.* 1975, 66, 338.
Levander, O. A. *Proc. Symp. Selenium-Tellurium Environ.* 1976, 26.
Mondragon, M. C.; Jaffe, W. G. *Arch. Latinoam. Nutr.* 1976, 26, 341.
Morris, W. C.; Levander, O. A. *J. Nutr.* 1970, 100, 1383.
National Research Council, Agricultural Board, Committee on Animal Nutrition, Subcommittee on Selenium (J. E. Oldfield, Chairman) *Selenium in Nutrition*; National Academy of Sciences: Washington, DC, 1971.
Olson, O. E.; Palmer, I. S. *J. Food Sci.* 1984, 49, 446.
Pipponen, S.; Liukkonen-Lilja, E.; Kuusi, T. *Z. Lebensm. Unter. Forsch.* 1983, 177, 257.
Pipponen, S. *Kem. Kemi.* 1984, 11, 180.
Randoïn; Legalli; Duchene In *Rations Equilibrees*, 8th ed.; Foucher, E. Ed.; Hygiene: Paris, 1969; Vol. I, p 35.
Rosenfield, I.; Beath, O. A. *Selenium, Geobotany, Biochemistry, Toxicity and Nutrition*; Academic: New York, 1964.
Schwarz, K.; Foltz, C. M. *J. Am. Chem. Soc.* 1957, 79, 3292.
Thompson, J. N.; Erdoby, P.; Smith, C. *J. Nutr.* 1975, 105, 274.

Received for review July 10, 1986. Revised manuscript received February 3, 1987. Accepted October 15, 1987.

Green Leaf Headspace Volatiles from *Nicotiana tabacum* Lines of Different Trichome Morphology

Roger A. Andersen,* Thomas R. Hamilton-Kemp, John H. Loughrin, Charles G. Hughes, David F. Hildebrand, and Tommy G. Sutton

Volatile compounds from fully expanded whole leaves of four greenhouse-grown vegetative (green) tobacco lines were isolated by entrainment in purified air followed by Tenax trapping, characterized by capillary GC and GC-MS, and quantified by capillary GC. Identified headspace compounds, which comprised about 50% of the number of GC peaks and weight of the total estimated volatiles, were (*E*)- β -ocimene, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, linalool, β -caryophyllene, (*E*)- β -farnesene, solanone, methyl salicylate, nicotine, and neophytadiene. Yields of total estimated volatile compounds among the replicated leaf samples of the tobacco lines ranged from 6.6 to 38.5 ng/g wet weight. The means of amounts of volatile components that were believed to be emitted as a result of wound-induced lipoxygenase-hydroperoxide lyase activities were higher for analyses performed with 5-L entrainment flasks than with 12-L flasks. The leaf surface emissions for individual volatile compounds are discussed in terms of genetic differences including leaf trichome morphologies among Tobacco Introduction (TI) 1112, TI 1406, and TI 1068 and KY 14 burley. The results obtained do not support the view that the majority of headspace volatiles are emitted from exudate-secreting glandular heads of leaf trichomes. However, there were significant differences in yields of some compounds among genetic lines of tobacco.

There have been relatively few reports on the occurrence, quantities, and biogenesis of volatile compounds in green vegetative tobacco as compared with cured tobacco. It is recognized, however, that knowledge about the composition of volatiles in tobacco prior to harvest could be

useful in the understanding of the biogenesis of compounds in postharvest tobacco that affect the quality of leaf and may play a role in plant-pest interactions. The occurrence of at least 25 compounds in steam distillates and five compounds in the headspace of burley tobacco stalk and their quantitative analyses were recently reported (Andersen et al., 1986). Burton and Kasperbauer (1985) showed that the composition of some volatile components in green burley leaf at harvest changed during air-curing. Several reports have appeared on volatiles in terms of the effects of cultural practices on cured tobacco (Weeks and Seltmann, 1986), composition in flue-cured tobacco (Lloyd

USDA-ARS, MSA (R.A.A., T.G.S.), Departments of Agronomy (R.A.A., D.F.H., T.G.S.) and Horticulture (T.R.H.-K., J.H.L.), and Tobacco and Health Research Institute (C.G.H.), University of Kentucky, Lexington, Kentucky 40546.