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Fatty acid quantification in different types of cookies with emphasis on *trans* Fatty Acids

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ABSTRACT. The objective of this work was to determine the centesimal composition and quantify the major fatty acids in the most produced cookies, filled cookies (28%), salty cookies (22%), butter cookies (12%), and wafers (4%), with emphasis on *trans* fatty acids. Three brands (A, B, C) of each kind of cookie were investigated. The results are given in brand alphabetic order. The *Trans* fatty acid results are given in g per 30 g portion of cookies, as recommended by Anvisa. Chocolate-flavored cookies presented *trans* fatty acid values of 0.055, 0.086, and 0.154 g. The amounts of *trans* fatty acids in salty cookies were 0.045, 0.115, and 0.051 g. Butter cookies have *trans* fatty acid values of 0.013, 0.054, and 0.010 g. The chocolate wafers were analyzed and presented the following amounts of *trans* fatty acids: 0.100, 0.170, and 0.301 g. The results show that the quantity of *trans* fatty acids in cookies is decreasing as a result of the modification of processes and/or raw materials. Only brand C wafer had *trans* fatty acid above the Brazilian regulation limits.

Keywords: saturated fatty acids, cookies, *trans* fatty acids.

Quantificação de ácidos graxos em diferentes tipos de biscoitos com ênfase nos ácidos graxos *trans*

RESUMO. O objetivo deste trabalho foi determinar a composição centesimal e quantificar os ácidos graxos presentes em maior quantidade nos biscoitos mais consumidos: biscoitos recheados (28%), biscoitos salgados (22%), biscoitos amanteigados (12%) e biscoitos *wafers* (4%), com ênfase nos ácidos graxos *trans*. Estes biscoitos são amplamente consumidos e estão presentes em 98% dos lares brasileiras. Foram investigadas 3 marcas (A,B,C) de cada tipo de biscoito. Os resultados são apresentados em ordem alfabética das amostras. Os resultados de ácidos graxos *trans* são dados em g por 30 g de porção de biscoito como recomendado pela ANVISA. Nos biscoitos aromatizados com chocolate os valores de ácidos graxos *trans* foram de 0,055; 0,086 e 0,154 g. Em biscoitos recheados esses resultados foram de 0,045; 0,115, e 0,051 g. Os biscoitos amanteigados apresentaram quantidades de ácidos graxos *trans* de 0,013; 0,054, e 0,010 g e biscoitos *wafer* de chocolate 0,100; 0,170, e 0,301 g. Os resultados indicam que as quantidades de ácidos graxos *trans* em biscoitos diminuíram, como resultado de modificações de processos e de matéria prima. Somente os biscoitos *wafer* de chocolate da marca C, apresentou valores superiores de ácidos graxos *trans* aos permitidos pela legislação brasileira.

Palavras-chave: ácidos graxos saturados, biscoitos, ácidos graxos *trans*.

Introduction

Although cookies are not considered a staple such as bread, they are largely accepted and consumed by people of all ages, being present in 98% of the Brazilian homes. They are produced in large scale so that the product has a long shelf life and can be broadly distributed (GUTKOSKI et al., 2007).

According to the manufacturers' trade union (SIMABESP, 2009), the cookies sector would close 2008 with an increase in production of 4.1%, going from 1,131 thousand tons in 2007 to 1,177 tons in 2008, and with an estimated increase of 2.2% for 2009 (ANIB, 2008)

This increase (4.1%) corresponds to 46 thousand tons of cookies or an annual production of a factory with 1.2 thousand employees. With this production, Brazil stands out as the second largest world market of the sector, second only to the United States. In terms of consumption, Brazil ranks 12th in the world, with an average consumption of 6 kg of cookies per capita per year (SINDIMASSAS, 2009).

Filled cookies are the sales leaders with 28% of the market, followed by salty cookies (22%), butter cookies (14%), and wafers (4%) (4%) (NIGRO, 2008).

According to Marins et al. (2008), the food package label is the main source of nutritional information on products for the consumer, but the consumers have low trust on the label information, criticizing the technical language, the excess advertisement in several media, and the lack of information on potentially allergenic food components.

Anvisa (BRASIL, 1978, 2005a) issued two resolutions in 2003, RDC no. 359 – Technical Regulation of Portions of Packed Foods for Nutritional Labeling Purposes and RDC no. 360 – Technical Regulation of Nutritional labeling of Packed Foods, that made the information of energetic value and the following nutrients mandatory: carbohydrates, proteins, total fat, saturated fat, *trans* fat, food fibers, and sodium.

These resolutions allow a maximum difference tolerance of 20% between product label and actual values. This specification does not meet the Consumer Protection Code requirements, but considers the inevitable variation in raw material composition and the alterations that may occur due to processing, as well as the need to use composition tables of foods of other countries for some foods/preparations.

Relative to *trans* fatty acids, the researchers' attention for their adverse effects was raised by the work of Mensink and Katan (1990), who demonstrated that the ingestion of large amounts of *trans* fatty acids increased the levels of low density lipoprotein (LDL), similarly to saturated fatty acids. However, *trans* fatty acids were also observed to reduce the levels of high density lipoprotein (HDL), which significantly alters the LDL to HDL ratio in relation to a diet in which *trans* fatty acids were substituted by saturated fatty acids (MARTIN et al., 2004, 2007). Epidemiologic and metabolic studies also indicate that the inadequate ingestion of saturated and *trans* fatty acids increases the risk of cardiovascular diseases (ASCHERIO et al., 1999, 2006; MOZAFFARIAN et al., 2004).

According to Anvisa (BRASIL, 2005a), products made with hydrogenated vegetable fat, margarines, or fat from ruminants contain large amounts of *trans* fatty acids. Therefore, these products must be analyzed. They will be considered *trans* free (0 g *trans*) if they have values equal or lower than 0.2 g per food portion. The reference cookie portion value is 30 g (BRASIL, 2001), which was calculated based on the average quantity of food that would be normally consumed by healthy individuals over 5 years of age in good nutritional condition for a healthy diet. According to the Department of Health and Social Security (DHSS, 1994) ratios between the summation of polyunsaturated fatty acids and the summation of saturated fatty acids lower than 0.45 must be considered little healthy, particularly in what concerns cardiovascular diseases. In a study by Sugano and Hirahara (2000), this ratio ranged from 0.8 to 1.2:1 in Japanese diet in the last years.

This work aimed to evaluate the amount of the main types of cookies produced and consumed in Brazil, with emphasis on *trans* fatty acids.

Material and methods

Sampling

The types of cookies analyzed were: filled (chocolate flavored), salty cookies, butter cookies, and wafer (chocolate flavored), which were bought at local shops. Three brands of each type of cookie were analyzed. The samples were constituted of three lots, which were ground and homogenized, vacuum-packed, and stored at -18°C for analysis.

Physical-chemical analysis

The centesimal composition analysis was done in triplicate, according to the AOAC (2005) methodology. The moisture content was determined in an oven at 105°C for 4h. The ash content was determined in a muffle at 550°C for 6h. The Crude protein contents were determined by the Micro-kjeldahl method based on the total nitrogen content. The total lipids were determined by the Bligh and Dyer (1959) method. For the determination of the fatty acid profile, the lipids were transesterified by the Hartman and Lago (1973) method modified by Maia and Rodrigues-Amaya (1993).

Analysis and quantification of fatty acid methyl esters

The fatty acid methyl esters were analyzed on a gas chromatography (Varian, USA) equipped with a flame ionization detector and fused silica capillary column (100 m long, 0.25 mm, and 0.39 μm i.d., *Varian*, USA). The column temperature was programmed to start with 165°C for 12 min., heating rate of 40°C $min.^{-1}$ up to 180°C, which was kept for 15 min., followed by a heating rate of 15°C $min.^{-1}$ up to 240°C, which was kept for 18.6 min., totaling 50 min. of chromatographic analysis. The injector and detector temperature was 230°C. Ultrapure gas flows (*White Martins*) of 1.4 mL $min.^{-1}$ for the carrier gas (H_2), 20 mL $min.^{-1}$ for the make-up gas (N_2), 30 and 300 mL $min.^{-1}$ for the flame gas, H_2, and synthetic air, respectively, were used. The sample split ratio used was 1:80. Sample volumes of 2 µL were injected in triplicate. The fatty acids were identified by comparison of the retention times with those of fatty acid methyl ester standards (*Sigma*) co-eluted (spiked) with the

samples and by equivalent chain length (ECL). The peak areas were determined using the software *Star* (*Varian*). The results are given as percentage of the normalized area of fatty acids. Polyunsaturated fatty acids (PUFA) were quantified in mg g^{-1} of total $lipids^{-1}$ by internal standardization and using fatty acid methyl ester standards. The methyl ester of tricosanoic acid (23:0, methyl-tricosanoate) was used as an internal standard.

The calculations were made following the methodology of Joseph and Ackman (1992) with equation:

$$PUFA\ (mg\ g^{-1}) = [(Ax \times Mp \times Fcx) / (Ap \times MA \times Fc)]$$

where:

Ax = PUFA area;

Ap = Internal standard area;

Fcx = Theoretical factor of correction of PUFA;

Mp = Mass of internal standard added to the sample in milligrams;

MA = Mass of total lipid sample in grams;

Fc = Factor of conversion to express the results in mg of fatty acid per gram of total lipids for PUFA methyl esters.

The analytes were identified by comparison of retention times of the sample constituents with that of a mixture of fatty acid methyl esters from Sigma-Aldrich (189-19).

Statistical analysis

The results were submitted to variance analysis (ANOVA) at 5% probability and the means were with software Statistica version 7.0 (STATSOFT, 2004).

Results and discussion

The percent values of moisture, ashes, proteins and total lipids are given in Table 1.

According to the Brazilian commission for Food Norms and Standards (CNNPA), Resolution no. 263 (23), the maximum moisture for cookies is 14%. For all the purchased cookies, it was lower than 6%.

The same resolution establishes that the ash content cannot be higher than 3.0%. The salty cookies of brands A and C presented values 11 and 8% higher than the recommended values. In relation to crude protein contents (BRASIL, 2005b), butter cookies are classified as raised dough, as they present raw protein contents around 11%, while salty cookies are classified as soft dough, with approximate protein contents of 9%. In relation to the low crude protein content, filled cookies and wafers are classified as hard dough.

Table 1. Centesimal composition of cookies.

Type	Brand	% Moisture	Ashes (%)	Crude Protein (%)	Total Lipids (%)
Filled (F)	A	2.51 ± 0.04^{bcd}	1.60 ± 0.03^{ab}	7.31 ± 0.24^{c}	15.21 ± 1.0^{c}
	B	2.36 ± 0.06^{cd}	2.04 ± 0.02^{ab}	4.55 ± 0.40^{de}	20.75 ± 0.48^{b}
	C	4.56 ± 0.02^{ab}	2.27 ± 0.04^{ab}	6.58 ± 0.33^{cd}	14.98 ± 0.38^{c}
Salty (S)	A	3.78 ± 0.01^{abc}	3.33 ± 0.08^{a}	9.46 ± 0.34^{b}	13.22 ± 1.01^{cd}
	B	5.68 ± 0.02^{a}	1.99 ± 0.03^{ab}	8.67 ± 0.28^{bc}	15.21 ± 0.99^{c}
	C	4.62 ± 0.09^{ab}	3.24 ± 0.01^{a}	10.11 ± 0.21^{b}	11.24 ± 0.50^{d}
Butter (A)	A	3.50 ± 0.08^{abc}	1.86 ± 0.02^{ab}	13.07 ± 0.23^{a}	8.01 ± 0.86^{e}
	B	2.85 ± 0.04^{bcd}	1.58 ± 0.03^{ab}	12.48 ± 0.39^{a}	8.24 ± 0.87^{e}
	C	2.79 ± 0.05^{bcd}	1.23 ± 0.02^{ab}	13.71 ± 0.30^{a}	5.14 ± 0.64^{f}
Wafers (W)	A	0.84 ± 0.02^{d}	0.87 ± 0.03^{b}	4.92 ± 0.27^{de}	15.21 ± 0.81^{c}
	B	1.33 ± 0.04^{d}	0.92 ± 0.03^{b}	3.67 ± 0.25^{e}	27.65 ± 1.01^{a}
	C	1.17 ± 0.02^{d}	0.90 ± 0.07^{b}	4.98 ± 0.31^{de}	28.86 ± 0.58^{a}

Results given as mean of triplicate analysis of three lots (n = 9). Values with equal letters are not significantly different at 5% confidence by Tukey's test.

Only filled cookies presented a significant difference in protein content in the same type of cookie ($p \leq 0.05$) between brands A and B. Brand C had an intermediate value, thus being similar to both. For total lipids, all types of cookies of the different brands presented significant differences between two brands of the same type of cookie. The major fatty acids found in the lipid extracts of cookies of brands A, B, and C were palmitic acid (16:0), and estearic acid (18:0), oleic acid (18:1n-9), and linoleic acid (18:2n-6), respectively, as shown in Table 2.

Table 2. Major fatty acids in cookies (values in % of total lipids).

Type	Brand	16:0	18:0	18:1n-9	18:2n-6
Filled	A	38.72 ± 0.45^{a}	8.73 ± 0.40^{d}	33.81 ± 0.46^{c}	10.73 ± 0.26^{f}
	B	33.76 ± 1.47^{d}	14.44 ± 0.40^{bc}	20.80 ± 0.46^{f}	19.71 ± 0.10^{c}
	C	26.45 ± 0.64^{f}	17.37 ± 0.44^{a}	28.19 ± 0.86^{d}	11.233 ± 0.31^{ef}
Salty	A	38.57 ± 0.62^{a}	4.20 ± 0.06^{g}	39.06 ± 0.39^{a}	15.65 ± 0.11^{d}
	B	38.96 ± 0.61^{a}	6.29 ± 0.17^{efg}	20.70 ± 0.54^{f}	26.21 ± 0.56^{a}
	C	35.98 ± 0.42^{bc}	6.74 ± 0.13^{def}	36.71 ± 0.68^{b}	15.17 ± 0.08^{d}
Butter	A	35.99 ± 0.10^{bc}	4.53 ± 0.06^{fg}	33.48 ± 0.05^{c}	0.16 ± 0.05^{e}
	B	37.72 ± 0.16^{ab}	6.65 ± 0.06^{de}	21.45 ± 0.10^{f}	23.67 ± 0.14^{b}
	C	39.74 ± 0.28^{a}	4.28 ± 0.01^{g}	40.19 ± 0.19^{a}	12.77 ± 0.08^{e}
Wafers	A	35.33 ± 0.78^{cd}	12.95 ± 0.37^{c}	29.83 ± 0.18^{d}	10.24 ± 0.16^{f}
	B	29.75 ± 0.50^{e}	16.23 ± 0.28^{ab}	25.34 ± 0.23^{e}	15.57 ± 0.24^{d}
	C	11.52 ± 0.38^{g}	12.74 ± 0.21^{c}	25.81 ± 0.38^{e}	2.18 ± 0.21^{g}

Results given as mean of triplicate analysis of three lots (n = 9). Values with equal letters are not significantly different at 5% confidence by Tukey's test.

The World Health Organization (WHO, 1995) recommends a maximum ingestion of *trans* of 1% of the diet energetic value. According to this criterion, each gram of fat corresponds to nine calories; thus, an adult who consumes 2,000 calories a day should not consume more than 2 g of *trans* fatty acids a day.

In relation to the *trans* fatty acid summation, Figure 1 shows that brand C wafers had values higher than the Brazilian regulation values (g portion $food^{-1}$). Aued-Pimentel et al. (2009) also found high values for *trans* fatty acids in wafers. However, there is a tendency for the manufactures to substitute or modify processes and raw materials to decrease the quantity of *trans* fatty acids in their products.

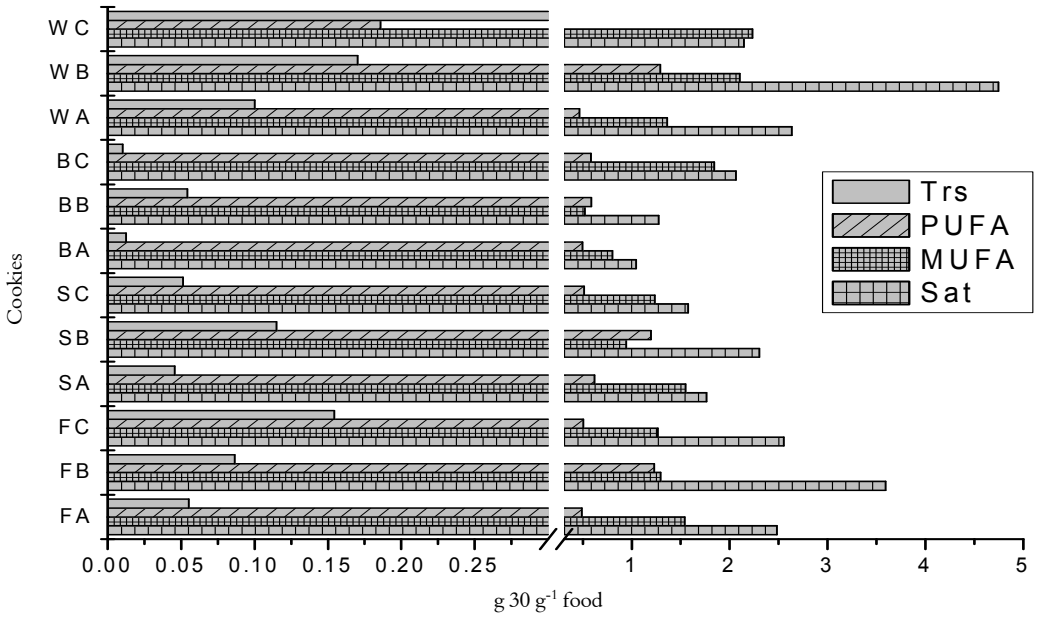

Figure 1. Summation of *trans* fatty acids (Trs), summation of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (Sat) in g per 30-g portion of cookies.

A two-year survey of specialized suppliers made by the Brazilian association of the cookie Industry (Associação Nacional da Indústria de Biscoitos, ANIB) (in partnership with the dough food industry union and the Simabesp demonstrated that 65% of the cookies available in the Brazilian market are already *trans* free (NUTRITOTAL, 2008).

In a study by Martin et al. (2007) of salty coolies produced between 2002 and 2303 and sold in Brazil, the summation of *trans* fatty acids in total lipids in five different brands was equal to 19.85% on average. In the present work, the mean percentage for the same type of cookie was 0.99%.

These results must be evaluated according to Anvisa's resolution RDC no. 360 of Dec. 23, 2003, which determines that the manufactures must inform the amount of *trans* fatty acids per portion of food in the product labels; however, it does not establish the limit percent daily values (%DV) of *trans* fat.

Nevertheless, this resolution gave manufacturers a period of grace until 2006, as the companies that did not comply with the regulation would just received educational communications without any penalties. Only from 2007 on would they be considered as breaking sanitary regulations and be fined according to Federal Act 6437 of 1997.

The comparison of the results of Martin et al. (2005) of salty cookies between 2002 and 2004 and of the present study of 2009 demonstrates a clear difference in the amounts of *trans* fatty acids. Aued-Pimentel et al. (2009), also found reduced values in brazilian biscuits for *trans* fatty acids.

According to Wagner et al. (2008), in Austria and Germany, the *trans* content in fast food, such as pizza, hamburger, French fries, instant soup etc., decreased considerably in 10 years from over 15% to less than 1%.

An investigation of foods consumed in Costa Rica in a period of 10 years showed that the concentration of *trans* fatty acids in soybean refined oil dropped from 20 to 1.5% (BAYLIN et al., 2007). Haytowitz et al. (2008) reported that in 2002, the *trans* content in margarines with 80% total fat was 19.7 g 100 g^{-1} of food, while in 2006 it was 14.8 g 100 g^{-1} food.

All these studies demonstrate a gradual reduction in *trans* fatty acids both in cookies and in the raw materials. Some manufacturers have increased the amount of saturated fatty acids, which also are considered harmful, particularly concerning cardiovascular diseases, to reduce the amount of *trans* fatty acids (ASCHERIO et al., 1999, 2006; MOZAFFARIAN et al., 2004).

The summation of saturated fatty acids in brand B wafers, and filled and salty cookies were higher than those of brands A and C. Only the butter cookies of brand B had values slightly lower than that of brand A.

The ratio between the summation of polyunsaturated fatty acid and saturated fatty acids was higher than 0.45 only for salty cookies (B) and butter cookies (A and B). The other cookies were considered little healthy, particularly in respect to cardiovascular diseases, according to the recommendations of the DHSS (1994).

In the study by Martin et al. (2005) of salty cookies produced and sold in Brazil between 2002 and 2003, the ratio of the summation of polyunsaturated fatty acids and saturated fatty acids in relation to the total lipids was 1.02 on average. In the present study, this ratio was 0.41 on average.

Conclusion

The quantity of *trans* fatty acids present in cookies has been decreasing as a result of modification of processes and/or raw materials. Only one of the studied brands has a type of cookie with *trans* fatty acid above the Brazilian regulation limits.

The quantities of the main constituents of cookies in general must be observed in Brazil, particularly in relation to constituents that may damage to health.

These values may indicate the substitution of *trans* fatty acids by saturated fatty acids to obtain a product with similar consistency for the manufacture modifications introduced with the reduction of *trans* fatty acids in foods. However, we point out that saturated fatty acids are related to the increase in cardiovascular diseases, as they raise the LDL level.

Trans-free labeled products must still be observed in respect to possible damages to health.

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