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Short communication

Trans fatty acids in commercial cookies and biscuits: An update of Portuguese market

Luísa Alexandra Teixeira Santos, Rebeca Cruz, Susana Casal*

REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal

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ABSTRACT

Cookies and biscuits are a recognized source of *trans* fatty acids (TFA). Aware of its consumption worldwide, an update on TFA content was taken in 2012, in a total of 50 samples commercialized in Portugal. Despite the absence of specific Portuguese legislation, TFA amounts in cookies and biscuits are generally low, with TFA amounts lower than 0.1 g per 100 g, and an average of 0.6% in the extracted fat in 49 of the 50 samples analyzed. Unfortunately, one sample presented 27% of TFA in the lipids, highlighting that the problem is still present. Also, a high prevalence of saturated fatty acids was observed, as high as 92.4% in the fat (53.0% on average). This fact is also a major health concern, particularly when the reformulation of these products in the last years (2006–2012) was effective regarding TFA reduction but seemed to have occurred at expenses of increasing SFA, thus reducing the potential beneficial effect achievable by replacing with *cis*-unsaturated fats, as generally recommended.

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1. Introduction

Trans fatty acids (TFA) in food derive from two different sources: industrial and natural. Natural TFA are produced as a result of microbial transformation of unsaturated fatty acids in ruminant animals and so exist at low levels in some products, such as dairy products. On the other hand, the major process contributing to the formation of industrial TFA (IP-TFA) is partial hydrogenation of vegetable oils, aiming to increase their stability and versatility. Thermal processes such as deodorization of edible oils and frying also lead to the formation of TFA, but in much smaller quantities (Bhardwaj, Passi, & Misra, 2011).

The consumption of IP-TFA has recognized negative health effects, as they cause harmful alterations in plasma lipid levels (raising low density lipoprotein cholesterol – LDL – and decreasing high density lipoprotein cholesterol – HDL), systemic inflammation, endothelial function, visceral adiposity and insulin resistance, contributing significantly to an increased risk of cardiovascular and other chronic diseases (Mozaffarian & Willett, 2007).

Over the last few years, a number of approaches have been initiated worldwide to reformulate food products and reduce the ingestion of partially hydrogenated fats, either voluntary or imposed by legal restrictions of TFA content in raw materials or food portions. Denmark was the first country to adopt legislation defining a limit of 2% on the level of IP-TFA that can be used in food manufacture, back in 2003. Furthermore, Food and Drug Administration Agency requires mandatory labeling of TFA content in foods containing 0.5 g or more per serving, since 2006. However, most countries in the European Union, Portugal included, rely on food producers to voluntarily reduce the amounts of IP-TFA in foods (L'Abbé, Stender, Skeaff, Ghafoorunissa, & Tavella, 2009). So, it is imperative to monitor the lipid composition of food groups that are potentially major sources of TFA in diet to find out if the expected decline is occurring (Skeaff, 2009). Several surveys have been published in recent years on this matter, and yet data on the Portuguese market is still restricted.

This study intended to update the knowledge about the cookies and biscuits commercialized in Portugal. This particular food class is internationally recognized as an important source of *trans* fat and has an elevated relevance in human diet, as well as on the Portuguese one. Besides the TFA content, total fat content and lipid fractions (saturated fatty acids, SFA; monounsaturated fatty acids, MUFA; and polyunsaturated fatty acids, PUFA) are also fundamental from the nutritional point of view along with their effective labeling, in order to verify the current reality of this market in Portugal and analyze their evolution in recent years.

* Corresponding author.
E-mail address: sucasal@ff.up.pt (S. Casal).

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2. Materials and methods

2.1. Sampling

A total of 50 samples were purchased from national supermarkets, during September 2012. Aware of the differences in the terminology used to classify these products all around the world, we made no distinction between cookies and biscuits but, instead, divided them into five groups based on their appearance and composition. The first group ("Covered/Filled" – #1 to #14) included all the "sandwich-type" biscuits with a layer of cream between the two parts as well as those covered with cream or chocolate-based covers. The second group ("Wafer" – #15 to #21) included wafer cookies that, despite being also "sandwich-type" cookies have a higher mass proportion of cream filling. The butter-type cookies and biscuits ("Butter" group – #22 to #25), due to their particular fat source, relevant when TFA are under discussion was assembled as the third group. Puff-based biscuits and cookies made also a separate group ("Puff" – #26 to #28) due to the particularity of having a recognized high fat content in the light mass. The "Plain" group included all the simple cookies, not included in the previous groups, as oatmeal biscuits or Marie biscuits (#20 to #50).

Samples were homogenized and grinded, and finally stored at 4 °C in the dark until the analyses were performed. Nutritional information was obtained from the labels, as well as information of the fat ingredients used.

2.2. Fat extraction

Fat was extracted from 500 mg of the homogenized sample with organic solvents (cyclohexane and 2-propanol, Sigma, Spain), based on Cruz et al. (2013) and Smedes (1999). An internal standard (triundecanoin, Fluka) was added for total fatty acid quantification, used as an estimative of the total fat content. The solvents were left overnight at 4 °C during the first extraction. The final cyclohexane layer was taken to dryness under a nitrogen stream and the residual extracted lipids were used directly for the fatty acid analysis as described below.

2.3. Fatty acids analyses

Fatty acid profile was determined by gas chromatography. A base-catalyzing transesterification was used to convert fatty acids glycerides into fatty acid methyl esters (FAMEs) in accordance with ISO 12966-2:2011, using 2 M KOH/methanol. After centrifugation (3000 rpm, 5 min), supernatant was transferred to a vial and injected in the chromatograph.

A Chrompack (CP 9001) gas chromatograph with flame ionization detection and a CP-Sil 88 column (50 m × 0.25 mm; Varian, USA) were used. The temperature of the injection port was 230 °C and of the detector was 250 °C. The carrier gas was helium. The oven had a temperature gradient from 120 to 200 °C. The analyses were performed in accordance with ISO 12966-2:2011 and ISO 15304:2002.

The identification of the fatty acid methyl esters was based on comparison of the retention times of sample peaks with those of a commercially available FAME mixture and individual standards from diverse suppliers (Supelco – USA, Larodan fine Chemicals – Sweden and Nu-Chek Prep, Inc, USA). The total amount of fatty acids was calculated from the peak area of the internal standard. The relative proportion of each fatty acid was obtained by normalization of the peak areas. The SFA include C6:0; C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0; C20:0, C22:0 and C24:0. The MUFA correspond do the sum of C14:1; C16:1, C17:1, C18:1, C20:1 and C22:1, while PUFA were the sum of C18:2, C18:3, C20:2, C20:3 and C22:2, all positional isomers included under the same designation. The *trans* isomers were grouped apart, as total TFA, and include all the *trans* isomers of C18:1, C18:2 and C18:3 superior to 0.05%, as detailed in ISO 15304:2002. Conjugated linoleic acid, eluting after C18:3, despite being calculate for authenticity purposes in the butter cookies, was not included in the TFA content.

2.4. Statistical analysis

The results are presented as mean values and standard deviation from duplicate analysis of each sample. Aiming an analysis of variance between all five sample groups, normal distribution of the residuals and the homogeneity of variances were evaluated through the Shapiro–Wilk's test (sample size = 50) and the Levene's test, respectively. Afterward, all dependent variables were studied using a one-way ANOVA, subjected or not to Welch's correction, depending if the requirement of the homogeneity of variances was verified or not. Furthermore, if a statistical significant effect was verified, post hoc tests, Duncan and Dunnett T3, were also applied for means comparison, depending if equal variances were assumed or not.

Additionally, analysis of variance between "Plain" group versus remaining samples was performed by Kruskal–Wallis test, since normal distribution of the residuals was not confirmed by Shapiro–Wilk's test for the latter.

In order to assess the evolution of fatty acids profile, between 2006 and 2012, a Pitman–Morgan's test was conducted followed by a paired-sample t-Student test or Wilcoxon signed-rank test, if homogeneity of variances were verified or not, respectively.

Statistical analyses were performed at a 5% significance level, using SPSS software, version 21.0 (IBM Corporation, New York, USA).

3. Results and discussion

3.1. Total fat and fatty acid profile

The results obtained for the 50 individual samples are detailed in Fig. 1 and global average is compiled in Table 1. The fat content was highly variable, ranging from 8.2% (#50) to 45.0% (#29) when expressed as the sum of total fatty acid per 100 g of fresh weight sample (FW), with a mean of 22.5% (Table 1). The TFA content, the main purpose of this study, ranged from 0.11% (#19) to 27.4% (#21) in the extracted lipids, with a global average of 1.12% (Table 1), being 0.80% from the C18:1 *trans* isomer and the remaining 0.32% from C18:2 *trans* isomers. Indeed, only one sample had high amounts of total TFA, with 27.4% (#19), a wafer-type with chocolate filling, and the TFA average is reduced to 0.59% just by discounting it. Apart from this sample, only three others had amounts superior to 2% of TFA in the fat, samples #22 to 24, being both butter cookies, therefore with an expected content of natural TFA within these amounts. The conjugated linoleic acid, eluting after linolenic acid, was not included in the total TFA amounts, accounting for further 0.40%, on average. As positive health effects are attributed to these fatty acids (Bhardwaj et al., 2011), they were not included in the total TFA sum, evaluated in the present study with the purpose of detecting industrially hydrogenated fat solely. Still, based on the TFA content reported and the presence of conjugated linoleic acid isomers, particularly the *cis*-9,*trans*-11 one, sample #25 was not consistent with the label information for a butter cookie, with probably only low butter amounts as an ingredient and other fat source as major one.

When expressed on a fresh weight basis, sample #19 had 5.7 g of TFA per 100 g, but all the remaining 49 samples had equal or lower

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