DETERMINATION OF β-CAROTENE CONCENTRATION IN ORANGE AND APPLE JUICE AND IN VITAMIN SUPPLEMENTED DRINKS

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abstract: Many natural antioxidants from fruit and vegetables are beneficial food components; they protect food from rancidity and have the potential of reducing oxidative damage in humans. In this study the values of β-carotene concentration in orange and apple juice and in vitamin supplemented drinks were obtained by HPLC analysis, using methods with saponification and without saponification of samples. The amount of β-carotene in orange juice samples ranged from 0.053 - 0.069 μg/ ml; in apple juice: 0.014-0.023 μg/ ml and in vitamin supplemented drinks: 0.13-0.19 μg/ ml. This method uses small volumes of solvent and mild saponification conditions, it is rapid and convenient to use.

Introduction

Many natural antioxidants from fruit and vegetables are beneficial food components, they protect food from rancidity and have the potential of reducing oxidative damage in humans [1]. Antioxidants have also been of interest to chemists and health professionals because they may help the body to protect itself against damage caused by reactive oxygen species [2-4]. β - Carotene is commonly known as a radical scavenger and a physical scavenger of singlet oxygen and is believed to play an important role in the inhibition of initial stages of lipid peroxidation. The β - carotene content of different foods is of nutritional importance and of additional interest since β - carotene is believed to have a protective role against cancer [1]. Several methods [4-8] have been used for determination of β - carotene in fruit and vegetables. In Tab. 1 are presented the characteristics of some methods for carotenoids determination in vegetables, fruit and juice. Analysis of carotenoid levels frequently

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employs HPLC because of its ability to distinguish between similar geometrical structures of carotenoids. Its rapidity and the small amount of sample required also makes it suitable for routine analysis of samples. Extraction of analytes from the sample matrix is an important step, prior to the HPLC analysis [9].

A literature review has shown that procedures for extraction of antioxidants from foods involved various types of solvents, solvent combinations and procedures. The relative efficiency of the existing methods has not yet been evaluated.

Tab. 1. **Characteristics of some methods for carotenoids determination in vegetables, fruits and juice**

	Ben-Amotz et al. (1998)	Taugbodhitham et al. (1998)	Pupin et all (1999)	Marx et al. (2000)	Furtado et al. (2004)
Sample	Vegetables and fruits (0.51 g)	Tomato juice (2 ml)	Orange juice (5 ml)	Carot juice (1-5 ml)	Vegetables and fruits (0.5 - 1 g)
Conditions	5 ml tetrahydrofuran (TFH) and methanol (1:1,v/v) internal standard	0.05g $Mg(CO_3)_2$ 35 ml ethanol;hexane (4:3, v/v)	50 ml ethyl acetate BHT (0.004%) transf. through anhydrous sodum sulphate (50g)	acetone and hexane (1:1, v/v) 50 ml NaCl (10%, w/v) 50 ml water BHT (0.1%)	750 µl ascorbic acid (20%, w/v) 250 µl internal standard (10 µl/ml *trans*-β-apo-8′-carotenal in ethanol)
	10 ml hexane 2 ml NaCl (10 %) 200 µl methilene chloride	12.5 ml ethanol 12.5 ml hexane 50 ml NaCl (10%) 50 ml water	50 ml methanol (0.004% BHT) 100 ml NaCl (1M) 75 ml ethyl acetate BHT (0.004%) 50 ml ethyl acetate BHT (0.004%)	sodium sulfate (2g)	1000 µl ethanol *Saponification:* 2000 µl KOH (60%) , 45 ^{0}C 15 min *Extraction:* 2000 µl hexane

Experimental part

Materials and methods

All used chemicals were of analytical or HPLC grade. Ultra-pure water generated by the Milli-Q system was used. All standards compounds were purchased from Sigma.

Working standards solution were prepared daily by diluting a stock solution of standards. The concentration of working solutions was calculated from its extinction coefficient.

HPLC separation was performed with a HPLC system (Shimadzu, Japan) equipped with a solvent Degasser (Degasys DG 1310, Tokyo, Japan), an autosampler (Spark Midas, Holland) and a Hypersil TM 5 µm ODS (4.6 ×150 mm Analytical column).

Two different detectors, one UV detection (785 UV/VIS detector) and one using fluorescence detection (PERKIN-ELMER Luminescence spectrometer LC 300) were used in the HPLC analysis. The data was collected and integrated with a GynkoSoft Chromatography DS Version 5.30.

The mobile phase was acetonitrile - tetrahydrofuran - methanol - ammoniumacetate (68,4 % (v:v) : THF 22.0 % (v:v) : 6.8 % (v:v) : 2.8 % (v:v) (1% (w:v)).

Detection wavelength was set at 450 nm with flow rate at 1.5 ml/min.

Sample preparation

First, in order to avoid possible degradation, the samples were extracted directly with solvent without saponification. Aliquots (2-5 g) of juice were extracted 5 min with acetone : hexane (4:6). After the extraction, the solvent was evaporated to dryness under a stream of nitrogen and the residue was reconstituted with 1 ml of eluent solution and was colled in a screw-cap vial for HPLC analysis.

Determination of β-carotene, including alkaline saponification, consisted in the treatment of sample extraction with 1000 µl aqueous potassium hydroxide solution (60%, w/v) for 15 min in a 45^0C water bath. Following saponification, each sample was extracted with 1000 µl hexane. The samples were vortexed for 3 min, centrifuged at 1500×g for 5 min, and the organic hexane layer was decanted into an evaporating tube. This procedure was repeated and the second hexane extract was combined with that from the first. The hexane was then evaporated to dryness with a stream of nitrogen gas. The remaining residue was reconstituted with 1 ml of eluent solution. Aliquots of 20 µl were used for HPLC analysis.

Results and discussions

The chromatograms and the content of β-carotene in standard solution, in orange and apple juice and in vitamin supplemented drinks are shown in Fig. 1 - 6.

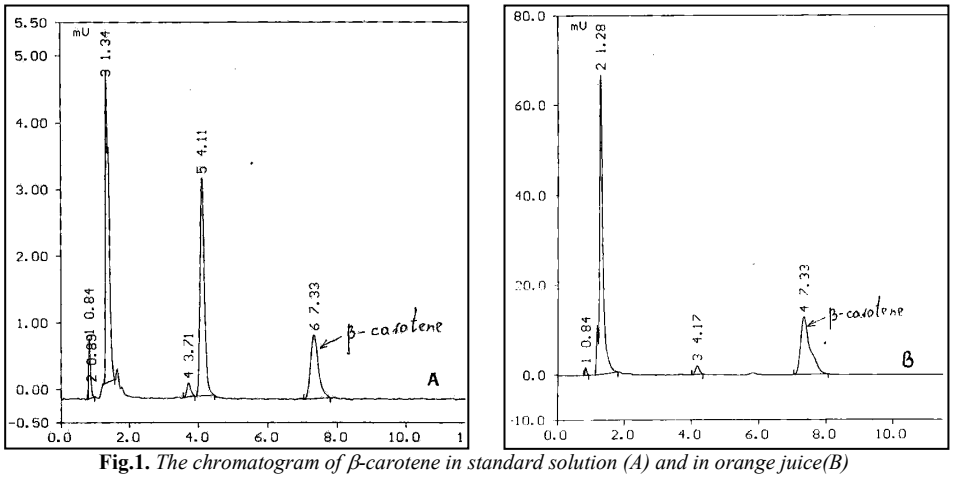

Fig.1. *The chromatogram of β-carotene in standard solution (A) and in orange juice(B)*

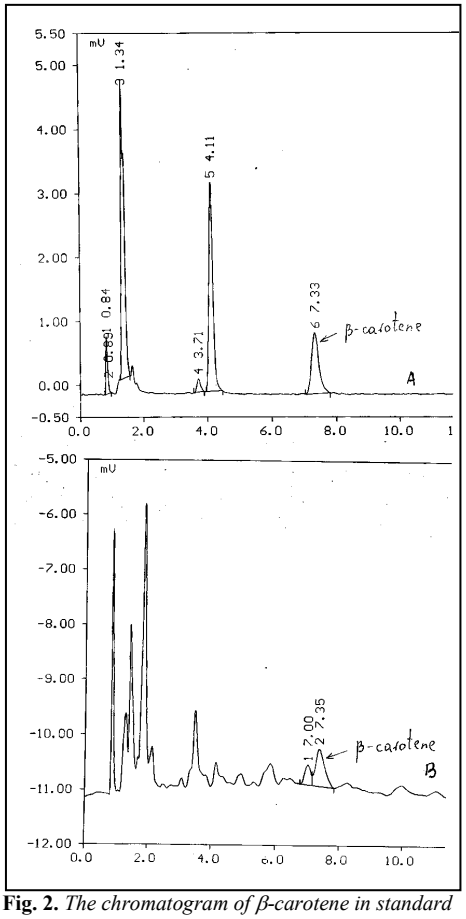

Fig. 2. *The chromatogram of β-carotene in standard solution (A) and in apple juice*

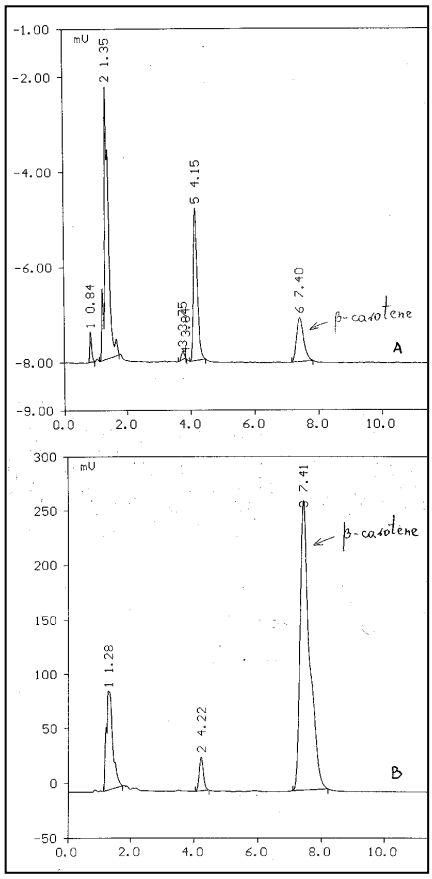

Fig. 3. *The chromatogram of β-carotene in standard solution (A) and in vitamin supplemented drink (B)*

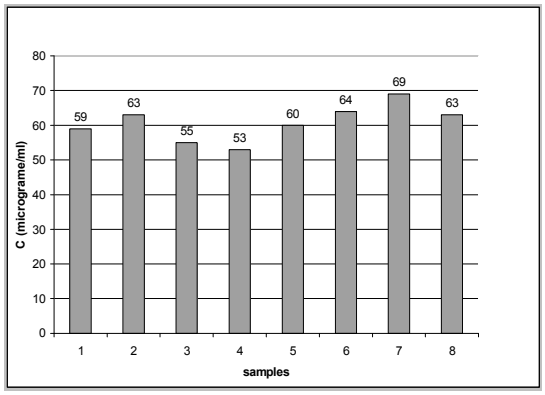

Fig. 4. *The β-carotene content of orange juice samples*

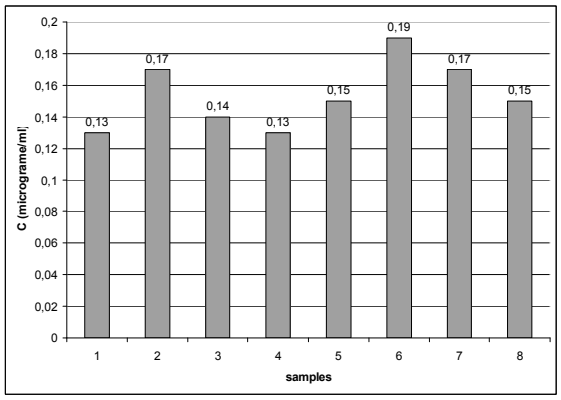

Fig. 5: *The β-carotene content of apple juice samples*

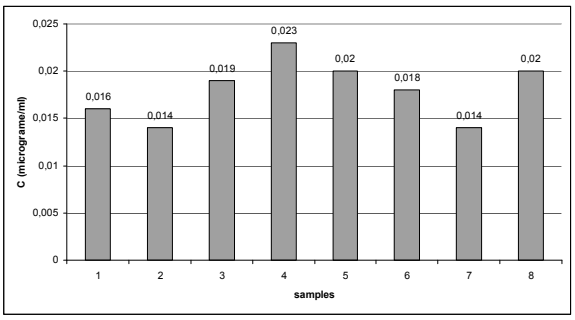

Fig. 6: *The β-carotene content of vitamin supplemented drinks samples*

Identification of carotenoids was based on retention times and comparison with a pure standard (β-carotene).

The amount of β-carotene in orange juice samples ranged from 0.053 - 0.069 μg/ ml; in apple juice: 0.014-0.023 μg/ ml and in vitamin supplemented drinks: 0.13-0.19 μg/ ml.

Carotenoids are compounds very sensitive to light, heat, air and other variables, consequently their determination, involving steps of extraction, saponification and chromatography, can be accompanied by degradations and loss. For this reason it is important to make a careful evaluation of the analytical procedure and the validation of the response so as to avoid causes of variation and inaccuracies.

Traditionally the extractions for antioxidants determination have been performed manually and the consumption of organic solvent in these extraction procedures is high. Legislation concerning reduction in use of organic solvents, especially chlorinated ones, and increasing labour costs make it necessary to develop fast, automated and environmentally friendly analysis methods.

Conclusion

In spite of the great number of publications dealing with carotenoid analysis in juice, fruit and vegetables, there is no generally accepted method for the isolation and determination of carotenoids. As a consequence, results obtained by various investigations and studies are difficult to compare.

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