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Recovered lipids from prickly pear [*Opuntia ficus-indica* (L.) Mill] peel: a good source of polyunsaturated fatty acids, natural antioxidant vitamins and sterols

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Abstract

Compositions and concentrations of fatty acids, lipid classes, sterols, fat-soluble vitamins and β-carotene were determined in extracted lipids from prickly pear [*Opuntia ficus-indica* (L.) Mill] peel. Total lipids (TL) recovered were found to be 36.8 g kg^{-1} (on dry weight basis). The level of neutral lipids was the highest, followed by glycolipids and phospholipids, respectively. Among the TL, linoleic acid was the dominating fatty acid, while oleic and palmitic acids were estimated to be in relatively equal amounts. Compared with the neutral lipids, the polar fractions were generally characterised by higher percentages of saturated fatty acids and lower percentages of unsaturated fatty acids in all subclasses. Concerning trienes, γ-linolenic acid was present at 8.60% of TL, while α-linolenic acid was present at 0.69%. Recovered lipids were characterised by a high percentage of unsaponifiables (12.8% TL) and found to be a rich source of vitamin E and sterols. Free sterols accounted for ca. 29% of the total unsaponifiables, wherein β-sitosterol and campesterol were the major sterols. In terms of vitamin E, α-tocopherol constitutes about 80.0% of total vitamin E present, the rest being β-, γ- and δ-tocopherols in decreasing order. Moreover, lipids under investigation were characterised by a high levels of β-carotene and vitamin K_1. The information obtained in the present investigation is useful for characterising lipid of prickly pear peel and further chemical and nutritional investigations of prickly pear peel. The results are also important for industrial utilisation of the major by-product of the fruit.

Keywords: Prickly pear peel; *Opuntia ficus-indica*; Fatty acids; Lipid classes; Sterols; Tocopherols; β-Carotene; Vitamin K_1

1. Introduction

The processing of many fruits results in the accumulation of large quantities of by-products. Proper utilisation of this by-product could reduce waste disposal problems and serve as a potential new source of fats and proteins for use in food and feed (Kamel & Kakuda, 2000). The prickly pear cactus [*Opuntia ficus-indica* (L.) Mill] has a global distribution and is an important nutrient and food source. About 1500 species of cactus are in the genus *Opuntia* and many of them produce edible and highly favoured fruits. The Prickly pear is a member of the Cactaceae family and widely distributed in Mexico, much of Latin America, South Africa and the Mediterranean area. In the Mediterranean countries, stems (cladodes) are not a usual nutritional source for humans, but the fruit are largely consumed (Butera et al., 2002; Gurbachan & Felker, 1998; Lee, Kim, Kim, & Jang, 2002; Pimienta-Barrios, 1994). Low water exigency favours the extension of prickly production and, under optimal conditions, annual production can reach 50 t/ha (Barbera, Inglese, & Pimienta-Barrios, 1995; Dominguez-Lopez, 1995; El-Kossori, Villaume, El-Boustani, Sauvaire, & Mejean, 1998). The fruit is a many-seeded berry with a thick peel enclosing a delicately flavoured very seedy pulp. Mexicans have used *Opuntia* leaves and fruits for their medicinal benefits, such as for treating arteriosclerosis, diabetes, gastritis and hyperglycemia. The fruits are important sources of vitamins for local people at the natural growth sites of the plant. Both nopal and fruit are consumed as fresh vegetables, added to casseroles, cooked, or used in salads, syrups and juices

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(Gurbachan & Felker, 1998; Lee et al., 2002). Besides its traditional use in human nutrition, the plant is mainly used for cattle feed, in field management and for the production of carminic acid (Stintzing, Schieber, & Carle, 2001).

The nutrients and chemical composition of the prickly pear (*Opuntia ficus-indica*) fruit have been reported (Dominguez-Lopez, 1995; El-Kossori et al., 1998; Sawaya & Khan, 1982; Stintzing, Schieber, & Carle, 2000, 2001). Aspects covered have included: contents of pigments and flavour compounds, particularly betalains, polyphenols, nutritionally important components of the fruit (amino acids, sugars, organic acids, vitamins, minerals, fibre) and seeds (oil composition), as well as its potential uses in functional foods. It is known that the fruit contains vitamin C; studies on other antioxidant components in peel, however, are lacking. The lipid content in the peel fraction was reported to be 2.43% (on dry weight basis) without enough information about extraction conditions and the degree of ripeness of the fruit (El-Kossori et al., 1998). Although peel makes up about 40% of the whole fruit weight and is subsequently the major by-product, no detailed data about the lipid constitution of prickly pear peel are yet available. One of the major needs within the prickly pear industry is the development of new processed prickly pear products to utilise prickly pear as well as the fruit by-products. In the present study, we have analysed the peel lipids, to obtain an informative profile which will serve as a basis for further detailed chemical investigation and nutritional evaluation of the prickly pear peel. The results, furthermore, will be important as an indication of the potentially nutraceutical and economical utility of prickly pear peel as a new source of edible lipids.

2. Materials and methods

2.1. Materials

Freshly harvested (November 2001) prickly pear (*Opuntia ficus-indica*) fruits were obtained from a local market, Berlin (Germany) and were processed within 24–48 h. Neutral lipid (NL) standards were from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for glycolipids (GL) identification, monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), cerebrosides (CER), steryl glucoside (SG) and esterified steryl glucoside (ESG) were of plant origin (plant species unknown) and purchased from Biotrend Chemikalien GmbH (Köln, Germany). Standards used for phospholipids (PL) identification, phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) from Bovine liver, phosphatidylcholine (PC) from Soybean, phosphatidylglycerol (PG), *lyso*-phosphatidylcholine (LPC) and *lyso*-phosphatidylethanolamine (LPE) from egg yolk were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for sterols (ST) characterisation, β-sitosterol, stigmasterol, lanosterol, ergosterol, campesterol, Δ5-avenasterol and Δ7-avenasterol, were purchased from Supelco (Bellefonte, PA, USA). Standards used for vitamin E (α-, β-, γ- and δ-tocopherol), β-carotene and vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) characterisation were purchased from Merck (Darmstadt, Germany). Reagents and chemicals used were of the highest purity available.

2.2. Methods

2.2.1. Extraction of total lipids (TL)

Intact fruits were carefully selected according to the degree of ripeness which measured by fruit colour (red to purple), the pH value of the pulp (pH 6.05) and the total titratable acidity (0.39%). Fruits were brushed under distilled water, air-dried and hand-peeled. Peel fraction (84.7% moisture) was lyophilised (Alpha 1–5, Martin Christ, Osterode am Harz, Germany) to 10–25% of the original weight. Lyophilised peel was ground (Analysenmühle A10, Janke & Kunkel GmbH, Staufen Br., Germany), then the lipids isolated using a chloroform/methanol extraction procedure (Yang & Kallio, 2001). The sample was homogenised in methanol (50 ml) for 1 min in a blender, chloroform (100 ml) was added and homogenisation was continued for a further 2 min. The mixture was filtered and the solid residue resuspended in chloroform/methanol (2:1, v/v, 150 ml) and then homogenised for 3 min. The mixture was filtered again and washed with fresh solvent (2:1, v/v, 150 ml). The combined filtrates were cleaned with a repeat addition of 0.2 volumes of 0.75% aqueous sodium chloride solution. The whole was thoroughly mixed without shaking, the layers allowed to separate and the chloroform layer recovered. The purified lipids were collected in a flask and subsequently treated with sodium sulphate to remove traces of water; then, after filtration, the extract was taken to dryness on a rotary evaporator at 40 °C. TL recovered were weighed and stored under chloroform at −20 °C until analysed.

2.2.2. Column and thin-layer chromatography of lipid classes

2.2.2.1. Fractionation of lipid classes and subclasses. TL were separated into the different classes by elution with different polar solvents over a glass column (20 mm dia × 30 cm) packed with a slurry of activated silicic acid (70–230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, w/v). NL were eluted with three-times the column volume of chloroform. The major portion of GL was eluted with five-times the column volume of

acetone and that of PL with four-times the column volume of methanol. The amount of the lipid classes obtained was determined by gravimetry. By means of thin-layer chromatography (TLC) on Silica gel F_{254} plates (thickness = 0.25 mm; Merck, Darmstadt, Germany), a further characterisation of the GL and PL subclasses was carried out, with the following solvent system chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v). For the characterisation of NL subclasses, Silica gel F_{254} plates were developed in the solvent system *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v). For the detection of the lipids, the thin-layer plates were sprayed with the following agents: for the marking of all lipids, with sulphuric acid (40%), for the marking of GL with α-naphthol/sulphuric acid, and for the marking of PL with the molybdate-blue reagent (Kates, 1972). Each spot was identified with lipid standards and their reported retention factor (R_f) values. For fatty acid characterisation of subclasses, individual bands were visualised under ultraviolet light, scraped from the plate and recovered by extraction with chloroform/methanol (2:1, v/v). Fatty acid composition of NL, GL and PL as well as their subclasses was determined by GLC/FID as described below.

2.2.2.2. Quantitative determination of lipid subclasses. For the quantitative determination of NL subclasses, individual bands were scraped from the plate and recovered by extraction with 10% methanol in diethyl ether, followed by diethyl ether. Data presented are the averages of three gravimetric determinations. For the quantitative estimation of GL subclasses, the acetone fraction, obtained by column chromatography, was separated by TLC in the above given solvent system. The Silica gel regions with the corresponding GL subclasses were scraped out followed by hexose measurement, photometrically at 485 nm, using the phenol/sulphuric acid method in the acid-hydrolysed lipids (Southgate, 1976). The percent distribution of each component was obtained from the hexose values. From the extinction values, the quantitative amount was determined and related to their portion of the GL fraction. The determined portion was set into relation with the amount of TL, which had been separated by column chromatography into the main lipid fractions. For the determination of the PL, the methanol fraction from column chromatography was also separated by TLC in the above given solvent system and, after scraping off the individual PL subclasses, brought to reaction with the hydrazine sulphate/sodium molybdate reagent at 100 °C for 10 min and photometrically analysed at 650 nm according to the AOCS method [1990; Fisestone (Ed.)]. From the obtained extinction values, via a calibration chart for phosphorus, the amount of PL was calculated. The individual values were put into relation to the PL fraction (methanol fraction from column chromatography) and to the amount of TL.

2.2.3. Gas liquid chromatography (GLC) analysis of fatty acid methyl esters

Fatty acids were transesterified into methyl esters (FAME) using N-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany) according to the procedure reported by Arens, Schulte, and Weber (1994). FAME were identified on a Shimadzu GC-14A equipped with flame ionisation detector (FID) and C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas helium was 0.6 ml/min and a split value ratio of 1:40. A sample of 1 μl was injected on a 30 m×0.25 mm×0.2 μm film thickness Supelco SP™-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperatures were set at 250 °C. The initial column temperature was 100 °C, programmed by 5 °C/min to 175 °C and kept 10 min at 175 °C, then at 8 °C/min to 220 °C and kept for 10 min at 220 °C. A comparison between the retention times of the samples and those of authentic standard mixture (Sigma, St. Louis, MO, USA; 99% purity specific for GLC), run on the same column under the same conditions, was made to facilitate identification.

2.2.4. High temperature gas liquid chromatography analysis of sterols (ST)

Separation of ST was performed after saponification of the lipid samples without derivatization according to Ramadan and Mörsel (2002a). TL (250 mg) were refluxed with 5 ml ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were first extracted three-times with 10 ml of petroleum ether; the extracts were combined and washed three-times with 10 ml of neutral ethanol/water (1:1, v/v) and then dried overnight with anhydrous sodium sulphate. The extract was evaporated in a rotary evaporator at 25 °C under reduced pressure; then ether was completely evaporated under nitrogen. GLC analyses of unsaponifiable residues were carried out using a Mega Series (HRGC 5160, Carlo Erba Strumentazione; Milan, Italy) equipped with FID. The following parameters were tested and found useful: GC column: ID phase DB 5, packed with 5% phenylmethylpolysiloxan (J&W scientific; Falsom, CA, USA), 30 m length, 0.25 mm i.d., 1.0 μm film thickness; carrier gas (helium), flow 38 ml/min (split–splitless injection was used). Detector and injector were set at 280 °C. The oven temperature was kept constant at 310 °C and the injected volume was 2 μl. The repeatability of the analytical procedure was tested and the relative standard deviation of three repeated analyses of a single sample was < 5%. Quantitative analyses were performed with a Shimadzu (C-R6A Chromatopac; Kyoto, Japan) integrator.

2.2.5. Normal phase high performance liquid chromatography (NP–HPLC) separation, identification and quantification of fat-soluble vitamins (FSV) and β-carotene

2.2.5.1. Procedure. NP–HPLC was selected to avoid extra sample treatment (e.g. saponification) according to Ramadan and Mörsel (2002b). Analysis was performed with a solvent delivery LC-9A HPLC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250×4 mm i.d. LiChrospher-Si 60, 5 μm, column (Knauer, Berlin, Germany). Separation of all compounds was based on isocratic elution when the solvent flow rate was maintained at 1 ml/min at a column back-pressure of about 65–70 bar. The solvent system selected for tocopherols elution was isooctane/ethyl acetate (96:4, v/v) with detection at 295 nm. Isooctane/isopropanol (99:1, v/v) mixture was used to elute β-carotene (detection at 453 nm) and vitamin K_1 (detection at 244 nm). Twenty microlitres of the diluted solution of TL in the selected mobile phase were directly injected into the HPLC column. FSV and provitamin A were identified by comparing their retention times with those of authentic standards.

2.2.5.2. Preparation of standard curves. Standard solutions were prepared by serial dilution to concentrations of approximately 5 mg ml^{-1} of vitamin E, 0.7 mg ml^{-1} of β-carotene and 1.4 mg ml^{-1} of vitamin K_1. Standard solutions were prepared daily from a stock solution, which was stored in the dark at −20 °C. Twenty microlitres were injected and peaks areas were determined to generate standard curve data.

2.2.5.3. Quantification. All quantitation was by peak area using a Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from six concentration levels by linear regression. Based on the established chromatographic conditions, repeated injections of different concentrations of the standard FSV and β-carotene were made three-times onto the HPLC system. Injections, in triplicate, were made at each concentration for both standards and samples. All work was carried out under subdued light conditions. All the experiments were repeated at least thrice when the variation on any one was routinely less than 5%.

All experimental procedures were performed in triplicate and their mean values (±standard deviation) obtained.

3. Results and discussion

3.1. General

Currently prickly pear production is increasing. Because the fruit can withstand prolonged drought, it is considered as a potential alternative crop for hot regions (Essa & Salama, 2002). Millions of hectares of marginal land in arid parts of the world, where irrigation cannot be supplied, can succeed as food producers when improved cultivars of prickly pear are available (Knight, 1980). In fact, for a plant to be suitable for lipid production, it must meet the following two criteria; (1) the lipid content must reach the minimum for commercially viable exploitation, (2) the plant must be suitable for high acreage cultivation. The only exceptions are plants that contain lipids unique in their composition or with properties that cannot be found elsewhere (Bockisch, 1998). The percentages of peel and edible pulp in prickly pear fruit are about 40 and 60%, respectively. In our study, it was found that peel contains a considerable amount of lipids (36.8 g kg^{-1}). These results were in relative agreement with the results of El-Kossori et al. (1998) who reported that the chemical composition of prickly pear skin was 2.43% lipids, 8.30% protein, 7.12% starch and 27.6% ethanol-soluble carbohydrate without available information about the degree of ripeness of fruit which may greatly affect the yield of TL. The levels of recovered lipids, moreover, may depend on fruit cultivar and also on fruit processing or storage conditions.

3.2. Levels of lipid classes and subclasses

A suitable combination of chromatographic procedures on Silica gel were used to obtain major lipids classes and subclasses of prickly pear peel. The proportion of TL, lipid classes and subclasses presented in prickly pear peel as well as R_f values of these subclasses are shown in Tables 1–3. Among the TL present in the peel, the level of NL was the highest (63.3% of TL), followed by GL (26.2% of TL) and PL (8.75% of TL), respectively. Subclasses of NL in peel extract contained triacylglycerol (TAG), esterified sterols (STE), free sterols (ST) diacylglycerol (DAG), monoacylglycerol (MAG) and free fatty acids (FFA) in decreasing order. A significant amount of TAG was found (ca. 60.0% of total NL) followed by a relatively high level of STE (ca. 10.8% of total NL) and ST (ca. 5.85% of total NL), while DAG, MAG and FFA were recovered at lower levels. Subclasses of GL in prickly pear peel were sulphoquinovosyldiacylglycerol (SQD), digalactosyldiglycerides (DGD), cerebrosides (CER), sterylglycosides (SG), monogalactosyldiglycerides (MGD) and esterified sterylglycosides (ESG) as shown in Table 2. The proportions of each component were estimated by the lipid-carbohydrate determination. Among the various reagents used for total carbohydrate estimation, phenol is the most popular and apart from its high sensitivity, a further advantage is the equal response of hexose and sulpholipids when measuring the absorbance at 485 nm. In contrast, the colour developed with anthrone has different

adsorption maxima for hexose (620 nm) and sulpholipids (590 nm). SG and CER were the prevalent components and made up about a half of the total GL followed by ESG as the third major subclass. SQD, MGD and DGD were together comprised about one-third of total GL. The average human daily intakes of GL have been reported to be 140 mg of ESG, 65 mg of SG, 50 mg of CER, 90 mg of MGD and 220 mg of DGD (Sugawara & Miyazawa, 1999). Therefore, it is noteworthy that prickly pear peel lipid could be an excellent and a complete

Table 1
Levels and fatty acid compositions of total lipids (TL) and neutral lipids (NL) in prickly pear peel

	Relative content (%)[b]						
Compound	TL	Total NL	MAG	DAG	TAG	FFA	STE
g kg^{-1} TL		633±4.08	13.7±1.25	28.5±2.03	380±3.26	2.53±0.36	68.3±2.56
R_f values×100[a]			14	39	79	56	95
Fatty acid							
C12:0	0.71±0.15	0.53±0.12	2.17±0.25	0.42±0.14	0.30±0.16	0.74±0.11	1.11±0.32
C14:0	1.95±0.25	1.45±0.21	4.60±0.35	0.95±0.13	1.07±0.22	1.48±0.17	1.73±0.16
C16:0	23.1±1.98	22.5±1.86	32.3±2.45	29.7±2.33	17.7±1.46	27.4±2.32	24.0±1.38
C16:1*n*-7	2.48±0.22	1.66±0.15	0.45±0.09	1.43±0.12	0.93±0.17	1.23±0.24	1.54±0.26
C18:0	2.67±0.21	2.23±0.19	1.77±0.13	1.97±0.14	2.25±0.21	1.25±0.12	2.15±0.23
C18:1*n*-9	24.1±2.15	24.6±1.95	22.1±1.88	23.5±1.75	21.3±1.63	21.6±1.22	26.3±1.36
C18:2*n*-6	32.3±2.14	33.7±2.22	27.5±2.56	30.7±2.44	35.6±2.34	32.7±2.26	33.7±2.62
C18:3*n*-6	8.60±1.04	8.40±1.22	6.21±1.09	7.55±1.14	13.7±1.22	9.20±1.53	5.19±1.01
C18:3*n*-3	0.69±0.06	1.02±0.54	0.43±0.11	0.98±0.16	0.94±0.16	1.05±0.18	0.75±0.18
C22:0	0.50±0.05	0.37±0.04	0.22±0.04	0.39±0.05	0.35±0.06	0.29±0.03	0.31±0.04
C24:0	0.41±0.04	0.49±0.07	0.22±0.02	0.37±0.06	0.75±0.08	0.31±0.06	0.42±0.05
C22:2	0.93±0.08	1.04±0.13	0.75±0.09	0.88±0.06	2.41±0.56	0.95±0.24	0.91±0.23
C26:0	0.35±0.04	0.40±0.06	0.31±0.05	0.31±0.03	0.67±0.08	0.25±0.04	0.33±0.05
C24:1*n*-9	1.21±0.26	1.61±0.22	0.97±0.19	0.85±0.16	2.03±0.31	2.55±0.46	1.56±0.33
U/S[c]	2.36	2.57	1.40	1.93	3.33	2.15	2.32

MAG, monoacylglycerols; DAG, diacylglycerols; TAG, triacylglycerols; FFA, free fatty acids; STE, sterol esters.
[a] Solvent system used in TLC development: *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v).
[b] Results are given as the average of triplicate determinations±standard deviation.
[c] Ratio of unsaturated to saturated fatty acids.

Table 2
Levels and fatty acid compositions of glycolipids (GL) in prickly pear peel

	Relative content (%)[b]						
Compound	Total GL	SQD	DGD	CER	SG	MGD	ESG
g kg^{-1} TL	262±4.45	32.5±2.08	18.8±1.22	55.5±2.89	63.4±3.24	26.2±2.21	48.7±3.05
R_f values×100[a]		6	17	29–35	41	64	76
Fatty acid							
C12:0	0.70±0.18	0.95±0.12	0.77±0.15	0.67±0.11	nd	0.59±0.09	0.68±0.16
C14:0	1.34±0.19	1.29±0.21	1.19±0.09	1.20±0.11	nd	2.80±0.26	1.73±0.17
C16:0	29.8±2.11	39.5±2.56	27.9±2.11	41.0±2.68	nd	32.7±1.89	31.7±1.56
C16:1*n*-7	1.56±0.23	1.45±0.28	1.41±0.27	1.63±0.31	nd	0.96±0.33	1.28±0.36
C18:0	2.63±0.36	2.49±0.39	2.73±0.35	2.61±0.29	nd	1.97±0.41	3.51±0.56
C18:1*n*-9	18.9±1.55	15.9±1.14	19.5±1.26	10.9±1.06	nd	16.8±1.21	17.8±1.09
C18:2*n*-6	25.3±2.67	22.6±2.53	25.6±2.49	21.2±1.87	nd	23.7±2.21	25.6±2.63
C18:3*n*-6	13.5±1.23	9.63±0.98	14.6±1.07	15.3±1.02	nd	13.3±0.96	12.9±0.92
C18:3*n*-3	0.34±0.09	0.32±0.04	0.49±0.08	0.25±0.02	nd	0.22±0.03	0.36±0.08
C22:0	0.45±0.11	0.49±0.12	0.37±0.14	0.49±0.15	nd	0.37±0.14	0.46±0.12
C24:0	1.01±0.36	1.09±0.36	1.05±0.31	0.95±0.29	nd	0.89±0.27	0.93±0.24
C22:2	0.47±0.16	0.56±0.18	0.39±0.19	0.44±0.13	nd	0.39±0.06	0.37±0.07
C26:0	3.10±0.54	2.88±0.41	3.05±0.36	2.57±0.39	nd	5.31±0.66	1.73±0.31
C24:1*n*-9	0.90±0.05	0.85±0.03	0.95±0.04	0.78±0.05	nd	0.77±0.06	0.95±0.07
U/S[c]	1.56	1.05	1.69	1.02	–	1.24	1.45

SQD, sulphoquinovosyldiacylglycerol; DGD, digalactosyldiacylglycerol; CER, cerebrosides; SG, steryl glucoside; MGD, monogalactosyldiacylglycerol; ESG, esterified steryl glucoside.
[a] Solvent system used in TLC development: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v).
[b] Results are given as the average of triplicate determinations±standard deviation (nd = not detected).
[c] Ratio of unsaturated to saturated fatty acids.

Table 3
Levels and fatty acid compositions of phospholipids (PL) in prickly pear peel

	Relative content (%)[b]							
Compound	Total PL	PS	LPC	PI	LPE	PC	PG	PE
g kg^{-1} TL	87.5±3.01	20.6±2.05	2.80±0.56	4.97±0.85	1.84±0.39	24.8±2.22	12.9±1.12	15.5±1.50
R_f values×100[a]		4.7	7	11	15	20	23	30
Fatty acid								
C12:0	1.17±0.12	1.48±0.15	1.22±0.11	1.02±0.09	1.85±0.12	1.30±0.08	0.72±0.06	0.65±0.05
C14:0	2.28±0.23	1.26±0.19	1.52±0.16	2.09±0.24	1.28±0.16	2.08±0.29	2.15±0.21	3.09±0.31
C16:0	30.2±3.01	33.5±3.12	35.1±3.24	23.5±2.98	23.1±2.21	30.7±2.41	36.8±3.11	33.2±3.12
C16:1*n*-7	3.04±0.41	4.69±0.44	2.40±0.31	3.11±0.36	5.40±0.71	3.07±0.36	3.11±0.32	2.07±0.29
C18:0	2.81±0.26	2.13±0.19	3.38±0.28	3.71±0.31	4.98±0.51	2.01±0.21	1.22±0.17	1.27±0.19
C18:1*n*-9	19.2±1.05	17.4±1.12	17.6±1.25	20.8±1.09	19.9±1.11	20.1±1.21	19.3±1.23	18.6±1.16
C18:2*n*-6	28.0±2.12	26.6±2.16	23.9±2.14	34.7±3.15	28.5±3.07	27.3±2.88	26.5±2.59	27.6±2.64
C18:3*n*-6	9.91±1.21	7.31±0.98	9.66±1.03	8.56±0.79	10.2±1.16	9.16±0.99	8.10±0.87	10.3±1.04
C18:3*n*-3	0.62±0.11	0.53±0.09	0.63±0.07	0.71±0.05	0.52±0.05	0.63±0.08	0.42±0.03	0.75±0.04
C22:0	0.93±0.10	0.75±0.04	0.95±0.05	0.88±0.08	0.85±0.06	0.95±0.11	0.91±0.13	0.73±0.06
C24:0	0.24±0.02	0.21±0.03	0.26±0.05	0.23±0.06	0.19±0.03	0.25±0.05	0.18±0.02	0.25±0.02
C22:2	0.35±0.07	0.31±0.05	1.40±0.12	0.21±0.05	0.45±0.08	0.32±0.09	0.22±0.05	0.23±0.04
C26:0	0.25±0.05	0.21±0.03	0.40±0.04	0.16±0.02	0.34±0.05	0.22±0.02	0.17±0.01	0.20±0.03
C24:1*n*-9	1.00±0.12	3.62±0.42	1.58±0.21	0.32±0.05	2.44±0.69	1.91±0.71	0.20±0.03	1.06±0.08
U/S[c]	1.61	1.50	1.26	2.14	2.02	1.64	1.36	1.52

PS, phosphatidylserine; LPC, *lyso*-phosphatidylcholine; PI, phosphatidylinositol; LPE, *lyso*-phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamin.

[a] Solvent system used in TLC development: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v).

[b] Results are given as the average of triplicate determinations±standard deviation.

[c] Ratio of unsaturated to saturated fatty acids.

source of GL in diet. PL subclasses in peel extract were separated into seven fractions via TLC. Phosphorimetry of the TLC fractions (Table 3) revealed that the predominant PL subclasses were PC, followed by PS, PE and PG, respectively. About one-third of the total PL was as PC and a quarter was as PE, while PI and *lyso*-phospholipids were isolated in smaller quantities.

3.3. Fatty acid profile of lipid classes and subclasses

Fatty acid profiles of TL, lipid classes and subclasses are presented in Tables 1–3. Fourteen fatty acids were identified in peel extract, wherein the analysis of FAME gave the proportion of linoleic, followed by oleic and palmitic as the major fatty acids, together comprising more than 75% of total identified FAME. A striking feature of the peel lipids was the relatively high level of polyunsaturated fatty acids (PUFA), especially trienes, in which γ-lionlenic fatty acid (GLA, C18:3*n*-6) was estimated to be at higher levels than α-lionlenic fatty acid (ALA, C18:3*n*-3). Furthermore, long-chain fatty acids (behenic C22:0, lignoceric C24:0, docosadienoic C22:2, cerotic C26:0 and nervonic C24:1) were in relatively lower amounts. Fatty acids in neutral lipids and polar lipids differed significantly from each other; linoleic acid was the main fatty acid in neutral fractions while palmitic acid was the abundant one in polar fractions. The ratio of unsaturated fatty acids to saturated fatty acid (U/S ratio), therefore, was higher in neutral fractions than in the corresponding polar fractions. Among the trienes (GLA and ALA), GL resembles PL in the higher content of this type of PUFA, while trienes were at relatively lower levels in the corresponding NL. The fatty acid profile of peel extract shows the lipids to be a good source of the nutritionally essential oleic acid as well as PUFA. A great deal of interest has been focussed on the few oils that contain PUFA, especially GLA. The source of natural GLA are few and at present only borage (21–25%), evening primrose, hemp and hopseed oils are well known. Fruit seeds belonging to the Ribes family have recently been shown to contain significant levels of GLA, including black currant oil (15–18%), red currant oil and gooseberry oil (Kamel & Kakuda, 2000). Moreover, interest in the PUFA, as health-promoting nutrients has expanded dramatically in recent years. A rapidly growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Finley & Shahidi, 2001; Riemersma, 2001). The fatty acid composition and high amounts of PUFA makes the prickly pear peel lipids important for nutritional applications.

3.4. Sterol composition

Fig. 1 presents GLC/FID chromatogram of sterols in prickly pear peel. Identification and content of the sterols in the chromatograms are summarised in Table 4. Peel

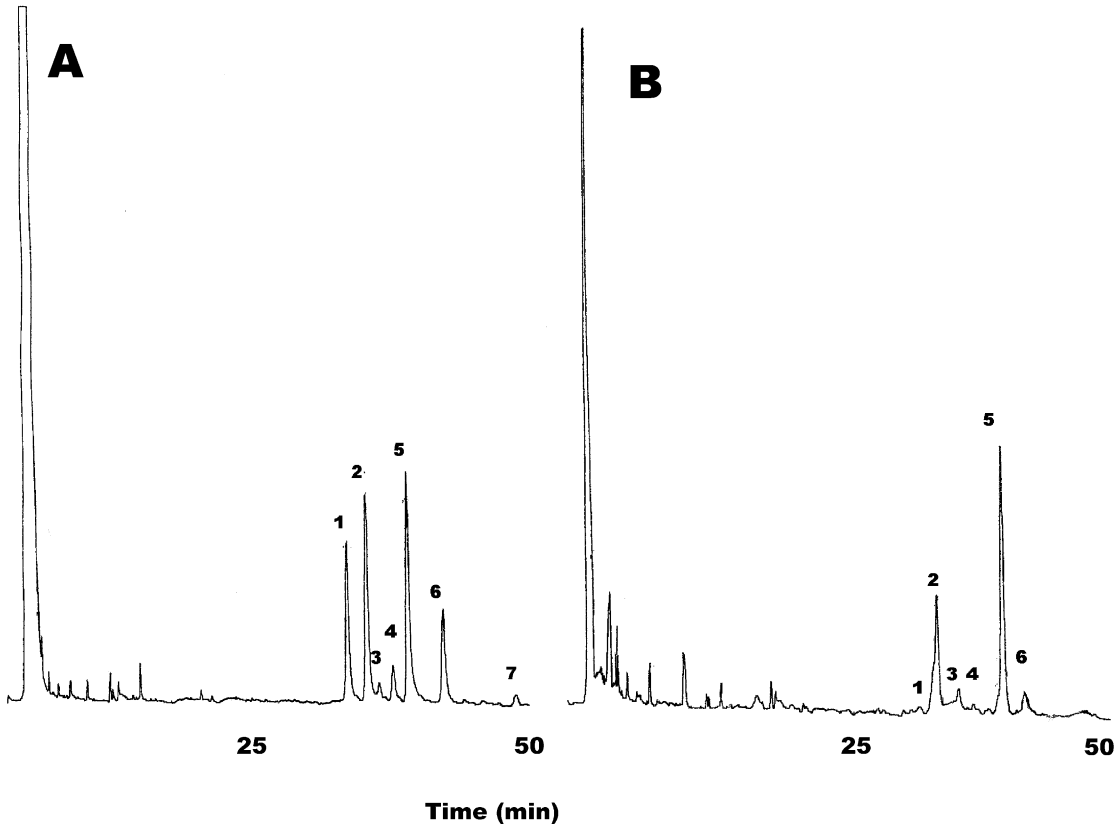

Fig. 1. GLC/FID separation of intact sterols standard (A) and unsaponifiables from prickly pear peel (B) by direct injection of reference mixture and/or unsaponifiables into GLC system without derivatisation. **1**, ergosterol; **2**, campesterol; **3**, stigmasterol; **4**, lanosterol; **5**, β-sitosterol; **6**, Δ5-avensterol; **7**, Δ7-avenasterol.

lipids are characterised by high amount of unsaponifiables (128 g kg^{-1} TL), of which ca. 29% were sterols. Six compounds were present; the sterol marker was β-sitosterol which comprised ca. 57.0% of the total ST content. The next major component was campesterol and these two major components constituted ca. 80% of the total ST. Other components, e.g. stigmasterol and Δ5-avenasterol, were presented in approximately equal amounts (ca. 6.0% of total ST). Among the different plant sterols, sitosterol and sitostanol have been most intensively investigated with respect to their physiological effects in man. Many beneficial effects have been shown for the two sterols (Yang, Karlsson, Oksman, & Kallio, 2001). Lanosterol and ergosterol were present at a lower levels, while Δ7-avenasterol was not detected in the peel unsaponifiable residues. Phytosterols, in general, are of interest, due to their antioxidant activity and impact on health. Recently, phytosterols have been added to vegetable oils as an example of a successful functional food (Ntanios, 2001).

Table 4
Levels of sterols, β-carotene and fat-soluble vitamins in prickly pear peel

Compound	g kg^{-1} TL
Ergosterol	0.68±0.22
Campesterol	8.76±2.31
Stigmasterol	2.12±0.42
Lanosterol	1.66±0.32
β-Sitosterol	21.1±2.55
Δ5-Avenasterol	2.71±0.33
Δ7-Avenasterol	nd[a]
Total ST content	37.0±2.55
α-Tocopherol	17.6±1.55
β-Tocopherol	2.22±0.45
γ-Tocopherol	1.74±0.31
δ-Tocopherol	0.26±0.12
Total vitamin E	21.8±1.98
β-Carotene	2.54±0.46
Vitamin K_1	1.09±0.32

Results are given as the average of triplicate determinations±standard deviation.

[a] Not detected.

3.5. Fat-soluble vitamins (FSV) and β-carotene composition

The nutritionally important components, such as carotenes and tocopherols (vitamin E), improve stability of the oil. In addition, carotenoids, as singlet oxygen quenchers, protect oils from photo-oxidation, whereas their role in autoxidation is associated with the presence of tocopherols (Psomiadou & Tsimidou, 2001). Tocopherols are the major lipid-soluble, membrane-localised

antioxidants in humans. Deficiency of these compounds affects many tissues in mammalian and bird models (Nelson, 1980). Vitamin E deficiency in man causes defects in the developing nervous system of children and hemolysis in man (Sokol, 1996). Eipdemiologic studies suggest that people with lower vitamin E and other antioxidant intakes may be at increased risk for certain types of cancer and for atherosclerosis (Gey, Puska, Jordan, & Moser, 1991; Rimm, Stampfer, Ascherio, Giovannucci, Colditz, & Willett, 1993). It is also suggested that supplementation with antioxidants may decrease the risk of these and other degenerative processes (Kallio, Yang, Peippo, Tahvonen, & Pan, 2002). Tocopherols in vegetable oils, moreover, are believed to protect PUFA from peroxidation (Kamal-Eldin & Andersson, 1997). Data about the qualitative and quantitative composition of vitamins E, K_1 as well as β-carotene, are summarised in Table 4. In our investigation, the NP–HPLC technique was used to eliminate column contamination problems and allow the use of a general lipid extraction for FSV as well as β-carotene isolation (Ramadan & Mörsel, 2003). Thus, saponification of lipid samples was not required, which allowed shorter analysis time and greater vitamin stability during analysis. An exemplary chromatogram of tocopherols in the peel extract is presented in Fig. 2. All four tocopherol isomers were present and the contents of individual tocopherols are shown in Table 4. Vitamin E level was extremely high in the peel lipids and α-tocopherol constituted ca. 80.5% of the total analytes, the rest being β-tocopherol (ca. 10.2%), γ-tocopherol (ca. 8.00%) and δ-tocopherol (ca. 1.20%). α-Tocopherol is the most efficient antioxidant of these compounds. β-Tocopherol has 25–50% of the antioxidative activity of α-tocopherol and γ-tocopherol 10–35% (Kallio et al., 2002). Despite general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue in vivo, however, studies indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness in vitro, especially when compared to γ-tocopherol (Kamal-Eldin & Appelqvist, 1996). High amounts of vitamin E detected in the examined lipids may contribute to the great oxidative stability of these lipids. In this study, evaluation of carotenoid levels is restricted to β-carotene which was measured in markedly large amounts in peel lipids (2.54 g kg^{-1}). This high level could be responsible for the orange hues of prickly pear peel lipids. The level of pigments, however, depends on the stage of fruit ripeness, the extraction process and storage conditions. Thus, lipids extracted from the older fruits contained a higher level of carotene pigments and lipids from younger fruits a higher content of chlorophyll pigments. However, it seems that chlorophyll did not disappear completely in ripe fruits. Table 4 shows that a substantial amount of vitamin K_1 (phylloquinone) was estimated in peel lipids (1.09 g kg^{-1}). The phylloquinone requirement of the adult human is extremely low (Suttie, 1985). However, relatively few

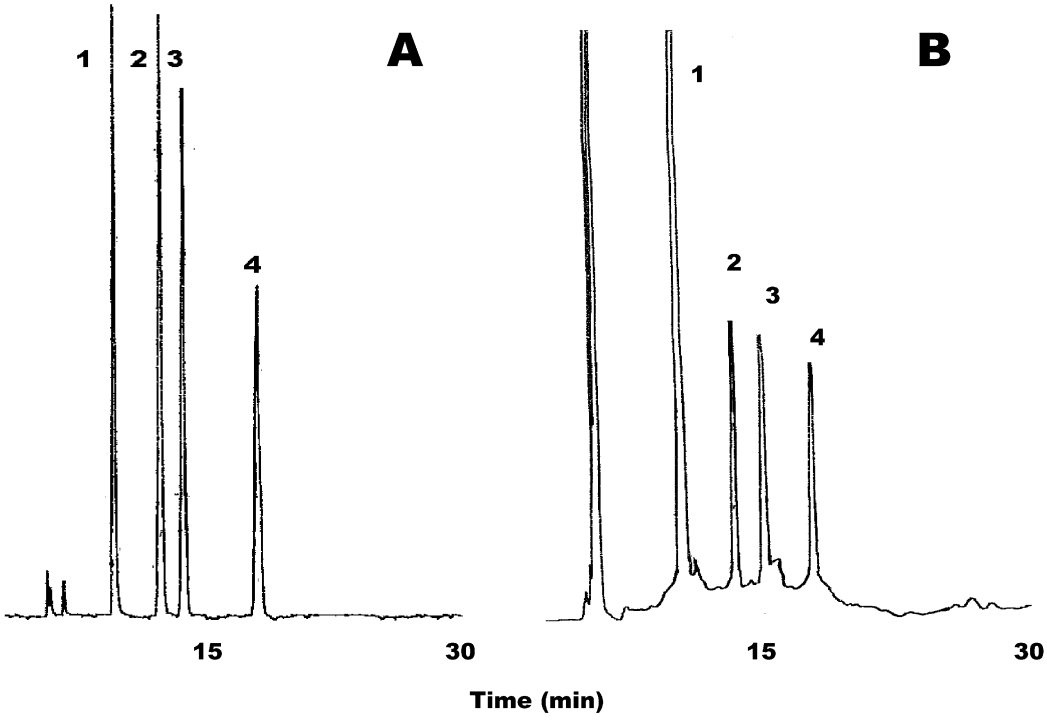

Fig. 2. Simultaneous isocratic NP–HPLC elution profiles of tocopherols standard (A) and tocopherols present in prickly pear peel (B) by direct injection of lipids into HPLC system. Detection was at 295 nm using *iso*-octane/ethyl acetate (96:4, v/v) as a mobile phase. **1**, α-tocopherol; **2**, β-tocopherol; **3**, γ-tocopherol; **4**, δ-tocopherol.

values for dietary items are available. Addition of vitamin K_1 rich lipids in the processing of foods that are otherwise poor sources could make them potentially important dietary sources of this vitamin.

4. Conclusions

Improved knowledge of the composition, analysis and properties of prickly pear peel would assist in efforts for industrial application of this fruit. Agro-waste products from the prickly pear industry, namely peel and seeds, can be used as sources of edible oil. Data about prickly pear seed oil are available; on the other hand, there are no reports in the literature about composition of peel lipids. The peel gives a considerable yield of lipids, but it is necessary to have a sizeable juice industry to have a profitable lipid production. It seems that peel lipids are rich sources of essential fatty acids and lipid-soluble antioxidants. Moreover, recovered lipids could be suitable for commercial exploitation as a source of lipids for food use, soap manufacture or production of cosmetics. High levels of glycolipids means that the peel could be a suitable and valuable source of corresponding glycolipid concentrates. Tocopherols and sterols, at the level estimated, may be of nutritional importance in the application of the fruit.

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