occurred during the resin cleanup (mainly in the effluent and the washing). The average recoveries obtained by the method B were, therefore, slightly lower than those obtained by the method A.

Nitrate and nitrite levels detected in some 15 whey powder samples are given in Table III. Of these, the first eight samples were of Canadian origin and they were known to have been manufactured without the addition of any nitrate. The remaining samples were of foreign origin, and they were made by a process which involved the addition of nitrate as an additive to the cheese milk. As expected, all the Canadian samples were negative except one which contained very low levels of nitrate. All the samples prepared by the nitrate addition process contained high levels of nitrate. Some of the samples were analyzed by both the methods (A and B), and the two sets of results agreed quite well, mostly within ±10%. No foreign normal (made without nitrate additive) whey powders were analyzed in this study. They would be expected to contain similar levels of nitrate and nitrite as the domestic varieties.

It must be emphasized that these whey powders containing excessively high levels of nitrate are exclusively used as animal feeds and not meant to be used for human consumption. Therefore, no health hazards to humans are expected from such products.

Since both the nitrate-free and nitrate-rich whey powders look alike, inadvertent mixing or mislabeling is possible. It would be advisable, therefore, to mark or stain such nitrate-rich whey powders with some acceptable food colors, thereby reducing the chance of such accidental mix-ups and eliminating any possible health hazard to infants consuming whey powder containing baby food.

Preliminary results suggest that this technique would be extremely useful in analyzing laboratory animal diets (e.g., rat chow) for nitrate and nitrite. These animal feeds also produce strongly colored extracts which make them difficult to analyze by the method A.

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Composition of Polar Lipids in Carrot Roots

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Lipids extracted from carrot roots have been fractionated on a silicic acid column into neutral lipids (NL), glycolipids (GL), and phospholipids (PL), and the composition of the polar lipids have been studied by chemical analysis after thin-layer chromatographic (TLC) development. In root samples of different origin the total lipid content at harvest varied from 225 to 340 mg/100 g of fresh root, but the percent composition of the three main lipid fractions was nearly similar: NL represented over 60% of the total lipid and the percentages of GL and PL were respectively 13–21% and 16–21%. The most abundant lipid classes in the PL fraction were phosphatidylcholines and phosphatidylethanolamines which together amounted to 65% of this fraction. The PL fraction contained 1.0% aldehydogenic lipids. The main part of GL consisted of digalactosyl and monogalactosyl diglycerides. The quantitative composition of polar lipids was in general very similar to that in many other nonphotosynthetic plant storage tissues.

Lipids in carrot root have received little attention, although their composition and changes are included among possible quality factors in carrot products. On the other hand, environmental conditions, which have an influence on yield, and different constituents of carrot have been studied extensively. Most research on carrot lipids has been applied to carotenes because of their importance as previtamins. Since the early work of Hanahan and Chaikoff (1947), only occasional published information has been available on the polar lipids of carrot (Hölzl, 1965). Quite recently, lipid composition from carrot root tissue cultures has been reported (Kleinig and Kopp, 1978) and it has been also compared with certain major lipids from the root material (Gregor, 1977).

In the present paper the authors present the composition of polar lipid classes in carrot roots, to supplement their earlier report, which was mostly concerned with the composition of neutral lipids (Soimajärvi and Linko, 1973).

EXPERIMENTAL SECTION

Materials. Five different samples of carrot, *Daucus carota* cv. Feonia Hunderup S 64 LH, roots were used. Samples A and B originated from a farm in Köyliö, southwestern Finland, sample C from another farm in southwestern Finland, and samples D and E from the Agricultural Research Station of Laukaa, central Finland. The mean fresh weights of roots are given in Table I. The roots were harvested at the normal time in September or October and stored at 4 °C for 1–2 weeks before use. The growth season of carrots was limited by natural environmental factors as the temperature, and it was 19 weeks in samples A–C and 16 weeks in samples D and E.

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Table I. Main Lipid Fraction Content of Carrot Roots[a]

sample, code and year: cultivation place:	A1970 1	B1971 1	C1972 2	D1973 3	E1974 3
mean root weight, g	162	151	177	55	40
total lipids, mg/100 g	225.8	253.7	247.0	339.0	340.0
lipid fractions, mg/100 g					
neutral lipids	143.3	161.3	151.9	213.1	217.6
glycolipids	47.2	33.9	47.3	61.1	51.4
phospholipids	35.4	53.6	49.8	64.8	70.9
lipid fractions, %					
neutral lipids	63.4	63.7	61.0	62.5	64.0
glycolipids	20.9	13.4	19.0	18.2	15.1
phospholipids	15.7	21.2	20.0	19.3	20.9

[a] The figures are mean values of three-four replicate extractions, at harvest.

Extraction and Fractionation of Lipids. Carrots were picked at random to form a sample of 2–3 kg, and from each sample three–four replicate extractions were made. For each replicate, one–five carrots of average size were taken to a subsample of 150–350 g. The roots were cut into 0.5 cm^3 cubes. The cubes were mixed thoroughly and 100 g of freshly cut cubes were extracted with a 2:1 (v/v) chloroform–methyl alcohol mixture. The crude lipids were purified on a column of Sephadex LH-20 gel, and the eluate was passed through a column of Chelex 100 ion-exchange resin in sodium form. The total lipids recovered in this way were fractionated into NL, GL, and PL by using chromatography on a silicic acid column. The procedures have been described in detail previously (Soimajärvi and Linko, 1973).

TLC and Analyses of Polar Lipids. Lipid classes of the PL and GL were separated by TLC on silica gel plates (precoated with Kieselgel 60 from E. Merck AG or corresponding homemade plates of silica gel G). The chromatograms of the PL, or GL, were developed in direction x with a 65:30:4 (v/v) chloroform–methyl alcohol–7 M ammonium hydroxide mixture, and in direction y with 170:25:25:6 (v/v) chloroform–methyl alcohol–glacial acetic acid–water mixture (Nichols, 1964). To detect the PL spots, the reagent of Vaskovski and Svetashev (1972), for phosphorus, was sprayed, and the lipids were identified on specific reactions to ninhydrin or Dragendorff's reagent, or by chromatography of their partial hydrolysis products according to Dawson (1967). To determine the PL composition, lipids were extracted from scraped spots, and total phosphorus was analyzed by the method of Chen et al. (1956). The GL spots were detected with a sequential periodate–Schiff reagent spray, or by water spraying. The quantitative GL determination was based on sugar analysis from lipid hydrolysate and it was usually performed already after the first development. The lipid sugar content was determined as hexoses with anthrone reagent as described previously (Soimajärvi and Linko, 1973).

To determine the aldehydogenic PL content long-chain aldehydes released by acid hydrolysis were analyzed as their p-nitrophenylhydrazones (Rapport and Alonzo, 1960).

RESULTS AND DISCUSSION

Total Lipid Content and Lipid Fraction Content. The mean lipid content of carrot in different samples varied from 225 to 340 mg/100 g of fresh root (Table I); the coefficient of variation of three–four replicates was in each sample from 4 to 9%. Since the dry matter of carrots varied from 12.1 to 12.5% the total lipid content was equal to 1.8–2.7% of the dry weight. The lipid content of the three samples (A–C) coming from southwestern Finland was nearly equal, but lower than that of samples D or E, coming from central Finland. Another difference between the samples was that the mean fresh weight of roots was considerably higher in samples A–C than in samples D or E (Table I). The differences of the samples in the lipid content may be derived from many factors, as differences in the root size, which is dependent on environmental conditions and the length of the growth season.

Table II. Composition of Polar Lipids of Carrot Root[a]

lipid	mol % of lipid P	mg/100 g of fresh root[b]	mol % of lipid sugar
phosphatidylcholines	39	29	
phosphatidylethanolamines	26	18	
phosphatidylglycerols	11	8	
phosphatidylinositols	8	6	
phosphatidylserines	6	4	
phosphatidic acids	4	2	
other phospholipids[c]	3	2	
recovery of phospholipids[d]	97	69	
digalactosyl diglycerides		34	63
monogalactosyl diglycerides		14	22
cerebrosides		4	5
steryl glycosides		3	3
other glycolipids[e]		3	3
recovery of glycolipids		58	96

[a] The figures are mean values of four replicate extractions, at harvest, determined by phosphorus and hexose analyses after TLC separation. [b] Molecular weights of the respective stearoyl derivates were applied. [c] Assumed mol wt 775. [d] Includes 1.0% aldehydogenic lipids. [e] Assumed to contain 25% of sugar.

The amount of the NL of different samples, as mg/100 g of fresh weight, varied about 1.5 times and that of polar lipids about 2 times (Table I). However, the variation in the relative composition of these lipid fractions was considerably smaller. The NL percentage in each sample was nearly the same. Thus, the main difference in the relative lipid composition was that of polar lipids (Table I). The percentage of the PL was in four samples higher than the respective percentage of the GL.

Composition of Polar Lipids. The most prominent PL classes were phosphatidylcholines (PC) and phosphatidylethanolamines (PE) which together formed the major part of this lipid fraction (Table II). Also phosphatidylglycerols (PG), phosphatidylinositols (PI), phosphatidylserines (PS), and phosphatidic acids (PA) were found among the PLs. The group of so called other PLs in Table II included small amounts of tentatively identified diphosphatidylglycerols and lyso forms of PC and PE. In the analysis of aldehydogenic lipids, the amount of p-nitrophenylhydrazones corresponded to 1.0% of total PL when the main molecular weight of respective lipids of 800 is assumed.

Galactosyl diglycerides were predominant glycolipids and their amounts were of the same quantity as those of major PL classes (Table II). Also small amounts of cerebrosides, steryl glycosides, and unidentified GL spots were observed.

Müller (1977) detected that in fresh carrot root phospholipid-bound choline amounts to 47–83 μg/g; this corresponds to 26–46 mg of PC/100 g, which is compatible with our results. Hölzl (1965) reported that of the PL of lyophilized carrot 20% was PE, 19% PC, 18% PI, and 9% cardiolipins, but at the same time as much as 27% PA and 7% polyphosphatidic acids. Evidently, different techniques in sample preparation and possibly in analysis explain part of the differences between this composition and our results.

Gregor (1977) obtained significantly lower lipid content (0.4–0.5 mg/g fresh weight) than that of other authors or our result, by repeated extraction of TCA homogenates from two carrot cultivars with chloroform–methyl alcohol mixture (2:1, v/v). In these extracts also the relative distribution of triglycerides and phospholipids (1:0.7) was different from that in our material.

Plasmalogens are common minor components of animal lipids but they have been detected also in certain plants as in pea seeds (Wagenknecht, 1957; Kaufman et al., 1970) and in different beans (Kuroda and Takahashi, 1975). In the present study the origin of aldehydes detected in the PL hydrolysate was not determined, but interpretation of plasmalogen nature of aldehydogenic PLs may be based on specificity and correct use of respective fractionation and analysis methods. The presence of aldehydogenic lipids in carrot suggests that small amounts of plasmalogens might occur also in other plants.

The total lipid content and the relative amounts of NL, GL, and PL, and also the composition of polar lipid fractions in carrot roots, appears in general very similar to that in many other nonphotosynthetic plant storage tissues containing little fat, such as sugar beet (Beiss, 1969), sweet potato (Walter et al., 1971), and potato tuber (Galliard, 1968). Although these equivalent tissues have very similar lipid composition, carrot root lipids differ significantly from carrot leaf lipids. Tevini (1976) reported that in green and yellow carrot leaves galactosyl diglycerides are predominant lipids, amounting to about 80% of polar lipids. Furthermore, the relative amount of MGDG and PG in leaf lipids is remarkably higher than that in root lipids. The essential differences between carrot leaf and root lipids are similar to general differences between the lipids of leaves and different nonphotosynthetic tissues in many plants (e.g., Hitchcock and Nichols, 1971).

The lipid pattern of carrot suspension culture cells grown at 25 °C in the dark (Kleinig and Kopp, 1978) differs from that in our root material most in the relatively low galactolipid content (6.4% of total lipids), evidently because of different plastid content. The composition of major PLs seems to be similar in root material and in cell cultures, but the latter contain less PG which is also typical to plastids (2% of total PL) and PS (trace amount).

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Papain-Catalyzed Synthesis of Methionine-Enriched Soy Plasteins. Average Chain Length of the Plastein Peptides

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A plastein was synthesized from a peptic soy protein hydrolyzate and ^{14}C-labeled methionine ethyl ester as substrates and with papain as a catalyst. The resulting water-insoluble plastein contained 14.3% of covalently linked methionine. By partial or complete oxidation of the plastein-linked methionine, it was converted into a water-soluble peptide mixture. This mixture was shown by gel chromatography on Sephadex G-25 to consist of peptide species of the same size as the substrate peptides used for plastein synthesis. Radioactive methionine had been incorporated into the entire range of substrate peptides. By chemical means the average chain length was established to be about six residues for both substrate and product peptides. No high-molecular-weight species were formed during plastein synthesis.

The plastein reaction is the protease-catalyzed conversion of soluble peptides into a water-insoluble product. The enzymatic reaction involves cleavage and resynthesis of peptide bonds. Synthesis of peptide bonds was claimed to occur by condensation of α-amino and α-carboxyl groups of peptides with elimination of water (Wieland et al., 1960; Yamashita et al., 1974), as well as by transpeptidation (Horowitz and Haurowitz, 1959).

For the food chemist it is of interest to know if plasteins are high-molecular-weight products or lower peptides. Is the enzymatic conversion from the soluble to the water-

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