Physiologically Significant Carotenoids and their Common Food Sources in Czech Population

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Carotenoids are a group of lipid soluble pigments with antioxidative, photoprotective, and anticarcinogenic properties. In a random group of 187 volunteers the carotenoid content and composition in the serum was analyzed using HPLC on the reversed phase. Within the group evaluated, lutein was found to be the major serum carotenoid (mean 52.97 %, SD = 8.32 %), followed by β-carotene (10.77 %, SD = 1.53 %), lycopene (8.32 %, SD = 1.65 %), and α-carotene (4.40 %, SD = 0.71). Mean β-carotene level in the studied individuals was found smaller (0.406 ± 0.165 μmol dm^{-3}) than the lower limit of physiological range (0.6—10 μmol dm^{-3}). The considerable seasonal differences of total serum carotenoids with the strong minimum in winter (January—March) and graduated growth to maximum in summer—autumn were observed. Since the exclusive supply of the whole group of the carotenoids are the foods (mainly fruit and vegetables), several common foods (potatoes, apples, carrots, green vegetables) as potential sources were analyzed. Except the carrot, the major inland supplies are potatoes and green vegetables, both in raw and boiled form, which contain predominantly lutein. However, apples, one of widely used all the year round fruits, are rather poor source of carotenoids (about 1000 times lower than carrot). With regard to unfavourable ratio of β-carotene/lutein in the serum it will be necessary to enrich conventional diet predominantly for the natural sources rich in β- and α-carotenes. Within inland foods, carrots were the major contributor of those (82.4 mg β-carotene, 30.68 mg α-carotene per 100 g). As additional sources can serve also the above-mentioned green vegetables. In the part of calendar year poor of inland raw fruits (peach, apricot) and vegetables (pepper, tomato) the complementary carotenes supplies will be needed.

Carotenoids are brightly coloured pigments occurring in plants, yellow to red in colour, that are introduced into the human body predominantly through the ingestion of vegetables and fruits. To date, almost 600 carotenoids have been identified. Of these, approximately 60 have provitamin A activity, the most notable being β-carotene. There are two major carotenoid families, the class of hydrocarbons (carotenes, provitamin A) and their oxygenated derivatives (xantophylls): monohydroxycarotenoids with proA activity such as cryptoxanthin, and dihydroxycarotenoids with nonproA activity, such as lutein [1].

Carotenoids are important factors in human health. The essential role of β-carotene and others as the main dietary source of vitamin A has been known for many years. More recently, protective effects of carotenoids against serious disorders such as cancer or heart disease have been recognized, and have stimulated intensive research into the role of carotenoids as antioxidants [2]. Free radicals may be responsible for cell membrane damage, DNA mutation and lipid (per)oxidation, all of which may lead to arthritis, hardening of the arteries, strokes, cataracts, heart disease, and many of the diseases we call "degenerative" [3].

Carotenoids are present in the human being, both in blood and tissues [1]. In several studies about 20 regularly occurring carotenoids were reported [4—7]. Of these, five, namely, α- and β-carotenes, cryptoxanthin, lutein, and lycopene, were the main components identified in human serum [8]. However, in most well-nourished persons, only about 1 % of the total body pool of carotenoids (100—150 mg) is found in the serum. It seems that individual organs selectively retain different carotenoids based on their protective needs [9]. In thyroid, kidneys, spleen, liver, heart, and pancreas, β-carotene and lycopene were the predominant carotenoids. In the adrenals and testes, however, lycopene clearly predominated. In fat and ovaries, lutein and β-carotene were most abundant. The macula, which is the area of highest visual discrimination within the retina, contains mainly lutein and zeaxanthin [10]. Interestingly, no β-carotene was found within the retina. Additionally, individual carotenoids have different solubility charac-

teristics, as well as different free radical scavenging activities. For instance, while β-carotene is particularly efficient as a singlet oxygen-radical quencher, lycopene and γ-carotene have approximately double activity [9]. While β-carotene has well documented antitumour effects, often causing regression and re-differentiation of established cancers, α-carotene is up to 10 times more effective [11, 12].

β-Carotene is the best known of carotenoids. In January 1996 the National Cancer Institute (USA) released the results of two anticipated antioxidants/β-carotene studies [13, 14]. In the multicenter Carotene and Retinol Efficacy Trial (CARET) lung cancer prevention study, β-carotene (and vitamin E) did not appear to have any effect on health. The β-carotene and vitamin A used in these studies were in the form of synthetic supplements, and in many epidemiological studies that show positive results, these vitamins were in the form of natural products [2, 15]. Additionally, the whole group of pigments were more efficient at inhibiting degenerative disease progression than pure individuals. The protective effect of carotenoids is most probably the result of a combination of various biological activities.

Many fruits and vegetables have high concentrations of carotenoid compounds. Carotenoids occur naturally in carrots, tomatoes, butter, cheese, pepper, green and leafy vegetables (*i.e.* spinach, kale, broccoli, and green beans), palm oil, corn kernels, and red salmon. However, in the Czech Republic, there is not complete information about carotenoid content in several of all the year round widely used (potato, apples), seasonal (green vegetables) and/or exotic foods and about suitable natural inland sources of these nutrients. The objectives of this study were to evaluate a total and individual carotenoid content of some traditionally frequently consumed carotenoid-rich foods both in raw and thermally processed form. Parallelly, a carotenoid level in the group of volunteers was measured and its relationship with food composition was evaluated. The monitoring of season influences on the content of carotenoids in foods in order to assess the actual dietary intake of this nutrients through the calendar year was performed.

EXPERIMENTAL

Chemicals

Carotenoids (α- and β-carotenes, lutein, lycopene) were purchased from Sigma Chemical Co. (Sigma-Aldrich Co., CZ). HPLC grade solvents (methanol – Merck, water, chloroform, petroleum ether, and acetone – Sigma-Aldrich Co., CZ) were used without further purification. The mobile phases were degassed by sonication for 10 min prior to use.

Stock solutions of carotenoids (10 mmol dm^{-3} α- and β-carotenes, 4 mmol dm^{-3} lutein, 2 mmol dm^{-3} lycopene) were prepared in absolute ethanol prior to calibration. Working standard solutions of carotenoids were prepared daily in absolute ethanol.

Group of Patients

The random study group included 187 volunteers (105 women, 82 men) aged 25—77 years, who did take any synthetic carotenoid supplement 4 weeks before blood sample was taken. Number of subjects analyzed in individual months was 12—19.

Blood Sample Preparation

The serum (2 cm^3), deproteinized with absolute ethanol (10 cm^3), was extracted twice with 25 cm^3 of petroleum ether. After evaporation, the residue was dissolved in 500 mm^3 of methanol. Samples (100 mm^3) were injected onto C18 Nucleosil 100 column that had been equilibrated with solvent mixture methanol—water ($\varphi_r = 95$ 5) at 1 cm^3 min^{-1}

Food Product Sampling

10 g of food sample (raw or boiled vegetable, raw fruit) was suspended and homogenized with 50 cm^3 of acetone. The mixture was filtered through Whatman No. 1 filter paper on a Büchner funnel. The filtrate (30 cm^3) was extracted twice with 25 cm^3 of petroleum ether. The petroleum ether extracts were combined and dried under vacuum. After evaporation, the residue was dissolved in absolute ethanol. Samples (10 mm^3 valve) were filtered and injected onto C18 Nucleosil 100 column that had been equilibrated with solvent mixture methanol—water ($\varphi_r = 95$ 5).

Within five sorts of the carrots analyzed, two were imported ("baby" carrot is distributed as a component of frozen vegetable mixtures, Holland carrot is delivered in raw state) and three were inland sorts (Moravian carrot I from South Moravia, Moravian carrot II from East Moravia, and Moravian carrot III from Brno). In the five of the most common sorts of apples a total content of carotenoids and a proportion of individual representatives presented was analyzed. Three sorts of potatoes from distinct regions of the Czech Republic were analyzed; *i.e.* potato sort I from South Moravia, potato sort II from East Moravia, and potato sort III from South Bohemia. With the aim to analyze influences of thermal processing on carotenoid content in potato extracts, one half of potato III sort was extracted in raw state, the other was boiled for 25 min in distilled water and then extracted according to the procedure described above.

The three samples from each of the five sorts of apples, carrots, and three sorts of potatoes, respectively, were extracted and analyzed for carotenoids content. This sampling scheme was repeated for each of further analyzed fruit and vegetable sources.
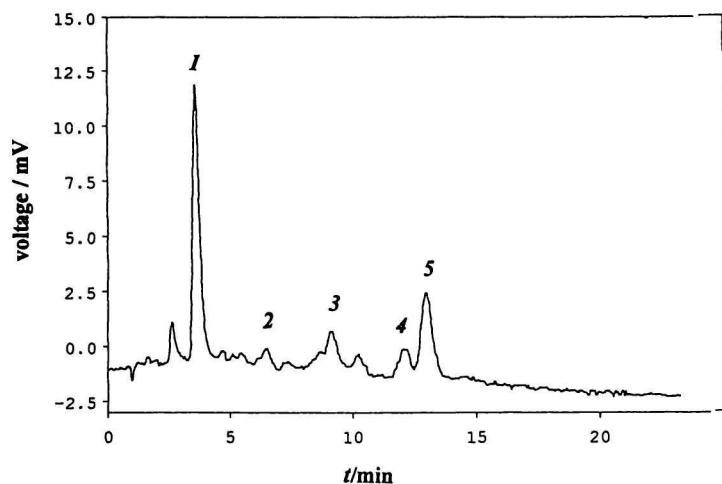
Fig. 1. A HPLC chromatogram of the carotenoids presented in the human serum. The monitoring wavelength was 450 nm. Peak description: *1.* lutein, *2.* cryptoxanthin, *3.* lycopene, *4.* α-carotene, *5.* β-carotene.

Table 1. Concentration of Individual Carotenoids in the Human Serum

	Lutein	Lycopene	α-Carotene	β-Carotene
$\dfrac{c \text{ (mean)}}{\mu\text{mol dm}^{-3}}$	2.260	0.335	0.166	0.406
$\dfrac{\text{SD}}{\mu\text{mol dm}^{-3}}$	0.495	0.135	0.074	0.165

Methods

The chromatographic system consisted of ECOM (Prague) apparatus: high-pressure analytical pump LCP 4020, gradient programmer GP 5, analytical injection loop valve type C, UV VIS variable wavelength detector LCD 2084 and LCO 101 column oven. Microfilters of PTFE were used for filtering a mobile phase. For filtration of all samples before injection sample filters (PTFE) were used. The operating conditions (both for the serum and food samples) included a C18 Nucleosil 100 stainless steel column (particle size 5 μm) 4.6 mm $\times$ 150 mm and the guard column 4.6 mm $\times$ 30 mm (Lachema, Brno) along with a solvent system of methanol—water ($\varphi_r = 95$ 5) pumped at a flow rate of 1.0 cm^3 min^{-1}. The isocratic elution was carried out at 45 °C for 15—20 min. Visible detection was achieved at $\lambda = 450$ nm. Data processing (integration) of analyses was assessed using a CSW integrator v.1.7. (Chromatographic Station Software) of DataApex Co. (Prague).

The amount of total carotenoids was expressed as the total area of HPLC chromatogram (mV s^{-1}), the fractions of individual carotenoids were evaluated as the ratio (in %) of peak area (mV s^{-1}) and total area. Data were evaluated using external standards. Calibration curves based on peak area were established for each physiological carotenoid with the exception of cryptoxanthin (*i.e.* α-carotene, β-carotene, lutein, lycopene) and were used to determine concentration.

RESULTS

Evaluation of a Group of Patients

Fig. 1 presents a typical HPLC chromatogram of the carotenoids of the human serum in a random sample of a Czech population. Fig. 2 illustrates mean distribution of individual representatives of physiologically significant carotenoids. Mean concentrations of lutein, lycopene, α-carotene, and β-carotene obtained in a study group are summarized in Table 1. With regard to limited concentration of cryptoxanthin in the most of serum samples the concentration of this derivative was not estimated. Within the group of patients evaluated, lutein was found the major serum carotenoid (mean 52.97 % of total carotenoids, SD = 8.32 %), followed by β-carotene (mean 10.77 %, SD = 1.53 %), lycopene (mean 8.32 %, SD = 1.65 %), and α-carotene (mean 4.40 %, SD = 0.71 %). Mean β-carotene level in the studied individuals (Table 1) was found smaller than the lower limit of published physiological range, *i.e.* 0.6—10 μmol dm^{-3} [8, 9]. The course of considerable seasonal differences of total serum carotenoids is introduced in Fig. 3, with the strong minimum in winter (January—March) and
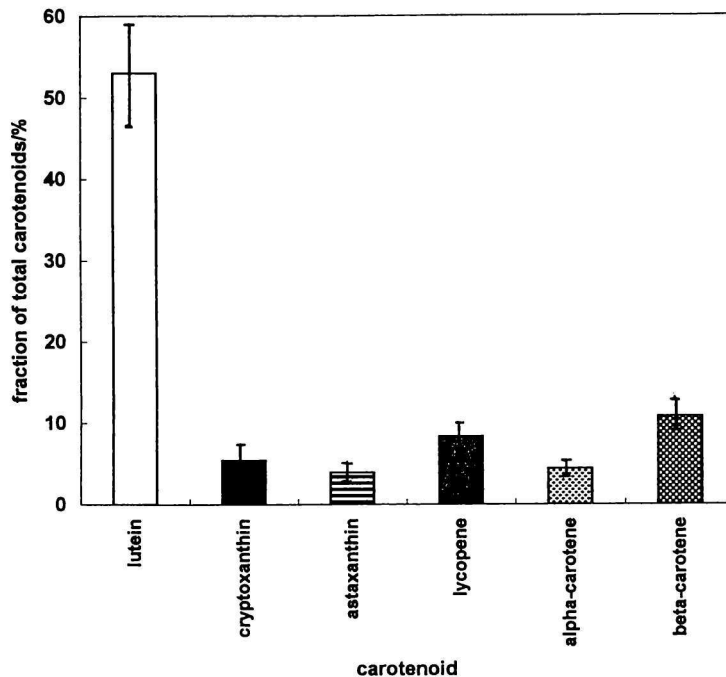
Fig. 2. Mean proportion of individual carotenoids found in the human serum. The values are expressed as the fraction of total carotenoids (fraction of total area in mV s^{-1} at 450 nm).
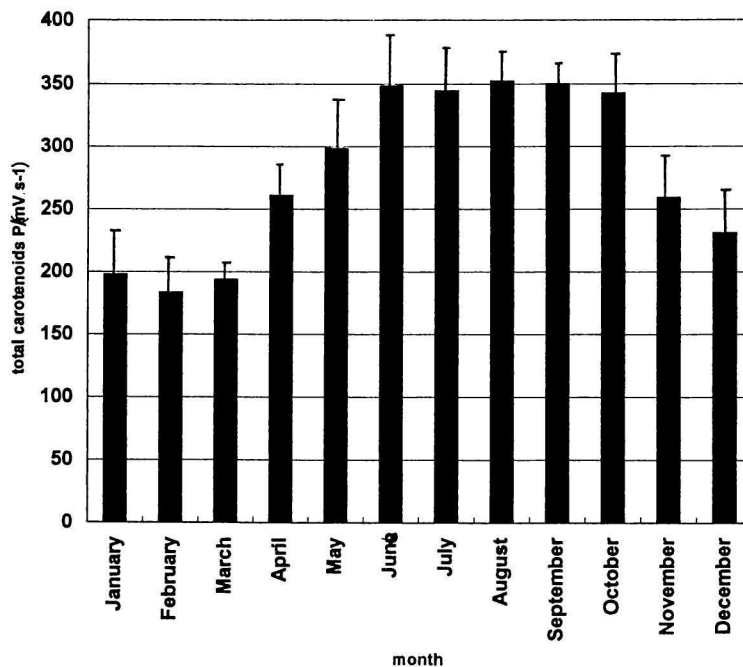

Fig. 3. Seasonal differences of the mean amount of total carotenoids in the human serum. The course of monthly changes of the mean amount of total carotenoids in the serum (total area of chromatogram ($P/(\text{mV s}^{-1})$)) at 450 nm) through the calendar year.

graduated growth to maximum in summer and autumn (June—October).

Analysis of the Food

In the scope of evaluation of potential carotenoid food sources widely accessible in our geographic conditions primarily were analyzed the most frequently consumed vegetables and fruits: carrots, potatoes, and apples.

Fig. 4 presents a standard chromatogram of the carrot extracted by the described procedure. The
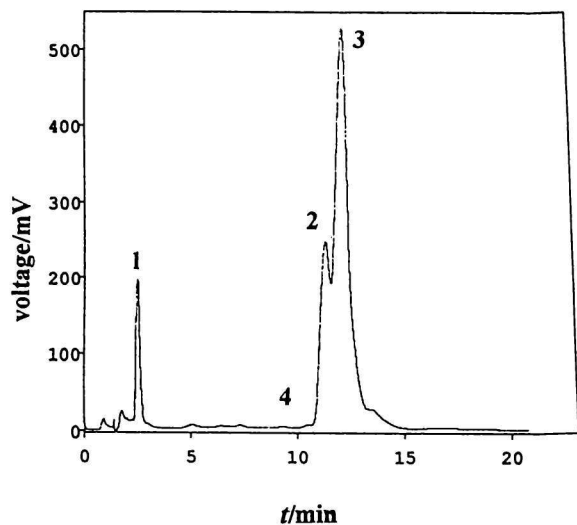
Fig. 4. A HPLC chromatogram of the extract from carrot. Peak description: *1.* lutein, *2.* α-carotene, *3.* β-carotene, *4.* lycopene.

major carotenoid here is β-carotene, followed by α-carotene and bý a small amount of lutein and lycopene. The content and constitution of individual carotenoids were analyzed in five distinct sorts of carrot commonly commercially obtained on trade (Fig. 5). Among those two sorts were imported (frozen "baby" carrot, Holland carrot) and three sorts pertained to inland (South Moravian carrot I, II, West Moravian carrot III). Within the sorts analyzed, the

proportion of individual carotenoids was very similar. The mean ratio of β-carotene α-carotene lutein lycopene was 75.77 % (SD = 2.77 %) 23.82 % (SD = 2.73 %) 2.08 % (SD = 0.98 %) 0.24 % (SD = 0.07 %). However, substantial differences in total carotenoid content were observed between the sorts, for instance the amount of carotenoids in "baby" carrot was 3.74 times higher and in Holland carrot 1.36 times higher than in the best of inland carrots species analyzed.

Apples are taken for one of the most frequently used fruits in our region. While evaluating five different sorts of apples, we found substantially (about 1000 times) lower amount of total carotenoids when compared with the carrot. The individual representatives of carotenoids present substantially depended on the sort of the fruit (Fig. 6). Within the sorts analyzed, the highest content of β-carotene was found in the Golden Delicious species (0.62 mg per 100 g, SD = 0.098 mg per 100 g).

Three sorts of potatoes originated from different regions of the Czech Republic were analyzed. Lutein was the major carotenoid present in all of sorts of potatoes tested in a raw state (Fig. 7a). After thermal processing (boiling in distilled water for 25 min) further carotenoids, *i.e.* α- and β-carotenes and more lutein were detected in a potato extract (Fig. 7b). Obviously, these representatives were released from the bound forms commonly present in the foods. Similar increase (1.5—2 times) in the carotenoid content after boiling was observed also in other inland vegetables:
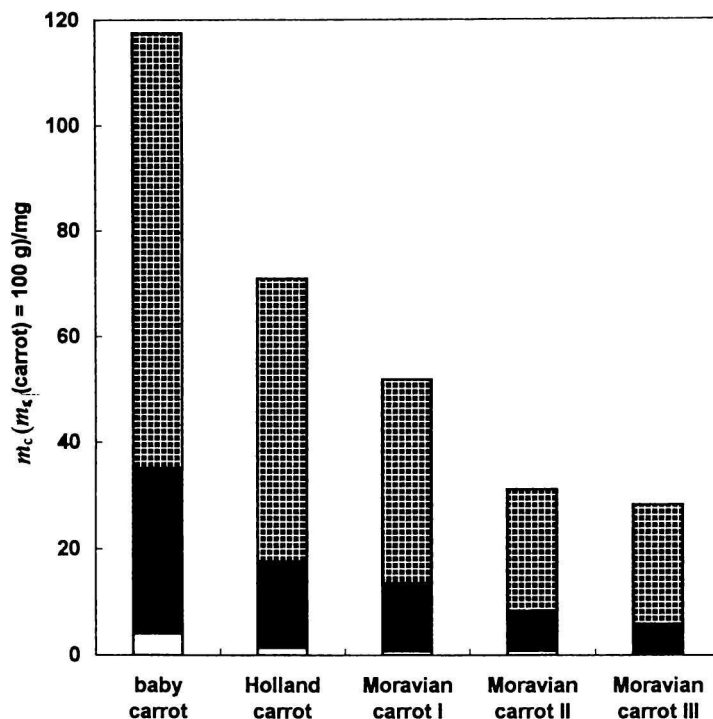

Fig. 5. Content and proportion of physiological carotenoids in several sorts of carrot. ▦ β-Carotene, ■ α-carotene, ☐ lutein.
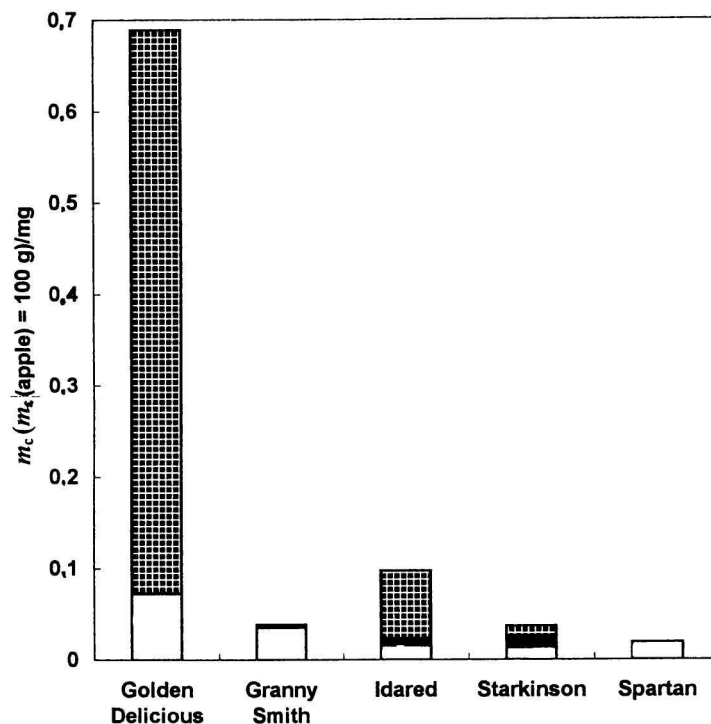
Fig. 6. Analysis of carotenoid content in several sorts of apples. ▦ α-, β-Carotenes, ■ lycopene, ☐ lutein.

green beans, peas, and leek. Cultivation conditions may slightly influence the total content of carotenoids, but not the ratio of the individual representatives.

In the last part of the work presented a route-identification analysis of several additional food sources was performed with regard to evaluate the proportion of individual physiological carotenoids both in widely consumed vegetables (Fig. 8) and in fruits (Fig. 9). Within the food analyzed, the main contributors of lutein were potatoes, green beans, spinach, pepper, kiwi, and pineapple. Except tomatoes, pink grapefruits, red currant, and pepper can serve as a dietary lycopene sources. The major contributor of α-carotene was the above-mentioned carrot. The additional contributors included pepper, grapefruits, mandarins, and oranges. Carrots, mustard, pepper, grapefruits, kiwi, pineapple, oranges, and many leafy vegetables (peas, green beans, spinach, leek) were the main sources of β-carotene.

DISCUSSION

From all members of the carotenoid family, 50 to 60 are in our foods [16]. Many fruits and vegetables contain at least one member, if not more, of the carotenoids. They literally are food colouring that gives many foods their yellow, orange, and red colour. They also are found in green, leafy vegetables, but the colour is masked by the green of chlorophyll. Foods, which are high in carotenoid content, are of interest because of the demonstrated association between con-

sumption of fruits and vegetables and reduced risk of several serious diseases in humans. Limited analytical data on the carotenoid content of some traditional food sources are available in food bases and data bases; however, they are often reported only in terms of vitamin A activity. In this study several frequently used food sources were extracted and some physiological carotenoids were individually identified and quantified by the reversed-phase HPLC according to simple methodology developed in our laboratory. The carotenoids that were detected included lutein, cryptoxanthin, lycopene, α-carotene and β-carotene. The same carotenoids were analyzed in the study group of 187 individuals to slightly indicate the major dietary contributors to the carotenoid intake in Czech population.

HPLC is the best analytical method available for the analysis of carotenoids not only in the serum, but also in the foods. Several reversed-phase HPLC procedures have been described for the determination of carotenoids [17], simultaneously with tocopherol and retinol or carotenoids only. Quantitation is usually done by calibrating with stock solutions in solvents [18]. However, some of the methods published are time-consuming or use an important flow-rate, others use multiple solvent systems, which require an additional delay for column reequilibration. In the present work a simple rapid modification of methodology for evaluation of carotenoids in biological samples was proved. The wavelength used for monitoring of carotenoids (450 nm) corresponds to absorption max-
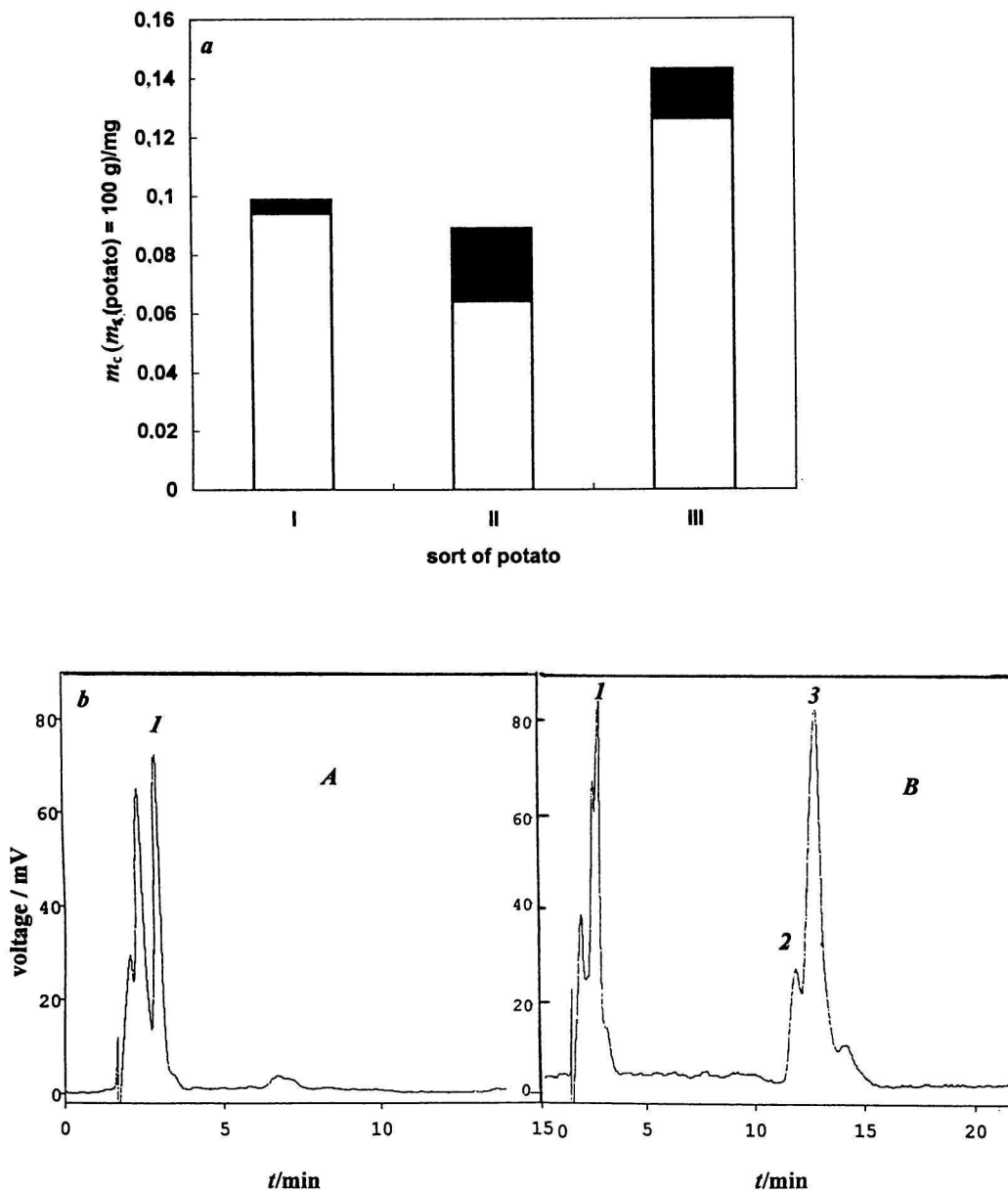
Fig. 7. *a*) The content of physiological carotenoids in several sorts of potatoes. ■ α-, β-Carotenes, □ lutein. *b*) Chromatogram of the extract of raw (*A*) and boiled (*B*) potato. Peak description: *1.* lutein, *2.* α-carotene, *3.* β-carotene.

imum of major serum carotenoids, *i.e.* lutein and β-carotene. Similar conditions and extract procedures were used both for the serum and food analysis, so that the results may be compared. Unfortunately, with regard to complex composition of samples analyzed, we have any acceptable internal standard to indicate the extent of losses as the result of extraction and chromatography. In our recent work, we have verified mean 100 % retinol recovery from the human serum using the same extraction procedure, *i.e.* twice petroleum ether extraction [19]. However, in previous study a differential behaviour of carotenoids to extraction has been described [20]. This also may explain why inclusion of an internal standard, which did not behave

identically with respect to all the carotenoids, failed to correct for differences in the efficiency of several extraction procedures. It is possible that some losses of total carotenoids occurred during food samples preparation, but the aim of this part of present study was above all demonstration of the real proportion of physiologically significant carotenoids in chosen foods.

Within our group of volunteers, lutein was the most abundant carotenoid in the serum (Table 1). Recent evidence suggests that plasma lutein is better correlated than either β-carotene or lycopene with its respective carotenoid intake [21]. In Czech population, mainly in infants or in deficient patients, of the usual plasma carotenoids, only lutein was measur-
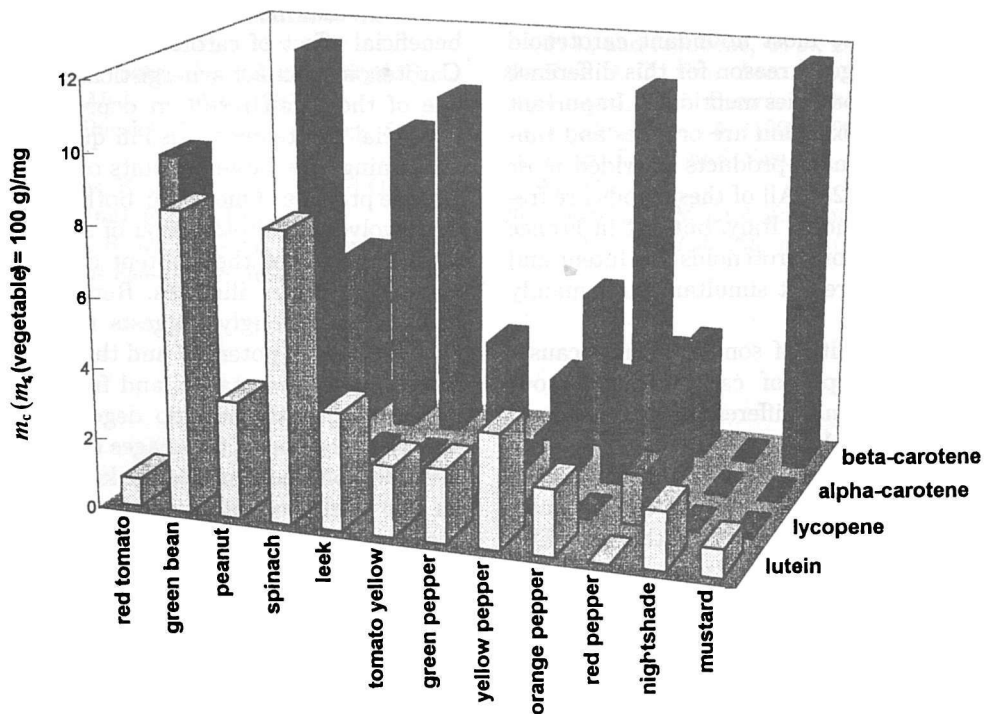
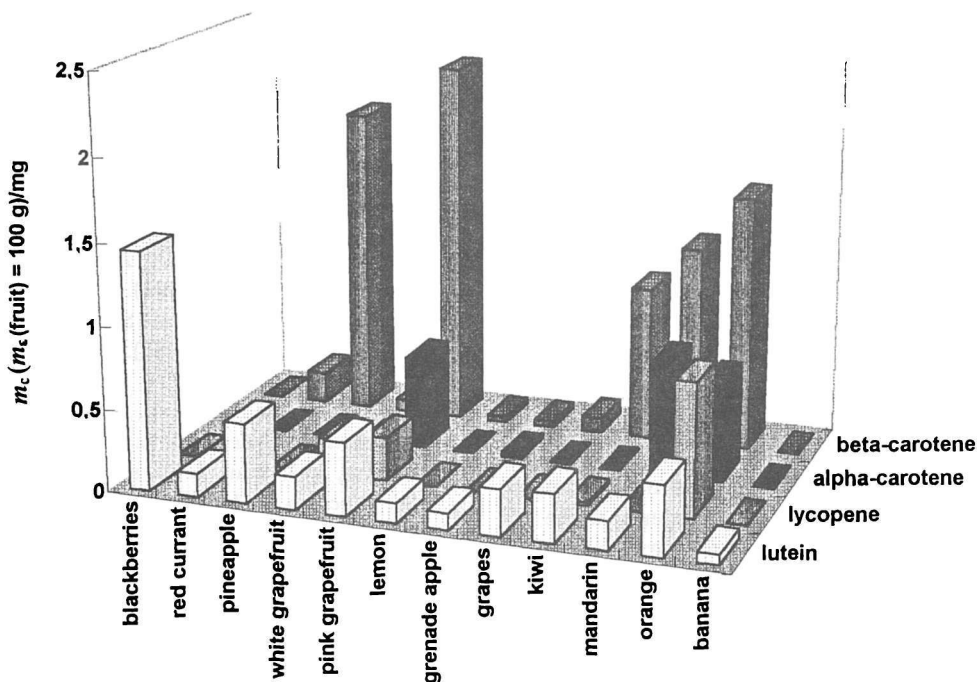**Fig. 8.** Content of physiological carotenoids in several vegetables.

Fig. 9. Content of physiological carotenoids in several fruits.

able in all samples and was correlated with retinol. According to our recent results, the close correlation between plasma lutein and retinol [19] suggests that the observed increase in retinol over the summer—autumn season may be attributable to an increased availability of yellow and green vegetables and fruits.

Therefore, plasma lutein can serve as a better marker of vegetable intake and vitamin A status than either β-carotene or lycopene (19). The similar results in subjects from some European countries were observed, mainly in UK, Ireland, and France [22]. On the other hand, Spain and Italy show the lowest lev-

els of β-carotene, along with the highest levels of β-cryptoxanthin, while the most abundant carotenoid was lycopene [18]. The good reason for this difference is food composition in countries mentioned. Important contributors of β-cryptoxanthin are oranges and tangerines, tomato and tomato products provided most of the dietary lycopene [23]. All of these foods are frequently used in Spain and in Italy, but not in France and UK, where the major carotenoids are lutein and β-carotene, which are present simultaneously mainly in green vegetables.

The marked seasonality of some products causes wide differences in supply of carotenoids. In our study, considerable seasonal differences of total serum carotenoids were observed (Fig. 3). Based on the seasonality, the frequency of use and the nutrient content of selected locally produced foods can be valuable and important nutrient factor in the diet. Unfortunately, apples, one of widely used all the year round fruits in our country, are relatively poor source of carotenoids. It seems, that the major inland contributors to carotenoid content for the future remain potatoes and green and leafy vegetables, both in raw and boiled form, which contain predominantly lutein (Figs. 7 and 8). With regard to unfavourable ratio of β-carotene/lutein and very low mean β-carotene level evaluated in our study group (Table 1) it will be necessary to enrich conventional diet predominantly for the natural sources rich in β- and α-carotenes, respectively. Within inland foods, carrots were the major contributor both of α- and β-carotenes (Fig. 5). As additional accessible sources of β-carotene can serve also the above-mentioned green vegetables. In the part of calendar year poor in inland raw fruits (peach, apricot) and vegetables (pepper, tomato) the complementary carotenes sources will be needed (*i.e.* exotic fruit, imported vegetable – see Figs. 8 and 9).

Within the synthetic supplies, for years synthetic β-carotene was the only choice of supplementation. Till this time, the marketplace is not yet awash in different β-carotene (and other physiological carotenoids, respectively) supplements from varied natural sources. Synthetic products claim benefits from containing both *trans-* and *cis-*isomers of β-carotene, while ignoring the importance of non-β-carotene carotenoids. Many of the epidemiological studies on carotenoids show that men with high blood levels of a variety of carotenoids (not one or two), had a lower risk of heart disease even if their blood cholesterol levels were high [24]. The large, randomized clinical trials of only β-carotene in primary prevention show no effect and potential for harm associated with the use of β-carotene. There are inconclusive and insufficient epidemiological and clinical trial data with regard to the role of supplemented β-carotene in cardiovascular protection [25], but in other studies the beneficial effect of the dietary intake of fruits and vegetables rich in carotenoids is supported [26]. It appears,

however, that no single dietary factor explains the beneficial effect of carotenoids on the human health. Carotenoids can act synergistically, but large doses of one of them can result in depressed levels of other beneficial carotenoids. Also in question is the logic of consuming very large amounts of purified β-carotene.

The practice of medicine, both past and present, often involves the prescription of specific foods (almost always plants) or their potent derivatives, to treat a wide spectrum of illnesses. Review of the epidemiological data strongly suggests that plant foods also have preventive potential and that consumption of the raw and fresh vegetables and fruits is lower in those who subsequently develop degenerative diseases. At almost every one of the stages of the disease process, identified phytochemicals are known to be able to alter the likelihood of progression – occasionally in a way that enhances risk but usually in a favourable direction. Thus, carotenoids act as antioxidants, essentially disabling the carcinogenic potential of specific compounds, but they also may alter membrane structure and integrity, they can suppress DNA synthesis and enhance differentiation. The carotenoids found together in vegetables may have a synergistic effect on biological processes, alternatively, other unmeasured factors in these foods may also influence risk.

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