

## **2012: No trans fatty acids in Spanish bakery products.**

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## **ABSTRACT**

Trans fatty acids (TFA) are strongly correlated with an increased risk of cardiovascular and other chronic diseases. Current dietary recommendations exclude bakery products from frequent consumption basically due to their traditionally high content of TFA.

The aim of this work was to analyze the lipid profile of different bakery products currently commercialized in Spain and with a conventionally high fat and TFA content. Premium and store brands for each product were included in the study.

No significant amounts of TFA were found in any of the analyzed products, regardless the brand. TFA content ranged between 0.17g and 0.22g/100g product (mean=0.19g/100g product). Expressed on percentage of fatty acids, the maximum value was 0.87g/100g fatty acids and the mean value was 0.68%. These data are significantly lower than those observed in previously published papers for these type of products, and pointed out the importance of updating food composition databases in order to accurately estimate the real and current intake of TFA.

**KEYWORDS:** trans fatty acids, bakery products, lipid profile, cakes, food composition data.

## 1. INTRODUCTION

It is generally accepted that *trans* fatty acids (TFA) are directly related to coronary heart disease (CHD) and altered lipid profile, among other diseases (Willet et al., 1993; Mozaffarian, Aro & Willett, 2009). Consumption of TFA increases the plasma cholesterol concentration of low-density lipoprotein (LDL) like saturated fatty acids (SFA) do and decreases high-density lipoprotein (HDL), compared with consumption of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (de Roos, Bots & Katan, 2001; Mensink, Zock, Kester & Katan, 2003). Some TFA have been proved to calcify cells and cause inflammation of the arteries, so as to inhibit the enzyme which converts arachidonic acid to an eicosanoid that is necessary to prevent blood clots in the arteries and veins (Kummerow, 2009). Also TFA have been related with an increased risk of cancer, although the results are inconclusive due to their long-term effect in the organism (Slattery, Benson, Ma, Schaffer & Potter, 2001; Kim et al., 2006; Chajès et al., 2008).

In Western Europe, the TRANSFAIR study estimated that the intake of TFA ranged between 1.5 to 5.4g/day (Hulsholf et al., 1999). In United States, the daily intake of TFA for men was estimated by the Food and Drug Administration (FDA) to be nearly 7g/day, and for women almost 5g/day (FDA, 2003).

The Danish food authorities were, in 2003, the first worldwide to have adopted legislation on the industrial use of trans fatty acids in foods, defining a limit on the level of TFA not more than 2g of TFA per 100g of fats or oil in the product as sold to the final consumer (Danish Food Authorities, 2003). On the other hand, the FDA requires the declaration of the amount of TFA present in foods, including dietary supplements, on the nutrition label (FDA, 2003), defining trans fatty acids as “the sum of all the fatty

acids with at least one non-conjugated double bond in the trans configuration". This rule took effect on January 2006, and a sharper decline in TFA intake was expected, due to reformulation of products and increased consumer awareness (Craig-Schmidt, 2006). As well as in the United States, food reformulation has also been one of the objectives of the European Union to promote health (European Commission, 2007; Webster, 2009), and it is hypothesised that, in consequence, processed foods are nowadays being modified by the food industry.

Since the TRANSFAIR study, carried out in 1998, and after the general recommendations of reducing the content of TFA in foods as a consequence of the scientific evidence of their negative effects, only few papers in indexed journals present updated data on the presence of these fatty acids in traditionally high-trans content foods. In the TransSwissPilot study, Richter, Shawish, Scheeder, and Colombani (2009) found that fine bakery products (31 samples analyzed between February 2006 and January 2007) showed, on average, 6.07% of TFA (0.32-16.97g/100g fatty acids) which represented a mean of 1.20g/100g food. Those authors concluded that still a substantial amount of products contained a total amount of TFA higher than 2% of fat. Lehner (2007) reported that 63% of bakery products in Austria had also TFA proportions >2%. Kuhnt, Baehr, Rohrer, and Jahreis (2011) analyzing different types of bakery products and confectioneries (chocolates and biscuits) from the German market found great variations, with means around 4.5 % for bakery products and 1.9% for confectioneries. These authors found that, in particular, unpackaged bakery products showed two fold higher TFA contents compared to packaged products. However, they pointed out that consumers cannot distinguish between products of similar appearance having high and low TFA contents on the bases of price, smell and taste.

The hypothesis of this work is that, in the last years, the use of high trans fatty acids lipid sources in Spanish bakery products has been sharply decreased, so the current intake of total trans fatty acids content through these type of bakery products is much lower than that reported some years ago. The study analyzes six different types of products traditionally considered as important suppliers of TFA. For each type of product, one premium brand and two store brands were analyzed, in order to assess a potential effect of price on the TFA content. The confirmation of this hypothesis is particularly interesting for those epidemiological studies that use food composition databases obtained some years ago to establish current intakes of TFA.

## **2. MATERIALS AND METHODS**

### *2.1. Samples and sample preparations*

Samples were purchased in regular commercial outlets for consumer products. Six products were selected on basis of their traditional high content in fats and trans fatty acids: two traditional Spanish bakery products similar to sponge cakes (sobaos and mini-ensaimadas), mini-puff pastry palms, mini-croissants, mini-chocolate croissants and chocolate nut spread. For each product, two store brands (brands 1 and 2) and one premium brand (brand 3) were selected. For each brand, samples from four different batches were purchased, and each one was analyzed in quadruplicate. Samples from each batch were homogenized, frozen and stored at -20°C until the analysis were performed. Nutrition information was obtained from the labels (energy, fat, lipid fractions) and information of ingredients used and serving size declared was also obtained (table 1).

### *2.2. Analysis of samples*

The moisture determination on all products was carried out by AOAC official method 950.46 (2002a). Total fat was determined by an extraction with petroleum ether (AOAC 960.39, 2002b) in a Soxhlet type apparatus Büchi model B-811 Extraction System. The method of Folch, Lees & Stanley (1957) was used for the extraction of lipids, where the determination of the fatty acid composition was carried out.

Fatty acid profile was determined in the lipid extracts by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAME) (AOAC 969.33, 2002c). A Perkin-Elmer Autosystem XL gas chromatograph fitted with a capillary column SPTM - 2560 (100 m x 0.25 mm x 0.2  $\mu$ m) and flame ionization detection was used. The temperature of the injection port was 250° C and of the detector was 260° C. The oven temperature was programmed at 175° C during 10 min and increased to 200° C at a rate of 10° C/min, then increased to 220° C at a rate of 4° C/min, which was kept for 15 min. The carrier gas was hydrogen, and the pressure was 20.5 psi. Split flow was 120 cm/s. The identification of the fatty acid methyl esters was done by comparison of the retention times of the peaks in the sample with those of standard pure compounds. Individual methylated standards from Sigma (St. Louis, MO, USA) were used for the saturated, monounsaturated, cis polyunsaturated fatty acids and the trans t-Palmitoleic C16:1  $\Delta$ 9t, Elaidic C18:1  $\Delta$ 9t, Brassidic C20:1  $\Delta$ 13t. For Linoleic acid isomers, the mixture Linoleic acid cis/trans isomers (50% of C18:2 $\Delta$ 9t,12t; 20% of C18:2 $\Delta$ 9c,12t and C18:2 $\Delta$ 9t,12c; 10% of C18:2 $\Delta$ 9c,12c) from Sigma was used. The order of elution in the case of mixtures of isomers (Linoleic acid cis/trans isomers) was also taken into account (Sigmaldrich.com – FAME Application guide), and spiking the sample with each standard individually was finally used for confirming the identification. The quantification of individual fatty acids was based on the internal standard method, using heptadecanoic acid methyl ester (Sigma, St. Louis,

MO, USA). Elaidic acid eluted very closely to other C18:1trans isomers (possibly  $\Delta 6$  to  $\Delta 12$ ), which are all located before Oleic acid (C18:1  $\Delta 9c$ ). Quantification for all these C18:1 trans isomers was done as the sum of all of them.

### 2.3. Statistical analysis

A one way Anova test and the Tukey *b* posteriori test were used to determine significant differences among the different brands and products. SPSS version 15.0 was used (SPSS inc. Chicago, Illinois, USA). Significance level of  $P \leq 0.05$  was used for all evaluations.

## 3. RESULTS

Table 8 shows for every analyzed product, the fat content for the two store brands and the premium brand. Mean values were: 33.54% for chocolate nut spread, 29.95% for mini-puff pastry palms, 29.05% for mini-chocolate croissants, 25.72% for mini-croissants, 25.08% for mini-ensaimadas and 24.78% for sobaos. Differences among the three brands, although statistically significant in some cases, were quantitatively small. The greatest fat range among brands (7.8g/100g) was shown for mini-puff pastry palms, with the premium brand showing the highest fat content. On the contrary, the premium brand showed the lowest fat content in the case of chocolate nut spreads. Table 8 also summarizes the sum of the different types of fatty acids analyzed by gas chromatography in the samples, whose detailed profile, expressed in g/100g total fatty acids are reported in tables 2 to 7. The results of these tables showed that, in four products (mini-ensaimadas, mini-puff pastry palms, mini-croissants and mini-chocolate croissants) the SFA fraction was higher than 50% of the total fatty acids. Sobaos showed a 46% of SFA, corresponding the lowest amount (44.27%) to the premium brand, and chocolate nut spread only a 26.65% of SFA, with the highest amount in this

case (33.41%) shown in the premium brand. The major fatty acids in this fraction were mainly palmitic acid and also stearic acid. Sobaos and chocolate nut spreads had lower presence of palmitic acid (30.81% and 21.68%, respectively) than the rest of products, where this acid ranged from 45.08% to 47.06%. For stearic acid, ensaimadas (7.13%) and chocolate nut spread (3.59%) were slightly different from the rest of products that ranged from 5.32% to 5.81%.

Chocolate nut spread showed the highest amount of MUFA, reaching a mean of 54.85%, whereas the rest of the products showed percentages between 29%-34.5%. The highest percentages of PUFA corresponded to sobaos (21.27%) and chocolate nut spread (17.86%). It can be pointed that linoleic acid was the main PUFA in all products (ranging from 11.92% to 21.01% of total fatty acids), and only in store brands of chocolate nut spread, certain amount of  $\alpha$ -linolenic acid was detected.

Considering all products, the mean TFA content ranged between 0.59%-0.87% of fat, for mini-puff pastry palms and sobaos, respectively. Only samples from the two store brands of sobaos showed percentages of TFA around 1% (table 2). The contribution of ~~elaidic~~ C18:1 trans isomers, c-t linoleic and t-c linoleic to the TFA fraction was similar in all products, except for chocolate nut spread, where ~~elaidic~~ C18:1 trans isomers were approximately two fold the value of the other two trans isomers. Regarding the distinction among brands, premium brands showed the lowest TFA content in the case of sobaos (table 2), mini-ensaimadas (table 3) and mini-puff pastry palms (table 4). No TFA differences were noticed between the premium brand and the store brands in mini-chocolate croissants (table 6) and chocolate nut spreads (table 7). In mini-croissants (table 5), it was one of the store brands that showed the highest TFA content among the three. It is also worthy to be mentioned that no differences in TFA content were either detected depending on the information given in



the labelling of the product about the absence or not of partially hydrogenated fats in the formulations.

Regarding the amount of the different types of fatty acids expressed over 100g of product (Table 8), the mean for TFA ranged 0.17-0.22g/100g product, finding slightly lower amounts for premium brands compared to store brands, only in the case of sobaos and chocolate nut spreads. The average fatty acid ratio  $[(SFA+TFA)/(MUFA+PUFA)]$  (FA ratio) ranged from 1.13 to 1.32, except for sobaos and chocolate nut spreads, in which they were lower than one.

#### **4. DISCUSSION**

TFA amounts were low in all analyzed products, giving, on average,  $0.68\pm 0.15$ g/100g of fatty acids and  $0.19\pm 0.04$ g/100g of product. These values are far from the limit established by the Danish legislation, which is the only one in force nowadays in Europe regarding trans levels in foods. Only slightly lower values were found in premium brands compared to store brands for two of the products (sobaos and chocolate nut spreads), leading to conclude that the brand seems not to be a relevant factor to be taken into account when evaluating the TFA in bakery products. Comparing these average data with those shown in Spanish food composition tables obtained within the TRANSFAIR study (Moreiras, Carbajal, Cabrera & Cuadrado, 1999) and other works on Spanish bakery products (Parcerisa, Codony, Boatella & Rafecas, 1999; Vicario, Griguol & Leon-Camacho, 2003) a great decrease can be observed.

According to the World Health Organization (WHO), the consumption of TFA should never exceed 1% of the total energy intake (World Health Organization, 2003). One serving of the analyzed products (see weight in table 1) would supply between 0.03 and 0.09g TFA, depending on the product. Considering an average 2000kcal daily

energy supply and the consumption of one serving of any of the products, these values would account for a TFA energy supply ranging from 0.01% and 0.04%. As these types of products are supposed to be among the richest in TFA content within the context of a total diet, these results confirm the low TFA supply of the currently marketed bakery products in Spain. Furthermore, according to the FDA regulation, they could be labelled as "Not a significant source of *trans* fat", as less than 0.5g TFA/serving was detected.

From the nutritional standpoint, one problem related to the TFA reduction strategy is the potential increase of SFA. Mozaffarian, Jacobson and Greenstein (2010) pointed out that reformulations of products removing partially hydrogenated oils would be expected to produce health benefits, even if these oils were replaced with animal fats or tropical oils, and if they suppose an increase of *cis*-unsaturated fats over saturated fats would maximize health benefits. Data shown in that work pointed out a decrease in the total amounts, not only of TFA but also of the summatory of TFA+SFA in the last years in United States supermarket and restaurant foods. In our study, it can be observed that SFA were, except for the chocolate nut spread, between 46%-56% of fatty acids, giving rise on average, to a total supply around 11.4-16.7g/100g product. Thus, the sum of TFA and SFA reached values around 11.6-16.9g/100g product.

Vicario et al. (2003) analyzing the fatty acid profile of Spanish bakery products (croissant, chocolate cake, sponge cakes, swiss rolls and swiss rolls with chocolate) found mean values of 43.27% and 3.68% for SFA and TFA, with a ratio S+T/M+P (*cis*) (FA ratio) of 1.45. A previous work with similar products (Parcerisa et al., 1999), showed mean values of 52.80% and 6.51% for SFA and TFA, respectively, being the FA ratio of 1.46. In our samples, the FA ratio ranged from 1.13-1.32 for four of the products (mini-ensaimadas, mini-puff pastry palms, mini-croissant and mini-chocolate croissants), it was 0.88 for sobaos and, as low as 0.38 in the case of chocolate nut

spread. So, it can be concluded that, in general, the high amount of SFA observed in most of the bakery products still gave rise to quite high FA ratios, so the total fatty acid profile is not too healthy keeping a low supply of unsaturated fatty acids.

Our study highlights the significant decrease of TFA content in some bakery products commercialized in Spain despite the absence of specific legislation on the limitation of its presence in foods. In consequence, studies carried out to evidence correlations between the TFA intake and the risk or development of diseases have to take into account current data on the supply of these TFA from food. Mayneris-Perxachs, Bondia-Pons, Molto-Puigmarti, Pairo, Castellote, and Lopez-Sabater (2010) analyzed the FA profile in plasma taken from 516 volunteers in 2003 and examined the correlations between plasma levels of TFA with their dietary sources obtained from the DIETECA database (Moreiras, Carbajal, Cabrera & Cuadrado, 2003). This study evidenced not only the importance of having good biomarkers of dietary intakes, but also the importance of having updated food composition databases, so that real values for nutrients intake can be obtained.

According to Skeaff (2009), determining the TFA amounts consumed in the population is a particularly difficult task using traditional methods of dietary assessment as food composition databases with TFA data are either nonexistent or incomplete in most countries. Moreover, it could be added that these food composition tables should be periodically revised to incorporate new data of reformulated processed foods, which are being launched into the market as a real response to new nutritional guidelines, that promote strategies such as the salt reduction or lipid profile modifications. In this sense, an initiative is taking place to develop a single online platform with up-to-date food composition data across Europe (EuroFIR project).

In summary, the study reported here demonstrates a considerable decrease in TFA amounts in Spanish bakery products in the last years, as well as no important differences between store brands and premium ones. Nevertheless, more studies are definitively needed to cover a wider range of bakery products and other processed foods. An accurate dietary intake estimation based on food frequency questionnaires can only be done if the tools used for that purpose are properly up-dated.

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## TABLES CAPTIONS

Table 1. Label information of analyzed products: Energy values (kcal/100g), fat content (g/100g product), main lipid fractions (g/100g product), source of lipids and weight of serving.

Table 2. Fatty acid composition of sobaos (g fatty acid/100g total fatty acid mean  $\pm$  standard deviation).

Table 3. Fatty acid composition of mini-ensaimadas (g fatty acid/100g total fatty acid mean  $\pm$  standard deviation).

Table 4. Fatty acid composition of mini-puff pastry palms (g fatty acid/100g total fatty acid mean  $\pm$  standard deviation).

Table 5. Fatty acid composition of mini-croissants (g fatty acid/100g total fatty acid mean  $\pm$  standard deviation).

Table 6. Fatty acid composition of mini-chocolate croissants (g fatty acid/100g total fatty acid mean  $\pm$  standard deviation).

Table 7. Fatty acid composition of chocolate nut spread (g fatty acid/100g total fatty acid mean  $\pm$  standard deviation).

Table 8. Results obtained from the analysis of samples: moisture, total fat and lipid profile (g/100g product), ratio (S+T)/(M+P) and the sum S+T.