TITLE: "Consequences of microwave heating and frying on the lipid fraction of chicken and beef hamburgers"

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ABSTRACT

Two types of commercial hamburgers were analyzed in order to evaluate the effect of two applied cooking methods on the lipid fraction and on the cholesterol oxidation process during heating. Microwaves hardly modified the fatty acid profiles of both chicken and beef hamburgers, whereas frying with olive oil increased oleic and eicosapentaenoic acids and decreased linoleic and docosahexaenoic acids in both types of products. Frying improved the w6/w3 fatty acids ratio in beef hamburgers from 10.67 (raw) to 5.37 (fried). Total COPs increments were 5.3-6.1 fold with microwaves and 1.5-2.6 fold with frying. Chicken hamburgers, raw and cooked, had a COPs content twice as much as the corresponding beef ones.

INTRODUCTION

Food safety of is one of the most important goals in food science nowadays. COPs are known from many years ago as compounds with adverse biological effects. Cytotoxic effects have been demonstrated in different types of cells and they are mediated by different mechanisms including apoptosis *(1, 2, 3, 4, 5).* However their implications with the atherosclerotic process have probably been the most widely studied. Since 1987, when Jacobson hypothesised that the high COPs level in Indian ghee was the cause of the high incidence of atherosclerosis in Indian immigrant populations *(6),* many researches have established the relationship between plasma COPs level, and more specifically oxidized-LDL, and the development of atherosclerosis (*7, 8, 9, 10, 11*).

Although COPs can be formed in the organism, it has been demonstrated that they can also be absorbed in the intestine from the diet. The few data available at the moment point out that the mean COPs absorption rate is around 30%, reaching for some of them higher levels (42% in 7β-hydroxycholesterol) *(7).* This significant absorption rate and the mentioned adverse effects lead to think that it is important to increase the knowledge on COPs not only in relation to the mechanisms of their negative health effects, but also in relation to the real presence of this type of compounds in food. Some authors have even pointed out the need to define an acceptable daily intake level for COPs *(7).*

Meat and meat products have to be taken into account as COPs´ suppliers. Although levels of COPs in raw meats do not seem to be too high *(12, 13, 14),* the cooking methods can significantly increase the cholesterol oxidation increasing the total COPs amounts *(12, 15, 16, 17, 18, 19, 20).* Cooking processes can also affect other lipid compounds of meats, especially the fatty acids, changing the nutritional value of cooked products in relation to raw samples *(21, 22, 23).* Moreover, it is stated that the presence

of more unsaturated fatty acids enhances the cholesterol oxidation intensity (*24, 25*). It can also be thought that different cooking methods, as a consequence of using different times and temperatures of processing could lead to modifications in the lipid fraction. Microwaves heating, one of the most usual cooking method currently used, is known to cause greater alterations in edible fats than conventional heating, although not many data are available yet on its consequences for the composition and nutritional quality of food *(26, 27).*

Hamburgers are a very popular type of fast food highly present in the occidental diet with significant cholesterol amounts. Although the nutritional value of hamburgers dealing with the lipid fraction has been studied especially in relation to the raw matter used *(28, 29),* there are very few data in relation to the effect of heating on their lipid fraction *(27, 30).* Lercker and Rodriguez-Estrada *(31)* in a review on the presence of 7-ketocholesterol in different food products concluded that attention should be focussed on the quality of the raw materials and the overall product technology in order to reduce cholesterol oxidation, which, according to these authors, is higher in beef and meat products compared to the rest of samples.

The aim of this work was to analyse the influence of the use of two household cooking methods, microwave heating and frying with olive oil, on the lipid fraction of beef and chicken hamburgers, with particular attention to the formation of cholesterol oxidation products.

MATERIAL AND METHODS

Four different batches of beef and chicken hamburgers were purchased in different supermarkets at different days in order to obtain representative samples. They were analyzed in raw and after being cooked using two different technologies (microwaves and frying with olive oil). Microwave treatment was carried out during 3 min at 900W. Internal temperature of samples at the end of the process was 100ºC. Frying was carried out in a pan with 10ml olive oil during 3 min each side. Temperature of oil when process started was 180ºC. Final internal temperature of hamburgers was 85-90ºC.

Chemical analysis

Moisture analysis was carried out according to the AOAC method *(32).* Cholesterol analysis was done according to Kovacs et al. *(33).* Fat content was obtained according to ISO-1443 (*34*). Total lipid was extracted using chloroform/methanol (2:1,v/v) according to Folch et al. (*35*) procedure. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters *(36).*

COPs. Extraction of lipids and saponification.

Saponification, purification and derivatization of COPs were made according to the method III of Guardiola et al. *(37).* Approximately 1g of fat was added to a flask containing 10ml of 1M KOH in methanol and 1ml (20μg/ml) of internal standard (IS: 19- hydroxycholesterol) and kept stirring at room temperature during 20h to complete the cold saponification. The unsaponificable material was extracted with diethyl ether. The whole organic extract was washed with water and filtered through anhydrous sodium sulphate. Then it was recovered in a round-bottom flask, and the solvent was evaporated using a rotatory vacuum evaporator at 30ºC. A purification was made with

silica cartridges (Sep-Pack Vac 6cc SPE, Waters, Millpore, Beldford MA, USA) using different proportions of hexane/diethyl ether and finally COPs were recovered with acetone/methanol (60:20, v/v). A derivatization to obtain the trimethyl silyl ethers of COPs was performed. Cholesterol oxides were identified and quantified by a Hewlett-Packard 6980 GC coupled to a 5973 Mass selective Detector (Palo Alto, Wilmington, USA). Column used was a HP-5MS column (30m X 250μm X 0.25 μm) and helium was the carrier gas (1ml/minute). The chromatographic conditions were as follows: initial column temperature at 80ºC, held for 1 min and programmed to 250ºC at a rate of 10ºC/min and final column temperature of 280ºC at a rate of 4ºC/min and held for 20 min. The injector temperature was 250ºC and the inlet pressure was 23.2 psig; mass range, m/z=50/550 amu (atomic mass units); solvent delay was 20 minutes. Calibration curves were developed for six cholesterol oxides: Cholest-5-en-3β,7α-diol (7α-hydroxycholesterol), Cholest-5-en-3β,7β-diol (7β-hydroxycholesterol), Cholest-5-en-3β-25-diol (25-hydroxycholesterol), 5,6α-Epoxy-5α-Cholestan-3β-ol (α-epoxycholesterol), 3β-Hydroxycolest-5-en-7one (7-ketocholesterol) and 5α-Cholestane-3β, 5, 6β-triol (cholestanetriol). All these standards were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA), except for 7α-hydroxycholesterol and 19-hydroxycholesterol, purchased from Steraloids (Steraloids INC, Newport, USA). Different concentrations (2.5, 5, 10, 20, 40 and 80μg/ml) of mixtures of all the standards were analyzed in the TIC (total ion chromatogram) mode. The identification of the COPs in the sample was made by checking their retention time and their mass spectra (using the HPCHEM Wiley 275 6th Edition library) with those of the standard compounds.

Quantification of the each COPs was made by means of their characteristic ion, by taking into account its proportion on the molecule, and multiplying the areas obtained

for these ions by their corresponding factor. The characteristic ions selected were: 457 for 7α-hydroxycholesterol, 458 for 7β-hydroxycholesterol, 271 for 25-hydroxycholesterol, 366 for α-epoxycholesterol, 472 for 7-ketocholesterol and 403 for cholestanetriol.

Data analysis

Four hamburgers, one from each species and batch (beef and chicken) were analyzed from each of the different technologies assayed. Each parameter was determined four times in each sample. Means and standard deviations are shown.

One-way analysis of variance (ANOVA) with a posteriori Tukey b test was carried out for each type of hamburger in order to analyze statistical differences among the cooking methods applied ($P \leq 0.05$). A Student t test was done to analyze the statistical differences between species both in raw and cooked samples.

Software used was SPSS 9.0 for Windows (SPSS Inc., Chicago, Ill.).

RESULTS AND DISCUSSION

Both microwaves and frying significantly decreased the moisture of beef and chicken hamburgers (Table 1). Microwaves is not expected to modify the total lipid content of food, whereas frying could modify it (*18, 23, 38*). However, other authors have found lipid losses in hamburgers after frying and after cooking with microwaves (*27*). Microwaves did not increase the lipid percentage and frying slightly increased it only in chicken. The effects over cholesterol amount were found to be significant, increasing with both types of technologies. In relation to the type of meat, no significant differences were found for raw samples or for cooked samples in fat percentages.

In spite of the absence of significant differences found in the total lipid percentages between species and between applied technologies, some interesting qualitative differences were found in the fatty acid profiles. In chicken hamburgers no changes were observed with microwaves in 12 fatty acids including oleic, linolenic, eicosapentaenoic and docosahexaenoic acids (Table 2). When chicken samples were fried 10 fatty acids showed significant changes in their concentrations. Oleic and eicosapentaenoic acids increased and linoleic and docosahexaenoic acids decreased. The number of fatty acids affected by cooking in beef hamburgers was higher (Table 3). With microwaves treatment only 8 fatty acids did not change, including oleic and eicosapentaenoic acids. Linoleic, linolenic and docosahexaenoic acids decreased. Frying caused greater modifications in the fatty acid profile with only 4 fatty acids without significant changes. In these fried samples oleic, linolenic and eicosapentaenoic acids increased whereas linoleic and DHA showed a decrease. Sánchez Muñiz et al. (*39*) proposed that fatty acid changes in foodstuff during frying was a consequence of fatty acid gradients. Hamburgers were fried with olive oil, which explained the observed increases found for oleic acid.

The type of meat has a notable influence on the fatty acid profile. It is known that chicken meat has a higher unsaturation degree than beef meat. Comparing the fatty acid profiles for beef and chicken hamburgers it can be observed that significant differences were found for most of them (Table 4). In other works different fatty acids profiles were observed for raw hamburgers depending on the type of meat *(28, 29)*. Chicken hamburgers, raw and cooked, showed significantly higher amounts for oleic, linoleic and linolenic acids in relation to those elaborated with beef. Myristic, palmitic and stearic acids were on the contrary, more abundant in beef hamburgers than in chicken ones. No significant differences were found for EPA and DHA except for fried chicken hamburgers, which showed lower DHA amounts than fried beef ones. So it can be concluded that differences in the fatty acid profile of raw hamburgers, consequence of the different type of meat, are maintained in cooked hamburgers.

The observed changes as a consequence of the cooking process can not be considered quantitatively relevant taking into account the total amounts of the different fatty acids fractions in raw and cooked samples (Table 5). From a quantitative point of view, total amounts of SFA, MUFA and PUFA (g/100g product) were hardly affected, as also reported by Rodriguez-Estrada et al. *(27*), although some statistical differences were detected. The use of microwaves did not modify the w6/w3 ratio in any case. It decreased the unsaturated / saturated fatty acids (U/S) in beef hamburgers, whereas no modification was observed in chicken hamburgers. In fried samples U/S ratio showed increases that, especially in chicken hamburgers, could be considered as beneficial from a nutritional point of view (increase from 1.64 to 2.11). w6/w3 ratio, which is considered to be too high in the nowadays diet *(40),* showed a decrease in beef hamburgers with frying (from 10.67 to 5.37).

In relation to the COPs analysis (Table 6), in raw beef hamburgers only 7-ketocholesterol was detected and also 7β-hydroxycholesterol in raw chicken hamburgers. Maor et al. *(41*) pointed out that the major oxysterol in arterial macrophages was found to be 7-ketocholesterol (51% of total oxysterols). 7β-hydroxycholesterol has been shown to be a good marker of lipid peroxidation in vitro *(42)* and in vivo *(43)* and a potential predictor of progression of carotid atherosclerosis *(44).* The percentages of 7-ketocholesterol were 100% and 46.53%, in raw hamburgers of beef and chicken, respectively, decreasing with cooking as a consequence of the increase of the rest of COPs. Zubillaga and Maerker *(45)* found that 7-ketocholesterol constituted more than 50% of the oxidation products in raw samples of veal, beef, pork and chicken tissues. Quantitatively, 7-ketocholesterol only increased significantly in beef hamburgers with heating when microwaves was used as the cooking method. In the case of chicken, also frying caused a significant increase of 7-ketocholesterol, reaching significantly higher amounts in fried chicken than in fried beef hamburgers ($p< 0.01$). Rodriguez-Estrada et al. *(27)* analysing 7-ketocholesterol in raw hamburgers found much more amounts for this compound (25.2 ppm in lipids). In that work raw samples showed significantly higher values of 7-ketocholesterol than samples cooked with different technologies, including microwaves. However, most of the researches have observed an increase in total COPs, including 7-ketocholesterol, when meats are cooked *(30, 46, 47, 48).* Echarte et al. *(18)* found that 7-ketocholesterol increased its level with frying 8-12 times and constituted more than 65% of total COPs in raw and cooked samples.

7β-hydroxycholesterol was, quantitatively, the second compound in microwave-treated beef and chicken hamburgers. Larkeson et al. *(30)* found in raw hamburgers not only 7β-hydroxycholesterol and 7-ketocholesterol, but also 7α-hydroxycholesterol and 5,6α-

epoxycholesterol, reaching a total COPs amount of 5.5 ppm in lipids. 7α-hydroxycholesterol showed the highest increase with cooking, especially with microwave treatment reaching the highest amount among all the COPs analysed except for fried beef hamburgers. 25- hydroxycholesterol has been found as one of the most cytotoxic COP on human hematopoietic progenitor cells *(48).* It was the only analyzed compound not detected in any sample (only traces in microwave-treated beef hamburgers). Cholestanetriol only appeared in fried samples, being its level significantly higher in chicken samples ($p < 0.001$). As beef samples show cholestanetriol and no α-epoxyde was detected, the cholestanetriol might be synthesised from β-epoxycholesterol, which was not analysed in this work.

Significant differences were found for total COPs among the cooking methods and types of meat. Total COPs amounts expressed in ppm on product (hamburger) did not reach 3ppm in any case. These data are similar to values obtained by Grau et al. *(50)* in cooked chicken meat (3.26ppm) and to those found in other works for raw beef meat (0.5-3.4ppm) (*51, 52*) and lower than for fried pork loin (around 9ppm) (*18*). Comparing beef and chicken hamburgers, the last showed in both raw and cooked samples the highest total COPs amounts and also higher increments of COPs than beef hamburgers with cooking. This fact can be attributed to the higher lipid oxidation potential of cooked chicken muscles compared to beef muscles due to their higher PUFA content, as pointed out by Rhee et al. *(53).* Only 7-ketocholesterol showed lower amounts in raw chicken than in raw beef and no differences were found for this compound between the samples subjected to microwaves. In the rest of samples, every COP showed higher amount in chicken than in beef hamburgers.

Conchillo et al. *(54)* analyzing the effect of grilling and roasting on cholesterol oxidation in breast chicken found that total COPs increased 4-4.5 fold with cooking. In

the analyzed hamburgers the increases were 5.3-6.1 fold with microwaves and 1.5-2.6 fold with frying. Microwaved chicken hamburgers showed a percentage of oxidation of 0.36%, being the rest of values lower than 0.2%, considered low by Lercker and Rodríguez -Estrada *(31).* All these data pointed out that microwaves caused the highest cholesterol oxidation.

Table 1. Moisture, lipid and cholesterol amounts of raw and cooked beef and chicken hamburgers.

	Beef			Chicken			LS		
	Raw	**Microwaves**	**Fried Olive oil**	**Raw**	**Microwaves**	**Fried Olive oil**	**R**	**M**	**F**
Moisture (%)	68.24 ± 0.69^{c}	62.74 ± 0.62^{b}	61.66 ± 0.38^{a}	66.19 ± 0.72^{b}	61.63 ± 0.54^{a}	61.35 ± 0.18^{a}	**	*	Ns
Lipid (%)	10.33 ± 0.63^{a}	10.02 ± 0.92^{a}	10.56 ± 0.43^{a}	10 ± 0.63^{a}	10.67 ± 0.24^{ab}	11.47 ± 0.65^{b}	Ns	Ns	Ns
Cholesterol (mg/100g)	60.72 ± 2.85^{a}	69.04 ± 3.62^{b}	70.61 ± 3.50^{b}	60.99 ± 4.51^{a}	73.3 ± 2.43^{b}	75.49 ± 4.77^{b}	Ns	Ns	Ns

For each parameter and species, different letters denote significant differences among cooking technologies ($p<0.05$). LS: level of significance for the Student t test to compare species using the same technology. R (raw), M (microwaves), F (fried with olive oil). Ns: not significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Table 2. Fatty acid composition of raw and cooked chicken hamburgers (g/100g fatty acids).

	Raw Chicken	*Microwaves*	*Fried Olive oil*
Lauric	0.10 ± 0.002^{a}	0.10 ± 0.001^{a}	0.10 ± 0.02^{a}
Myristic	1.28 ± 0.02^{a}	1.2 ± 0.07^{a}	0.93 ± 0.03^{a}
Palmitic	24.65 ± 0.13^{b}	25.16 ± 0.19^{c}	23.49 ± 0.16^{a}
T-Palmitoleic	0.39 ± 0.01^{a}	0.37 ± 0.01^{a}	0.36 ± 0.02^{a}
Palmitoleic	3.89 ± 0.04^{b}	3.73 ± 0.07^{b}	3.44 ± 0.15^{a}
Stearic	11.13 ± 0.05^{b}	11.54 ± 0.05^{c}	10.83 ± 0.02^{a}
Oleic	44.73 ± 0.19^{a}	44.72 ± 0.17^{a}	47.53 ± 0.16^{b}
Elaidic	0.17 ± 0.02^{a}	0.16 ± 0.01^{a}	0.15 ± 0.008^{a}
T-Linoleic	0.06 ± 0.004^{a}	0.05 ± 0.01^{a}	0.08 ± 0.03^{a}
Linoleic	11.91 ± 0.05^{c}	11.03 ± 0.03^{a}	11.48 ± 0.09^{b}
Arachidic	0.21 ± 0.05^{a}	0.19 ± 0.10^{a}	0.25 ± 0.04^{a}
Linolenic	0.74 ± 0.06^{a}	0.64 ± 0.008^{a}	0.64 ± 0.06^{a}
Behenic	0.29 ± 0.04^{a}	0.56 ± 0.1^{b}	0.21 ± 0.04^{a}
Arachidonic	0.10 ± 0.02^{b}	0.27 ± 0.02^{c}	0.04 ± 0.006^{a}
Brassidic	0.24 ± 0.02^{b}	0.05 ± 0.01^{a}	0.32 ± 0.13^{c}
Eicosapentaenoic	0.04 ± 0.007^{a}	0.04 ± 0.009^{ab}	0.07 ± 0.03^{b}
Docosahexaenoic	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}	0.03 ± 0.009^{a}

For each parameter, different letters denote significant differences among cooking methods ($p<0.05$).

Table 3. Fatty acid composition of raw and cooked beef hamburgers (g/100g fatty acids).

	Raw Beef	***Microwaves***	***Fried Olive oil***
Lauric	0.13 ± 0.01^{b}	0.10 ± 0.003^{a}	0.10 ± 0.001^{a}
Myristic	2.84 ± 0.04^{b}	2.87 ± 0.08^{b}	2.69 ± 0.03^{a}
Palmitic	26.04 ± 0.47^{b}	26.35 ± 0.34^{b}	25.11 ± 0.01^{a}
T-Palmitoleic	0.33 ± 0.01^{a}	0.47 ± 0.01^{b}	0.30 ± 0.07^{a}
Palmitoleic	4.30 ± 0.04^{a}	4.79 ± 0.1^{b}	4.73 ± 0.006^{b}
Stearic	14.75 ± 0.10^{a}	16.54 ± 0.17^{c}	15.04 ± 0.008^{b}
Oleic	39.38 ± 0.17^{a}	39.26 ± 0.34^{a}	43.17 ± 0.05^{b}
Elaidic	4.71 ± 0.03^{c}	4.01 ± 0.1^{b}	3.68 ± 0.02^{a}
T-Linoleic	0.16 ± 0.02^{b}	0.10 ± 0.02^{a}	0.12 ± 0.01^{a}
Linoleic	6.07 ± 0.45^{b}	4.15 ± 0.03^{a}	4.07 ± 0.005^{a}
Arachidic	0.11 ± 0.06^{a}	0.13 ± 0.04^{a}	0.22 ± 0.006^{b}
Linolenic	0.42 ± 0.07^{c}	0.27 ± 0.07^{a}	0.59 ± 0.01^{b}
Behenic	0.37 ± 0.16^{a}	0.39 ± 0.07^{a}	0.33 ± 0.01^{a}
Arachidonic	0.11 ± 0.009^{a}	0.31 ± 0.01^{b}	0.02 ± 0.00^{a}
Brassidic	0.11 ± 0.007^{ab}	0.06 ± 0.00^{a}	0.19 ± 0.02^{b}
Eicosapentaenoic	0.04 ± 0.005^{a}	0.06 ± 0.01^{a}	0.09 ± 0.00^{b}
Docosahexaenoic	0.09 ± 0.02^{b}	0.06 ± 0.02^{a}	0.06 ± 0.01^{a}

For each parameter, different letters denote significant differences among cooking methods ($p<0.05$).

Table 4. Student t test for every fatty acid analyzed between the two species (chicken and beef) in the different types of samples.

	Raw	***Microwaves***	***Fried Olive oil***
Lauric	*	Ns	Ns
Myristic	***	***	**
Palmitic	**	**	***
T-Palmitoleic	***	***	Ns
Palmitoleic	***	***	***
Stearic	***	***	***
Oleic	***	***	***
Elaidic	***	***	***
T-Linoleic	*	**	Ns
Linoleic	***	***	***
Arachidic	*	Ns	Ns
Linolenic	**	***	***
Behenic	Ns	Ns	**
Arachidonic	Ns	*	**
Brassidic	*		***
Eicosapentaenoic	Ns	Ns	Ns
Docosahexaenoic	Ns	Ns	**

Ns: Not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 5. Fatty acids fractions (g/100 g product) and ratios for the different analyzed hamburgers.

	Beef			**Chicken**		
	Raw	***Microwaves***	***Fried Olive oil***	***Raw***	***Microwaves***	***Fried Olive oil***
SFA	4.39b	4.63c	4.30a	3.76b	3.81b	3.44a
MUFA	4.52b	4.41a	5.06c	4.86a	5.17b	5.84c
PUFA	0.69b	0.49a	0.51a	1.29a	1.29a	1.40b
PUFA+MUFA/SFA	1.18b	1.06a	1.3c	1.63a	1.69a	2.09b
w3	0.06a	0.04a	0.08b	0.08a	0.08a	0.09a
w6	0.64b	0.45a	0.43a	1.20a	1.20a	1.31b
w6/w3	10.67b	11.25b	5.37a	15a	15a	14.56a

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Within each type of meat, different letters in the same raw denote significant differences ($p<0.05$) among cooking methods.

Table 6. Cholesterol oxides content found in each type of hamburger (μg/g fat).

	Beef			Chicken			LS		
μg/g fat	***Raw***	***Microwaves***	***Fried Olive oil***	***Raw***	***Microwaves***	***Fried Olive oil***	***R***	***M***	***F***
7α-Hydroxy cholesterol	Nd[a]	4.58 ± 0.32[c]	0.83 ± 0.13[b]	Nd[a]	12.67 ± 1.42[c]	2.93 ± 0.09[b]	-	***	***
7β-Hydroxy cholesterol	Nd[a]	3.68 ± 0.18[b]	0.86 ± 0.07[a]	2.14 ± 0.13[a]	6.43 ± 0.37[b]	2.25 ± 0.12[a]	ns	***	***
7-Keto cholesterol	2.31 ± 0.26[b]	3.44 ± 0.03[c]	0.95 ± 0.1[a]	1.87 ± 0.21[a]	4 ± 0.53[c]	2.53 ± 0.38[b]	*	ns	**
α-Epoxy cholesterol	Nd[a]	0.56 ± 0.01[b]	Nd[a]	Nd[a]	1.53 ± 0.13[c]	0.65 ± 0.04[b]	-	***	***
Cholestanetriol	Nd[a]	Nd[a]	0.77 ± 0.1[b]	Nd[a]	Nd[a]	2.39 ± 0.15[b]	-	-	***
25- Hydroxy cholesterol	Nd	traces	Nd	Nd[a]	Nd[a]	Nd[a]	-	-	-
Total COPs	2.31[a]	12.26[c]	3.41[b]	4.01[a]	24.63[c]	10.75[b]	***	***	***
Total COPs (ppm in hamburgers)	0.23[a]	1.23[c]	0.36[b]	0.40[a]	2.63[c]	1.23[b]	***	***	***
% of Cholesterol Oxidation	0.04	0.18	0.05	0.06	0.36	0.16			

For each parameter and species, different letters denote significant differences among culinary technologies. LS: level of significance for the Student t test to compare species using the same technology. R (raw), M (microwaves), F (fried with olive oil). Ns: not significant ($p>0.05$); *$p<0.05$; **$p<0.01$; ***$p<0.001$.
Nd: non detected.

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