ELSEVIER

Food Chemistry 79 (2002) 141–144

Food Chemistry

www.elsevier.com/locate/foodchem

Control of nutritional labels in beverages with added vitamins: screening of β-carotene and ascorbic acid contents

M. Rodríguez-Comesaña, M.S. García-Falcón, J. Simal-Gándara*

Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology, Ourense Campus, University of Vigo, E-32004 Ourense, Spain

Received 20 September 2001; received in revised form 19 December 2001; accepted 19 December 2001

Abstract

Simple, rapid and inexpensive methods were used for the determination of β-carotene and ascorbic acid, while checking recovery and precision with samples, and calibration data and detection levels with standards. These methods involved direct injection of the beverage into an HPLC with an UV-Visible detector. The results obtained with 13 beverages, with different β-carotene and ascorbic acid levels added, did not show good agreement between the vitamin levels on the labels and the levels determined. Found levels, in virtually all drinks, were much higher than those stated on the nutritional label. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Beverages with added vitamins; Antioxidants; β-carotene; Ascorbic acid; HPLC-UV

1. Introduction

The levels of the essential antioxidant vitamins, in contrast to other antioxidative defences, are determined mainly by their dietary supply. This is the reason for a rapidly growing market of vitamin-enriched foods, mainly beverages. Major vitamins for enriching beverages are the antioxidant vitamins A, C and E. Vitamin A is usually added as pro-vitamin A (β-carotene), vitamin C, as ascorbic acid, and vitamin E, as α-tocopheryl acetate. Antioxidant vitamins can counteract the oxidising effects of lipids by scavenging free radicals which have been found to be major promoters of certain diseases (Sies, 1991). Great interest has been focused on β-carotene and ascorbic acid, particularly because of their likely role in the prevention of coronary heart disease and cancers (Hercberg et al., 1998, 1999; Omenn, Goodman & Thornquist, 1996; Simon, 1992). Our aim was to evaluate the suitability of the nutritional label with regard to the antioxidant vitamins in beverages enriched with β-carotene and ascorbic acid, taking into consideration that these are natural chemicals in many of the fruit juices composing the beverages. The case of the addition of tocopheryl acetate was not studied because, generally, the chemical forms of vitamin E in most fruit juices are different tocopherols and tocotrienols but not the mentioned ester.

The principal problems associated with the determination of vitamins in foods are the low detection levels and the diversity of potential interferents present in food. Reversed-phase or normal-phase high performance liquid chromatography (HPLC) are the techniques of choice in samples such as those based on fruits and vegetables (Abushita, Hebshi, Daood, & Biacs, 1997; Bureau, Razungles, Baumes, & Bayonove, 1998; De la Cruz García, González Castro, Oruña Concha, López Hernández, Simal Lozano, & Simal Gándara, 1998, 1999; Kurilich et al., 1999; Lee & Coates, 1999; Osuna-García, Wall & Waddell, 1998; Pérez, Olías, Espada, Olías, & Sanz, 1997; Pinheiro Sant'Ana, Stringheta, Cardoso Brandao, & Cordeiro de Azeredo, 1998; Romero Rodríguez, Vázquez Odériz, López Hernández & Simal Gándara, 1992; Wimalasiry & Wills, 1983). In this study we use two isocratic quality control HPLC methods for the rapid analysis of β-carotene and ascorbic acid in vitaminised beverages. The methods have several advantages: high sample throughoutput, low solvent consumption and short sample preparation time. These methods have been successfully applied to the final separation and determination of vitamins in a variety of beverages. We have found that levels in virtually all drinks are much higher than those stated on the nutritional label.

* Corresponding author. Tel.: +34-988-387000; fax: +34-988-38-7001.

E-mail address: jsimal@uvigo.es (J. Simal-Gándara).

PII: S0308-8146(02)00121-8

2. Materials and methods

2.1. Chemicals and standard solutions

Trans-β-carotene and L-ascorbic acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade tetrahydrofuran (THF) and methanol, together with analytical grade sulphuric and glacial acetic acids, were supplied by Panreac (Barcelona, Spain). HPLC grade water was from a Milli-Ro water purification system (Millipore, Bedford, USA).

Independent vitamin Standard stock solutions containing β-carotene at 1000 mg/l in THF, and ascorbic acid at 10 000 mg/l in diluted sulphuric acid, at pH 2.2, were prepared and stored at 4 °C in amber glass bottles to prevent photolysis. Intermediate and standard solutions were prepared by appropriate dilutions of existing stock solutions.

2.2. Apparatus

All HPLC measurements were taken using a Thermo Separation Products P2000 binary pump, equipped with a Thermo Separation Products UV2000 ultraviolet-visible detector. The chromatographic data were collected and processed using the Chrom-Card software.

2.3. Determination of β-carotene and ascorbic acid in vitaminised beverages

The optimized instrumental parameters for the chromatographic analysis of β-carotene and ascorbic acid are detailed in Table 1. The quantification of the antioxidant vitamins by HPLC used the chromatographic peak area. Recovery estimation was not necessary because samples were directly analysed without any pre-analytical step, such as extraction, clean-up or concentration. The precision of the method was determined by applying the full procedure to three identical samples of three representative vitaminised beverages with a low, medium and high level of vitamins according to their respective calibration ranges. Detection limits were determined experimentally with standards as the smallest detectable concentrations corresponding to a signal-to-noise ratio of three, and quantification limits were considered about three times higher levels than the detection limits (ACS, 1980). The confirmation of analyte identity by HPLC is based on the scanning abilities of the ultraviolet detector.

2.4. Investigation of commercial beverages with added β-carotene and ascorbic acid

Thirteen commercial samples of refreshing beverages with added vitamins were analysed. The samples included 100% fruit juices, beverages of a low content in fruit juices, with or without milk, nectars, and energetic and isotonic drinks (Table 2). According to nutritional labelling regulations (EEC Council Directive 90/496, 1990) foodstuff components with vitamin A activity should be expressed as retinol. Once samples were in the laboratory, packages were opened, samples were poured into amber glass bottles, caps were screwed on, and closed bottles were kept under refrigerated conditions. After homogenisation, samples were analysed by direct injection into the HPLC system ($n=2$). It was necessary to dilute some of them to keep the vitamin level within the calibration range.

Table 1
Instrumental parameters and performance of the proposed methods for the determination of β-carotene and ascorbic acid in vitaminised beverages

	β-carotene	Ascorbic acid
Injection	20 μl	20 μl
Guard column	Pelliguard LC-18 40 μm (5 cm × 4.6 mm i.d.) (Supelco) Note: refill each 200 analysis	None
Analytical column	Spherisorb 12% ODS 5 μm (15 cm × 4 mm i.d.) (Sugelabor)	Kromasil NH_2 5 μm-100 Å (25 cm × 4.6 mm i.d.) (Teknokroma)
Columns temperature	50 °C	30 °C
Mobile Phase	Tetrahydrofuran/water (75/25)	Acetic acid in water (0.1 M)
Flow	0.8 ml min^{-1}	1.5 ml min^{-1}
Detection wavelength	455 nm	250 nm
Detector range	0.4	0.5
Detector rise yime	1 s	1 s
Retention time	3.8 min	9.3 min
Linear range	1–300 (mg/l)	4–600 (mg/l)
Regression equation	$y=757043\,x+131360$ ($n=7$)	$y=745913\,x+700662$ ($n=7$)
r^2	>0.9997	>0.9997
Precision	$1.6\pm1.1\%$ ($<2.5\%$)	$2.1\pm1.5\%$ ($<3.5\%$)
(RSD%)	(with samples 5, 11 & 12)	(with samples 1, 10 & 13)
LOD & LOQ	0.3 & 1.0 (mg/l)	1.2 & 4.0 (mg/l)

Table 2
β-carotene and ascorbic acid levels ($n=2$) obtained in the analysis of different vitaminised beverages

Sample	Description	β-carotene level (mg retinol/l)		Ascorbic acid level (mg/l)	
		In label	By HPLC	In label	By HPLC
1	Dairy drink, including juices such as orange, pineapple, passion fruit, carrot, mango, guava, apricot and papaya	1.2	16.5±0.3	96	89±3
2	Dairy drink, including juices such as orange and pineapple	1.2	4.5±0.1	90	193±7
3	Dairy drink, including juices such as orange, carrot, lemon, apricot, passion fruit, pineapple and acerola	1.2	6.6±0.1	90	292±6
4	Nectar, including juices such as orange, carrot and lemon	3.2	49.2±0.5	240	298±6
5	Beverage with a low content of fruit juices such as orange, carrot and lemon	2.4	40.8±0.4	180	343±7
6	Beverage of a low content in fruit juices such as orange, carrot and lemon	2.4	32.3±0.3	180	308±7
7	Nectar, including juices such as orange, carrot and lemon	1.2	40±0.7	90	294±6
8	100% pineapple juice	1.2	1.0±0.1	400	341±7
9	Nectar, including juices such as pear, apple, orange, lemon, passion fruit, pineapple, banana, peach, plum and mango	3.5	42.3±0.4	150	221±5
10	100% juice, including juices such as orange, apple, grape, pineapple, pear, passion fruit, lemon, guava, mango, peach, banana and papaya	4.0	51.5±0.5	300	348±7
11	Nectar, including juices such as apple, orange, pineapple, lemon, apricot, grape, passion fruit, banana, mango, guava, papaya and kiwi fruit	1.5	16.3±0.3	188	221±5
12	Isotonic and energetic drink	3.5	3.2±0.1	100	140±5
13	Beverage with milk of a low content in orange juice	1.2	13.5±0.2	300	590±6

3. Results and discussion

The development of chemical and instrumental methods for the separation, identification and quantitative analysis of food nutrients has become extremely important, and has allowed the food industry and academic and governmental institutions to assess the nutritional value of food and food products and their nutritional labelling. We have optimised HPLC conditions for the direct analysis of β-carotene and ascorbic acid in different vitaminised beverages. Thirteen refreshing drinks, with different levels of β-carotene and ascorbic acid added, were then selected to investigate the degree of correspondence between the label and the vitamin levels found (Table 2).

Results showed no good agreement between the vitamin levels on the labels and the levels determined; in virtually all drinks higher values were found, even with a factor of 30 for β-carotene. This can be explained because, in most of the beverages, there were β-carotene- and ascorbic acid-rich fruits, among the ingredients, that positively contributed to their added levels. This assessment was confirmed by the fact that the most simple samples, 8 and 12, show the best agreement between the vitamin levels determined and the levels on the label. In a previous work (Torres Sequeiro, García Falcón, & Simal Gándara, 2001), we also analysed riboflavin and pyridoxine in similar beverages, finding good correspondence between labels and contents, because the ingredients used did not naturally contain those vitamins. Given the biological variability in the vitamin content of the fruits and vegetables used in these products, and variable losses during processing, manufacturers would always under-claim the amount in to ensure that the product never provided less than the labelled amount. Therefore, it seems that their labels only reflect the added levels of those vitamins and do not include the natural levels of those vitamins in the juices.

4. Conclusions

The versatility of HPLC as an analytical tool makes it ideal for analytical quality control and for research and development laboratories in the food and beverage industry. The determination of the antioxidants, β-carotene and ascorbic acid, was performed in a short time

and using a low volume of solvent. The analysis proved not to affect stability, as indicated by the method precision. Detection and quantification limits were shown to be satisfactory in the analysis of very different vitaminised beverages available in the market.

The results obtained confirm the need for serious control of the nutritional labelling of such foodstuffs. The labels should reflect the real contents of vitamins in the foodstuff since this is important for specific groups of people (e.g. pregnant women and sporting professionals).

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