Proximate composition and quantification of fatty acids in breaded chicken steak

Composição centesimal e quantificação de ácidos graxos em steak empanado de frango

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Abstract

The aim of this work was to analyze the fatty acid and proximate composition of five brands of breaded chicken steak (A, B, C, D, and E) by accurate chromatographic quantification and to compare the experimental results with food label nutrition facts. The protein values of all brands were in agreement with the Brazilian regulation values, except for that of sample E, which presented the highest lipid content. Thirteen fatty acids were identified in the studied brands and the major ones were oleic acid, linoleic acid, and palmitic acid. The polyunsaturated fatty acid/saturated fatty acid ratios were within the values considered appropriate for human health; however, the high n-6/n-3 ratios found can result in an unbalanced intake of these fat acids. Only samples D and E can be considered trans free according to the regulations. The comparison of the analyses' results and the food label nutrition facts showed little variation in protein values. Nevertheless, most brands underestimated their lipid and energy levels. Brand B and C labels declared "free of trans", but the obteined results showed levels excedding those especified by regulation to be considered trans free values.

Keywords: chicken steak; composition; fatty acid; nutritional labeling.

Resumo

O objetivo deste estudo foi analisar a composição centesimal e de ácidos graxos de cinco marcas de *steak* empanado de frango (A, B, C, D e E). As análises de ácidos graxos foram realizadas por cromatografia gasosa e técnicas de quantificação absoluta. Os resultados experimentais foram comparados com valores constantes nos rótulos. Os valores encontrados para proteína de todas as marcas estavam em acordo com o estabelecido pela legislação brasileira, exceto o da amostra E, a qual apresentou o maior teor lipídico. Treze ácidos graxos foram identificados nas marcas avaliadas, sendo os majoritários os ácidos: oleico, linoleico e palmítico. As razões dos ácidos graxos poli-insaturados/ácidos graxos saturados encontraram-se dentro dos valores considerados adequados para a saúde humana, porém, as elevadas razões n-6/n-3 encontradas podem resultar em um desequilíbrio na ingestão destes ácidos graxos. Apenas amostras D e E podem ser consideradas livres de trans de acordo com a legislação vigente. A comparação entre os resultados das análises e os valores constantes nos rótulos apresentaram pequenas variações nos teores de proteína, entretanto, a maioria das marcas subestimou os teores de lipídios e valor energético. Os rótulos das marcas B e C declaravam-se livre de trans, entretanto os resultados obtidos apontam teores acima dos valores permitidos pela legislação para serem consideradas livre de trans. *Palavras-chave: steak de frango; composição; ácidos graxos; informação nutricional.*

1 Introduction

Changes in human eating habits have required new industrialized food products to meet the demand of quick home preparation foods (NEVES; CHADDAD; LAZZARINI, 2002). Restructured breaded steak stands out for its easy preparation and as an accessible protein source. In Brazil, the interest in poultry products has been growing year after year (FRANCISCO; NASCIMENTO; LOGUERCIO, 2007), and recent studies have shown a noticeable increase in the consumption of breaded chicken steak (NUNES et al., 2006).

Breaded chicken steak is produced mainly from mechanically separated poultry meat, chicken skin and chest flap, which is homogenized, formed, breaded, pre-fried, and cooked (BRASIL, 2001). Breading decreases water loss during frying and helps to prevent lipid oxidation thus increasing product shelf life (NUNES et al., 2006).

Highly industrialized foods have been pointed as the main responsible for the increased energetic value of western diets (FRENCH; STORY; JEFFERY, 2001). Their excessive consumption is directly linked to overweight and higher incidence of obesity, cardiovascular disturbs, and other diseases (LOBANCO, 2007). This has led consumers to pay attention to nutrition facts in food labels, particularly to lipid and trans fatty acid contents, as well as the energetic value (PINHEIRO et al., 2007), factors that have become decisive in the choice of products.

According to the Brazilian Surveillance Agency (Agência Nacional de Vigilância Sanitária, ANVISA), food labels must inform, among other facts, protein, total fat, saturated fat, and trans fat contents (BRASIL, 2003). The nutritional data may be given as "non-significant amount per portion" when the values

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are below the limits recommended. In the case of trans fat, this limit is 0.2 g per portion, and there is no recommended daily value for this type of fat.

The accurate and reproducible analysis of food fatty acid profile is of growing importance. The results are frequently expressed as weight percent (normalized area) in nutrition studies. One disadvantage of area normalization is error propagation due to the strong interdependence of the results. Thus, if one fatty acid is estimated wrongly (or omitted when unknown), the results of the other fatty acids are affected. The use of an internal standard, generally 17:0 (methyl heptadecanoic) or 19:0 (methyl nonadecanoic), is recommended to reduce errors. The official methods of the Association of Analytical Chemists and the American Oil Chemists' Society provide clear guidelines for the accurate quantification of fatty acids and stipulate the use of 23:0 (methyl tricosanoic) as an internal standard, as well as for the use of wax-type capillary columns and the flame ionization detector (FID) correction factor (ACKMAN; SIPOS, 1964; VISENTAINER; FRANCO, 2006). Among the advantages of the use of standard 23:0 to quantify fatty acids, are stability, low cost, accessibility, and the fact that the 23:0 elute separately and close to the sample components (EDER, 1995).

Thus, the objective of this study was to analyze the fatty acid composition by accurate quantification as well as the proximate composition of five brands of breaded chicken steak widely commercialized in Brazil and to compare the results of the analyses with food label nutrition facts.

2 Material and methods

2.1 Sampling

Breaded chicken steak samples of five different brands were characterized as A, B, C, D, and E in this work. Three lots of each brand were purchased from the local market in Maringá, Paraná State - Brazil, homogenized, and stored in freezer at -18 °C for later analysis.

2.2 Analyses

Moisture, ash, and protein contents were determined in accordance with Cunnif (1998). The total lipids were extracted by the Bligh and Dyer (1959) method. The values of carbohydrates (nifext) were calculated according to Brasil (1998) considering the following energy conversion factors in calculation: carbohydrate 4 kcal.g⁻¹ (17 kj.g⁻¹), protein 4 kcal.g⁻¹ (17 kj.g⁻¹), and lipid 9 kcal.g⁻¹ (37 kj.g⁻¹). Analytical grade reagents were used and chemical analyses were performed in triplicate.

2.3 Fatty acid methyl esters

Fatty acid methyl esters (FAME) were prepared by methylation of total lipids (TL), as described by Hartman and Lago (1973). The methyl esters were separated by gas chromatography in a Varian model 3380 equipped with flame ionization and cyanopropyl capillary column (100 m \times 0.25 d.i., 0.25 μ m film thickness, CP-7420 Varian, EUA). The gas flow

rates used were 1.2 mL/minute carrier gas (H₂), 30 mL/minute make-up gas (N2), and 300 mL/minute flame gases (H2 and synthetic air, respectively). The sample splitting rate was 1:100 and the samples $(2 \mu L)$ were injected in triplicate. The operation parameters were as follows: detector temperature 225 °C, injection port temperature 245 °C, column temperature 197 °C for 23 minutes, programmed to increase at 20 °C/minute to 225 °C and kept at this temperature for 15 minutes. The peak areas were determined by Workstation 5.0 (Varian) acquisition program. For the fatty acid identification, retention times were compared to those of standard methyl esters (Sigma, St. Louis, MO, USA). Quantification (in mg fatty acid/g of total lipids) was made against tricosanoic acid methyl ester from Sigma (USA) as an internal standard (23:0), as described by Joseph and Ackman (1992). Theoretical FID (flame ionization detector) correction factor (VISENTAINER; FRANCO, 2006) values were used to obtain the concentration values. Fatty acid contents are reported in mg.g⁻¹ of total lipids by using the following Equation 1:

$$FA = \frac{A_X W_{IS} CF_X}{A_{IS} W_X CF_{AE}} \tag{1}$$

Where FA is mg of fatty acids per g of total lipids, A_x is the peak area (fatty acids), $A_{\rm IS}$ the peak area of internal standard (IS) methyl ester of tricosanoic acid (23:0), $W_{\rm IS}$ is the IS weight (mg) added to the sample (in mg), W_x is the sample weight (in mg), CF_x is the theoretical correction factor, and $CF_{\rm AE}$ is the conversion factor necessary to express the results as mg of fatty acids rather than as methyl esters.

The results were recalculated from $mg.g^{-1}$ to g fatty acid $100 g^{-1}$ breaded chicken steak.

2.4 Statistical analysis

The results were submitted to variance (ANOVA) analysis and Tukey's test (5% probability) using the Statistica 5.0 software (STATSOFT, 1995).

3 Results and discussion

3.1 Analyses

The moisture values (Table 1) ranged from 42.56 to 53.78%, while the ash content did not differ between the samples. Protein contents were higher for samples B, C, and D, and lower for sample E (8.79%), which was below the set limit in Brasil (2001). The low protein content observed in sample E may be related to the use of inappropriate amounts of source raw materials, such as breast flaps and soybean protein.

Sample A lipid content (10.43%) was the lowest and significantly different from those of the other samples. Sample E had the highest content (18.56%). The lack of reference lipid content values can explain the high results in the analyzed breaded chicken breast samples. Thus, it is suggested the specification of a minimum total lipid content. The lipid fraction of breaded chicken breast derives from two main sources according to the label ingredients: chicken skin and the soybean oil used in frying.

No significant difference was observed in carbohydrate contents among the studied brands. The energetic value of breaded chicken steak ranged between 225.63 and 312.54 kcal $100~{\rm g}^{-1}$ sample.

3.2 Fatty acids

A total of 13 fatty acids were identified in the studied brands (Table 2): oleic (18:1n-9), linoleic (18:2n-6), and palmitic (16:0) acids in larger amounts, as also reported by Tanamati et al. (2007) for breaded chicken steak.

Figure 1 presents an indentified chromatogram with fatty acid methyl esters of a breaded chicken steak sample (brand B).

With regard to the saturated fatty acids (SFA), brand D had the largest content, while brand C had the largest amount of polyunsaturated fatty acids (PUFA) (Table 2). The PUFA/SFA ratio of brands A, B, C, and E were within the HMSO (1994)

recommendation, which establishes that amounts lower than 0.45 are not suitable to consumption considering cardiac diseases. Brand D presented a lower amount.

Although the PUFA/SFA ratios of most brands were within the limits considered appropriate to foods, the n-6/n-3 ratio (Table 2) indicates an unbalance between n-6 (omega-6) and n-3 (omega-3) series fatty acids. The n-6/n-3 ratios of breaded chicken steak were between 22.87 and 38.90, which are much higher than the maximum limit of 4.0 recommended by Enser et al. (1998). The high n-6/n-3 ratios are related to the high linoleic acid (LA) contents of the n-6 series derived from the soybean oil (MILINSK et al., 2008; MARTIN et al., 2008) used in the industrial pre-frying step. During home preparation, breaded chicken steak is generally fried, which further increases the LA amount. The unbalance between n-6/n-3 series fatty acids is closely related to several diseases, such as obesity, cardiovascular diseases, nervous system disorders, and cancer,

Table 1. Proximate composition of breaded chicken steak expressed in g.100 g⁻¹ food.

	A	В	С	D	Е
Moisture	$53.78^a \pm 0.67$	$47.37^{ab} \pm 3.02$	$46.89^{ab} \pm 2.70$	$49.57^{ab} \pm 1.16$	$42.56^{\text{b}} \pm 5.37$
Ash	$2.87^{a} \pm 0.09$	$2.63^{a} \pm 0.11$	$2.27^{a} \pm 0.17$	$2.52^{a} \pm 0.61$	$2.51^{a} \pm 0.14$
Protein	$10.15^{ab} \pm 0.82$	$11.44^{a} \pm 0.85$	$11.35^{a} \pm 0.42$	$11.26^a \pm 1.32$	$8.79^{b} \pm 0.49$
Total lipid	$10.43^{a} \pm 1.07$	$15.89^{b} \pm 0.26$	$15.94^{b} \pm 2.24$	$15.14^{b} \pm 1.58$	$18.56^{b} \pm 0.84$
Carbohydrate	$225.53^a \pm 6.55$	$279.44^{a} \pm 10.76$	$267.30^{a} \pm 10.65$	$283.08^{a} \pm 16.09$	$312.54^{a} \pm 25.79$

Values are the average of triplicate sample analysis results with the respective standard deviations. Means followed by different letters in the same line are statistically different (p < 0.05) by Tukey's test.

Table 2. Fatty acids in g.100 g⁻¹ of breaded chicken steak.

Fatty acids	Brand						
	A	В	С	D	Е		
14:0	$0.03^{a} \pm 0.002$	$0.04^{ab} \pm 0.014$	$0.05^{ab} \pm 0.007$	$0.07^{\rm b} \pm 0.024$	$0.05^{ab} \pm 0.007$		
16:0	$1.24^{a}\pm0.144$	$1.87^{ab} \pm 0.313$	$2.24^{bc} \pm 0.153$	$2.80^{\circ} \pm 0.537$	$2.17^{bc} \pm 0.104$		
6:1n-7	$0.16^{a} \pm 0.010$	$0.30^{a} \pm 0.091$	$0.32^a \pm 0.123$	$0.16^a \pm 0.021$	$0.19^a \pm 0.016$		
8:0	$0.44^{a} \pm 0.050$	$0.49^{a} \pm 0.123$	$0.55^a \pm 0.013$	$0.53^a \pm 0.117$	$0.51^a \pm 0.047$		
8:1 9 <i>t</i>	$0.38^a \pm 0.052$	$0.35^{a} \pm 0.301$	$0.29^a \pm 0.277$	$0.03^{a} \pm 0.017$	$0.15^a \pm 0.060$		
8:1n-9	$2.56^{a} \pm 0.236$	$3.06^a \pm 0.973$	$3.19^a \pm 0.587$	$3.11^a \pm 0.474$	$2.64^a \pm 0.163$		
8:1n-7	$0.08^{a} \pm 0.007$	$0.11^a \pm 0.038$	$0.14^a \pm 0.065$	$0.07^a \pm 0.008$	$0.10^a \pm 0.020$		
8:2 9 <i>c</i> ,12 <i>t</i>	$0.10^{\mathrm{a}} \pm 0.008$	$0.12^a \pm 0.081$	$0.10^a \pm 0.081$	$0.00^{a} \pm 0.006$	$0.06^a \pm 0.020$		
8:2n-6	$1.48^{a} \pm 0.065$	$2.60^{b} \pm 0.507$	$3.31^{b} \pm 0.571$	$1.36^{a} \pm 0.176$	$3.53^{b} \pm 0.472$		
0:0	$0.02^a \pm 0.001$	$0.03^{\mathrm{a}} \pm 0.007$	$0.02^a \pm 0.002$	$0.02^a \pm 0.004$	$0.02^a \pm 0.001$		
8:3n-3	$0.06^{a} \pm 0.003$	$0.08^a \pm 0.030$	$0.09^a \pm 0.036$	$0.06^a \pm 0.002$	$0.11^a \pm 0.031$		
0:1n-9	$0.03^{\mathrm{a}} \pm 0.002$	$0.03^{\mathrm{a}} \pm 0.014$	$0.03^a \pm 0.009$	$0.02^{a} \pm 0.005$	$0.03^a \pm 0.001$		
2:0	$0.03^{\mathrm{a}} \pm 0.002$	$0.04^{\mathrm{a}} \pm 0.015$	$0.03^a \pm 0.014$	$0.02^a \pm 0.002$	$0.04^{a} \pm 0.008$		
FA	$0.48^{\mathrm{a}} \pm 0.045$	$0.47^{\rm a} \pm 0.380$	$0.40^a \pm 0.356$	$0.03^{a} \pm 0.023$	$0.21^a \pm 0.076$		
FA	$1.75^{a} \pm 0.195$	$2.47^{ab} \pm 0.402$	$2.90^{b} \pm 0.145$	$3.45^{b} \pm 0.675$	$2.79^{b} \pm 0.084$		
IUFA	$1.54^{a}\pm0.068$	$2.67^{b} \pm 0.522$	$3.39^{b} \pm 0.538$	$1.41^{a} \pm 0.177$	$3.64^{b} \pm 0.497$		
UFA	$2.83^{a} \pm 0.250$	$3.50^{a} \pm 1.107$	$3.67^a \pm 0.782$	$3.37^a \pm 0.502$	$2.96^a \pm 0.146$		
-3	$0.06^{a} \pm 0.003$	$0.08^{a} \pm 0.030$	$0.09^a \pm 0.036$	$0.06^a \pm 0.002$	$0.11^a \pm 0.031$		
-6	$1.48^{\mathrm{a}} \pm 0.065$	$2.60^{b} \pm 0.507$	$3.31^{b} \pm 0.571$	$1.36^a \pm 0.176$	$3.53^{b} \pm 0.472$		
UFA/SFA	$0.88^{\mathrm{a}} \pm 0.072$	$1.08^{ac} \pm 0.145$	$1.17^{ac} \pm 0.140$	$0.41^{b} \pm 0.061$	$1.30^{\circ} \pm 0.142$		
-6/n-3	$23.84^a \pm 0.272$	$34.57^a \pm 17.512$	$38.90^a \pm 20.947$	$22.87^{a} \pm 2.311$	$32.31^a \pm 6.649$		

Means followed by different letters in the same line are significantly different (p < 0.05) by Tukey's test. TFA: trans fatty acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 and n-6: omega-3, and omega-6.

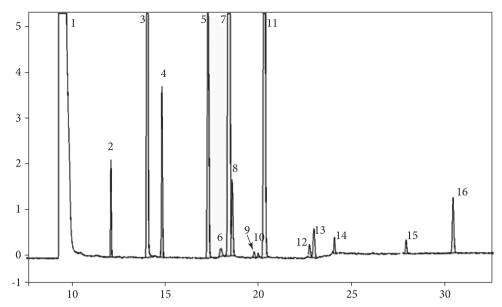


Figure 1. Fatty acid methyl esters of sample of breaded chicken steak (brand B). (1) Solvente; (2) 14:0; (3) 16:0; (4) 16:1n-7; (5) 18:0; (6) 18:1t9; (7) 18:1n-9c; (8) 18:1n-7; (9) 18:2 9t12c; (10) 18:2 9c12t; (11) 18:2n-6; (12) 20:0; (13) 18:3n-3; (14) 20:1n-9; (15) 22:0; (16) 23:0.

whose incidence have climbed to alarming levels with the increased consumption of industrialized foods (MARTIN et al., 2006).

All evaluated brands had trans fatty acids in their composition, and brand A presented the highest level - $0.48~\rm mg.100~\rm g^{-1}$ of chicken steak.

Among the raw materials used for the formulation breaded chicken steak the skinned carcass (equivalent to the mechanically separated poultry meat and skin) has no trans fatty acids in its lipid composition, and the skinless breast has a content of 0.03% in 100 g of edible parts (OLIVO et al., 2006), which represents an insignificant portion of the lipid content of the final product.

Therefore, the presence of trans fatty acids in the final product can be attributed to the hydrogenated vegetable fat used in the industrial pre-frying since at this stage the breaded chicken steak loses moisture and fat aggregated to its composition. Therefore, brands that claim to be trans-fat free probably use hydrogenous fats with low or no trans fatty acids at the pre-frying stage; however, the lack of control of the parameters time and frying temperature of, with the inappropriate replacement of fat in industrial deep fryers, may trigger the process of trans-fatty acid formation, as observed by Sanibal and Mancini-Filho (2004).

However, if the product is prepared by frying, trans fatty acids from the oil used in the food preparation may be incorporated (SANIBAL; MANCINI-FILHO, 2002).

The high standard deviation values observed in Table 2 for fatty acids in brands B, C, D, and E result from the differences in lipid composition within the same brand analyzed lots. Those variations indicate that different raw materials were used in the manufacture of the same brand product.

3.3 Comparison between analysis results and food label nutrition facts

Figure 2 compares the total lipid, trans fatty acids $(g^{-1}.100 g^{-1})$, and the energetic values $(kcal^{-1}.100 g^{-1})$ from breaded chicken steak labels and the analysis results.

According to the Technical Regulation of Portions of Packaged Food for the Purpose of Nutritional Labeling (BRASIL, 2003b), preparations of meat with flour or breaded meat must present 130 g per serving with a tolerance range of \pm 30% for the claim regarding the nutritional value in 1 serving of the product. The portions evaluated were 116, 125, 120, 125, and 130 g for brands A, B, C, D, and E, respectively.

Brands B and C claimed trans free in disagreement with the analysis results, which exceeded the trans free regulation limits in effect in Brasil (2003a).

A major variation in protein contents was not observed. Nevertheless, most brands underestimated their lipid contents, and brand E presented the highest difference from its label claim. The pronounced difference between the values of lipids in the label and those obtained in the analysis is mainly related to the pre-industrial frying process, and the level of absorption of product's hydrogenated vegetable fat is generally related to the quality of the breaded flour, time and temperature of frying, and level of hydrogenated vegetable fat used in the industrial fryer. An inadequate control of one of those parameters can result in a final product with a higher content of lipids than expected. To a lesser extent, another factor that may be responsible for the observed differences is the proportion of raw materials such as the use of more chicken skin than the other ingredients, which may result in a higher concentration of lipids.

A substantial difference in energetic values in brands B, D, and E was observed due to the high lipid contents, which contribute significantly to the energetic value.

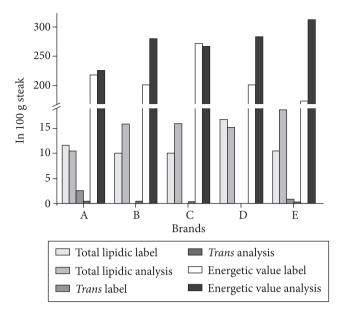


Figure 2. Comparison between label and analysis results values of total lipid, trans fatty acid $(g.100g^{-1})$ and energetic value (Kcal.100g⁻¹) of breaded chicken steak.

Research involving food labels can inform on possible divergences between the label nutrition facts and actual food analysis results.

Several comparative studies in the literature, Demiate, Konkel and Pedroso (2001), which evaluated milk fudge and found nonconformities with protein and starch contents, Lobanco (2007) analyzed several industrial products and observed that the food label nutrition facts diverged from the laboratory results. Marins, Jacab and Peres (2008) demonstrated the dissatisfaction of a large number of consumers regarding food label information, which was often considered wrong or even incomplete (TANNUS et al., 2001). Thus, it is necessary a more strict control of such information by the food industry.

4 Conclusions

The studied breaded chicken steak brands presented a large difference in raw material fatty acid composition. Among the analyzed brands, one brand presented smaller protein content than the recommended by regulations and a higher lipid content, resulting in high energetic values. The high n-6/n-3 ratios of all samples indicate that these products are inappropriate for a healthy diet. In the comparison of laboratory results and food label information, the greatest differences were found in the total lipid content, trans fatty acids and energetic values.

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