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Plant sterol oxides in functional beverages: Influence of matrix and storage

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ABSTRACT

Three plant sterol (PS)-enriched beverages, milk based fruit juice (MFJPS), fruit juice (FJPS) and milk beverage (MPS), were stored at 4, 24, or 37 °C and analysed at regular time intervals of 2 months until 6 months. PS stability was analysed from the production of phytosterol oxidation products (POPs). The β-sitosterol oxides (7α/7β-hydroxy, β/α-epoxy, triol, and 7-keto) and campesterol oxides (β/α-epoxy, and 7-keto) were detected in all beverages and at all storage times and temperatures. Total POP contents followed the order MPS ≫ FJPS > MFJPS. In general, the beverages showed low PS oxidation levels (<0.17%). Predictive models of POP content versus storage time were established. These models explain total POP content by over 75% and individual POP content by over 50%. We propose 7-ketositosterol and 7-ketocampesterol as PS oxidation markers during storage of beverages of this kind.

1. Introduction

Plant sterols (PS) are currently used as functional food ingredients due to their capacity to reduce low-density lipoprotein (LDL)-cholesterol levels. PS consumption (2 g/day) results in a cholesterol reduction of approximately 10%, and the European Atherosclerosis Society Consensus Panel has concluded that functional foods with PS may be considered for patients with high cholesterol levels at intermediate and low global cardiovascular risk who do not qualify for drug treatment (Gylling et al., 2014). In addition, PS have been described as anti-inflammatory and anticancer compounds (García-Llatas & Rodríguez-Estrada, 2011; Marangoni & Poli, 2010). 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 PS-enriched spreads in the European Union, several more approvals have been issued for the addition of PS to other food categories, such as milk-based fruit beverages (Commission Decision 2004/336/EC of 31 March 2004/336/EC).

During processing and storage it is essential to maintain the quality and safety of PS-enriched foods, since PS are susceptible to oxidation like all unsaturated lipids, and a decrease in initial PS content could occur as a result (Kamal-Eldin & Lampi, 2008). One way of improving the oxidative stability of PS used for enrichment would be to include them in a matrix with natural antioxidant compounds such as milk based fruit beverages.

On the other hand, the compounds resulting from PS oxidation (phytosterol oxidation products (POPs)) could exert toxic effects qualitatively similar to those of cholesterol oxidation products (COPs), which have been extensively studied and are now known to be implicated in the initiation and progression of major chronic diseases (atherosclerosis, neurodegenerative processes, diabetes, etc.) (Alemany, Barberá, Alegría, & Laparra, 2014; García-Llatas & Rodríguez-Estrada, 2011; Vanmierlo, Husche, Schött, Pettersson, & Lütjohann, 2013).

Regarding POP contents in PS-enriched dairy matrixes, only two studies have been made involving PS-enriched whole milk powder stored for 12 months and PS-enriched non-fat milk stored for 6 months (Soupas, Huikko, & Lampi, 2006), and a phytosterol enriched milk subjected to Schaal oven conditions (equivalent to 1 month of storage at room temperature) (Menéndez-Carreño, Ansorena, & Astiasarán, 2008). Another storage study has been made of PS-enriched margarine stored for 18 weeks at 4 °C and 20 °C (Rudzinska, Przybylski, & Wasowicz, 2014).

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Only three studies published by our group have evaluated PS stability and POP contents in functional beverages. Quantification has been made of POPs from two fruit beverages and two milk-based fruit beverages enriched with two different plant sterol sources (free PS from tall oil and esterified PS from vegetable oils), elaborated on a laboratory scale. Only POPs from β-sitosterol were detected. No differences in β-sitosterol oxidation percentage were recorded when free or esterified PS were used as the enrichment source (Alemany-Costa et al., 2012).

In a later study involving beverages of this kind but produced on an industrial scale and enriched with free PS from tall oil, again only POPs from β-sitosterol were detected – though the total POPs content was 10-fold lower than when preparation was on a laboratory scale. This indicates that the beverages manufactured on an industrial scale have a lesser oxidation stage (Alemany et al., 2013).

In the PS-enriched functional beverages employed in this work, phytosterol stability and antioxidant parameters were assessed during 6 months of storage at 4 °C, 24 °C, and 37 °C (González-Larena, Cilla, García-Llatas, Barberá, & Lagarda, 2012). The results showed that the studied storage time and temperatures did not affect the PS contents in the samples. However, if part of the PS were oxidised to POPs, these could be present in very low concentrations (in the μg/100 g range), which would not imply a drop in the amount of PS.

Since storage can increase the POP contents, and in view of the fact that this phenomenon has not been previously evaluated in any PS-enriched functional beverage, the present study was carried out to determine the influence of the matrix, temperature and storage time upon the POP contents in these beverages, in order to confirm that they are adequate PS vehicles during their shelf life.

2. Materials and methods

2.1. Chemicals and reagents

Standards used were cholest-5-ene-3β, 19-diol (19-hydroxycholesterol) (purity: 95%) used as internal standard (IS) in POPs quantification, (24R)-methylcholest-5-en-3β-ol (campesterol) (purity: 98.6%), cholest-5-ene-3β,7α-diol (7α-hydroxycholesterol) (purity: 98.6%) and (24S)-ethylcholest-5,22-dien-3β-ol-7-one (7-ketostigmasterol) (purity: 98.6%), purchased from Steraloids (Newport, RI, USA). (24S)-Ethylcholest-5,22-dien-3β-ol (stigmasterol) (purity: 95%), cholestane-3β,5α,6β-triol (cholestanetriol) (purity: 98%), cholest-5-ene-3β,7β-diol (7β-hydroxycholesterol) (purity: 95%), 5α,6α-epoxycholestan-3β-ol (α-epoxycholesterol) (purity: 80%), 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). (24R)-Ethylcholest-5-en-3β-ol (β-sitosterol, for POPs obtaining 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 sulphate, acetone, 2-propanol, and anhydrous pyridine were purchased from Merck & Co., Inc. (Whitehouse Station, NJ, USA). KOH was from POCH S.A. (Gliwice, Poland), KCl from Panreac (Barcelona, Spain), hexane from J.T. Baker (Deventer, The Netherlands), and butylated hydroxytoluene (BHT) was from Sigma Chemical Co. (St. Louis, MO, USA). Silylating reagents: hexamethyldisilazane (HMDS) 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

Three beverages enriched with free microcrystalline PS from tall oil (0.8 g PS/100 mL beverage or 0.68 g PS/100 g beverage) as a source of PS were manufactured in a pilot plant by Hero Spain, S.A. Since PS are insoluble in water, they were supplied in microencapsulated powder form suitable for use in low-fat beverages. Milk based fruit beverage (MFJPS) composed of skimmed milk, tangerine fruit juice from concentrate, banana puree, grape juice from concentrate, and PS; fruit beverage (FJPS) composed of tangerine fruit juice from concentrate, banana puree, grape juice from concentrate, PS and water to substitute skimmed milk; and milk beverage (MPS) composed of skimmed milk PS, and water to substitute fruit juices were used. The beverages were heat treated at 100–115 °C for 15–30 s to obtain a microbiologically stable foodstuff, and were packed under aseptic conditions in sterile plastic containers. The nutritional composition, per 100 g, of the MFJPS, FJPS and MPS was: 72, 30, and 48 kcal, 2.7, 0.5, and 1.6 g of proteins, 14.3, 6.1, and 2.5 g of carbohydrates, and 0.4, 0.2, and 0.2 g of fat (excluding PS), respectively. Samples were analysed just after manufacture (time 0) and were then stored at 4 °C, 24 °C or 37 °C, and analysed at regular time intervals of 2 months up until 6 months. The storage time of 6 months was chosen because the manufacturer of the PS-enriched beverages indicated that within this period the organoleptic properties of the samples are preserved. In addition, this is the common and usual turnover period for products of this kind at sales points, where storage is limited to no more than 6 months. Mean PS contents (g/100 g of beverage) of the samples were: β-sitosterol 0.486–0.556, sitostanol 0.077–0.088, campesterol 0.034–0.040, stigmasterol 0.010–0.011, and campestanol 0.008–0.009 (González-Larena et al., 2012).

2.3. Analysis of phytosterol oxidation products (POPs)

Lipids were extracted according to the procedure of González-Larena et al. (2012). Briefly, an amount of sample (15 g) providing approximately 120 mg of PS was taken. Lipids were extracted with 80 mL of a chloroform: methanol mixture (1:1, v/v) containing 0.05% of BHT (used as antioxidant to avoid POPs artifact formation during saponification) and homogenised with a Polytron homogenizer (PT 2000, Kinematica AC, Switzerland) for 3 min at 250 W. After adding 40 mL of chloroform and mixing again with the Polytron, the sample was filtered (Whatman No. 1, 90 mm, Maidstone, England) through a Buchner funnel. To the filtrate, 20 mL of 1 M KCl solution was added and refrigerated overnight. Then, after phase separation, the chloroform phase was concentrated in a rotary evaporator and dried with nitrogen. The lipid fraction was then reconstituted with hexane: 2-propanol (4:1, v/v), dividing the sample into four aliquots for saponification.

Next, the method described by González-Larena et al. (2011) was applied. Briefly, IS (10 μg of 19-hydroxycholesterol) was added to the extracted lipids. Cold saponification was performed at room temperature with 10 mL of methanolic 1 N KOH, in darkness and under continuous agitation in an orbital shaker (IKA KS26, Staufen, Germany) at 150 rpm for 18–20 h. The unsaponifiable material was extracted with diethyl ether. A fraction (1/2) of the unsaponifiable material was purified by solid-phase extraction (Si-SPE, 3 mL/500 mg, Supelco, Bellefonte, PA, USA), and the acetone fraction obtained was then subjected to derivatization by silylation with HMDS/TMCS in anhydrous pyridine (2:1:5). The trimethylsilyl ether (TMSE) derivatives obtained were dissolved in hexane, filtrated (syringe-driven Millex-FH filter unit, 1 mL, 0.45 μm Millipore, Milford, MA, USA), and evaporated with nitrogen. For chromatographic analyses, the TMSE derivatives were dissolved in 40 μL of hexane, and 1 μL was injected into a Trace GC-Ultra ITQ ion trap 900 gas chromatograph (GC)–mass spectrometer