Carotenoids and Retinoids in Finnish Foods: Cereal and Bakery Products

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ABSTRACT

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The carotenoid and retinoid composition of 22 cereal and bakery products was analyzed using a high-performance liquid chromatographic method. There was little, if any vitamin A activity in the cereal products. Retinoids (originating from added ingredients) were present only in bakery products. The amount of β-carotene varied from <1.0 to 170 μg/100 g of fresh product. The predominating carotenoid in flours and some other milling products was lutein (and zeaxanthin), a vitamin A inactive xanthopyll; its concentration in corn products was 3 mg/100 g. The corn products and wheat germ also contained zeaxanthin, but this carotenoid could not be quantified separately from lutein. Only traces of α-carotene and cryptoxanthin were found in cereal products. The amount of vitamin A in bakery products was dependent on their ingredients. Both all-*trans*- and 13-*cis*-retinol were present in most bakery products. The vitamin A content, expressed as retinol equivalents of bakery products, was below 69 μg/100 g.

The carotenoids found in wheat are mainly xanthopylls, such as lutein and its esters, and the total carotenoid content ranges from 280 to 530 μg/100 g depending on the variety (Lepage and Sims 1968, Wildfeuer and Acker 1968). Small quantities of β-carotene and some other carotenoids are found in durum wheat, where the carotene fraction is only 1% of the total carotenoid content (Wildfeuer and Acker 1968). Compared to the endosperm and bran fractions, the wheat embryo contains a high concentration of carotenoids (Chen and Geddes 1945).

Flours and other milling products do not generally contain significant amounts of vitamin A. Yellow corn has some vitamin A value due mainly to its β-carotene and cryptoxanthin content (Kläui and Bauernfeind 1981). In Finland, cereal products are not enriched with carotenoids or retinoids as they are in some other countries. Cereals do contain xanthopylls; however, only a few are vitamin A active components (Bauernfeind 1972).

In this study 22 cereal and bakery products were analyzed for carotenoids and retinoids, and their vitamin A activity was determined. The present study is part of a food composition study carried out to produce defined, updated data on the carotenoid, retinoid, fatty acid, and triglyceride contents of Finnish foods. The food composition study is related to a joint U.S.-Finland project sponsored by the National Cancer Institute. The project consists of human studies of nutrition and cancer in which the main emphasis is on a β-carotene and vitamin E lung cancer intervention trial. A comprehensive and reliable food composition file is essential for nutritional calculations forming the basis of the epidemiological studies now under way at the National Public Health Institute, Helsinki.

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MATERIALS AND METHODS

Sampling

Cereal and bakery products (Tables I and II) were purchased from 10 different stores representing the four major wholesale food chains (about 90% of total sales) in the Helsinki area. Equal weights of food from each store were composited. The subsample sizes were as follows: 2 dl for items in Table I excluding "Talkkuna" (200 g), corn flakes (6 dl), popcorn (20 g), and peanuts (150 g), slices (1/12 of a loaf) for breads, one whole biscuit or crisp bread, half a meat pie cut into cubes, and a quarter of Karelian pie or sponge cake. The 10 subsamples of each food item were mixed together, divided into portions of approximately 100 g, vacuum packed in

polyethylene-nylon laminate bags, and stored at −20°C until analyzed (generally for two to three weeks). Durum wheat flour (1 kg) was obtained from a local importer (Finnish Sugar Ltd.).

Extraction

For analysis, the frozen food samples were homogenized in a blender (flour samples required no homogenizing). Aliquots (10 g) of sample homogenates were saponified with 20 ml (or 40 ml for meat pie and sponge cake) of potassium hydroxide (100 g KOH + 100 ml H_2O) in ethanolic solution (50 ml) (Piironen et al 1984). Ascorbic acid (1 g) was used as an antioxidant. Carotenoids and retinoids were extracted using a mixture of hexane and diethylether (70:30). Under these conditions the recovery of β-carotene was 94% and that of retinol 99%. The entire extraction procedure has been described elsewhere (Ollilainen et al 1988).

High-Performance Liquid Chromatography (HPLC)

Two Varian Vista 5500 liquid chromatographs (Varian, USA) were each equipped with a Varian UV-200 detector and a Varian 4270 integrator. In the nonaqueous reversed-phase (NARP) chromatography of carotenoids (Nelis and De Leenheer 1983), a Zorbax ODS 5–6 μm, 25 × 0.46 cm i.d. column (DuPont) was preceded by a 5 × 0.46 cm i.d., 35–50 μm Spherisorb ODS 2 guard column (Phase Sep, UK). The elution mixture was acetonitrile, dichloromethane, and methanol (70:20:10) and the flow rate was 2 ml/min. The temperature was 30°C and the wavelength for detection 450 nm. For the normal phase chromatogram of retinoids, a LiChrosorb Si60 5-μm, 25 × 0.46 cm i.d. column (Merck, Germany) was used. The mobile phase was 3% isopropanol in *n*-hexane and the flow rate was 1 ml/min. The temperature was 30 °C and the wavelength for detection 325 nm. All solutions were injected via full loop, approximately 55 μl.

The concentrations of the standard stock solutions of various carotenoids or all-*trans*-retinol were determined spectrophotometrically (Ollilainen et al 1988). The extinction coefficients ($E^{1\%}_{1cm}$) used were α-carotene, 2,725 at 446 nm in hexane; all-*trans*-β-carotene (and 15-*cis*-β-carotene), 2,592 at 453 nm in hexane; β-cryptoxanthin, 2,470 at 452 nm in hexane; lutein, 2,550 at 445 nm in ethanol; zeaxanthin, 2,480 at 452 nm in ethanol; and all-*trans*-retinol (and 13-*cis*-retinol), 1,832 at 325 nm in ethanol. All standard solutions were stored in the dark at −70°C under nitrogen.

The quantification of carotenoids and retinoids was based on an external standard method. Three replicate analyses of the pooled material were done per item. The mean, standard deviation, and coefficient of variation were calculated. A *Q* test was used to reject the error results (Fritz and Schenk 1979). The analysis was repeated in duplicate (sweet wheat bread) or triplicate (meat pie) in cases where the coefficient of variation exceeded 10%. Lutein and the possible zeaxanthin were quantified as lutein using lutein as the standard. The peak of 13-*cis*-retinol was quantified using all-*trans*-retinol standard. The UV-spectra of lutein, zeaxanthin, all-*trans*-β-carotene and 15-*cis*-β-carotene of some individual samples were obtained via a diode array technique (Hewlett Packard liquid chromatographic system available in the National Public Health Institute of Finland). The carotenoids were identified via comparison with the spectrum of the correspondent standard, except for the 15-*cis*-β-carotene, which was tentatively identified

TABLE I
Carotenoids and Vitamin A (retinol equivalents, RE) in Flours, Other Milling Products, and Special Products

Item	μg/100 g Fresh Product		
	β-Carotene	Lutein[a]	RE[b]
Wheat meal	4.3 ± 0.4[c]	220 ± 4.3	0.7
Wheat flour[d] (approximately 0.7% ash)	<1.0	190 ± 3.4	0.0
Wheat flour, durum	9.3 ± 0.6	480 ± 4.3	1.6
Wheat flour[e] (1.2–1.4% ash)	5.3 ± 0.2	220 ± 1.7	0.9
Wheat bran	5.5 ± 0.2	240 ± 3.2	0.9
Wheat germ	62 ± 1.3	790 ± 23	10
Barley meal	<1.0	160 ± 6.5	0.0
Rye meal	6.5 ± 0.6	210 ± 7.0	1.1
Oats, rolled	<1.0	180 ± 2.6	0.0
Talkkuna, a roasted meal product[f]	4.3 ± 0.2	110 ± 3.1	0.7
Corn flakes	170 ± 5.6	2,700 ± 80	28
Popcorn	63 ± 1.1	1,300 ± 15	11
Peanuts	2.0 ± 0.1	14 ± 1.3	0.3

[a] May contain some zeaxanthin.
[b] Calculated as μg/RE/100 g = μg all-*trans*-retinol + (μg 13-*cis*-retinol × 0.75) + (μg β-carotene × 0.167) + (μg cryptoxanthin × 0.083).
[c] Standard deviation of the three replicate analyses.
[d] Extraction rate of wheat approximately 78%.
[e] A flour rich in aleuronic fraction.
[f] Mixture of oat, barley, and pea.

TABLE II
Carotenoids, Retinoids, and Vitamin A (retinol equivalents, RE) in Bakery Products

Item	μg/100 g Fresh Product				
	β-Carotene	Lutein[a]	All-*trans*-Retinol	13-*cis*-Retinol	RE[b]
Wheat bread	<1.0	71 ± 0.5[c]	...	...	0.0
Wheat bread, dark	<1.0	75 ± 2.6	...	...	0.0
Rye bread	<1.0	78 ± 3.2	...	...	0.0
Crisp bread	<1.0	84 ± 1.2	...	...	0.0
Karelian pie[d]	16 ± 1.4	14 ± 1.3	30 ± 2.1	5.9 ± 0.5	37
Meat pie[e]	6.2 ± 0.8	43 ± 4.0	7.2 ± 0.7	...	8.2
Biscuit	14 ± 0.6	75 ± 0.5	32 ± 2.0	5.9 ± 0.4	39
Sweet wheat bread	19 ± 1.3	76 ± 7.3	43 ± 2.9	9.5 ± 0.3	53
Sponge cake, with fruit filling[f]	120 ± 8.3	66 ± 2.4	38 ± 0.9	7.3 ± 0.5	69

[a] May contain some zeaxanthin.
[b] Calculated as μg RE/100 g = all *trans*-retinol + (μg 13-*cis*-retinol × 0.75) + (μg β-carotene × 0.167) + (μg cryptoxanthin × 0.083).
[c] Standard deviation of three (for sweet wheat bread five and for meat pie six) replicate analyses.
[d] Ingredients: rye and wheat flour, rice, butter, milk powder.
[e] Ingredients: wheat flour, rice, ground beef, dietary fats and oils.
[f] Contains cryptoxanthin, 70 ± 3.2 μg/100 g.

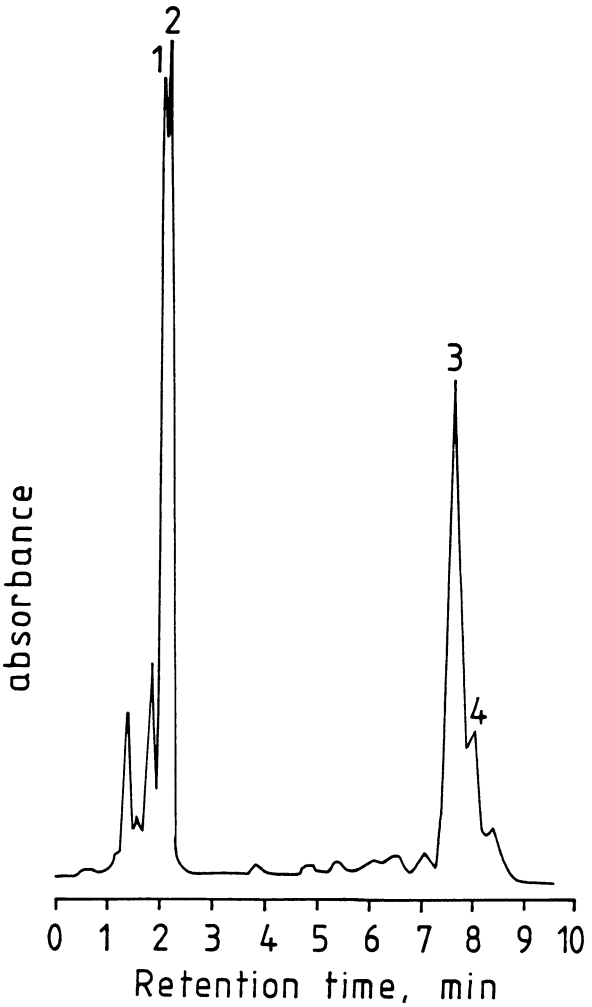

Fig. 1. Nonaqueous reversed-phase chromatogram of wheat germ carotenoids. Peak identification: lutein (**1**); zeaxanthin (**2**); all-*trans*-β-carotene (**3**); 15-*cis*-β-carotene (**4**). The attenuation was changed from 2^4 to 2^2 between the elution of xanthophylls and carotenes.

according to the characteristics of its spectrum.

The individual carotenoid and retinoid values were converted into retinol equivalents (RE) according to the guidelines of the Food and Nutrition Board (FNB 1980). The vitamin A activity of 13-*cis*-retinol was assumed to be 75% of all-*trans*-retinol (Ames et al 1955). Since the tentative 15-*cis*-β-carotene was not resolved in every sample, its peak area was added to that of all-*trans*-β-carotene.

Reagents

HPLC-grade solvents (Rathburn, Scotland), acetonitrile, dichloromethane, and methanol were used without further purification. Hexane (HPLC-grade, Rathburn) used in the liquid chromatography was dried with anhydrous sodium sulfate (Merck, Germany) and filtered through a 0.45-μm membrane (Millipore, France). Ascorbic acid, *n*-hexane for spectrophotometry (Uvasol), isopropanol, and sodium chloride, all analytical grade, were obtained from Merck. Ethanol (94 and 99%, Alko, Finland), crystalline BHT (2,6-di-*tert*-butyl-*p*-cresol) from Sigma Chemical Co., and potassium hydroxide (EKA Kemi, Sweden) were used. All carotenoid and retinoid standards were purchased from Hoffman-LaRoche Ltd., Switzerland.

RESULTS AND DISCUSSION

The carotenoid composition of flours and other milling products is given in Table I, and the carotenoid and retinoid composition of bakery products are given in Table II. According to Fortman and Joiner (1971), wheat flour contains between 295 and 410 μg of carotenoids per 100 g, and the amount of carotenoids in durum wheat can be as high as 730 μg/100 g. In the present study the predominating carotenoid in flours, other milling products, special products, and in most bakery products was lutein, possibly containing some zeaxanthin. Its amount in wheat flour varied from 190 to 480 μg/100 g depending on the milling process. The lower rate of extraction (lower ash content), the lower the carotenoid content of the flour. The highest amount of lutein was found in durum wheat flour, which also contained some β-carotene. According to the present data, the vitamin A content of Finnish cereal products was somewhat lower than that presented earlier (Turpeinen 1983).

The NARP chromatogram of wheat germ carotenoids is given in Figure 1. Lutein and zeaxanthin were quantified as lutein, 790 μg/100 g, because the two xanthopylls were poorly resolved. Also, β-carotene (62 μg/100 g) and traces of both α-carotene and cryptoxanthin were present. Wheat germ has a vitamin A-value of 10 RE/100 g. The amount of lutein in wheat bran was about 30% of that in wheat germ. The amount of β-carotene in wheat flour ranged from traces to 9.3 μg/100 g. Of all cereal flours, barley meal contained the smallest amount of carotenoids.

In corn flakes and popcorn, both lutein and zeaxanthin were present. Also, β-carotene and cryptoxanthin were found, although the latter was not quantified. The vitamin A value of corn flakes was 28 RE/100 g and that of popcorn 11 RE/100 g.

No retinoids were present in bread made from plain flour and water. Lutein (and possibly zeaxanthin) was the only carotenoid found in bread, the range being 71–84 μg/100 g. Karelian pie, meat pie, and sweet bakery products contained lutein (and zeaxanthin), β-carotene, all-*trans*-, and 13-*cis*-retinol. The retinoids in these products were derived from ingredients such as fat, egg, and milk. In the sponge cake with fruit filling, cryptoxanthin (70 ± 3.2 μg/100 g) was also present. Corn flakes and sponge cake with fruit filling contained more than 100 μg/100 g of β-carotene.

In Finland, 24% of the daily caloric intake is received from cereal products (Agric. Econ. Res. Inst. 1985) of which about three-fifths are bakery products with added fat and other vitamin A-containing ingredients (Salovaara 1986). In recent years, the per capita consumption of cereal products has remained at a constant level of approximately 70 kg/year (Salovaara 1986).

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