Food Chemistry 133 (2012) 678–682

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Brominated vegetable oil in soft drinks – an underrated source of human organobromine intake

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ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form 18 November 2011
Accepted 19 January 2012
Available online 31 January 2012

Keywords:
Organobromine compounds
Brominated vegetable oil
Soft drink
Food additives
Human exposure

ABSTRACT

In North America brominated vegetable oil (BVO) is frequently used as a clouding agent for soft drinks. BVO containing soft drinks have a market share of about 15% in the USA. In our study we analysed several soft drinks from North America for BVO and calculated exposures from our results and consumption data. Based on a mean content of 8 ppm in BVO-containing soft drinks, the average daily human intake of BVO exceeds the intake of other organobromine compounds, e.g., polybrominated diphenyl ethers (PBDEs), by >4000 times for adults and >1000 times for children. By comparing the patterns of the brominated fatty acids (BFA) in the soft drinks, we were able to distinguish three BVO products used by three soft drink manufacturers. The analysis also revealed that the predominant BFAs in BVO are the Br_2-18:0 (bromination product of oleic acid) and Br_4-18:0 (bromination product of linoleic acid).

1. Introduction

Organobromine compounds are widely used in industry. Compounds such as polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) are all widely used as brominated flame retardants (BFRs). The high volumes of BFRs used coupled with their persistence and lipophilicity led to their distribution in the environment and bioaccumulation in the food chain (de Wit, 2002). Their uptake (Toms, Hearn, Sjödin, & Mueller, 2011) is a cause for concern due to the potential toxic effects of individual compounds (Johnson-Restrepo & Kannan, 2009). These concerns have led a number of countries to either ban or regulate the use of BFRs (Guerra, Alaee, Eljarrat, & Barceló, 2011). However, some 70 organobromine compounds are currently in use or have been patented for use as BFRs. Brominated vegetable oil (BVO) belongs to this group as patents have been issued in Germany as BFRs (HAWO Ökologische Rohstoffe GmbH, 2002) and a patent application has been submitted in the USA (Kruyper et al., 2009).

BVO is of particular interest because it is regularly used as a food additive in soft drinks from North America. BVO serves as a solubility-transmitter and clouding agent e.g., for citrus oils and other lipophilic compounds (Bockisch, 1993; Chanamai & McClements, 2000). Since lipophilic ingredients such as citrus oils are insoluble in water and their density is generally lower than 1 g/cm^3 (normally 0.91–0.95 g/cm^3 (Myers, 1991), they would gradually separate from the water phase. By the addition of bromine to the double bonds of unsaturated fatty acids in vegetable oil (mainly soy oil) the specific gravity is significantly increased to about 1.33 g/cm^3 (American Chemical Service, 2011). By blending BVO with the lipophilic ingredients, the density can be adjusted to that of a soft drink (1.0 (diet)–1.06 (sugar containing) g/cm^3) (Gillies MT, 1973).

For this purpose, BVO is used in three of the top 10 selling soft drinks in the U.S. BVO has reportedly been used as a food additive since 1931 (PepsiCo, 2011) while it was classified as safe by the U.S. FDA in 1958 (Conacher, Chadha, & Sahasrabudhe, 1969). Subsequent toxicity studies by the Canadian Food and Drug Directorate (1969) led the FDA to remove BVO from the Generally Recognised as Safe (GRAS) list, and interim usage levels were established at 15 ppm (Turner, 1972). In European and most other countries, BVO has been prohibited for decades, while its use was just recently discontinued in Japan (Shibata, 2010). An evaluation of BVO by the WHO in 1970 could not draw any conclusions because of a lack of suitable toxicological data (FAO, 1970). For instance, chronic toxic effects have not been studied and long-term studies were not available so that formal acceptable daily intake (ADI) values could not be determined (FAO, 1970). However, it was found that bromine is accumulated in body lipids of rats, pigs and humans (Crampton, Elias, & Gangolli, 1971; Farber, Ritter, & Weinberger, 1976; Jones, Tinsley, Wilson, & Lowry, 1983; Munro, Hand, Middleton, Heggtveit, & Grice, 1972). Even shorter chain metabolites of brominated fatty acids were identified (Jones et al., 1983). High levels of lipid-bound bromine were reported

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doi:10.1016/j.foodchem.2012.01.058

especially in children up to the age of 15. In the 1970s, Crampton et al. (1971) determined a much higher lipid-bound bromine level in adults from the United Kingdom (BVO permitted at 80 ppm until September 1970) than in Germany (BVO use prohibited) and the Netherlands (BVO banned in 1950). Conacher, Hartman, and Chadha (1970) showed that BVO are degraded enzymatically in the same way as common vegetable oils. In rats, progressive cardiac lesions have been reported at high doses of BVO but not in control groups fed unbrominated lipids (Munro et al., 1972). Moreover, myocardial cellular degeneration and, to some extend, edema and necrosis was observed when BVO was fed (Jones et al., 1983).

Early cases of bromism were initially associated with consumption of common bromine containing drugs such as sleeping aids, anti-epilepsy drugs in the 1930s and 1940s (Horowitz, 1997). More recently, a person who daily consumed 2–4 l BVO-containing soft drink suffered from severe bromism, which is characterised by symptoms such as headache, fatigue, ataxia, and memory loss which progressed over 30 days (Horowitz, 1997). Another more recent case of toxic effects caused by BVO containing soft drinks was reported by Jih, Khanna, & Somach (2003). The patient was diagnosed with bromoderma showing symptoms similar to those reported in literature in association with intoxication by brominated swimming pool disinfectants (Fitzgerald, Wilkinson, Bhaggoe, Beck, & English, 1995), exposure to methyl bromide (Hezemans-Boer et al., 1988), and halogen acne after severe intoxication with PBBs (Chanda, Anderson, & Glamb, 1982). In this case the patient daily consumed 8 l of a BVO containing soft drink for several months. The patient's serum bromine level of 9.6 mg/l was about twice the normal level (<5 mg/l) (Müller et al., 1999; Versieck & Cornelis, 1980).

Quantification of BVO has been only scarcely carried out and most published data predates the 15 ppm limit established in North America in the 1970s (Chadha, Lawrence, & Conacher, 1986; Conacher et al., 1969; Turner, 1972). Although BVO is a permitted food additive, the exact composition is not known. U.S. regulatory agencies (CFR, 2011) do not clearly define the vegetable oil to be used for bromination. Accordingly, BVO-containing soft drinks list brominated vegetable oil or brominated soybean oil on the content list.

The goal of our study was to determine the composition of BVO used in soft drinks and to determine their quantities in typical products. To this end, we used liquid–liquid extraction, transesterification, and GC/MS for quantification. Synthesised standards were used as internal standards and for peak identification. Eventually, we wanted to estimate average and worst case scenario exposures for U.S. citizens with a focus on children because they suffer most from accumulation in body fat tissue as mentioned above.

2. Materials and methods

2.1. Chemicals

Standards of BFA methyl esters were synthesised in our laboratory (see Fig. 1). For the bromination, 50 mg of the fatty acid was dissolved in 10 ml of toluene and 10 ml of 0.1 M aqueous bromine solution (5.2 ml bromine in 1 l saturated sodium bromide solution) were added. After shaking for 30 min in the absence of light, excessive bromine was removed by addition of 1 M sodium thiosulfate solution. After common cleanup procedures, purities of >98% (according to GC/MS) were obtained. Namely, 9,10-dibromooctadecanoic acid (Br_2-18:0) was obtained by the bromination of oleic acid (18:1(9)), 10,11-dibromoheptadecanoic acid (Br_2-17:0) was obtained by the bromination of 10-heptadecenoic acid (17:1(10)) and the ethyl ester of Br_2-18:0 was obtained after ethylation of Br_2-18:0. Mixtures of BFA methyl esters derived from linoleic acid (18:2(9,12)) and α-linolenic acid (18:3(9,12,15)) were also synthesised for identification purposes, i.e., Br_4-18:0 Br_6-18:0 and Br_2-18:1 isomers. A qualitative standard of 9,10-dibromohexadecanoic acid (Br_2-16:0) was obtained after bromination of palmitoleic acid (16:1(9)). Chemicals used for extraction and cleanup, namely diethyl ether (⩾99%), ethanol (⩾99%) and sodium chloride (p.a., ⩾99.5%) were from Carl Roth (Germany); *n*-hexane (HPLC grade, ⩾95%) and methanol (HPLC grade, ⩾99.85%) were from Th. Geyer (Germany) while sulphuric acid (96–98%) was from BASF (Germany). Key chemicals for the synthesis of BFAs were 16:1(9), (90–95%) and sodium thiosulfate (p.a., ⩾99.5%) from Sigma–Aldrich, (Germany), 17:1(10) (⩾99%) from Larodan (Sweden), 18:1(9) (65–88%), 18:2(9,12) methyl ester (⩾96%), 18:3(9,12,15) methyl ester (⩾96%), sodium bromide (p.a., ⩾99%) and toluene (GC grade) from Merck (Germany) and bromine (p.a., ⩾99.5%) from Sigma–Aldrich, (Germany).

2.2. Instruments

BVO was analysed after conversion of the fatty acids into methyl or ethyl esters. A Hewlett–Packard 6890 gas chromatograph in combination with a 5973A mass spectrometer (Agilent, Waldbronn, Germany) operated in the electron ionisation mode was used for analysis. Injections of 1 µl were made in splitless mode with an Agilent 7683 automated liquid sampler. Injector, transfer line, ion source and quadrupole temperatures were set at 250 °C, 300, 230 and 150 °C, respectively. A VF-5MS column (25 m × 0.25 mm internal diameter, 0.25 µm film thickness, Varian, Darmstadt, Germany) was installed in the GC. Helium 5.0 (Sauerstoffwerke Friedrichshafen, Germany) with a constant flow of 1.2 ml/min was used as carrier gas. The fatty acid methyl esters were analysed in the

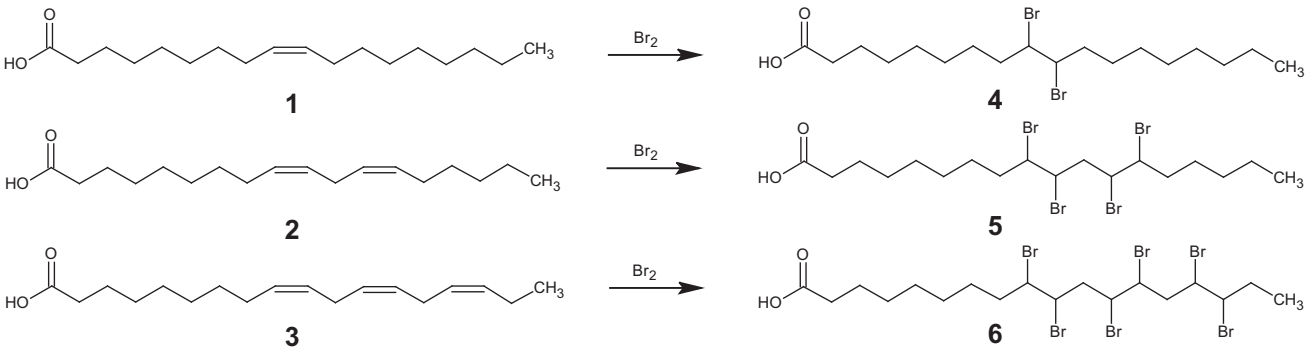

Fig. 1. Chemical structures of (1) oleic acid (18:1(9)); (2) linoleic acid (18:2(9,12) and (3) α-linolenic acid (18:3(9,12,15) and their bromination products (4) 9,10-dibromooctadecanoic acid (Br_2-18:0); (6) 9,10,12,13-tetrabromooctadecanoic acid (Br_4-18:0) and 9,10,12,13,15,16-hexabromooctadecanoic acid (Br_4-18:0).