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Original Research Article

Comparison of the nutritional composition and the concentrations of various contaminants in branded and private label yogurts

G. Cano-Sancho, G. Perelló, M. Nadal, J.L. Domingo *

Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain

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ABSTRACT

This study was aimed at comparing the nutritional and contaminant profiles among several of the most consumed trademarks of yogurts in Catalonia (Spain). The nutritional composition, the levels of aflatoxin M_1 (AFM_1), as well as the concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and various heavy metals were determined in six of the main trademarks of yogurts in Catalonia. To assess potential quantitative differences, a critical comparison between "private label" yogurts and branded yogurts was performed. AFM_1 was detected in six samples (33%), while the most detected heavy metals were As, Cd, and Pb. Slight differences were found between yogurt samples in some minerals and trace elements including Ba, Ca, Cu, Cr, Fe, K, and Mg. The WHO-TEQ values ranged between 0.006–0.008 and 0.003–0.012 ng/kg, for PCDD/Fs and PCB, respectively. To the best of our knowledge, this is the first study providing a transversal approach on the occurrence and co-occurrence of the major chemical contaminants in yogurt with a critical comparison between trademarks. The results do not show relevant differences on the nutritional composition, or on the levels of the assessed contaminants (chemicals and AFM_1) between "private label" and branded plain yogurts.

1. Introduction

Fermented milks and yogurts are typical constituents of the Mediterranean diet. Their beneficial effects and nutritional value have been thoroughly studied, and the results have been widely reported in the scientific literature (Astrup, 2014; Kimoto-Nira et al., 2014). The content of live lactic acid bacteria (LAB), *Lactobacillus bulgaricus* and *Streptococcus thermophilus* species, has been correlated with a wide range of positive health effects in clinical trials (Kimoto-Nira et al., 2014; Wang et al., 2013). Furthermore, yogurt is a rich source of dietary minerals including calcium (Ca), magnesium (Mg), potassium (K), phosphorous (P), and zinc (Zn), among others. Compared with milk, the concentrations of these minerals are higher in yogurt by nearly 50%. Yogurt is also a good source of riboflavin, niacin, vitamin B_6, and vitamin B_{12}, as well as an excellent source of essential amino acids of high biological quality, generally containing higher protein levels than milk (Germani et al., 2014). The proteolytic activity of LAB increases the digestibility of the proteins through a pre-digestion, which efficiently actives the aminoacids (El-Abbadi et al., 2014).

In spite of the beneficial effects of the frequent yogurt consumption, as for most other food items, the potential presence of undesirable chemical substances in foodstuffs is an issue of important concern, which has been increasing in recent years. A considerable number of studies have reported the presence of environmental pollutants (i.e., heavy metals, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), etc.) and contaminants of biological origin (i.e., aflatoxin M_1) (Arnich et al., 2009; Cano-Sancho et al., 2010; Martí-Cid et al., 2009; Martorell et al., 2011; Perelló et al., 2009, 2012, 2014).

Regarding environmental pollutants, a prolonged exposure to toxic elements such as arsenic (As), cadmium (Cd), mercury (Hg), or lead (Pb), which are frequently found in foodstuffs, can cause adverse effects to human health even at relatively low levels (Domingo, 1994; Sharma and Agrawal, 2005). In turn, due to their relevant potential bioaccumulation and toxicity, organochlorinated contaminants such as PCDD/Fs and PCBs are among the most

* Corresponding author. Tel.: +34 977759380.
E-mail address: joseluis.domingo@urv.cat (J.L. Domingo).

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known and investigated persistent organic pollutants (POPs). Both PCDD/Fs and PCBs were included at the 1998 UN-EC POP protocol (UNEP, 2008). Although human exposure to PCDD/Fs and PCBs occurs by various routes, food is the primary source (Llobet et al., 2008; Perelló et al., 2012). On the other hand, aflatoxin M_1 (AFM_1) is the main monohydroxylated derivative of aflatoxinB_1 (AFB_1), formed in the liver by means of cytochrome p450-associated enzymes. Aflatoxins are highly toxic, mutagenic, teratogenic, and carcinogenic (Cano-Sancho et al., 2010).

In 2013, yogurt was the most consumed dairy product in Spain, with 15.53 L/person the annual consumption of this product (MAGRAMA, 2013). Between 2009 and 2013, the consumption of dairy products in Spanish homes has increased by an 8.6% (MAGRAMA, 2013; MARM, 2009). Despite the growing variety of dairy products, including flavored recipes and formulations with added prebiotics and/or probiotic bacteria, the most consumed dairy product in Catalonia (Spain) is plain yogurt (MAGRAMA, 2013). In Spain, the market of dairy products in general, and that of yogurts in particular, is characterized by an oligopolistic situation, where the branded yogurts (BY) and the "private label" yogurts (PLY, also known as *white-label* products) hold equal market shares. As a result, the BY seek to differentiate themselves from PLY by means of high investments in nutritional and health research strategies that may raise the price of the final products (Baena and Rodríguez, 2013). As consumers face tighter economic constraints with respect to household food budget spending, determining the cost/benefit ratio of highly consumed food items becomes an issue of socioeconomic, as well as nutritional, interest.

The main objective of this study was to compare the nutritional and contaminant profiles between branded and private-label yogurts purchased in Catalonia. For this study, we determined the nutritional composition and the levels of AFM_1, PCDD/Fs, PCBs, heavy metals and minerals, in the six main yogurt brands sold in Catalonia.

2. Materials and methods

2.1. Sampling

In April 2014, plain yogurt samples from the six major producers (four PLY: "M1, M2, M3, and M4" and two BY: "M5 and M6") were purchased from supermarkets and large markets in Catalonia. The selected brands cover more 80% of the yogurt market in Catalonia, a rate that can be also applied to Spain, where these brands are also similarly distributed. For each brand/company, three composite samples were prepared by pooling 20 individual yogurts (125 g each) from three different production batches. Samples were stored until their subsequent analyses at −20 °C.

2.2. Analysis of nutrients

The ash determination was conducted by subjecting the samples at 525 °C in a muffle furnace (JP Selecta, Abrera, Barcelona, Spain) until a constant weight was obtained. Moisture content was determined by drying the samples at 105 °C in an air oven (JP Selecta, Abrera, Barcelona, Spain). Protein contents were estimated from the crude nitrogen content of the samples determined by the Dumas method (Jakob et al., 1995). Total fat contents were determined by the Soxhlet method (Manganiello et al., 2000), with the extract gravimetrically determined. Carbohydrates were calculated by difference with the sum of the ash, humidity, protein and fat content combined.

2.3. Analysis of aflatoxin M_1

AFM_1 was determined in each composite sample by competitive ELISA method RIDASCREENs Aflatoxin M_1 30/15 n°R1111 (Ridascreens, R-BiopharmAG, Darmstadt, Germany), according to the procedure described by R-Biopharm GmbH (Kanungo et al., 2014) with minor modifications. First 10 g of triturated and homogenized composite samples of yogurt were weighed and extracted with 40 mL of dichloromethane by shaking for 15 min on a magnetic stirrer and subsequently filtered. The determination was made photometrically at 450 nm on a microplate reader (LT-4000MS, Labtech, Uckfield, East Sussex, UK). The limit of detection was 25 ng/kg.

2.4. Analysis of PCDD/Fs and PCBs

The concentrations of the 17 most toxic PCDD/F congeners and 18 PCBs (including also 12 dioxin-like PCBs (DL-PCBs)) were determined according to the US EPA method 8290 for PCDD/Fs and the US EPA Method 1668 and JIS K 0311 for PCBs. Appropriate C_{13}-labeled extraction standards were added to the homogenized samples in order to control the sample preparation process. Samples were extracted using hexane/acetone (3:1 v/v, pesticide grade, Sigma–Aldrich, Steinheim, Germany) as solvent. The extracts were then concentrated to determine the concentrations of PCDD/Fs and PCBs. A multi-step sample clean-up was performed to remove the matrix, as well as the potential interfering components. The first stage was fat destruction by treatment of the sample solution with acid silica to breakdown the fat. The obtained extract was then subjected to a multilayer silica clean-up column in order to further remove the matrix. After the clean-up, the extract was eluted on a basic alumina column to separate the PCDD/Fs from the PCBs, and from interfering components, by applying different eluent solutions on the column. The PCDD/F and PCB fractions were separately collected and concentrated until near dryness. After adding 25 μL of the C13-labeled injection standards, the extracts were ready for the analysis. The final obtained PCDD/F and PCB extracts were injected and analyzed separately by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) on an Agilent 6890 Capillary Gas Chromatograph equipped with a DB5-MS capillary column and coupled to a Waters Autospec Ultima High Resolution Mass Spectrometer.

Following the chromatographic separation, the mass spectrometric parameters allowed to separate PCDDs, PCDFs, and PCBs between the different chlorination degrees, and between the C^{13}-labeled congeners and the native C^{12}-congeners. The mass spectrometer measured two selected ions per congener group for the native, as well as the labeled components (via "selected ion recording" at a resolution of 10,000). Quantification was carried out using the corresponding isotope-labeled compounds as internal standards (Llobet et al., 2008; Perelló et al., 2012). Toxic equivalents (TEQ) of the analyzed PCDD/Fs and DL-PCBs were calculated using the WHO-toxic equivalency factors (WHO-TEF) for dioxins and dioxin-like compounds (Van den Berg et al., 2006). The limits of detection (LOD, fresh weight) were 0.001–0.023 and 0.038–1.20 ng/kg, for PCDD/Fs and PCBs, respectively.

2.5. Analysis of trace elements

About 0.10 g of each composite sample were pre-digested with 3 mL of 65% nitric acid (Suprapur, Merck, Darmstadt, Germany), 3 mL of 30% hydrogen peroxide (Suprapur, E. Merck), and 2 mL of ultrapure water in Teflon vessels with a Milestone Start D Microwave digestion system (Milestone Srl, Sorisole, Italy). The characteristics of the selected program consisted of ten intervals of 5–10 min each for a total of 1 h 35 min, heating up to a maximum

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