demonstrates the necessary role of modern high-performance analytical techniques in assessing xenobiotic product purity for toxicological studies. In these cases, verification of toxicant purity must be approached rigorously; otherwise, the results of associated toxicological studies may be in error.

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Tocopherols and Tocotrienols in Finnish Foods: Vegetables, Fruits, and Berries

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This study is part of a survey carried out to determine the tocopherol and tocotrienol contents of Finnish foods. Tocopherols and tocotrienols from 75 commodities of vegetables, fruits, berries, and their respective processed products and those of almond, peanut, and mushrooms were analyzed by using an HPLC method. α-Tocopherol was the predominant compound in all other samples except in pea, pea products, bean, cauliflower, raspberry, and potato chips where γ-tocopherol predominated. Small amounts of β- and δ-tocopherols as well as α- and γ-tocotrienols were also found. The proportions of non-α-tocopherols and tocotrienols were highest in berries. The highest α-tocopherol values (>1 mg/100 g, fresh weight) among the fresh vegetables analyzed were found in dark green leafy vegetables and in sweet pepper. The α-tocopherol content of fruits ranged from 0.06 to 0.96 mg/100 g and that of berries from 0.56 to 4.14 mg/100 g. No tocopherols were found in mushrooms. α-Tocopherol was found to be quite stable in freezing as well as in canning and marmalade and jam making, but only small amounts of α-tocopherol were detected in juices.

Plants synthesize tocopherols (α-, β-, γ-, δ-tocopherols) and tocotrienols (α-, β-, γ-, δ-tocotrienols). No chlorophyll *a* containing higher plant tissue has been proved devoid of tocopherols (Booth, 1963). α-Tocopherol is the major tocopherol in chlorophyll-containing tissue, and it is localized in chloroplasts (Booth, 1963; Bucke, 1968; Newton and Pennock, 1971; Janiszowska and Korczak, 1980). Non-α-tocopherols are situated mainly in nongreen tissues such as vegetable oils, nuts, fungi, and cereal grains (Newton and Pennock, 1971), and in chlorophyll-containing tissue they are localized mainly outside the chloroplast (Booth, 1963; Newton and Pennock, 1971; Janiszowska and Korczak, 1980). Tocotrienols have been found in carrots (McLaughlin and Weihrauch, 1979; Leth and Andersson, 1982), kale and broccoli (Leth and Andersson, 1982), mushrooms (McLaughlin and Weihrauch, 1979), and vegetable oils and cereal grains (McLaughlin and Weihrauch, 1979; Bauernfeind, 1980; Piironen et al., 1986; Syväoja et al., 1986). However, generally only α-tocopherol is determined, and knowledge of the tocopherol and tocotrienol composition of vegetables, fruits, and berries is poor.

α-Tocopherol concentrations have been shown to be high in dark green tissues, moderate in fast-growing leaves, light green tissues, and colored fruits, and low in roots, etiolated tissues, and colorless fruits (Booth and Bradford, 1963). α-Tocopherol content is high (1.8–14.5 mg/100 g, fresh weight) in for example dandelion leaves, mint leaves, nettle leaves, spinach, parsley, and asparagus (Booth and Bradford, 1963; Leth, 1975; McLaughlin and Weihrauch, 1979). Large amounts of α-tocopherol were also found in wild blackberries (Booth and Bradford, 1963) and in pepper fruit (Kanner et al., 1979).

Variation in the α-tocopherol values of vegetables, fruits, and berries is caused, in addition to variation between species, by many other factors. One factor is the variety

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(McLaughlin and Weihrauch, 1979) and another the uneven distribution of tocopherols, while richer inedible parts, such as the dark green parts of brassicae and leeks or yellow senescent leaves, are variously discarded (Booth and Bradford, 1963). Maturity of the tissue, as well as growing conditions such as the weather, growing season, intensity of sunlight, and soil state, also has an influence on the tocopherol values (Booth and Bradford, 1963; Kanner et al., 1979).

In this study the most important vegetables, fruits, and berries consumed in Finland as well as their respective commercial processed products were analyzed for tocopherols and tocotrienols. Peanut, almond, and mushrooms were also included. The study belongs to a survey carried out to determine the tocopherol and tocotrienol contents of Finnish foods (Piironen et al., 1985; Piironen et al., 1986; Syväoja et al., 1985a–c; Syväoja et al., 1986).

EXPERIMENTAL SECTION

Sampling. Sampling of fresh vegetables, fruits, berries, and mushrooms was carried out during the summer and fall of 1982. Samples of vegetable, berry, and fruit products and those of peanut and almond were taken in February and March 1984.

Fresh vegetables, fruits, and berries do not pass through centralized industrial collection or distribution procedures. Consequently it is very difficult to obtain representative samples of the whole harvest. The samples of this study are more or less random, and they cannot be said to represent Finnish production as a whole. However, the results probably give a good picture of the general tocopherol levels of the vegetables, fruits, and berries produced in or imported to Finland.

Samples of fresh vegetables, fruits, and berries (generally five samples of 0.5–1 kg) were bought from retail stores, market places, market halls, or a wholesale store. Samples of noncultivated plants such as nettle, rose hip, a mixture of various mushrooms, and others were picked by the research group. Eight samples of each vegetable, fruit, and berry products and of peanut and almond products representing the most important producers and importers were bought from eight retail stores of the four major food chains in Finland.

Pretreatment. One pooled sample was made up for each item. Vegetables, fruits, berries, and mushrooms were trimmed according to common household practice, and only the edible portions were analyzed. The samples were cut with a knife, homogenized in a blender (Moulinex), and analyzed immediately in duplicate. The respective commercial products were homogenized and analyzed accordingly.

Analytical Method. Tocopherols and tocotrienols were analyzed by normal-phase high-performance liquid chromatography with fluorescence detection as previously described (Piironen et al., 1984; Piironen et al., 1985; Syväoja et al., 1985b). The samples were generally prepared by using the room-temperature saponification method (Piironen et al., 1984). For some fresh vegetables the solvent extraction method described by Thompson and Hatina was also applied (1979). When these two extraction methods were compared, no difference was found between the α-tocopherol values obtained for fresh vegetables. For berries especially the γ-tocopherol values were much higher by saponification than by solvent extraction.

Sample size was from 10 to 30 g, except 5 g for dill and parsley and 3 g for peanut and almond. The amount of 50% KOH in the saponification solution was 20 mL for fresh items, 10 mL for the commercial products, and 5 mL for peanut and almond. The other saponification conditions were as described earlier (Piironen et al., 1985).

Recoveries of α-, β-, γ-, and δ-tocopherols ($n = 4$) added to carrot and parsley samples were 95%, 93%, 97%, and 97% when the solvent extraction method was used while saponification method gave recoveries of 97%, 93%, 97%, and 52%, respectively. When the saponification method was tested for a rhubarb sample, recoveries of 107%, 107%, 103%, and 80% were received for α-, β-, γ-, and δ-tocopherols, respectively. Recoveries of α- and γ-tocopherols added to peanut samples ($n = 3$) were 104% and 99%.

The moisture content of the samples was determined by using the AOAC- (1980) approved Method No. 16.284.

RESULTS AND DISCUSSION

The tocopherol and tocotrienol compositions of the analyzed fresh vegetables, fruits, berries, and mushrooms and those of their respective commercial products and of peanut and almond are shown in Tables I–III. β- and δ-tocotrienols were not detected in any of the samples, and they are therefore not included in the tables.

α-Tocopherol was the predominant tocopherol in all other samples except in pea, pea products, bean, cauliflower, raspberry, and potato chips where γ-tocopherol predominated. Those vegetables in which α-tocopherol was the predominant compound also contained smaller amounts of γ-tocopherol, and some species also contained small amounts of β- and δ-tocopherols but tocotrienols were nearly absent. Also, fruits contained β- and γ-tocopherols as minor components, but tocotrienols were detected only in plum and grapes. The proportions of the non-α-tocopherols and tocotrienols were highest in berries: they contained considerable amounts of γ-tocopherol and also β- and δ-tocopherols and γ-tocotrienol. γ-Tocopherol was shown to be derived mostly from seeds.

In most of the previous studies only α-tocopherol has been determined in vegetables, fruits, and berries. The compositions found in this study are generally in accordance with the results of the few previous studies also including non-α-tocopherols and tocotrienols (Leth, 1975; Leth and Andersson, 1982; McLaughlin and Weihrauch, 1979; Bauernfeind, 1980). Yamauchi and Matsushita (1976) have, however, reported considerably higher δ-tocopherol values for vegetables than those found in this study.

The best sources of α-tocopherol among the samples of this study were found to be almond and peanut (Table I). The results obtained for those samples were close to the values previously reported (Leth 1975; McLaughlin and Weihrauch, 1979).

In accordance with the literature the highest α-tocopherol values (>1 mg/100 g, fresh weight) among the analyzed vegetables were found in the dark green leafy vegetables, spinach, nettle, parsley, and dill and in sweet pepper. The α-tocopherol values found for spinach (1.22 mg/100 g, fresh weight) and especially for nettle (1.66 mg/100 g) were, however, lower than previously reported (Booth and Bradford, 1963; Leth, 1975; McLaughlin and Weihrauch, 1979; Thompson and Hatina, 1979; Candlish, 1983).

The α-tocopherol contents of broccoli, Brussels sprouts, and lettuce were moderately high, but those of cabbages and cauliflower were extremely low. Booth and Bradford (1963) showed that the α-tocopherol content of the green outer leaves of cabbages was high but α-tocopherol was barely detectable in the almost colorless heart. α-Tocopherol content of the edible part of cabbages is therefore dependent upon the portion of outer leaves that is included. Booth and Bradford (1963) also found no α-to-

Table I. Tocopherol and Tocotrienol Contents of Vegetables, Mushrooms, Peanut, and Almond (Number of Samples: One Pooled Sample for Each Item) (See Text)[a]

item	moisture, %	tocopherols and tocotrienols, mg/100 g (fresh product)					
		α-T	α-T3	β-T	γ-T	γ-T3	δ-T
potato	77.6	0.05	–	–	–	–	–
carrot	90.0	0.36	0.04	tr	tr	–	–
celery root	85.9	0.50	0.08	0.02	0.07	–	–
parsnip	80.5	0.82	tr	0.03	0.02	–	–
red beet	86.2	0.05	–	0.01	–	–	–
radish	96.2	tr	–	–	–	–	–
rutabaga	88.8	tr	–	–	–	–	–
turnip							
yellow	89.9	–	–	–	–	–	–
white	93.9	–	–	–	–	–	–
broccoli	88.7	0.68	–	tr	0.14	–	–
Brussels sprout	84.3	0.40	tr	tr	tr	–	–
cauliflower	92.5	0.09	–	–	0.26	–	tr
chinese cabbage	95.7	0.24	–	–	tr	–	–
red cabbage	93.0	0.05	–	–	–	–	–
white cabbage	92.9	0.04	–	–	–	–	–
lettuce	95.5	0.63	–	tr	0.34	–	–
nettle	82.5	1.64	–	0.05	0.19	–	tr
spinach	89.0	1.22	–	–	–	–	–
rhubarb	94.8	0.25	–	tr	–	–	–
leek	90.6	0.34	–	–	0.02	–	–
onion							
red	85.7	0.04	–	0.01	0.01	–	–
yellow	85.5	0.04	–	0.01	0.01	–	–
dill	89.2	1.59	–	tr	0.22	–	–
parsley	85.8	3.58	tr	tr	1.23	tr	tr
bean	90.6	0.13	–	tr	0.32	–	0.03
pea	77.4	0.03	–	–	1.60	tr	0.04
tomato	95.4	0.66	–	tr	0.20	–	tr
sweet pepper	92.4	2.16	–	0.11	0.02	–	tr
cucumber	97.1	0.04	–	0.01	0.02	–	0.01
cantharelle	92.8	–	–	–	–	–	–
mushrooms, mixed	91.1	–	–	–	–	–	–
almond	4.0	26.44	0.24	0.17	0.76	0.02	0.02
peanut	6.4	10.89	–	0.27	8.39	–	0.17

[a] Abbreviations: T = tocopherol; T3 = tocotrienol; – = not detected; tr = trace.

Table II. Tocopherol and Tocotrienol Contents of Fruits and Berries (Number of Samples: One Pooled Sample for Each Item) (See Text)[a]

item	moisture, %	tocopherols and tocotrienols, mg/100 g (fresh product)					
		α-T	α-T3	β-T	γ-T	γ-T3	δ-T
apple, domestic flesh only	87.5	0.24	–	–	–	–	–
pear, fresh only	85.9	0.06	–	0.01	tr	–	–
grape	79.5	0.63	0.07	tr	0.14	0.06	–
orange	87.6	0.36	–	tr	tr	–	–
grapefruit	89.1	0.32	–	tr	tr	–	–
mandarin	89.8	0.32	–	tr	tr	–	–
banana	75.8	0.21	–	tr	tr	–	–
plum	86.2	0.85	tr	0.01	0.08	–	–
peach, flesh only	88.8	0.96	–	tr	0.05	–	–
blueberry	84.1	1.85	tr	tr	0.21	0.66	–
lingonberry	85.6	1.53	0.06	tr	0.13	0.42	–
cranberry	88.5	0.94	0.05	0.02	0.25	0.35	0.04
cloudberry	86.3	2.95	–	0.20	0.45	–	0.02
raspberry	84.4	0.88	–	0.15	1.47	–	1.19
strawberry	89.6	0.56	–	tr	0.15	–	tr
black currant	81.4	2.23	–	tr	0.83	–	tr
red currant	86.1	0.82	tr	0.16	0.32	–	0.15
gooseberry	87.0	0.73	–	tr	0.11	–	tr
rose hip	79.7	4.14	–	0.14	0.10	–	tr
sea buckthorn[b]	85.2	3.05	–	0.26	0.73	tr	tr

[a] Abbreviations: T = tocopherol; T3 = tocotrienol; – = not detected; tr = trace. [b] *Hippophae rhamnoides.*

copherol in the flowers of cauliflower, and so the α-tocopherol values received for cauliflower are determined by the green part included.

In roots and potato only traces of α-tocopherol were found, or tocopherols were not detected at all. However, roots of the plants belonging to the family Umbelliferaceae such as carrot, celery, and parsnip regularly contained higher amounts of α-tocopherol (0.36–0.82 mg/100 g). The tocopherol content of tomato was found to be moderately high, but the values found for onion, pea, bean, and cucumber were extremely low. McLaughlin and Weihrauch (1979), Bauernfeind (1980), and Yamauchi and Matsushita (1976) have also reported similar values.

The α-tocopherol content of fruits ranged from 0.06

Table III. Tocopherol and Tocotrienol Contents of Vegetable, Fruit, and Berry Products (Number of Samples: One Pooled Sample for Each Item) (See Text)[a]

item	moisture, %	tocopherols and tocotrienols, mg/100 g (fresh product)					
		α-T	α-T3	β-T	γ-T	γ-T3	δ-T
carrot, frozen	91.3	0.58	0.06	0.01	–	–	–
mixed vegetables, frozen	88.4	0.29	0.02	0.01	0.47	0.01	0.01
pea							
frozen	77.2	0.03	tr	–	1.38	0.04	0.03
dried	11.8	0.06	–	–	6.55	0.05	0.19
spinach, frozen	94.0	1.32	–	tr	0.09	–	–
red beet, pickled	84.4	0.06	–	tr	–	–	–
champignon, canned	91.8	–	–	–	–	–	–
potato chips	2.2	5.19	0.56	0.21	14.18	0.39	1.37
apple juice	90.0	–	–	–	–	–	–
orange juice	90.7	0.16	tr	tr	tr	–	–
black currant juice[b]	56.9	0.01	–	–	–	–	–
mixed juice[b]	59.5	tr	–	–	–	–	–
blueberry, frozen	87.4	1.81	tr	0.04	0.23	0.39	0.01
rose hip sauce, frozen	74.5	1.60	–	0.04	0.91	–	0.09
strawberry jam	40.9	0.08	–	tr	0.01	–	–
orange marmelade	42.1	0.20	–	tr	0.07	–	–
fruit cocktail, canned	82.1	0.85	–	0.01	0.04	–	–
peach, canned	82.8	1.95	0.01	0.01	0.05	–	–
pineapple, canned	84.0	0.04	–	tr	–	–	–
apricot, dried	30.2	6.24	0.05	0.13	0.17	–	0.20
raisin	22.4	0.32	–	tr	0.09	–	tr
prune	35.7	1.76	–	0.04	0.13	–	tr

[a] Abbreviations: T = tocopherol; T3 = tocotrienol; – = not detected; tr = trace. [b] Sweetened concentrates.

mg/100 g (fresh weight) for pear to 0.96 mg/100 g for peach. For most fruits the α-tocopherol values were about 0.2–0.4 mg/100 g. These results agree with the previous reports (McLaughlin and Weihrauch, 1979; Bauernfeind, 1980).

Berries were found to be good sources of α-tocopherol (range 0.56–4.14 mg/100 g, fresh weight). The values found in this study were slightly higher than reported by Booth and Bradford (1963) and McLaughlin and Weihrauch (1979) but close to the values reported by Leth (1975). Differences are probably caused by the differing varieties and growing conditions.

No tocopherols or tocotrienols were found in wild mushrooms. Previously small amounts of α-tocopherol, α-tocotrienol, and a fraction containing β- and γ-tocopherol have been found in mushrooms (McLaughlin and Weihrauch, 1979).

The α-tocopherol values of frozen vegetables and berries were comparable to those received for the fresh items, indicating that α-tocopherol is quite stable in freezing and during storage of frozen products. The α-tocopherol contents of frozen spinach and especially that of frozen carrots were even higher than those of fresh spinach and carrot. The reason was probably variation in the tocopherol content of carrots coming from different growing areas. The variation has been demonstrated in further studies (Piironen, 1984). In contrast to this study Leth and Andersson (1982) have reported considerable differences between the α-tocopherol contents of frozen and fresh vegetables. In their study, there was a 6-year period between the investigation of fresh and frozen vegetables. The results of this study indicated that α-tocopherol was also quite stable in jam and marmelade making and in canning. Hellendoorn et al. (1971) also showed that α-tocopherol was quite stable in the canning and in storage of canned products.

Compared with those of raw materials, the α-tocopherol contents of juices were extremely low. Especially low were the values for black currant juice and mixed juice. These are sweetened concentrates. They are sold in bottles, and their shelf life is long. The α-tocopherol contents of raisin and prune were also considerably lower than those of grape and plum when the values are compared on the dry matter basis.

In this study only one pooled sample for each item was analyzed, and the results do not give any information about variation in the tocopherol contents of vegetables, fruits, or berries. According to Booth and Bradfod (1963), the variation is so great that there is no typical value for the tocopherol concentration in a given plant. The mean tocopherol and tocotrienol values received in our unpublished studies concerning the variation of vitamin E content of carrot, tomato, blueberry, and strawberry have, however, been close to the results of this study (Piironen, 1984).

In Finland the average daily per capita consumptions of vegetables, fruits, and berries were in 1983 as follows: fresh potatoes, 163.8 g; other fresh vegetables, 77.6 g; fresh fruits, 125.8 g; fruit juices, 37.4 g; fresh and processed berries, 35.3 g (Agricultural Economic Research Institute, 1984). The consumption of carrots, tomatoes, cucumbers, and cabbages accounts for more than 60% of the total consumption of fresh vegetables [not including potatoes (Central Statistical Office of Finland, 1984)]. Based on the figures given by the Central Statistical Office, the estimate for the daily per capita intake of α-tocopherol from vegetables, fruits, and berries is 1.3 mg.

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Degradation of Atrazine by *Pseudomonas*: N-Dealkylation and Dehalogenation of Atrazine and Its Metabolites[1]

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Three species of *Pseudomonas* capable of utilizing atrazine as a sole source of carbon were isolated by enrichment from soil with a long history of atrazine application. Atrazine was metabolized via N-dealkylation with preferential formation of deisopropylatrazine over deethylatrazine. Two of the species were able to carry out the dechlorination of both deisopropylatrazine and deethylatrazine following incubation in glucose-supplemented mineral salts medium. The dehalogenation of atrazine and its metabolites as a bacterial degradation process is shown.

INTRODUCTION

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] is a widely used selective herbicide for weed control in corn. It has been shown that the metabolism of atrazine in soil involves hydroxylation, dealkylation, and ring cleavage (Esser et al., 1975). Phytotoxicity of the herbicide is destroyed by hydroxylation at the 2-position but not by dealkylation of either of the two alkylamino groups (Kaufman and Blake, 1970). Dealkylation reactions in soil have been shown mainly due to fungi (Couch et al., 1965; Kaufman and Blake, 1970; Wolf and Martin, 1975). Giardina et al. (1979) isolated a *Nocardia* species from a soil enrichment culture that can utilize atrazine as the sole source of carbon and nitrogen. Their metabolic studies with this organism showed both N-dealkylation and deamination of atrazine and the formation of 2-chloro-4,6-diamino-*s*-triazine (Giardina et al., 1980, 1982), which was reported as nonphytotoxic in bioassays with oats.

Dechlorination of atrazine to a nonphytotoxic product, hydroxyatrazine, occurs in soils treated with atrazine, but this has been attributed solely to chemical hydrolysis rather than resulting from microbial activity (Armstrong et al., 1967; Skipper et al., 1967; Kaufman and Blake, 1970). According to Knackmuss (1981) the bacteria have not evolved enzymatic systems for the direct hydrolysis of the aromatic carbon–halogen bond. Klages et al. (1981), however, have reported such activity in whole cells.

Very little is reported in the literature on the metabolism of atrazine by *Pseudomonas*. This is a ubiquitous genus, genetically well characterized, and shown to have a wide range of catabolic pathways including the capability to degrade oil spills and a large number of other herbicides (Wheelis, 1975; Chakrabarty, 1976; Clark, 1982; Haas, 1983). Bryant (1963) isolated three groups of *Pseudomonas* species from enrichment cultures of soil treated with seven *s*-triazine compounds. However, only one of the species could grow on atrazine as the sole source of carbon. The abundance of this *Pseudomonas* species in his enrichment culture was dependent on the presence of an ethylamino group at the 4-position of the *s*-triazine ring. Cook and Hutter (1981) have isolated and described *Pseudomonas* species capable of utilizing various *s*-triazines as the sole source of nitrogen. However, atrazine was not employed in their studies.

This study is a part of our research program involving the isolation of bacteria from the soil capable of degrading certain pesticides with a view to determine the intracellular localization of genes responsible for such biodegradation. The investigation reported in this paper describes the isolation of bacteria, belonging to the genus *Pseudomonas*,

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