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The effect of heat treatment on the cholesterol oxides, cholesterol, total lipid and fatty acid contents of processed meat products

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Abstract

The effects of heat treatment on the formation of cholesterol oxides and on alterations of fatty acid composition were investigated in processed meat products. Meatballs (beef), hamburger (beef and Chester), sausage (pork, chicken and Chester) and frankfurter (mixed meat, chicken and Chester) were analysed. There was no cholesterol oxide formation caused by heat treatment of the samples analysed. The fatty acid compositions, calculated as g/100 g sample, showed alterations only between the raw and grilled beef hamburger. Only the cholesterol levels were significantly changed when comparing the raw and grilled pork sausages and the raw and grilled Chester hamburger, the values being lower in the grilled samples. Also, the total lipid contents of grilled beef hamburgers were lower than the values.

Keywords: Cholesterol oxides; Cholesterol; Fatty acid; Effect of heat; Processed meat products

1. Introduction

During the past two decades, the relationship between diet and health has been widely studied and increasing numbers of consumers have been encouraged to improve their eating habits. Moreover, fat consumption, particularly that of saturated fat, is still considered to be excessive. According to the American Heart Association (2004), the daily ingestion of lipids for individuals with normal blood cholesterol levels, should be no more than 30% of the total calorie intake, that of saturated fat no more than 10% of the total calorie intake and cholesterol intake below 300 mg/day.

Processed meat products, such as sausage, frankfurter, salami, hamburger and meatballs, are highly appreciated by the population. However, in general, they are high cholesterol, total lipid and saturated fatty acid. In contrast, chicken products have relatively abundant polyunsaturated fatty acids, including the key *n*3 types. Lipids play an important role in food product quality, making them more desirable by improving the organoleptic properties of flavour, colour and texture. In addition, they confer nutritive value on the product, constituting a source of metabolic energy, essential fatty acids and fat-soluble vitamins. On the other hand, the lipid components are susceptible to attack by molecular oxygen, resulting in lipid oxidation with the generation of cholesterol oxides and alteration of fatty acids.

Some studies have evaluated the effect of heat treatment of meat and processed meat products and on the formation of cholesterol oxides, suggesting that time and temperature are determinant factors in this process, directly influencing the rate of oxidation (Chen, Chiu, & Chen, 1994; Echarte, Ansorena, & Astiasaran, 2001; Kesava-Rao, Kowale, Babu, & Bisht, 1996; Pie, Spahis, & Seillan, 1991). The cholesterol oxides are considered more prejudicial than cholesterol itself in the formation

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of arteriosclerotic plaque and as mutagenic, carcinogenic and cytotoxic agents (Guardiola, Codony, Addis, Rafecas, & Boatella, 1996; Kubow, 1990; Sevanian & Peterson, 1986).

The fatty acid composition of foods can also be altered by auto-oxidation, especially affecting foods containing unsaturated fatty acids (Li, Oshima, Shozen, Ushio, & Koizumi, 1994). Echarte et al. (2001) found modifications in the fatty acid profile of pork loin fried in sunflower oil, significantly increasing the polyunsaturated/saturated fatty acid ratio. This increase is interesting from the nutritional point of view since the British Department of Health (1994) recommended a minimum polyunsaturated/saturated fatty acid ratio of 0.45 for the diet as a whole. Currently, nutritionists are focussing on the type of polyunsaturated fatty acid and the *n*6/*n*3 balance in the diet. An insufficient *n*3 fatty acid intake negatively influences health and the ideal *n*6/*n*3 ratio is about 6:1 (Wijendran & Hayes, 2004).

Thus the objective of this study was to verify the effect of heat treatment on the formation of cholesterol oxides and alterations in the fatty acid composition of processed beef, pork, mixed meat, chicken and Chester meat products. These processed meat products are commercialised in Brazil and the heat treatment reproduced the traditional way of heating or cooking them.

2. Materials and methods

2.1. Sample preparation

Meatballs (beef), hamburger (beef and Chester), sausage (pork, chicken and Chester) and frankfurter (mixed meat, chicken and Chester) were bought in supermarkets in Campinas, São Paulo, Brazil and analysed. Chester is a new chicken species, in which 70% of the meat is concentrated in the breast and thighs. It was developed by Perdigão Industrial, a Brazilian company commercialising chicken meat and products. Mixed meat was called mixed because the meat composition consisted of beef, pork and mechanically deboned poultry meat. Table 1 shows the basic composition of these meat products.

Three batches of each product with different expiry dates were analysed. Each batch consisted of six packages of 500 g each with 12 units, except for the meatballs that had 20 units. Three packages of each product were analysed raw and three cooked.

The processed meat products were cooked according to the instructions on the packing, except for the frankfurter. The frozen meatballs were placed in a lightly greased (soybean oil) aluminium baking tray and cooked in a conventional gas oven pre-heated to 250 °C for 20 min. After 10 min, the oven temperature was reduced to 203 °C and, at the end of the 20 min, it was 208 °C. The temperature at the centre of the meatballs was determined before removing them from the oven, and was shown to be 92 ± 1 °C.

The frozen hamburgers were grilled on a plate at 165 °C, each side being grilled for 4 min. The final internal temperature was 98 ± 1 °C.

The pork sausages were grilled at 165 °C for 30 min, until the internal temperature reached 94 ± 1 °C. The chicken and Chester sausages were cooked in a conventional gas oven pre-heated to 240 °C and the sausages were baked for 25 min. After 10 min, the oven temperature was reduced to 205 °C and, at the end of the 25 min, the internal temperature of the sausages was 95 ± 1 °C.

The frankfurters were cooked in distilled water at 98 °C for 10 min, reaching a final internal temperature of 96 ± 1 °C.

Table 1
Basic composition of processed meat products

Products	Ingredients
Meatballs	
Beef	Beef, hydrogenated fat, breadcrumbs, vegetable protein, salt, natural condiments, monosodium glutamate and sodium erythorbate
Hamburger	
Beef	Beef, hydrogenated fat, vegetable protein, salt, natural condiments, monosodium glutamate and sodium erythorbate
Chester	Chester meat, vegetable protein, monosodium glutamate, spices and sodium erythorbate
Sausage	
Pork	Pork, bacon, pork fat, salt, soy protein, natural condiments, sodium nitrate and nitrite and sodium erythorbate
Chicken	Chicken meat, soy protein, salt, natural condiments, sodium nitrite, monosodium glutamate and sodium erythorbate
Chester	Chester breast, bacon, salt, starch, spices, sodium nitrite and nitrate and sodium erythorbate
Frankfurters	
Mixed meat	Mechanically deboned chicken meat, pork, beef, pork/chicken skin, bacon, pork giblets, vegetable protein, starch, salt, spices, sodium nitrate and nitrite and sodium erythorbate
Chicken	Chicken meat, mechanically deboned chicken meat, chicken fat, soy protein, salt, starch (max. 2%), natural condiments, monosodium glutamate, sodium nitrite and nitrate and sodium erythorbate
Chester	Chester meat, mechanically deboned Chester meat, vegetable protein, glucose, salt, starch (max. 2%), natural condiments, sodium nitrate and nitrite and sodium erythorbate

The temperatures were monitored by contact, using a calibrated Kane-Mav model Type K thermocouple.

The products were bought and analysed immediately and again after heat preparation. The processed products were ground and homogenised in a multiprocessor to obtain a homogeneous mass.

2.2. Methods

2.2.1. Extraction and chromatogrphy

The lipids were extracted according to Folch, Less, and Stanley (1957), the moisture content being determined according to AOAC (1990) and the apparent retention factor according to Murphy, Criner, and Gray (1975).

Aliquots of the lipid extracts were taken for the determination of total lipids by weighing after evaporation of the solvent, cholesterol and cholesterol oxides, by high performance liquid chromatography (HPLC), and the fatty acid composition by gas chromatography (GC).

2.2.2. Simultaneous determination of cholesterol and cholesterol oxides by HPLC

50 ml of the lipid extracts, content between 3 and 8 g of lipid, were dried in a rotary evaporator, saponified in the cold (10 ml of 1 N KOH in methanol for 18 h at 20 °C) and the non-saponifiable matter extracted with deperoxided diethyl ether (Baggio & Bragagnolo, 2004; Sander, Addis, Park, & Smith, 1989). The diethyl ether extract was dried in a vacuum evaporator and freed of solvent by using a nitrogen flush before dissolving in 2 ml of mobile phase and injecting into the HPLC (Baggio, Miguel, & Bragagnolo, 2005).

For HPLC, a Shimadzu chromatograph was used, equipped with a quaternary solvent delivery system (LC-10ATVP), rheodyne injector with a 20 μl loop, photodiode array (SPD-M10AVP) and refractive index (RID-10A) detectors, oven-heated column (CTO-10ASVP) and software (CLASS-LC 10). The analytical column was a Nova Pak CN HP, 300 × 3.9 mm column, 4 μm (Waters, USA), preceded by a Hypersil BDS CN 7.5 × 4.6 mm, 5 μm guard column. The column temperature was 32 °C. The mobile phase consisted of *n*-hexane/isopropanol (96 + 4) at a flow rate of 1.0 ml/min. Absorption spectra were taken from 200 to 400 nm, and the chromatograms at 210 nm.

Cholesterol, cholesta-3,5-dien-7-one, 20α-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, α- and β-epoxycholestrol and 7β-hydroxycholesterol were purchased from Sigma Chemical Company (St. Louis, USA). 7α-Hydroxycholesterol was obtained from Steraloids Inc. (Wilton, USA). HPLC grade *n*-hexane and isopropanol were obtained from Mscience (Darmstadt, Germany) and all other analytical grade solvents were from Merck (Darmstadt, Germany). The HPLC solvents were filtered through a 0.22 μm membrane filter Millipore (USA) under vacuum prior to use.

Quantification was done by external standardisation, with a concentration range from 0.5 to 2.22 mg/ml for cholesterol and from 0.5 to 64.0 μg/ml for the cholesterol oxides. Cholesterol and α- and β-epoxycholesterol were quantified using a refractive index detector, the cholesterol because it is better separated from interfering substances in this case and α- and β-epoxycholesterol because they do not absorb ultraviolet light. The other oxides were quantified using the photodiode array detector. The detection limits were 0.14 μg/g of the sample for 20α-hydroxycholesterol and 25-hydroxycholesterol, 0.12 μg/g of the sample for α- and β-epoxycholestrol and 7α- and 7β-hydroxycholesterol and 0.09 μg/g of the sample for 7-ketocholesterol and cholesta-3, 5-dien-7-one.

Identification of cholesterol and its oxides was performed by comparison of the retention times of the samples with those of the standards, co-chromatography and the characteristics of the absorption spectrum. Confirmation of the identity was carried out using a gas chromatograph–mass spectrometer (Baggio, Vicente, & Bragagnolo, 2002).

2.2.3. Determination of the fatty acid composition

One hundred milligrams of lipid were esterified with a solution of ammonium chloride and sulphuric acid in methanol (Hartman & Lago, 1973). Fatty acid methyl esters were separated in a gas chromatograph (Konik, model HRCG 4000A) equipped with a split injector (75:1), fused silica capillary column (50 m × 0.25 mm i.d., 0.20 μm film thickness of polyethylene glycol) (CP-SIL 88, Cromapak, Netherlands), flame ionisation detector and workstation (Borwin, France). The initial temperature of the column was 180 °C for 2 min and it was programmed to rise to 225 °C at 5 °C/min, the injector temperature was set at 270 °C and the detector temperature at 300 °C. The carrier-gas was hydrogen at a flow rate of 0.5 ml/min and nitrogen was used as the make-up gas at 30 ml/min. The fatty acids were identified by comparison of the retention times of the sample with those of the standards and by spiking. A total of 37 saturated, monounsaturated and polyunsaturated fatty acid standards (Sulpeco^{TM} 37 FAME Mix 47885-U, USA) were used to verify the identity and the accuracy of the method. Quantification was done as area percentages.

2.3. Statistical analysis

The results for cholesterol, total lipid and fatty acids were submitted to an analysis of variance (ANOVA). Tukey's test was used to compare the means at a 5% significance level.

3. Results and discussion

3.1. The effect of heat treatment on the total lipid and cholesterol in processed meat products

Table 2 shows the results for the determinations of moisture, cholesterol and total lipids in the raw and heat-prepared processed meat products.

On a wet weight basis, the cholesterol and total lipid contents of the frankfurters (mixed meat, chicken and Chester) were lower in the cooked samples due to water absorption during cooking, diluting the constituents. On the other hand, in the other samples, these contents were always higher in the baked and grilled samples than in the raw ones, due to the loss of water during the heat treatment, consequently increasing the concentration of these components.

On a dry weight basis, only the total lipid contents showed significant ($p < 0.05$) differences between the raw and grilled beef hamburger, the grilled hamburger showing lower results. In the remaining samples, the total lipid contents were always lower in the baked, grilled and cooked samples, although not significantly ($p > 0.05$) different. The apparent retention (calculation based on the component's content in the moisture-free raw and cooked foods, i.e. dry weight basis) of total lipids varied from 79% in grilled beef hamburger to 99% in cooked mixed meat and chicken frankfurters.

On a dry weight basis, the levels of cholesterol only showed significant ($p < 0.05$) differences between the raw and grilled pork sausages and between the raw and grilled Chester hamburger, being lower in the grilled samples. The cholesterol contents were always higher in the raw samples, although not significantly ($p > 0.05$) different. The apparent retention (AR) of cholesterol varied from 85% for the grilled beef hamburger to 99% in the cooked mixed meat frankfurters. Badiani et al., 2002 and Bragagnolo and Rodriguez-Amaya (2003) found higher values for AR for the cholesterol and total lipids in beef, but no data on their retention in meat products have been found.

No cholesterol oxides were found in any of the processed meat products, either in the raw or in the heat treatment samples. In this study, the heat preparation varied from 8 to 30 min, the times normally used to cook the products under analysis. As declared on the label (Table 1), all the processed meat products analysed contained the antioxidant sodium erythorbate (INS 360), spice and natural condiments in their composition, which probably protected the cholesterol against oxidation. According to Tai, Chen, and Chen (1999), the use of antioxidants in the formulation and appropriate packaging materials, presenting physical barriers against air and light, can impede the formation of cholesterol oxidation products. In addition, spices and natural condiments are natural antioxidants and their antioxidant

Table 2
Moisture (g/100 g), cholesterol (mg/100 g) and total lipid (g/100 g) contents of the raw and cooked processed meat products

Processed meat products	Moisture	Cholesterol		Total lipid	
	$M \pm SD$[a]	Wet basis $M \pm SD$[a]	Dry basis $M \pm SD$[a]	Wet basis $M \pm SD$[a]	Dry basis $M \pm SD$[a]
Meatballs					
Raw beef	63.5 ± 0.6a	25.7 ± 0.4b	70.4 ± 1.2a	9.8 ± 0.8a	26.9 ± 2.0a
Baked beef	58.6 ± 0.5b	27.8 ± 0.6a	67.0 ± 2.1a	10.2 ± 0.7a	24.5 ± 1.6a
Hamburger					
Raw beef	66.0 ± 2.0a	30.0 ± 0.1b	89.7 ± 6.5a	10.9 ± 1.6a	32.2 ± 3.6a
Grilled beef	55.0 ± 5.0b	34.4 ± 3.1a	76.5 ± 11.4a	11.6 ± 1.6a	25.5 ± 1.1b
Raw Chester	68.7 ± 0.2a	31.0 ± 1.3a	99.0 ± 4.0a	9.7 ± 0.9b	31.0 ± 3.2a
Grilled Chester	59.0 ± 1.4b	36.4 ± 0.7a	88.8 ± 2.1b	11.3 ± 1.0a	27.5 ± 2.1a
Sausage					
Raw pork	55.6 ± 2.3a	36.7 ± 0.3b	82.8 ± 4.7a	25.9 ± 3.0b	58.1 ± 4.6a
Grilled pork	37.0 ± 0.5b	45.8 ± 2.2a	72.7 ± 3.5b	31.9 ± 1.5a	50.7 ± 2.8a
Raw chicken	71.2 ± 1.0a	45.0 ± 1.8a	156.5 ± 9.0a	11.4 ± 0.7a	39.7 ± 3.3a
Baked chicken	66.6 ± 1.2b	46.3 ± 0.9a	138.8 ± 3.4a	12.3 ± 0.7a	36.8 ± 2.5a
Raw Chester	65.5 ± 0.4a	40.4 ± 1.3a	117.1 ± 4.6a	13.5 ± 0.1b	39.1 ± 0.5a
Baked Chester	61.9 ± 0.4b	42.4 ± 2.6a	111.5 ± 8.2a	14.6 ± 0.4a	38.4 ± 1.0a
Frankfurter					
Raw mixed	60.3 ± 0.8b	45.9 ± 11.4a	115.3 ± 30.1a	15.0 ± 0.5a	37.8 ± 2.2a
Cooked mixed	61.9 ± 1.5a	43.7 ± 11.4a	113.8 ± 29.2a	14.2 ± 0.3b	37.3 ± 1.8a
Raw chicken	61.4 ± 0.9b	61.9 ± 3.5a	160.7 ± 12.5a	13.7 ± 0.5a	35.5 ± 1.1a
Cooked chicken	62.6 ± 0.6a	58.2 ± 1.7b	155.3 ± 5.5a	13.2 ± 0.5a	35.2 ± 1.2a
Raw Chester	67.9 ± 0.8a	63.1 ± 4.2a	196.5 ± 12.8a	8.4 ± 0.8a	26.1 ± 2.0a
Cooked Chester	68.4 ± 0.9a	60.6 ± 4.8a	191.6 ± 13.4a	7.9 ± 0.6a	25.1 ± 1.6a

Values in the same column with the same letter do not present significant differences between the raw and cooked samples at the 5% level.
[a] Mean and standard deviation of three samples in duplicate.

properties in food have long been recognised (Chipault, Mizuno, Hawkins, & Lundberg, 1952). On the other hand, there are great discrepancies in the results for cholesterol oxides in foods found in the literature, on account of the considerable variation in methodologies, many of which lead to the formation of artefacts and quantification errors due to the presence of interfering substances (McCluskey & Devery, 1993; Park, Guardiola, Park, & Addis, 1996). During our analytical procedures, various measures were taken to impede the formation of artefacts, such as avoiding the incidence of light on the samples during extraction, carrying out saponification in an inert atmosphere (N_2), in the cold (20 °C) and in the dark and the use of peroxide-free solvents. Thus it can be assumed that the use of such precautions during the analysis, allied to the use of antioxidants in the formulation, adequate storage and a short period of heating of the processed meat products, avoided cholesterol oxidation.

Park and Addis (1985) also failed to find cholesterol oxides in hamburger, jerked beef and liver sausage. Rodriguez-Estrada, Penazzi, Caboni, Bertacco, and Lercker (1997) showed a decrease in the concentration of 7-ketocholesterol when hamburgers were submitted to different heat treatments. Torres, Pearson, Gray, and Ku (1989) showed that the concentrations of total cholesterol oxides in jerked beef were 2.5 times lower in samples prepared with refined salt and BHA/BHT than in those prepared with refined salt without the addition of BHA/BHT. Chen et al. (1994) observed that the cholesterol oxide content of bacon increased with heating time. The 7-ketocholesterol and cholesta-4, 6-dien-3-one levels increased to a maximum after 200 h, whilst the concentrations of α-epoxycholesterol, β-epoxycholesterol and 7β-hydroxycholesterol increased during the first 100 h and then decreased. Cholestanetriol was only detected after 20 h of heating of bacon and its content then increased, reaching a maximum after 200 h; 25-hydroxycholesterol was not found.

In contrast to what was observed in this study and in that of Rodriguez-Estrada et al. (1997), other studies, which also reproduced the traditional ways of preparing some foods, showed the production of cholesterol oxides during heat preparation, even when using a reduced heating time. However, the authors failed to report whether these samples contained antioxidant or not. Higley, Taylor, Herian, and Lee (1986) found high levels of 7α-hydroxycholesterol in cooked frankfurters (1640 μg/g), cholestanetriol in cooked frankfurters (1335 μg/g) and raw hamburger (1298 μg/g) and 22-hydroxycholesterol in cooked frankfurters (1869 μg/g) and raw hamburger (210 μg/g). Paniangvait, King, Jones, and German (1995) suggested that these workers may have overestimated the cholesterol oxide levels, probably due to the analytical methodology used. Larkeson, Dutta, and Hansson (2000) showed increases in the total cholesterol oxide contents of meatballs (50% beef + 50% pork) and beef hamburgers when fried at 150–160 °C. The total cholesterol oxides contents (7α- and 7β-hydroxycholesterol, 7-ketocholesterol, α- and β-epoxycholesterol and cholestanetriol) of the raw and fried meatballs, varied from 3.3 to 10.4 μg/g of lipid and of the hamburgers, from 5.5 to 6.7 μg/g of lipid. Osada, Hoshina, Nakamura, and Sugano (2000) found 7β-hydroxycholesterol + β-epoxycholesterol, α-epoxycholesterol, cholestanetriol and 7-ketocholesterol, respectively, in retort hamburger (beef) (10.92, 5.27, 3.41 and 9.09 μg/g), non-heated hamburger (beef) (8.06, 7.12, 2.63 and 9.73 μg/g) and sausage (pork) (4.74, 3.83, 3.68 and 7.29 μg/g).

3.2. Comparison of the total lipid and cholesterol levels in processed meat products

Considering the cooked processed meat products, the lowest level of total lipid content was found in the Chester frankfurters (7.9 g/100 g) and the highest level in the pork sausages (31.9 g/100 g). The results for the beef meatballs (10.2 g/100 g) and hamburgers (11.6 g/100 g for beef and 11.3 g/100 g for Chester) were very similar, although the beef products contained added hydrogenated fat. The pork sausages showed higher lipid levels than the chicken (12.3 g/100 g) and Chester (14.6 g/100 g) sausages, probably due to the presence of bacon and pork fat in the formulation. Of the frankfurters, the lowest lipid level was found in the Chester frankfurters and the highest in the chicken (13.2 g/100 g) and mixed meat (14.2 g/100 g) frankfurters. The mixed meat frankfurters contained pork and chicken skin, bacon and pork giblets in the formulation and the chicken frankfurters, chicken fat, which were responsible for the higher lipid levels.

Of the products analysed, the highest cholesterol level was found in the Chester frankfurters (60.6 mg/100 g) and the lowest in the beef meatballs (27.8 mg/100 g). In general, the products containing poultry meat presented higher levels than the other meat products. These results agreed with those reported by Bragagnolo and Rodriguez-Amaya (1995) who found more cholesterol in chicken than in pork and beef meat.

3.3. The effect of heat treatment on the fatty acid composition

Tables 3–5 present the fatty acid compositions (% of total identified) in raw and heat-prepared samples of beef meatballs and beef and Chester hamburgers (Table 3), pork, chicken and Chester sausages (Table 4) and mixed meat, chicken and Chester frankfurter (Table 5).

When the fatty acid contents were calculated in relation to the total lipid content of the samples, on a dry weight basis, it was shown that there was no significant

Table 3
Fatty acid compositions (% of total identified) in raw and cooked meatballs and hamburger

Fatty acids	Meatballs		Hamburger			
	Raw beef $M \pm SD$[a]	Baked beef $M \pm SD$[a]	Raw beef $M \pm SD$[a]	Grilled beef $M \pm SD$[a]	Raw Chester $M \pm SD$[a]	Grilled Chester $M \pm SD$[a]
C14:0	2.1 ± 0.1a	2.1 ± 0.1a	2.4 ± 0.2a	2.4 ± 0.3a	0.7 ± 0.0a	0.7 ± 0.0a
C15:0	1.1 ± 0.1a	1.1 ± 0.1a	1.0 ± 0.2a	1.0 ± 0.2a	0.4 ± 0.0a	0.4 ± 0.0a
C16:0	23.5 ± 0.5a	23.4 ± 0.3a	22.8 ± 0.3a	22.7 ± 0.2a	24.8 ± 0.1a	24.8 ± 0.5a
C17:0	0.8 ± 0.1a	0.8 ± 0.1a	1.0 ± 0.0a	1.0 ± 0.1a	0.2 ± 0.0a	0.2 ± 0.0a
C18:0	12.6 ± 0.3a	12.6 ± 0.9a	15.1 ± 0.5a	15.0 ± 0.5a	6.1 ± 0.1a	6.0 ± 0.1a
C20:0	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a
C21:0	tr	tr	0.4 ± 0.0a	0.4 ± 0.0a	tr	tr
C22:0	tr	tr	tr	tr	0.2 ± 0.0a	0.2 ± 0.0a
C24:0	tr	tr	tr	tr	0.1 ± 0.0a	0.1 ± 0.0a
C16:1*n*7	2.9 ± 0.3a	2.9 ± 0.4a	3.5 ± 0.4a	3.5 ± 0.5a	5.7 ± 0.2a	5.7 ± 0.2a
C17:1*n*7	0.5 ± 0.1a	0.5 ± 0.1a	0.5 ± 0.1a	0.5 ± 0.1a	0.3 ± 0.0a	0.3 ± 0.0a
C18:1*n*9 *trans*	5.9 ± 0.4a	5.9 ± 0.7a	3.6 ± 0.1a	3.7 ± 0.0a	0.2 ± 0.1a	0.2 ± 0.0a
C18:1*n*9	34.6 ± 1.5a	34.5 ± 2.9a	36.2 ± 0.8a	36.1 ± 1.9a	38.2 ± 0.1a	38.2 ± 0.2a
C20:1*n*11	0.6 ± 0.3a	0.7 ± 0.1a	0.4 ± 0.1a	0.4 ± 0.3a	0.3 ± 0.0a	0.3 ± 0.0a
C18:2*n*6 *trans*[b]	1.1 0.4a	1.1 ± 0.2a	1.2 ± 0.1a	1.2 ± 0.2a	tr	tr
C18:2*n*6	13.0 ± 1.1a	13.3 ± 0.8a	11.0 ± 1.9a	11.3 ± 2.2a	21.0 ± 0.1a	20.9 ± 0.2a
C18:3*n*6	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
C18:3*n*3	0.8 ± 0.1a	0.8 ± 0.1a	0.5 ± 0.1a	0.5 ± 0.2a	1.1 ± 0.0a	1.1 ± 0.0a
C20:2*n*6	tr	tr	tr	tr	0.1 ± 0.0a	0.1 ± 0.0a
C20:4*n*6	0.2 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.1a	0.2 ± 0.0a	0.5 ± 0.0a	0.5 ± 0.1a
SAT	47a	47a	48a	48a	33a	33a
MONO	39a	39a	40a	40a	44a	44a
PUFA	14a	14a	12a	12a	23a	23a
Total *n*3	0.8	0.8	0.5	0.5	1.1	1.1
Total *n*6	13.3	13.6	11.3	11.6	21.8	21.7
*n*6/*n*3	16.6	17.0	22.6	23.2	19.8	19.7
PUFA/SAT	0.3	0.3	0.25	0.25	0.7	0.7

Values in the same line with the same letter do not present significant differences between the raw and cooked samples at the 5% level.
[a] Mean and standard deviation of three samples in duplicate.
[b] (9*c*, 12*t* + 9*t*, 12*c*), tr = traces (<0.1% of total identified), SAT = saturated, MONO = monounsaturated, PUFA = polyunsaturated.

($p > 0.05$) difference between the raw and heat-treated samples, with the exception of the beef hamburgers, which showed significantly lower levels in the grilled samples. Rodriguez-Estrada et al. (1997), also found no significant difference in the percentages of saturated and monounsaturated fatty acids between raw and cooked beef hamburger samples, although there were significant differences for the polyunsaturated fatty acids. On the other hand, Echarte et al. (2001) found modifications in the fatty acid profile of pork loin fried in sunflower oil, significantly increasing the polyunsaturated/saturated fatty acid ratio.

3.4. Comparison of fatty acids in processed meat products

In decreasing order, the main fatty acids found in the samples of sausage (pork, chicken and Chester), frankfurter (mixed meat, chicken and Chester) and Chester hamburger, were: C18:1*n*9, C16:0, C18:2*n*6, C18:0 and C16:1*n*7, whilst the main fatty acids in the samples of beef meatballs and beef hamburger were: C18:1*n*9, C16:0, C18:0, C18:2*n*6 and C18:1*n*9 *trans*. Considering the total lipid content found in the samples, the fatty acids C18:1*n*9 (23.8 g/100 g sample), C16:0 (14.9 g/100 g sample) and C18:0 (6.6 g/100 g sample) were found in greater amounts in the pork sausages, C18:2*n*6 (9.4 g/100 g sample) in the mixed meat and chicken frankfurters, C16:1*n*7 (2 g/100 g sample) in the chicken sausages and C18:1*n*9 *trans* (1.6 g/100 g sample) in beef meatballs.

The *trans* isomer fatty acid C18:1*n*9 was found in all the samples analysed, varying from 0.02 g/100 g in the Chester frankfurters to 1.6 g/100 g in the beef meatballs. The *trans* isomer C18:2*n*6 was found only in beef meatballs and hamburger (0.3 g/100 g sample). The high levels of *trans* fatty acid C18:1*n*9 found in the meatball and hamburger samples can be attributed to the presence of hydrogenated fat in the formulations (Table 1). The *trans* isomers are natural components of animal origin fats, being formed by bio-hydrogenation, by the action of enzymes from the microbial flora present in the rumen of polygastric animals, including isomerases and hydrogenases (Smith, Dunkley, Franke, & Daiiking, 1978). In meat from non-ruminant animals, the amount of *trans* fatty acids is usually low and depends on the presence of *trans* fatty acids in the feed (Aro, Antoine, Pizzoferrato, Reykdal, & Van Popel, 1998).

Table 4
Fatty acid compositions (% of total identified) in raw and cooked sausages

Fatty acids	Raw pork	Grilled pork	Raw chicken	Baked chicken	Raw Chester	Baked Chester
	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]
C14:0	1.6 ± 0.0a	1.4 ± 0.1a	0.7 ± 0.1a	0.7 ± 0.1a	1.1 ± 0.1a	1.2 ± 0.0a
C15:0	0.1 ± 0.0a	0.2 ± 0.0a	0.4 ± 0.0a	0.5 ± 0.0a	0.4 ± 0.0a	0.3 ± 0.0a
C16:0	25.6 ± 0.9a	25.0 ± 0.4a	24.8 ± 2.6a	24.9 ± 0.5a	23.8 ± 0.8a	24.2 ± 0.8a
C17:0	0.4 ± 0.1a	0.4 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.1a	0.5 ± 0.0a	0.5 ± 0.0a
C18:0	11.4 ± 0.7a	11.4 ± 0.8a	6.6 ± 0.2a	6.7 ± 0.1a	8.6 ± 0.4a	8.5 ± 0.4a
C20:0	0.2 ± 0.0a	0.2 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a
C22:0	tr	tr	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
C24:0	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
C16:1*n*7	2.5 ± 0.2a	2.5 ± 0.2a	5.1 ± 0.3a	5.3 ± 0.1a	3.8 ± 0.2a	3.8 ± 0.3a
C17:1*n*7	0.4 ± 0.1a	0.4 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.0a	0.5 ± 0.0a	0.5 ± 0.0a
C18:1*n*9 *trans*	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a
C18:1*n*9	41.0 1.1a	41.5 ± 1.5a	36.8 ± 0.6a	36.5 ± 0.6a	39.8 ± 0.4a	39.5 ± 0.7a
C20:1*n*11	0.7 ± 0.1a	0.7 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.0a	0.6 ± 0.1a	0.5 ± 0.1a
C18:2*n*6	14.4 ± 1.7a	14.5 ± 1.9a	22.3 ± 1.0a	21.9 ± 0.8a	18.2 ± 0.7a	18.2 ± 0.7a
C18:3*n*6	tr	tr	0.2 ± 0.0a	0.2 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a
C18:3*n*3	0.6 ± 0.1a	0.6 ± 0.1a	1.2 ± 0.1a	1.1 ± 0.1a	0.9 ± 0.1a	0.9 ± 0.1a
C20:2*n*6	0.6 ± 0.1a	0.6 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.5 ± 0.0a	0.5 ± 0.0a
C20:4*n*6	0.3 ± 0.0a	0.4 ± 0.0a	0.7 ± 0.1a	0.7 ± 0.1a	0.6 ± 0.0a	0.6 ± 0.0a
SAT	39a	39a	33a	34a	35a	36a
MONO	45a	45a	42a	42a	45a	44a
PUFA	16a	16a	25a	24a	20a	20a
Total *n*3	0.6	0.6	1.2	1.1	0.9	0.9
Total *n*6	15.3	15.5	23.3	22.9	19.3	19.3
*n*6/*n*3	25.5	25.8	19.4	20.8	21.4	21.4
PUFA/SAT	0.4	0.4	0.8	0.7	0.6	0.6

tr = traces (<0.1% of total identified). SAT = saturated, MONO = monounsaturated, PUFA = polyunsaturated.
Values in the same line with the same letter do not present significant differences between the raw and cooked samples at the 5% level.
[a] Mean and standard deviation of three samples in duplicate.

The samples of grilled pork sausage showed the highest amounts of saturated (19.8 g/100 g) and monounsaturated (22.8 g/100 g) fatty acids. These values were lowest in Chester frankfurter, being 8 and 10.3 g/100 g of saturated and monounsaturated fatty acids, respectively. The concentration of polyunsaturated fatty acids was lowest in grilled beef hamburgers (3.1 g/100 g) and highest in cooked chicken frankfurter (10.6 g/100 g), the fatty acid C18:2*n*6 being that found in greatest amounts. In the present study, the ratio of the polyunsaturated fatty acids to the saturated fatty acids was lowest in the beef hamburger (0.25) and highest in the chicken frankfurter (1.0). The only *n*3 series fatty acid found was C18:3*n*3, the greatest concentrations being found in chicken frankfurters (0.7 g/100 g) and lowest in grilled beef hamburger (0.1 g/100 g). The *n*6/*n*3 ratio varied from 13.7 in raw chicken frankfurters to 25.8 in grilled pork sausages.

Larkeson et al. (2000) obtained similar amounts of saturated fatty acids (48.7%), greater amounts of monounsaturated ones (47.8%) and lower amounts of the polyunsaturated ones (3.5%) than those found in this study for meatballs (50% beef + 50% pork) and raw and fried beef hamburger. Pereira, Tarley, Matsushita, and Souza (2000) found lower amounts of saturated fatty acids in common sausages (34.1%), greater amounts in chicken sausages (35.6%) and similar amounts in Chester sausages (34.6%). They also found greater percentages of monounsaturated fatty acids for the three types of sausage: common (50.2%), chicken (50.2%) and Chester (50.1%) and similar amounts of polyunsaturated fatty acids in common sausages (15.7%) and lower amounts in chicken (14.2%) and Chester (15.4%) sausages, when compared to the percentages found in the samples of raw sausage (pork, chicken and Chester) analysed in this study.

With respect to the nutritional aspects, it was observed that the processed meat products analysed in this study presented total lipid contents greater than 5%, not therefore being classified as low-fat foods (Food Advisory Committee, UK). The cholesterol content varied from 28 to 61 mg/100 g in the cooked products, below the maximum recommended value of 300 mg cholesterol/day (American Heart Association, 2004) and no cholesterol oxides were found. In the pork sausage, beef meatball and beef hamburger, the polyunsaturated fatty acid/saturated fatty acid ratio was below the minimum value of 0.45, as recommended by the British Department of Health (1994) for the whole diet, whilst the other samples were above this value. The *n*6/*n*3 ratio varied from 13.7 to 25.8, i.e. higher than the adequate *n*6/*n*3 ratio of ~6:1 (Wijendran & Hayes, 2004). These deficiencies should be compensated by other components of the diet.

Table 5
Fatty acid compositions (% of total identified) in raw and cooked frankfurters

Fatty acids	Raw mixed meat	Cooked mixed meat	Raw chicken	Cooked chicken	Raw Chester	Cooked Chester
	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]	$M \pm SD$[a]
C14:0	0.7 ± 0.0a	0.6 0.0a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a
C15:0	0.2 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.1a	0.3 0.0a	0.4 ± 0.0a	0.4 ± 0.0a
C16:0	25.4 ± 2.0a	23.6 ± 2.3a	22.8 ± 0.4a	23.3 ± 0.4a	23.5 ± 0.1a	23.5 0.5a
C17:0	0.3 ± 0.1a	0.3 ± 0.0a	0.4 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1a
C18:0	6.0 ± 0.2a	6.4 ± 0.2a	6.0 ± 0.0a	5.9 ± 0.2a	6.3 ± 0.2a	6.3 ± 0.3a
C20:0	0.2 ± 0.0a	0.2 ± 0.0a	tr	tr	tr	tr
C24:0	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
C16:1*n*7	4.7 ± 0.5a	4.5 ± 0.4a	4.4 ± 0.2a	4.7 ± 0.4a	4.7 ± 0.2a	4.7 ± 0.2a
C17:1*n*7	0.2 ± 0.1a	0.2 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a	0.4 ± 0.1a	0.3 ± 0.1a
C18:1*n*9 *trans*	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a
C18:1*n*9	35.2 ± 1.3a	35.8 ± 0.9a	35.2 ± 1.4a	35.0 ± 1.7a	35.9 ± 0.4a	35.8 ± 0.2a
C20:1*n*11	0.2 ± 0.1a	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a
C18:2*n*6	24.5 ± 1.2a	25.4 ± 1.1a	26.6 ± 2.0a	26.4 ± 1.9a	24.6 ± 0.3a	24.6 ± 0.5a
C18:3*n*6	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
C18:3*n*3	1.5 ± 0.1a	1.7 ± 0.2a	2.0 ± 0.4a	1.9 ± 0.3a	1.5 ± 0.0a	1.5 ± 0.1a
C20:2*n*6	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a
C20:4*n*6	0.4 ± 0.1a	0.5 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a	0.9 ± 0.0a	0.9 ± 0.1a
SAT	33a	31a	30a	31a	32a	32a
MONO	40a	41a	40a	40a	41a	41a
PUFA	27a	28a	30a	30a	27a	27a
Total *n*3	1.5	1.7	2.0	1.9	1.5	1.5
Total *n*6	25.3	26.2	27.5	27.3	25.8	25.9
*n*6/*n*3	16.9	15.4	13.7	14.4	17.2	17.3
PUFA/SAT	0.8	0.9	1.0	1.0	0.8	0.8

tr = traces (<0.1% of total identified). SAT = saturated, MONO = monounsaturated, PUFA = polyunsaturated.
Values in the same line with the same letter do not present significant differences between the raw and cooked samples at the 5% level.
[a] Mean and standard deviation of three samples in duplicate.

4. Conclusions

In the samples analysed, heat treatment did not lead to the formation of cholesterol oxides. Considering the total lipid contents on a dry weight basis, the fatty acid compositions only presented significant differences between the raw and grilled beef hamburger samples. The cholesterol contents were shown to be lower in the grilled samples than in the raw samples only for pork sausage and Chester hamburger. Similarly, the total lipid contents were lower in the grilled samples than in the raw samples only for the beef hamburger.

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