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Simultaneous determination of cholesterol oxides, cholesterol and fatty acids in processed turkey meat products

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Abstract

Cholesterol oxides, cholesterol, total lipids and fatty acids were determined in the lipid extracts from eight processed turkey meat products (blanquet, frankfurter, ham, meatball, smoked breast, smoked ham and roule). Cholesterol and cholesterol oxides were determined simultaneously by high performance liquid chromatography using a diode array detector at 210 nm and a refractive index detector. Only two cholesterol oxides were identified, 7-ketocholesterol (not detected up to 184 μ g/100 g) and β -epoxycholesterol (not detected up to 450 µg/100 g). With the exception of the meatballs, hamburger and frankfurters, all the turkey meat products showed less than 5% fat, and could thus be considered as low fat foods. The cholesterol content varied from 32 to 43 mg/100 g, the ratio of polyunsaturated: saturated fatty acids varied from 1.1 to 1.8 and the ratio of ω 6: ω 3 from 18 to 28. Trans fatty acids were present in significant amounts in both hamburger and meatball.

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Keywords: Processed turkey meat products; Cholesterol oxides; Cholesterol; Total lipids; Fatty acids

1. Introduction

World consumption of turkey meat increased by 10% from 1997 to 2002 (USDA, 2001), due to its alleged characteristic of presenting low levels of cholesterol, total lipids and saturated fatty acids. Apart from this, good acceptability due to its neutral taste and smooth texture is another important factor responsible for its growing place on the market. The United States and the European Union are the greatest consumers (USDA, 2001). However, processed turkey meat products contain considerable amounts of polyunsaturated fatty acids (PUFA), and undergo heat processing and disintegration of the meat during processing (Novelli et al., 1998), making them highly susceptible to oxida-

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tion. On the other hand, the PUFA are considered to be anticholesterolemic, although the beneficial effects of PUFA depend on the ratio of omega 6×60 to omega 3 (o3) fatty acids. The recommended value is 4.0 for the diet as a whole (Department of Health, 1994).

Although cholesterol is a relatively stable compound, it can be oxidized under harsh conditions. Oxidation of cholesterol in muscle foods can be influenced by many factors such as processing temperature, storage time, packaging conditions and lipid composition (Paniangvait, King, Jones, & German, 1995). Smith (1987) suggested that hydroperoxides of polyunsaturated fatty acids, formed during lipid oxidation, might be necessary to initiate cholesterol oxidation, and unsaturated fat could increase the oxidation of cholesterol synergistically.

Eight common oxysterols have been identified in foods: 25-hydroxycholesterol, cholestanetriol, a- and β -epoxycholesterol, 7α - and 7β -hydroxycholesterol, 7-ketocholesterol and cholesta-3,5-dien-7-one, which is

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actually an artifact derived from 7-ketocholesterol during isolation (Finocchiaro & Richardson, 1983). These compounds possibly contribute to the initiation of the formation of arteriosclerotic deposits, principally cholestanetriol and 25-hydroxycholesterol (Kubow, 1990; Kumar & Singhal, 1991). Furthermore, these compounds have received much attention due to their undesirable biological effects such as cytotoxic, mutagenic and carcinogenic effects (Guardiola, Codony, Addis, Rafecas, & Boatella, 1996; Kubow, 1990; Sevanian & Peterson, 1986) and the inhibition of 3-hydroxy-3 methylglutaryl coenzyme A reductase, (HMG-CoA reductase), activity (Guardiola et al., 1996).

Since human plasma cholesterol is dependent not only on the dietary cholesterol level but also on the fatty acid composition, the simultaneous determination of these food components and oxysterols was carried out in processed turkey meat products.

2. Materials and methods

2.1. Materials

Cholesterol, cholesta-3,5-dien-7-one, 20a-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, a and b-epoxycholestrol and 7b-hydroxycholesterol were

Basic composition of processed turkey meat products

purchased from Sigma Chemical Company (St. Louis, USA). 7a-Hydroxycholesterol was obtained from Steraloids Inc. (Wilton, USA). A total of 37 saturated, monounsaturated and polyunsaturated fatty acid standards were purchased from Sulpeco (USA). HPLC grade nhexane and 2-propanol were obtained from Mscience (Darmstadt, Germany) and all other solvents analytical grade were from Merck (Darmstadt, Germany). The HPLC solvents were filtered through a $0.22 \mu m$ membrane filter Millipore (USA) under vacuum prior to use. Standard reference material (SRM 1546, meat homogenate) was obtained from NIST (Gaithersburg, USA).

2.2. Sample preparation

Eight processed turkey products were examined, these being blanquet, frankfurter, ham, meatball, smoked chest, smoked ham and roule. Blanquet and roule are chunked and formed turkey meat products (breast for blanquet and drumstick for roule). The basic compositions of each product are listed in Table 1. The samples were packaged in vacuum packs. Five different batches of each sample were acquired from supermarkets in Campinas, State of São Paulo, Brazil. Each batch, consisting of three units, was taken at random. The samples were ground and blended in a multiprocessor. Samples of 50 g were taken for analysis, each being carried out in duplicate.

Table 1

Product	Ingredients					
Blanquet	White breast meat, turkey fat, water, soy protein, malt dextrin, cassava starch, salt, natural spices, sugar, sodium polyphosphate (INS 452i), carrageenan (INS 407), sodium nitrite (INS 250) and sodium erythorbate (INS 316)					
Ham	Dark meat from whole drumstick, salt, malt dextrin, soy protein, natural spice, sugar, carmine natural color (INS 120), sodium nitrite (INS 250), carrageenan (INS 407), sodium polyphosphate (INS 452i), monosodium glutamate (INS 621) and sodium erythorbate (INS 316)					
Hamburger	Dark meat from drumstick, white breast meat, water, soy protein, malt dextrin, hydrogenated vegetable oils, natural spices, salt, sodium lactate (INS 325), sodium polyphosphate (INS 452i), natural pepper aroma and monosodium glutamate (INS 621)					
Frankfurter	Turkey meat, mechanically deboned poultry meat, soy protein, glucose, salt, starch, natural color, natural spices, sodium nitrite (INS 250), sodium polyphosphate (INS 452i), monosodium glutamate (INS 621)					
Meatball	White breast meat, dark meat from drumstick, water, soy protein, malt dextrin, hydrogenated vegetable oils, wheat flour, natural spices, breadcrumbs, vegetable protein, salt and sodium erythorbate (INS 316)					
Roule	Dark meat from drumstick, water, cassava starch, salt, natural spices, soy protein, malt dextrin, natural pepper aroma, carrageenan (INS 407), sodium polyphosphate (INS 452i), sodium nitrite (INS 250) and sodium erythorbate (INS 316)					
Smoked breast	White meat from whole breast, sugar, salt, malt dextrin, soy protein, natural spice, carrageenan (INS 407), sodium nitrite (INS 250), sodium polyphosphate (INS 452i) and sodium erythorbate (INS 316)					
Smoked ham	Dark meat from whole drumstick, salt, malt dextrin, soy protein, natural spice, sugar, carmine natural color (INS 120), sodium nitrite (INS 250), carrageenan (INS 407), sodium polyphosphate (INS 452i), monosodium glutamate (INS 621) and sodium erythorbate (INS 316)					

2.3. Lipid extraction

The lipids were extracted according to Folch, Less, and Stanley (1957). From this extract, an aliquot of 10 mL was taken and the total lipid content determined gravimetrically. Another aliquot (50 mL) was taken for the determination of cholesterol and cholesterol oxides by high performance liquid chromatography (HPLC). A further aliquot, containing approximately 100 mg of lipid was taken, and the fatty acid composition determined by gas chromatography (GC).

2.4. Simultaneous determination of cholesterol and cholesterol oxides by high performance liquid chromatography

Aliquots of the lipid extracts (50 mL) were dried in a rotary evaporator, saponified in the cold (10 mL of 1 N KOH in methanol for 18 h at 20 $^{\circ}$ C) and the non-saponifiable matter extracted with deperoxided diethyl ether (Baggio, Vicente, & Bragagnolo, 2002; 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 & Bragagnolo, 2004; Baggio et al., 2002).

For HPLC, a Shimadzu chromatograph was used, equipped with a quaternary solvent delivery system (LC-10ATVP), rheodyne injector with a 20 μ L 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, 4 lm column (Waters, USA), preceded by a Hypersil BDS CN 7.5×4.6 mm, 5 µm guard column, and the column temperature was $32 \degree C$. The mobile phase consisted of 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.

Quantification was done by external standardization, with a concentration range from 0.5 to 2.22 mg/mL for cholesterol and from 0.5 to $64.0 \mu g/mL$ for the cholesterol oxides. Characteristic chromatograms of the cholesterol and cholesterol oxide standards and of the blanquet samples can be seen in [Figs. 1 and 2.](#page-3-0) Cholesterol and β -epoxycholesterol were quantified using a refractive index detector ([Fig. 2](#page-4-0)), the cholesterol because it is better separated from interfering substances in this case and the b-epoxycholesterol because it does not absorb ultraviolet light. 7-ketocholesterol was quantified using the photodiode array detector. As the cholesterol peak was poorly separated ([Fig. 2\(b\)](#page-4-0)) the accuracy of its quantification was evaluated using certified reference meat material (SRM 1546, NIST) and recovery. Although peaks 8 and 9 were poorly separated, this was the best possible separation under the established

chromatographic conditions, since total peak separation failed to occur even when the proportions of the mobile phase were altered. The order of chromatographic elution of cholesterol and its oxides and their chemical and common names are given in [Table 2](#page-4-0).

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 (GC–MS) Hewlett Packard 6890 GC and 5973 MS. The GC/MS conditions were as follows: HP-5MS (Palo Alto, USA) fused-silica capillary column $(30 \text{ m} \times 0.25 \text{ mm} \text{ i.d., film thickness})$ 0.25 mm); carrier gas of helium at 0.7 mL/min; splitless injection at 290 \degree C. The oven temperature program was as follows: 110 °C (2 min), increasing to 235 °C at 40 °C/ min (5 min) and then at 1 °C/min to 310 °C (5 min); interface temperature, 320 °C. The mass spectrometers were operated under electron ionization conditions with an electron energy of 70 eV. The samples and standards of cholesterol and its oxides were derivatized according to Schmarr, Gross, and Shibamoto (1996).

Since no certified reference material for cholesterol oxides was available, the accuracy of the method was evaluated through coefficients of variation, recovery and detection and quantification limits. The accuracy of the cholesterol method was evaluated using certified reference material (SRM 1546, NIST, meat homogenized). The cholesterol content of the certified reference material was found to be 69.0 ± 1.0 mg/100 g, compared to the certified value of 75.0 ± 7.0 mg/100 g, showing good precision of the applied methodology. Recovery was carried out by adding 10 mg of cholesterol and 40 μ g of 7-ketocholesterol and β -epoxycholesterol to 50 g of the smoked breast. The percent recoveries for cholesterol, 7-ketocholesterol and β -epoxycholesterol were $90.2 \pm 0.2\%$, $91.2 \pm 0.0\%$ and $93.9 \pm 0.0\%$, respectively. The values for the coefficient of variation between duplicates of the samples varied from 0.0 to 0.03% and from 0.0% to 0.04% for β -epoxycholesterol and 7-ketocholesterol, respectively, demonstrating the good precision of the method. The detection and quantification limits for cholesterol, b-epoxycholesterol and 7-ketocholesterol were $0.72 \text{ mg}/100 \text{ g}$ and $2.40 \text{ mg}/100 \text{ g}$, $12 \text{ µg}/100 \text{ g}$ and 40 μ g/100 g and 9 μ g/100 g and 30 μ g/100 g, respectively, calculated according to Chairman et al. (1983). Apart from this, during the analysis care was also taken to avoid the formation of artifacts and it appears this did not occur since when fresh chilled turkey meat was analyzed, no cholesterol oxides were found.

2.5. Determination of the fatty acid composition

The dried lipid extract was esterified with a solution of ammonium chloride and sulfuric acid in methanol

Fig. 1. Typical HPLC chromatogram of the cholesterol and cholesterol oxide standards. Nova Pak CN column (4 µm 300×3.9 mm) with hexane/ isopropanol (96+ 4) as mobile phase at 1 mL/min: (a) photodiode array detector (b) refractive index detector. Peaks: (1) cholesterol, (2) diene, (3) 20a-hydroxycholesterol, (4) 25-hydroxycholesterol, (5) a-epoxycholesterol*, (6) b-epoxycholesterol*, (7) 7-ketocholesterol, (8) 7b-hydroxycholesterol (9) 7α -hydroxycholesterol. $*$, Only using the refractive index detector.

(Hartman & Lago, 1973). Fatty acid methyl esters were separated on a gas chromatograph (Philips, PU 4550) equipped with a split injector (100:1), fused silica capillary column (50 m \times 0.25 mm i.d., 0.20 µm film thickness of polyethylene glycol) (CP-SIL 88, Cromapak, Netherlands), flame ionization detector and workstation (Borwin, France). The column temperature was 180 $^{\circ}$ C (isothermal), the injector temperature was set at 270 ^oC and the detector temperature at 300 ^oC. 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 (SulpecoTM 37 FAME Mix 47885-U, USA) were used to verify the identity and the accuracy of the method. Quantification was done by normalization and transformation of the area percentage into mg/100 g of edible portion, using the lipid conversion factor (F) . An *F*-value of 0.945 was used for the processed turkey meat products based on Holland et al. (1994).

2.6. Statistical analysis

To verify the differences among the processed turkey meat products, the results for cholesterol, total lipid and fatty acids were submitted to an analysis of variance (ANOVA) at the 5% level of confidence.

3. Results and discussion

3.1. Total lipids and cholesterol

[Table 3](#page-5-0) shows the results for cholesterol and total lipids found in the samples of turkey meat products. The cholesterol content showed no significant difference between the products, varying from 32 mg/100 g in the blanquet and smoked breast to 43 mg/100 g in the

Fig. 2. Typical HPLC chromatogram of the cholesterol and cholesterol oxides in the blanquet. Nova Pak CN column (4 µm 300×3.9 mm) with hexane/isopropanol (96+4) as mobile phase at 1 mL/min (a) photodiode array detector (b) refractive index detector. Peaks: (1) cholesterol, (6) βepoxycholesterol*, (7) 7-ketocholesterol. *, Only using the refractive index detector.

Table 2 List of cholesterol oxides and their common names

Order of chromatographic elution ^a	Systematic name	Common name	
	$Cholest-5-ene-3\beta-ol$	Cholesterol	
	Cholesta-4,6-dien-3-one	Diene	
	Cholest-5-ene- 3β , 20α -diol	20α -Hydroxycholesterol	
4	Cholest-5-ene- 3β , 25 -diol	25-Hydroxycholesterol	
	5,6-α-Epoxy-5α-cholestan-3β-ol	α -Epoxycholesterol ^b	
6	$5,6 - \beta$ -Epoxy-5 β -cholestan-3 β -ol	β -Epoxycholesterol ^b	
	3β-Hydroxycholest-5-ene-7-one	7-Ketocholesterol	
	Cholesta-5-ene-3 β , 7 β -diol	7β-Hydroxycholesterol	
9	Cholesta-5-ene-3 β , 7 α -diol	7x-Hydroxycholesterol	

^a As shown in [Figs. 1 and 2](#page-3-0).
^b Only using the refractive index detector.

smoked ham. Although there was no significant difference amongst the products, the blanquet and smoked breast, made from white meat, presented lower values than the remaining products, all of which were formulated with dark meat.

The total lipid content varied from 1.1 ± 0.1 g/100 g in the smoked breast to 14 ± 2 g/100 g in the meatballs. There was no significant difference in total lipid content between the meatballs and the hamburger, nor between the samples of blanquet, roule, ham and smoked ham.

Table 3 Cholesterol (mg/100 g) and total lipid $(g/100 g)$ contents of the processed turkey meat products

Values in the same column with the same letter do not present significant differences (p < 0.05).
^a Mean and standard deviation of four samples in duplicate.
^b Mean and standard deviation of five samples in duplicate.

The smoked breast showed the lowest values for total lipids and cholesterol, whilst the greatest values were found in the meatballs and hamburger. The higher lipid values found in the meatballs and hamburger were due to the addition of hydrogenated vegetable fat, whilst those of the frankfurters were due to the addition of mechanically deboned poultry meat and those of the blanquet to the addition of turkey fat. The lipid content of the remaining products reflected the amount of fat present in the turkey meat. According to data in the literature, the lipid content of the white meat varies from 0.5% to 2.0% and that of the dark meat from 1.0% to 6.65% (Baggio et al., 2002; Nam, Du, Jo, & Ahn, 2001; USDA, 2002; Wong & Sampugna, 1993).

Higher cholesterol values were found in turkey ham according to both Al-Hassani, Hlavac, and Carpenter (1993) (68.2 mg/100 g) and the USDA Database (2002) (64 mg/100 g). King, Paniangvait, Jones, and German (1998) found higher values for cholesterol in some turkey meat processed products, these being $116±3$ mg/100 g for ham, $118±5$ mg/100 g for pastrami and 114 ± 15 mg/100 g for bologna. A similar value was reported for cholesterol in turkey sausages, by Pereira, Tarley, Matsushita, and Souza (2000). The value for li-

Table 4

Comparison between the values for total lipids found and those declared on the packages of the processed turkey meat products

^a Mean and standard deviation of five samples in duplicate.

pid in the ham was, however, similar to that described in the USDA Database (2002).

Comparing the total lipid and cholesterol contents of the turkey meat products obtained in this study with those of other processed meat products, it can be seen that, in general, the turkey products showed lower total

Table 5

Cholesterol oxide $(\mu g/100 \text{ g})$ contents of the processed turkey meat products

Samples ^a	7-Ketocholesterol $M^{\rm b}$	β-Epoxycholesterol Total oxides $M^{\rm b}$			
Blanquet					
$\mathbf{1}$	88	186	274		
\overline{c}	48	170	218		
3	51	nd	51		
$\overline{4}$	nd	119	199		
Frankfurter					
$\mathbf{1}$	113	nd	113		
\overline{c}	184	112	296		
3	71	130	201		
$\overline{4}$	70	97	167		
Ham					
$\mathbf{1}$	112	nd	112		
\overline{c}	62	249	311		
$\overline{\mathbf{3}}$	31	130	161		
$\overline{4}$	44	213	257		
Hamburger					
$\mathbf{1}$	82	nd	82		
\overline{c}	173	230	403		
3	35	nd	35		
$\overline{4}$	32	206	238		
Meatlball					
$\mathbf{1}$	50	339	389		
\overline{c}	119	nd	119		
$\overline{\mathbf{3}}$	44	nd	44		
$\overline{\mathbf{4}}$	65	nd	65		
Roule					
$\mathbf{1}$	100	140	240		
\overline{c}	38	242	280		
$\overline{\mathbf{3}}$	nd	450	450		
$\overline{4}$	67	192	259		
Smoked breast					
$\mathbf{1}$	nd	nd	nd		
\overline{c}	nd	181	181		
3	55	87	142		
$\overline{4}$	35	nd	35		
Smoked ham					
$\mathbf{1}$	140	92	232		
\overline{c}	47	154	201		
$\overline{\mathbf{3}}$	34	80	114		
$\overline{4}$	66	340	406		

^a Samples, four batches with different production dates.
^b Mean of duplicate determinations; nd, not detected (detection

limit ≤ 9 µg/100 g for 7-ketocholesterol and 12 µg/100 g for 5,6- β epoxycholesterol).

lipid contents, but the values for cholesterol were similar in some samples. Novelli et al. (1998) found values for mortadella and salame Milano samples (both are pork products) which varied from 22.74 to 35.97 g/100 g for lipids and from 34.29 to 138.14 mg/100 g for cholesterol. Pereira et al. (2000) found values for lipids of 11.4 and 9.54 g/100 g in chicken and chester sausages, respectively, and 43.6 mg/100 g for cholesterol in both products.

A comparison was made between the values found for total lipids in this study, and the values shown on the respective product packages [\(Table 4](#page-5-0)). It can be seen that the values are similar.

3.2. Cholesterol oxides

The total amount of cholesterol oxides varied from not detected in a sample of smoked turkey breast to 450 lg/100 g in the roule ([Table 5\)](#page-5-0). Of the cholesterol oxides analyzed (cholesta-3,5-dien-7-one, 20a-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, 7β and 7α -hydroxycholesterol and α and β -epoxycholesterol), only two were found in the turkey meat products analyzed, these being 7-ketocholesterol and β -epoxy-

Table 6 Fatty acid compositions $\left(\frac{0}{2} \text{ area}\right)$ of the processed turkey meat products

cholesterol, their identities being confirmed by mass spectroscopy.

The values obtained for the cholesterol oxides varied considerably between samples and between batches, and thus the values are presented separately ([Table 5](#page-5-0)). 7-ketocholesterol varied from not detected in the blanquet, smoked breast and roule to $184 \mu g/100 g$ in the frankfurter, and b-epoxycholesterol varied from not detected (meatballs, blanquet, hamburger, smoked breast, ham and frankfurter) to $450 \mu g/100 g$ in the roule. Some factors, such as cooking, mincing, packaging, irradiation and storage time and conditions, are important in the formation of cholesterol oxides. In their studies on heated meat products Münch and Arneth (2001) verified that curing salt possessed a strong effect in lowering oxidation in some products. Nam et al. (2001) observed that vacuum packaging of raw meats was sufficient to protect cholesterol and fatty acids from oxidation.

Higley and Taylor (1986) analyzed for cholesterol oxides in selected meat samples and found $8600 \mu g$ 100 g of cholestanetriol and traces of 22-S-hydroxycholesterol in the turkey bologna. Nam et al. (2001) found 7α plus 7 β -hydroxycholesterol (239.4 µg/100 g), and

A Mean and standard deviation of five samples in duplicate.
B 18:2 ω 6t (9c, 12t+ 9t, 12t); SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; t, trans; nd, not detected; values in the same line with the same letter do not present significant differences at the 5% level.

7-ketocholesterol (18 μ g/100 g) in raw, non-irradiated turkey leg meat after 7 days of storage in vacuum packaging. However, in cooked turkey leg meat, under the same conditions, six cholesterol oxides were found (Ahn, Nam, Du, & Jo, 2001) and the total amount of cholesterol oxides was higher. Baggio et al. (2002) found 7-ketocholesterol (33 to 765 μ g/100 g) and 7 β -hydroxycholesterol (nd to 370 µg/100 g) in different turkey meat cuts. Sander et al. (1989) found β and α -epoxycholesterol, 7-ketocholesterol and 7b-hydroxycholesterol in freeze-dried turkey meat, and the values obtained were always higher than those found in this study.

Although the meat products analyzed were not made from turkey meat, a comparison was made. Higher values of 7-ketocholesterol were found in eight commercial species of meat products (Osada, Hoshina, Nakamura, & Sugano, 2000), and similar values for 7-ketocholesterol in mortadella and Milano salami (Novelli et al., 1998), except for three samples. Lower values for β epoxycholesterol were found in raw hamburger (100% beef) and meatballs $(50\% \text{ pork} + 50\% \text{ beer})$ (Larkeson, Dutta, & Hansson, 2000). The following were also found, 7a-hydroxycholesterol (Larkeson et al., 2000), 7b-hydroxycholesterol (Novelli et al., 1998), 5,6a-epoxycholesterol, (Larkeson et al., 2000; Osada et al., 2000), cholestanetriol (Larkeson et al., 2000; Osada et al., 2000), 25-hydroxycholesterol (Novelli et al., 1998) and 20a-hydroxycolesterol (Schmarr et al., 1996).

3.3. Fatty acid composition

The main fatty acids found in the products were: $C18:1\omega$ 9, C18:2 ω 6, C16:0, C18:0 and C16:1 ω 7, as shown in [Table 6 and 7.](#page-6-0) The results obtained by normalization were transformed into mg/100 g of edible portion, using the lipid conversion factor (F) . An *F*-value of 0.945 (poultry) was used for the processed turkey meat products based on Holland et al. (1994). Higher values for $C18:1\omega$ 9, $C16:0$, $C18:2\omega$ 6, $C18:0$ and $C16:1\omega$ 7 were found in the meat ball, hamburger and frankfurter samples. With respect to the minor fatty acids, it can be seen that the fatty acids $C14:0$, $C18:3\omega3$ and $C20:2\omega6$ were lower in the smoked breast, $C20:4\omega$ 6, $C22:5\omega$ 3, $C22:5\omega 6$ in the blanquet, C15:0 in both, and C22:6 $\omega 3$ in hamburger. The *trans* fatty acids were significant in the hamburger, which presented significant values for

Table 7 Fatty acid compositions (% area) of the processed turkey meat products

Fatty acids	Roule		Blanquet		Meatball		Smoked breast	
	mg/100 g $M \pm DP^{A}$	% Area $M \pm DP^{A}$	mg/100 g $M \pm DP^{\bar{A}}$	% Area $M \pm DP^A$	mg/100 g $M \pm DP^A$	% Area $M \pm DP^{A}$	mg/100 g $M \pm DP^{\tilde{A}}$	$%$ Area $M \pm DP^A$
C12:0	$1.8 \pm 0.2c$	0.1 ± 0.0	$1.4 \pm 0.5c$	0.1 ± 0.0	$13.3 \pm 11.6a$	$0.1 \pm 0.0a$	$0.4 \pm 0.1c$	0.1 ± 0.0
C14:0	31.6 ± 1.5 b	$0.7 \pm 0.1a$	29.9 ± 2.4 b	0.6 ± 0.1 ab	$67.5 \pm 15.8a$	0.5 ± 0.1	$6.1 \pm 1.2c$	0.6 ± 0.0 ab
C15:0	30.6 ± 2.5 bc	0.7 ± 0.1 bc	$26.8 \pm 3.5c$	0.6 ± 0.0 bc	30.1 ± 7.4 bc	$0.2 \pm 0.0c$	$26.6 \pm 4.7c$	$2.6 \pm 0.7a$
C16:0	$1060 \pm 131b$	$23.3 \pm 0.6a$	$1169 \pm 74b$	$23.6 \pm 0.6a$	$2746 \pm 356a$	20.1 ± 1.1	$225 \pm 37c$	21.9 ± 1.1 ab
C18:0	$333 \pm 22b$	7.7 ± 0.8 bc	$340 \pm 12b$	6.9 ± 0.6 bcd	$788 \pm 159a$	$6.1 \pm 0.5d$	$96 \pm 6c$	$9.6 \pm 1.1a$
C20:0	nd	nd	$2.8 \pm 0.1c$	$0.1 \pm 0.0c$	$23.1 \pm 14.9a$	$0.2 \pm 0.0a$	nd	nd
C21:0	nd	nd	nd	nd	$18.8 \pm 15.9a$	$0.1 \pm 0.0a$	nd	nd
$C16:1\omega$ 7	$229 \pm 63b$	5.2 ± 0.9	$303 \pm 39b$	$6.1 \pm 0.4a$	$308 \pm 90b$	$2.4 \pm 0.4d$	$45 \pm 17c$	5.0 ± 0.6 bc
$C18:1\omega9t$	nd	nd	nd	nd	nd	nd	nd	nd
$C18:1\omega9$	1483 ± 276 cd	32.7 ± 4.4 de	$1918 \pm 249c$	$38.6 \pm 2.5c$	$6698 \pm 720a$	$52.8 \pm 2.5a$	$285 \pm 56e$	$28.1 \pm 2.4e$
$C20:1\omega11$	$9.1 \pm 1.3a$	$0.2 \pm 0.0a$	$3.8 \pm 0.8a$	$0.1 \pm 0.0c$	nd	nd	nd	nd
$C18:2\omega$ 6	$1155 \pm 119a$	$26.6 \pm 3.4a$	$1037 \pm 93b$	21.3 ± 1.8 b	$1820 \pm 246b$	$14.8 \pm 1.2c$	$281 \pm 33c$	$26.4 \pm 1.0a$
$C18:2\omega 6t^B$	nd	nd	$1.6 \pm 0.5c$	0.1 ± 0.0	$195.6 \pm 45.7a$	$1.4 \pm 0.4a$	nd	nd
$C18:3\omega3$	$50.3 \pm 4.5c$	$1.1 \pm 0.1a$	53.4 ± 6.7 bc	$1.0 \pm 0.1a$	$84.5 \pm 18.7ab$	0.6 ± 0.1	$11.4 \pm 2.0d$	$1.0 \pm 0.1a$
$C18:3\omega$ 6	4.4 ± 2.0 cd	0.1 ± 0.0 ab	7.6 ± 2.9 ab	$0.2 \pm 0.0a$	$12.4 \pm 7.2ab$	0.1 ± 0.0 ab	$0.7 \pm 0.1d$	0.1 ± 0.0
$C20:2\omega$ 6	3.4 ± 1.7 cd	0.1 ± 0.0 bc	$5.9 \pm 1.2ab$	0.1 ± 0.0	4.7 ± 1.7 ab	0.1 ± 0.0	$1.5 \pm 0.4d$	$0.2 \pm 0.0a$
$C20:4\omega$	52.8 ± 15.0 bc	1.2 ± 0.4	$30.0 \pm 6.7d$	0.6 ± 0.1	46.3 ± 7.6 bd	0.5 ± 0.0	$42.7 \pm 11cd$	$3.7 \pm 0.7a$
$C22:5\omega3$	$3.1 \pm 0.7a$	0.1 ± 0.0	$2.2 \pm 0.5a$	0.1 ± 0.0	$5.0 \pm 3.1a$	0.1 ± 0.0	$3.0 \pm 0.5a$	$0.3 \pm 0.0a$
$C22:5\omega$	$3.4 \pm 1.7a$	0.1 ± 0.0 bc	$1.9 \pm 0.8a$	0.1 ± 0.0	$3.1 \pm 1.2a$	0.1 ± 0.0	$3.1 \pm 0.6a$	$0.3 \pm 0.1a$
C22:603	$3.0 \pm 1.8a$	0.1 ± 0.0	1.5 ± 0.9 ab	0.1 ± 0.0	2.4 ± 0.7 ab	0.1 ± 0.0	$2.7 \pm 0.5a$	$0.3 \pm 0.0a$
SFA	1457.0	33	1571.5	32	3882.4	29	354.1	35
MUFA	1721.1	38	2224.8	45	7006.0	55	330.0	33
PUFA	1275.4	29	1139.5	23	1978.4	16	346.1	32
Total ω 3	56.4	1.3	57.1	1.2	91.9	0.8	17.1	1.6
Total co6	1219.0	28	1082.4	22	1886.5	16	329.0	31
SFA/PUFA	1.1		1.4		1.9		1.0	
ω 6/ ω 3	22		19		20			19

^A Mean and standard deviation of five samples in duplicate.

B 18:2 ω 6t (9c, 12t+9t, 12t); SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; t, trans; nd, not detected; values in the same line with the same letter do not present significant differences at the 5% level.

the trans fatty acids C18:1 ω 9 and C18:2 ω 6 (9c, 12t+ 9t, $12t$), and in the meatball, which presented the *trans* fatty acid $C18:2\omega$ 6. The *trans* fatty acid contents found in the hamburger and meatballs were probably due to the addition of hydrogenated vegetable oils. Insignificant *trans* fatty acids were found in some samples, which can be attributed to the poultry diet (Wong & Sampugna, 1993).

The percentage of saturated fatty acids varied from 29% in the meatballs to 35% in the hamburger and smoked breast, the fatty acid C16:0 being found in the greatest concentrations, followed by C18:0. Saturated fatty acids are considered to be hypercholesterolemic and the most worrying are lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids. According to Hayes, Pronczuk, Lindsey, and Diersen-Schade (1991), C14:0 is the most hypercholesterolemic fatty acid and C16:0 the least. Stearic acid (C18:0) has a neutral effect on the blood cholesterol level, since it is quickly converted into oleic acid (C18:1 ω 9), a monounsaturated fatty acid considered to be hypocholesterolemic. A reduction in saturated fatty acids is desirable since these acids, coming from the diet, raise the blood cholesterol level and that of LDL (low density lipoprotein) (Sinclair, 1993).

The lowest percentage of monounsaturated fatty acids was found in the smoked breast (33%) and the greatest in the meatballs (55%). The fatty acid found in greatest proportions was $C18:1\omega$ 9, followed by $C16:1\omega$ 7. The percentage of total polyunsaturated fatty acids was lowest in the meatballs (16%) and greatest in the smoked breast (32%) , the C18:2 ω 6 fatty acid being that found in greatest amounts.

Ferreira, Morgano, de Queiroz, and Mantovani (2000) found means of 35%, 38% and 26% of saturated, monounsaturated and polyunsaturated fatty acids, respectively, in processed turkey meat products. These values were, in general, similar to those found in this study, except that the values for saturated acids in the meatballs and hamburger were higher and the monounsaturated acids lower. Pereira et al. (2000) also found similar values to those found in this study for the saturated, monounsaturated and polyunsaturated fatty acids in turkey sausages.

4. Conclusions

Of all the samples analyzed, the smoked turkey breast showed the lowest values for cholesterol and total lipids and the highest percentage of total polyunsaturated fatty acids. The same principal fatty acids were found in the samples, although there were significant differences in the concentrations. From the results it can be concluded that from the nutritional point of view, with the exception of the meatballs, hamburger and frankfurters, all the turkey meat products showed less than 5% fat, and could thus be considered as low fat foods (Food Advisory Committee, 1990). The cholesterol content varied from 32 to 43 mg/100 g, being below the intake of 300 mg per day for the whole diet recommended by American Heart Association for the whole diet (2003). The ratio of polyunsaturated:saturated fatty acids varied from 1.1 to 1.8, being above the recommended minimum value of 0.45 and the ω 6: ω 3 was much higher than the maximum recommended value of 4.0 for the whole diet (Department of Health, 1994). This implies that it is necessary to compensate for this deficiency with other components of the diet. Trans fatty acids were present in significant amounts in hamburger and meatball and two oxysterols were found in the majority of the samples. However, the total oxide concentrations were below the levels considered necessary to give a toxic response in cell cultures (Guardiola et al., 1996).

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References

- Ahn, D. U., Nam, K. C., Du, M., & Jo, C. (2001). Effect of irradiation and packaging conditions after cooking on the formation of cholesterol and lipid oxidation products in meats during storage. Meat Science, 57, 413–418.
- Al-Hassani, S. M., Hlavac, J., & Carpenter, W. (1993). Rapid determination of cholesterol in single and multicomponent prepared foods. Journal of AOAC International, 76, 902-906.
- American Heart Association. (2003). Dietary Guidelines for Healthy American Adults. Available from [http://www.americanheart.erg/](http://www.americanheart.erg/Heart_and_Stroke_A_Z_Guide/dietg.html.) Heart and Stroke A Z Guide/dietg.html. Accessed May 2003.
- Baggio, S. R., & Bragagnolo, N. (2004). Validação da metodologia para determinação simultânea, por CLAE, de colesterol e óxidos de colesterol em produtos cárneos processados. Ciência e Tecnologia de Alimentos, 24, 64–70.
- Baggio, S. R., Vicente, E., & Bragagnolo, N. (2002). Cholesterol oxides, cholesterol, total lipid and fatty acid composition of turkey meat. Journal of Agricultural and Food Chemistry, 50, 5981–5986.
- Chairman, L. H. K., Crummett, W., Deegan, J. J., Libby, R. O., Taylor, J. K., & Wentler, G. (1983). Principles of environmental analysis. Journal of the American Chemistry and Society, 55, 2210–2217.
- Department of Health. (1994). Nutritional aspects of cardiovascular disease. Report on health and social subjects (Vol. 46). London: HMSO.
- Ferreira, M. M. C., Morgano, M. A., de Queiroz, S. C. N., & Mantovani, D. M. B. (2000). Relationships of the minerals and fatty acid contents in processed turkey meat products. Food Chemistry, 69, 259–265.
- Finocchiaro, E. T., & Richardson, T. (1983). Sterol oxides in foods: A review. Journal of Food Protection, 46, 917–925.
- Folch, J., Less, M., & Stanley, S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497–509.
- Food Advisory Committee. (1990). Report on review of food labelling and advertising. London: HMSO.
- Guardiola, F., Codony, R., Addis, P. B., Rafecas, M., & Boatella, J. (1996). Biological effects of oxysterols: Current status. Food Chemical Toxicology, 34, 193–211.
- Hartman, L., & Lago, R. C. A. (1973). Rapid preparation of fatty acid methyl esters from lipids. Laboratory Practies, 22, 475–481.
- Hayes, K. C., Pronczuk, A., Lindsey, S., & Diersen-Schade, D. (1991). Dietary saturated fatty acids (12:0, 14:0, C16:0) differ in their impacton plasma cholesterol and lipoproteins in nonhuman primates. American Journal of Clinical Nutrition, 53, 491–498.
- Higley, N. A., & Taylor, S. L. (1986). Cholesterol oxides in processed meats. Meat Science, 16, 175–188.
- Holland, B., Welch, A. A., Unwin, I. D., Buss, D. H., Paul, A. A., & Southgate, D. A. T. (1994). McCance and Widdowson's. The composition of foods (5th ed., pp. 8–9) London: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.
- King, A. J., Paniangvait, P., Jones, A. D., & German, J. B. (1998). Rapid method for quantitation of cholesterol in turkey meat and products. Journal of Food Science, 63, 382–385.
- Kubow, S. (1990). Toxicity of dietary lipid peroxidation products. Trends in Food Science and Technology, 1, 67–70.
- Kumar, N., & Singhal, O. P. (1991). Cholesterol oxides and atherosclerosis: A review. Journal of the Science of Food and Agriculture, 55, 487–510.
- Larkeson, B., Dutta, P. C., & Hansson, I. (2000). Effects of frying and storage on cholesterol oxidation in minced meat products. Journal of Americal Oil Chemistry and Society, 77, 675–680.
- Münch, S., & Arneth, W. (2001). Studies on the content of cholesterol oxides in heated meat products. Mitteilungsblatt, 40, 177–186.
- Nam, K. C., Du, M., Jo, C., & Ahn, D. U. (2001). Cholesterol oxidation products in irradiated raw meat with different packaging and storage time. Meat Science, 58, 431–435.
- Novelli, E., Zanardi, E., Ghiretti, G. P., Campanini, G., Dazzi, G., & Madarena, G., et al. (1998). Lipid and cholesterol oxidation in frozen stored pork, salame milano and mortadella. Meat Science, 48, 29–40.
- Osada, K., Hoshina, S., Nakamura, S., & Sugano, M. (2000). Cholesterol oxidation in meat products and its regulation by supplementation of sodium nitrite and apple polyphenol before processing. Journal of Agricultural and Food Chemistry, 48, 3823–3829.
- Paniangvait, P., King, A. J., Jones, A. D., & German, B. G. (1995). Cholesterol oxides in foods of animal origin. Journal of Food Science, 60, 1159–1174.
- Pereira, N. R., Tarley, C. R. T., Matsushita, M., & Souza, N. E. (2000). Proximate composition and fatty acid profile in Brazilian poultry sausages. Journal of Food Composition and Analysis, 13, 915–920.
- Sander, B. D., Addis, P. B., Park, S. W., & Smith, D. E. (1989). Quantification of cholesterol oxidation products in a variety of foods. Journal of Food Protection, 52, 109–114.
- Schmarr, H., Gross, H. B., & Shibamoto, T. (1996). Analysis of polar cholesterol oxidation products: Evaluation of a new method involving transesterification, solid phase extraction, and gas chromatography. Journal of Agricultural and Food Chemistry, 44, 512–517.
- Sevanian, A., & Peterson, A. R. (1986). The cytotoxic and mutagenic properties of cholesterol oxidation products. Food and Chemical Toxicology, 24, 1103–1110.
- Sinclair, A. J. (1993). Dietary fat and cardiovascular disease: The significance of recent developments for the food industry. Food Australia, 45, 226–239.
- Smith, L. L. (1987). Cholesterol autoxidation 1981–1986. Chemical Physical Lipids, 44, 87–125.
- USDA. (2001). FASonline. United State Department of Agriculture. Available from [http://www.fas.usda.gov/commodities.](http://www.fas.usda.gov/commodities)
- USDA. (2002). Nutrient Database for Standard Reference, Release 14, Poultry Products, Nutrient Data Laboratory, Agricultural Research Service: Beltsville, MD. Available from [http://www.nal.us](http://www.nal.usda.gov/fnic/foodcomp/Data/SR14/sr14doc.htm)[da.gov/fnic/foodcomp/Data/SR14/sr14doc.htm.](http://www.nal.usda.gov/fnic/foodcomp/Data/SR14/sr14doc.htm)
- Wong, M. K., & Sampugna, J. (1993). Moisture, total lipids, fatty acids and cholesterol in raw ground turkey. Journal of Agricultural and Food Chemistry, 41, 1229–1231.