Food Research International 48 (2012) 265–270

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Sterol stability in functional fruit beverages enriched with different plant sterol sources

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ARTICLE INFO

Article history:
Received 5 March 2012
Accepted 2 May 2012

Keywords:
Plant sterols
Phytosterol oxidation products
Gas chromatography
Phytosterol stability
Sterol oxidation
Functional foods

ABSTRACT

Two different plant sterol (PS) sources (free PS from tall oil and esterified PS from vegetable oils) were used for manufacturing two types of functional beverages (fruit and milk-based fruit beverages), and their PS and phytosterol oxidation product (POP) contents were determined. Gas chromatography–tandem mass spectrometry (GC–MS/MS) was used for identification and gas chromatography–flame ionization detection (GC–FID) for quantitation purposes. Brassicasterol, campesterol, campestanol, stigmasterol, β-sitosterol and sitostanol were the quantified PS, conforming a profile in order with current legislation. The relative percentages of PS differed according to the enrichment source involved, though the enrichment levels (g/100 g beverage) were of the same order (1.77 from tall oil and 1.84 from vegetable oils). Only POPs from β-sitosterol (the prevalent PS in the analyzed beverages) were detected — the predominant representative being 7β-hydroxysitosterol (39–58.5% of total POP content). The following POPs were quantified: 7α-hydroxy, β-epoxy, α-epoxy, and 7-ketositosterol, yielding a total POP content ranging between 42.9 and 57.4 mg/100 g of PS. No statistically significant differences ($p > 0.05$) in total and individual POP content according to the source of PS were found. The mean β-sitosterol oxidation percentage was <0.07%, which reflected a low PS oxidation extent, though manufacture was on a laboratory scale regardless of the PS source used in enrichment of the functional beverages. These functional drinks therefore can be regarded as healthy food products and as an adequate PS vehicle as well.

1. Introduction

Consumption of foods enriched with plant sterols (PS) (including phytosterols and phytostanols) may help reduce low-density lipoprotein (LDL)-cholesterol levels. PS consumption (2 g/day) results in a cholesterol reduction of approximately 9%, exerts beneficial effects upon other lipid variables and, in addition, PS have been described as anti-inflammatory and anti-cancer compounds (García-Llatas & Rodríguez-Estrada, 2011; Hernández-Mijares et al., 2010; Marangoni & Poli, 2010).

Plant sterols intake with the diet ranges from 150 to 440 mg/day, and can reach 1 g/day in vegans (García-Llatas & Rodríguez-Estrada, 2011). Since the diet alone is unable to offer the effective intake required to deliver the health benefits of PS, a large variety of commercial foods have been enriched with free or esterified PS, including spreads, which were the first commercial applications of PS-enriched foods. Since the approval of spreads in the European Union (EU), several more approvals have been issued for the addition of phytosterol to other food categories such as milk-based fruit beverages (European Food Safety Authority (EFSA), 2008). This kind of enrichment is a convenient and alternative way for consumers to obtain the daily recommended amount of PS for subjects with moderate hypercholesterolemia. In effect, these products can be regarded as healthy staple foods, since skimmed milk can be used for their formulation — thereby replacing milk fat with unsaturated fatty acids used in PS esterification. Furthermore, fruit juices can provide vitamins and antioxidants as well (Normen & Frohlich, 2004).

The main sources of PS for current functional foods and dietary supplements are tall oil, a by-product of the wood pulp industry, and vegetable oil deodorizer distillate. Tall oil contains a mixture of

Abbreviations: BHT, butylhydroxytoluene; COPs, cholesterol oxidation products; EU, European Union; Fb, fruit beverages; GC–FID, gas chromatography–flame ionization detection; GC–MS/MS, gas chromatography–tandem mass spectrometry; HMDS, hexamethyldisilazane; IS, internal standard; KCl, potassium chloride; KOH, potassium hydroxide; LDL, low-density lipoprotein; MFb, milk-based fruit beverages; POPs, phytosterol oxidation products; PS, plant sterols; Rf, response factor; SPE, solid-phase extraction; TMCS, trimethylchlorosilane; TMSE, trimethylsilyl ether.

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doi:10.1016/j.foodres.2012.05.005

free and esterified PS in a proportion of less than 15%, whereas free sterols are a major component (15–30%) of the deodorizer distillate fraction obtained from refining plant or vegetable oils (Fernandes & Cabral, 2007; Moreau, 2004).

Like all unsaturated lipids, phytosterols are liable to oxidation, giving rise to a family of compounds termed oxyphytosterols or phytosterol oxidation products (POPs), which are structurally similar to cholesterol oxidation products (COPs) (García-Llatas & Rodríguez-Estrada, 2011). While the negative biological effects of COPs have been extensively studied, and these compounds are now known to be implicated in the initiation and progression of major chronic diseases (atherosclerosis, neurodegenerative processes, diabetes, among others), the biological effects of POPs are still unclear and contradictory (García-Llatas & Rodríguez-Estrada, 2011; Hovenkamp et al., 2008; Otaegui-Arrazola, Menéndez-Carreño, Ansorena, & Astiasarán, 2010; Ryan, McCarthy, Maguire, & O'Brien, 2009). As has been recently reported, the presence of POPs in enriched foods may come from the PS source used for enrichment (González-Larena et al., 2011). Accordingly, several factors may contribute to their formation: the high temperatures involved in obtaining PS (Moreau, 2004), the different food characteristics (Ryan et al., 2009), and the processing conditions employed after the addition of these functional ingredients to different food matrices (Menéndez-Carreño, Ansorena, & Astiasarán, 2008; Soupas, Huikko, & Lampi, 2006).

Plant sterol content has been determined in several milk beverages enriched with free or esterified PS (Laakso, 2005; Menéndez-Carreño et al., 2008; Saraiva, Castilho, Martins, Noronha da Silveira, & Ramos, 2011; Soupas et al., 2006), and in orange juices fortified with sterol esters (Mezine, Zhang, Macku, & Lijana, 2003) and with a sterol concentrate (Clement, Hansen, Costin, & Perri, 2010). However, few studies to date have identified and quantified POPs in other foods, and the existing publications mostly focus on high lipid content matrixes (Bortolomeazzi, Cordano, Pizzale, & Conte, 2003; Dutta, 2002; Soupas, Huikko, Lampi, & Piironen, 2007). In the case of dairy matrixes, 7-ketositosterol contents have been determined as an indicator of phytosterol oxidation in milk-based infant foods (Zunin, Calcagno, & Evangelisti, 1998), and several POPs have been quantified in milk- and cereal-based infant foods (García-Llatas et al., 2008). Plant sterol stability in enriched dairy products was determined for the first time by Soupas et al. (2006), who reported POP contents in skimmed milk enriched with free or esterified PS. More recently, phytosterol oxides have been analyzed in commercial low-fat milk enriched with esterified phytosterols (Menéndez-Carreño et al., 2008).

To the best of our knowledge, no studies have been made to identify and quantify POPs in milk-based fruit beverages and fruit beverages, and since the biological effects of POPs are still unclear, the present study was designed to identify and quantify PS and POPs in such functional beverages enriched with free or esterified PS of different origins, with a view to confirming that these beverages are adequate PS vehicles.

2. Materials and methods

2.1. Reagents

Standards: 5β-cholestan-3α-ol (epicoprostanol) (purity: ≥95%) used as internal standard (IS) in PS determination, (24S)-ethylcholest-5,22-dien-3-ol (stigmasterol) (purity: 95%), (24R)-ethylcholest-5-en-3β-ol (β-sitosterol) (purity: 95%), 24α-ethyl-5α-cholestan-3β-ol (stigmastanol) (purity: 97.4%), cholest-5-ene-3β,7β-diol (7β-hydroxycholesterol) (purity: 95%), 5β,6β-epoxycholestan-3β-ol (β-epoxycholesterol) (purity: 98%) and cholest-5-en-3β-ol-7-one (7-ketocholesterol) (purity: 90%), were from Sigma Chemical Co. (St. Louis. MO, USA). Cholest-5-ene-3β,19-diol (19-hydroxycholesterol) (purity: 95%) used as IS in POP determination, cholest-5-ene-3β,7α-diol (7α-hydroxycholesterol) (purity: 98.6%), (24S)-ethylcholest-5,22-dien-3β-ol-7-one (7-ketostigmasterol) (purity: 98.6%) and (24R)-methylcholest-5-en-3-ol (campesterol) (purity: 98.6%) were purchased from Steraloids (Newport, RI, USA). (24R)-ethylcholest-5-en-3β-ol (β-sitosterol, for POP obtention by thermo-oxidation) (purity: 78.7% β-sitosterol, with campesterol and sitostanol traces) was obtained from Fluka (Buchs, Switzerland).

Chloroform, diethyl ether, methanol, anhydrous sodium sulfate, acetone, 2-propanol, and anhydrous pyridine were purchased from Merck & Co., Inc. (Whitehouse Station, NJ, USA). Potassium hydroxide (KOH) was from Poch, S.A. (Sowinskiego, Poland), potassium chloride (KCl) from Panreac (Barcelona, Spain), butylhydroxytoluene (BHT) from Sigma Chemical Co. (St. Louis. MO, USA), and hexane from J.T. Baker (Deventer, The Netherlands). Silylating reagents: hexamethyldisilazane (HMDS) was from Fluka (Buchs, Switzerland), and trimethylchlorosilane (TMCS) from Carlo Erba (Rodano, Italy). All reagents were of analytical grade. Ultrapure water was obtained by means of a Millipore Q water purification system (Milford, MA, USA).

2.2. Samples

Two fruit beverages (Fb) and two milk-based fruit beverages (MFb) were enriched with similar PS concentrations (1.7 g PS/100 g of beverage), using two different ingredients as source of PS (A: free PS from tall oil, and B: esterified PS from soybean, rapeseed, sunflower and corn oils). Since PS are insoluble in water, both ingredients were presented in microencapsulated powder form suitable for use in low fat beverages. Due to the different matrix characteristics, the products were made by two distinct processes in the Pilot Plant of the Hero Global Technology Center located in Alcantarilla (Murcia, Spain), at laboratory scale. Briefly, Fb were made by mixing the microencapsulated PS with the fruit juices using high-speed mixing equipment at 27,000 rpm (Polytron PT2000, Kinematica AC, Switzerland), two-step homogenization (GEA Niro Soavi S.p.A, Italy) at 150 bars (100 + 50), pasteurization at 90 °C during 30 s and hot filling (at 80 °C) in 100 mL plastic bottles. These samples contained concentrated tangerine juice, water, banana puree, grape concentrate and banana flavor. MFb were manufactured in a way similar to Fb, except that the milk was previously acidified with part of the fruit juices and stabilized with a high-methoxyl pectin in order to prevent protein flocculation. MFb contained reconstituted skimmed milk, concentrated tangerine juice, stabilizer (pectin) and aromas. The total PS concentrations were 1.773 g PS/100 g beverage and 1.842 g PS/100 g beverage for samples enriched with sources A and B, respectively. The nutritional information and physicochemical characteristics of the samples are detailed in Table 1.

2.3. Determination of plant sterols

Lipids were extracted according to the procedure of Boselli, Velazco, Caboni, and Lercker (2001). A weight of sample providing

Table 1
Fruit beverages: nutritional information and physicochemical characteristics per 100 mL.

	FbA	MFbA	FbB	MFbB
Energetic value (kJ/kcal)	340/80	304/72	354/83	311/73
Proteins (g)	0.3	2.7	0.3	2.7
Carbohydrates (g)	19.5	14.3	20.3	14.7
Lipids (g)	0.1	0.4	0.1	0.4
°Brix (20 °C)	21.0	18.2	21.9	18.7
Ascorbic acid (mg/100 mL)	30.0	30.0	30.0	30.0
Titratable acidity (g of citric acid)	1.3	1.2	1.3	1.2
PS source	Free PS from tall oil		Esterified PS from soybean, rapeseed, sunflower and corn oils	

FbA and FbB: fruit beverage enriched with plant sterol source A (tall oil) or B (vegetable oils), respectively; MFbA and MFbB: milk-based fruit beverage enriched with plant sterol source A or B, respectively; PS: plant sterols.