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Short Communication

Fatty acid composition including *trans*-fatty acids in selected cereal-based baked snacks from Lebanon

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ABSTRACT

Ten samples of biscuits (including plain, whole meal, cream-filled and chocolate-coated), 7 samples of cakes (including plain, chocolate and cream-filled), 4 samples of wafers (including plain, chocolate-filled, coated and chocolate-filled) and 3 samples of croissants (including plain and chocolate-filled) were collected from Lebanon, in 2006, and analyzed for their fatty acid composition including *trans* fatty acids (TFA) by $AgNO_3$-thin layer chromatography and gas chromatography. All samples contained TFA ranging between 0.7 and 25.8 g/100 g fat and 20 out of the 24 analyzed samples contained more than 2% TFA on fat basis. The ratios of *trans* 18:1/(*trans* 18:2 + *trans* 18:3) ranged between 4 and 15.3 thereby indicating formulation of the products with partially hydrogenated oils. The content of TFA in the samples showed similarities to those reported in similar product categories in developing countries. This study is the first to report data on the levels and spectrum of TFA in snack products in Lebanon and underlines the importance of developing effective policies for reducing the intake of TFA by populations in the Middle East.

1. Introduction

The *trans* fatty acids (TFA) fraction of foods comprises fatty acids with at least one double bond in the *trans* configuration. The sources of TFA in the diet are meat and dairy products derived from ruminants, cooking oils/fats and margarines stabilized by hydrogenation and/or formulated with partially hydrogenated oils, and *trans* isomers produced from their *cis* counterparts in the deodorization step during refining of vegetable oils and during cooking and frying (Menaa et al., 2013). The levels of TFA produced through thermal isomerization of *cis* fatty acids during deodorization and culinary processes are relatively low and do not contribute, significantly, to dietary intakes of TFA (Menaa et al., 2013). Ruminant TFA are produced by hydrogenation of polyunsaturated fatty acids (PUFA) in the rumen while industrially produced TFA are formed during hydrogenation, using different metal catalysts, of unsaturated vegetable and marine oils (Stender et al., 2008). The TFA content of ruminant fats ranges between 4% and 6% while industrially produced hydrogenated fats might contain up to 60% TFA of the total fatty acids (Stender et al., 2008). The wide range of TFA in industrially produced hydrogenated fats and oils is largely determined by the demands of the food industry for more stable and longer shelf-life fats and oils and the different degrees of fat plasticity required for product formulation to achieve the sensory attributes desired by consumers. Industrially produced partially hydrogenated fats and oils are used in deep-fat frying, production of margarines, and formulation of biscuits, cakes, cookies, crackers and savory snacks (Micha and Mozaffarian, 2008). The consumption of ruminant and industrially produced TFA is universally recognized to result in a decrease in high-density lipoprotein cholesterol (HDL-c), an increase in low-density lipoprotein cholesterol (LDL-c) and total cholesterol, and increases in LDL-c to HDL-c and total cholesterol to HDL-c ratios (Remig et al., 2010; Brouwer et al., 2013). These changes in serum lipids are strongly associated with higher risk of coronary heart disease (CHD).

The deleterious effects of TFA consumption on health prompted national and international bodies to issue recommendations aimed

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at reducing their levels in the diet. The FAO/WHO and the American Heart Association recommended that populations should limit their daily intake of TFA to <1% of energy (WHO, 2003; Remig et al., 2010). Many of the industrialized countries amended their nutrition labeling regulations to require mandatory declaration of TFA contents on the Nutrition Facts panel for foods and supplements (Menaa et al., 2013). The Danish government decreed that fats and oils with >2% industrially produced TFA will not be sold in Denmark after January 1, 2004 (Stender et al., 2008). A number of other countries passed legislations for removing TFA from the food supply and the US Food and Drug Administration (FDA) has proposed discontinuing the generally recognized as safe (GRAS) status of TFA (Stender et al., 2014). The enactment of regulations for decreasing TFA levels in foods coupled with increased awareness about the negative health effects of TFA and consumer demands for healthier food choices led to marked reductions in TFA levels in the food supply in the developed countries (Brouwer et al., 2013) with intakes of industrially produced TFA being eliminated in Denmark (Stender et al., 2008). In contrast, increased urbanization, adoption of increasingly western dietary habits, lack of effective regulation of TFA in foods, limited knowledge and ineffective communication of the detrimental health effects of TFA to consumers in the developing countries are largely responsible for the continued utilization of industrially produced hydrogenated fats and oils in product formulation and culinary applications (Butt and Sultan, 2009; Dixit and Das, 2012). In Lebanon, and other Middle Eastern countries, locally produced western-type snack foods are heavily consumed especially by children. Many of these products owe their structure and textural attributes to industrially produced hydrogenated fats and margarines used in their formulation. Further, quantification of TFA levels in these products provides a marker for the presence of TFA in other products (Stender et al., 2014). Accordingly, the determination of TFA levels in this product category is pivotal for the enactment of regulations on nutritional labeling and recommendations governing use of hydrogenated fats and oils in the food supply, product reformulation by the food industry and development of effective intervention strategies for reducing/eliminating TFA consumption. The objective of the present work was to determine the fatty acid composition of selected snack foods, with particular emphasis on TFA composition, marketed in Lebanon.

2. Materials and methods

2.1. Samples

Twenty-four baked confectionery products were collected in August and September 2006 from supermarkets in Lebanon. The samples comprised the most sold locally produced, and few regional, brands as indicated by the supermarkets' supervisors. The products are manufactured by large processors and distributed to the different areas in the country. The selected samples comprised: Biscuits (10 including plain, wholemeal, cream-filled and chocolate-coated), cakes (7 including plain, chocolate and cream-filled), wafers (4 including plain, chocolate-filled, chocolate-filled and coated with chocolate) and croissants (3 including plain and chocolate-filled). Three packages of each sample were pulverized in a food processor (Moulinex Type 320, Spain), combined into a composite sample for analysis and stored at −18 °C until analyzed.

2.2. Analyses

2.2.1. Fat extraction and preparation of fatty acid methyl esters (FAME)

The fat was extracted from the pulverized material and FAME were prepared according to AOAC procedures (AOAC, 2000, Official Methods 992.06 and 996.06). A homogenized subsample (2 g) was placed in a plastic centrifuge tube and pyrogallic acid (100 mg), to minimize fat oxidation, and 2 ml of a triglyceride internal standard solution (tritridecanoate $C_{13:0}$, 5 mg/mL in $CHCl_3$) were added. After the addition of ethanol (2 mL) and 8.3 *N* HCl (10 mL), the mixture was heated at 80 °C in a shaking water bath for 40 min. The tube was treated with ethanol (5 mL), cooled in tap water and the fat extracted, at room temperature, with diethyl ether (10 mL) and petroleum ether (10 mL; boiling point 40–60 °C). The extraction was repeated 3 times with 1:1 diethyl ether:petroleum ether and the ether extracts were combined. The ether was evaporated on a steam bath with the last traces of solvent being removed under a stream of nitrogen. The fat content was calculated by difference after allowing for a blank. The extracted fat was stored under nitrogen at −18 °C. The extraction of fat from the homogenized samples was carried out in duplicate.

The extracted fat was dissolved in chloroform (3 mL) and diethyl ether (3 mL), transferred into a glass vial and the solvent evaporated under a stream of nitrogen. Boron trifluoride (7% in methanol; 2 mL) and toluene (1.0 mL) were added and the glass vial was sealed and heated in an oven at 100 °C for 45 min with shaking every 10 min. The vial was left to cool to room temperature and double distilled water (5 mL), hexane (1 mL), and $Na_2S_2O_4$ (1 g) were added and the vial shaken for 1 min. The top layer was transferred into another vial containing 1 g $Na_2S_2O_4$. The vials containing the FAME, including FAME of the triglyceride internal standard, were kept at −18 °C. One FAME preparation was made from each of the duplicate fat extracts.

2.2.2. Analysis of fatty acids

The distribution of FAME was analyzed by gas chromatography (GC) of the total extract and after fractionation of the FAME by silver nitrate thin-layer chromatography ($AgNO_3$-TLC) to allow for better resolution and quantification of the C 18 isomers. Analyses were carried out on a ThermoQuest trace 2000 series gas chromatograph equipped with a ThermoQuest AS 2000 autosampler, a ThermoQuest ChromQuest data system (ThemoQuest Co, Milan, Italy) and a flame ionization detector (FID). An SP-2560 flexible fused capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness; Supelco, Sigma–Aldrich, Germany) was used for separation of FAME. For analysis of the total FAME extract, GC was conducted under the following operating conditions: injector temperature, 250 °C; detector temperature, 260 °C; holding at 140 °C for 10 min followed by a ramp to 240 °C at 4 °C/min and holding at this temperature for 25 min. One μL injections of the FAME mixture were made at a split ratio of 200:1 and helium was used as the carrier gas at a constant pressure of 250 kPa. The peaks for the fatty acids were identified according to the chromatogram of the FAME standard mixture (Supelco 37 component FAME mix, CRM47885, Sigma–Aldrich, Germany).

Fractionation of FAME by $AgNO_3$-TLC was carried out according to Ratnayake and Cruz-Hernandez (2009). FAME (500 μL) were applied on pre-coated glass plates, with a 0.2 mm layer of silica gel impregnated with 10% (w/v) $AgNO_3$ in acetonitrile (Analtech Inc., Newark, DE, USA), that had been activated by heating at 110 °C for 1 h. The plates were placed in toluene/*n*-hexane (9:1, v/v) in the dark for 1 h. After drying at room temperature, the plates were sprayed with Rhodamin B (0.05% in ethanol; Fluka, Germany) and dried at 80 °C. The separated bands were viewed under ultraviolet light (254 nm) and the bands containing the C 18:1 *trans* and the saturated fatty acids fractions were scrapped off together, thereby allowing for the use of the internal standard in the quantification of the C 18:1 isomers (Chardigny et al., 1996), into a vial containing diethyl ether (5 mL). The vials were allowed to stand for 30 min with frequent agitation and the resulting extracts were dried under a stream of nitrogen, re-dissolved in dichloromethane and stored