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Consequences of Microwave Heating and Frying on the Lipid Fraction of Chicken and Beef Patties

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Two types of commercial meat patties were analyzed to evaluate the effect of two applied cooking methods on the lipid fraction and the cholesterol oxidation process during heating. Microwave heating hardly modified the fatty acid profiles of both chicken and beef patties, whereas frying in olive oil increased oleic and eicosapentaenoic acids and decreased linoleic and docosahexaenoic acids in both types of products. Frying improved the $\omega 6/\omega 3$ fatty acids ratio in beef patties from 10.67 (raw) to 5.37 (fried). Total cholesterol oxidation product (COP) increments were 5.3−6.1-fold with microwave heating and 1.5−2.6-fold with frying. Chicken patties, raw and cooked, had a COP content twice as high as the corresponding beef ones.

KEYWORDS: Food safety; cholesterol; oxidation; fatty acids; hamburger

INTRODUCTION

Food safety is one of the most important goals in food science nowadays. Cholesterol oxidation products (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*−*5*). However, their implications with the atherosclerotic process have probably been the most widely studied. Since 1987, when Jacobson hypothesised that the high COP level in Indian ghee was the cause of the high incidence of atherosclerosis in Indian immigrant populations (*6*), much research has established the relationship between plasma COP levels, and more specifically oxidized low-density lipoprotein (LDL), and the development of atherosclerosis (*7*−*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 COP absorption rate is ∼30%, alhtough the rate for some COPs is higher (e.g., 42% in 7β-hydroxycholesterol) (*7*). This significant absorption rate and the mentioned adverse effects lead to the conclusion that it is important to increase the knowledge of 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 compound in foods. 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 suppliers of COPs. Although levels of COPs in raw meats do not seem to be too high (*12*−*14*), cooking methods can significantly increase cholesterol oxidation, increasing the total COP amounts (*12*, *15*−*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*−*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. Microwave heating, one of the most common cooking methods 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*).

Meat patties are a very popular type of fast food widely present in the occidental diet with significant cholesterol amounts. Although the nutritional value of meat patties 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 focused 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 other samples.

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

MATERIALS AND METHODS

Four different batches of beef and chicken patties were purchased in different supermarkets on different days in order to obtain representative samples. They were analyzed raw and after being cooked using two different technologies (microwave heating and frying in olive oil). Microwave treatment was carried out during 3 min at 900 W. The internal temperature of samples at the end of the process was 100 °C. Frying was carried out in a pan with 10 mL of olive oil during 3 min

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Table 1. Moisture, Lipid, and Cholesterol Amounts of Raw and Cooked Beef and Chicken Patties[a]

	beef			chicken			LS		
	raw	microwaved	fried in olive oil	raw	microwaved	fried in olive oil	R	M	F
moisture (%)	68.24 ± 0.69c	62.74 ± 0.62b	61.66 ± 0.38a	66.19 ± 0.72b	61.63 ± 0.54a	61.35 ± 0.18a	**	*	NS
lipid (%)	10.33 ± 0.63a	10.02 ± 0.92a	10.56 ± 0.43a	10 ± 0.63a	10.67 ± 0.24ab	11.47 ± 0.65b	NS	NS	NS
cholesterol (mg/100 g)	60.72 ± 2.85a	69.04 ± 3.62b	70.61 ± 3.50b	60.99 ± 4.51a	73.3 ± 2.43b	75.49 ± 4.77b	NS	NS	NS

[a] 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 (microwaved), F (fried in olive oil). NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2. Fatty Acid Composition of Raw and Cooked Chicken Patties (Grams per 100 g of Fatty Acids)[a]

fatty acid	raw chicken	microwaved	fried in olive oil
lauric	0.10 ± 0.002a	0.10 ± 0.001a	0.10 ± 0.02a
myristic	1.28 ± 0.02a	1.2 ± 0.07a	0.93 ± 0.03a
palmitic	24.65 ± 0.13b	25.16 ± 0.19c	23.49 ± 0.16a
T-palmitoleic	0.39 ± 0.01a	0.37 ± 0.01a	0.36 ± 0.02a
palmitoleic	3.89 ± 0.04b	3.73 ± 0.07b	3.44 ± 0.15a
stearic	11.13 ± 0.05b	11.54 ± 0.05c	10.83 ± 0.02a
oleic	44.73 ± 0.19a	44.72 ± 0.17a	47.53 ± 0.16b
elaidic	0.17 ± 0.02a	0.16 ± 0.01a	0.15 ± 0.008a
T-linoleic	0.06 ± 0.004a	0.05 ± 0.01a	0.08 ± 0.03a
linoleic	11.91 ± 0.05c	11.03 ± 0.03a	11.48 ± 0.09b
arachidic	0.21 ± 0.05a	0.19 ± 0.10a	0.25 ± 0.04a
linolenic	0.74 ± 0.06a	0.64 ± 0.008a	0.64 ± 0.06a
behenic	0.29 ± 0.04a	0.56 ± 0.1b	0.21 ± 0.04a
arachidonic	0.10 ± 0.02b	0.27 ± 0.02c	0.04 ± 0.006a
brassidic	0.24 ± 0.02b	0.05 ± 0.01a	0.32 ± 0.13c
erucic	0.19 ± 0.01a	0.21 ± 0.01a	0.54 ± 0.02b
eicosapentaenoic	0.04 ± 0.007a	0.04 ± 0.009ab	0.07 ± 0.03b
docosahexaenoic	0.07 ± 0.01b	0.06 ± 0.01b	0.03 ± 0.009a

[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 Patties (Grams per 100 g of Fatty Acids)[a]

	raw beef	microwaved	fried in olive oil
lauric	0.13 ± 0.01b	0.10 ± 0.003a	0.10 ± 0.001 a
myristic	2.84 ± 0.04b	2.87 ± 0.08b	2.69 ± 0.03a
palmitic	26.04 ± 0.47b	26.35 ± 0.34b	25.11 ± 0.01a
T-palmitoleic	0.33 ± 0.01a	0.47 ± 0.01b	0.30 ± 0.07a
palmitoleic	4.30 ± 0.04a	4.79 ± 0.1b	4.73 ± 0.006b
stearic	14.75 ± 0.10a	16.54 ± 0.17c	15.04 ± 0.008b
oleic	39.38 ± 0.17a	39.26 ± 0.34a	43.17 ± 0.05b
elaidic	4.71 ± 0.03c	4.01 ± 0.1b	3.68 ± 0.02a
T-linoleic	0.16 ± 0.02b	0.10 ± 0.02a	0.12 ± 0.01a
linoleic	6.07 ± 0.45b	4.15 ± 0.03a	4.07 ± 0.005a
arachidic	0.11 ± 0.06a	0.13 ± 0.04a	0.22 ± 0.006b
linolenic	0.42 ± 0.07c	0.27 ± 0.07a	0.59 ± 0.01b
behenic	0.37 ± 0.16a	0.39 ± 0.07a	0.33 ± 0.01a
arachidonic	0.11 ± 0.009a	0.31 ± 0.01b	0.02 ± 0.00a
brassidic	0.11 ± 0.007ab	0.06 ± 0.00a	0.19 ± 0.02b
erucic	0.27 ± 0.03a	0.25 ± 0.008a	0.34 ± 0.004b
eicosapentaenoic	0.04 ± 0.005a	0.06 ± 0.01a	0.09 ± 0.00b
docosahexaenoic	0.09 ± 0.02b	0.06 ± 0.02a	0.06 ± 0.01a

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

on each side. The temperature of the oil when the process started was 180 °C. The final internal temperature of the patties was 85−90 °C.

Chemical Analysis. Moisture analysis was carried out according to the AOAC method (*32*). Cholesterol analysis was done according to the method of 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 the 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 method III of Guardiola et al. (*37*). Approximately 1 g of fat was added to a flask containing 10 mL of 1 M KOH in methanol and 1 mL (20 μg/mL) of internal standard (IS = 19-hydroxycholesterol) and kept stirring at room temperature during 20 h to complete the cold saponification. The unsaponifiable material was extracted with diethyl ether. The whole organic extract was washed with water and filtered through anhydrous sodium sulfate. Then it was recovered in a round-bottom flask, and the solvent was evaporated using a rotatory vacuum evaporator at 30 °C. Purification was performed with silica cartridges (Sep-Pak Vac 6 cm^3 SPE, Waters, Millipore, Bedford, MA) using different proportions of hexane/diethyl ether, and finally COPs were recovered with acetone/methanol (60:20, v/v). A derivatization to obtain the trimethylsilyl 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, CA). The column used was an HP-5MS column (30 m × 250 μm × 0.25 μm), and helium was the carrier gas (1 mL/min). The chromatographic conditions were as follows: initial column temperature at 80 °C, held for 1 min, and programmed to 250 °C at a rate of 10 °C/min and finally to 280 °C at a rate of 4 °C/min, and held for 20 min. The injector temperature was 250 °C, and the inlet pressure was 23.2 psig; mass range, *m/z* 50−550 amu (atomic mass units); solvent delay was 20 min. Calibration curves were developed for six cholesterol oxides: cholest-5-ene-3β,7α-diol (7α-hydroxycholesterol), cholest-5-ene-3β,7β-diol (7β-hydroxycholesterol), cholest-5-ene-3β,25-diol (25-hydroxycholesterol), 5,6α-epoxy-5α-cholestan-3β-ol (α-epoxycholesterol), 3β-hydroxycolest-5-en-7-one (7-ketocholesterol), and 5α-cholestane-3β,5,6β-triol (cholestanetriol). All of these standards were purchased from Sigma Chemical Co. (St. Louis, MO) except for 7α-hydroxycholesterol and 19-hydroxycholesterol, which were purchased from Steraloids (Steraloids Inc., Newport, RI). Different concentrations (2.5, 5, 10, 20, 40, and 80 μg/mL) of mixtures of all the standards were analyzed in the total ion chromatogram (TIC) mode. The identification of the COPs in the sample was made by checking their retention times and their mass spectra (using the HPCHEM Wiley 275 6th ed. library) with those of the standard compounds.

Quantification of the each COP was made by means of its 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 patties, 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 patty in order to analyze statistical differences between the cooking methods applied ($p \leq 0.05$). A Student *t* test was done to analyze the statistical differences between species in both raw and cooked samples.

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

RESULTS AND DISCUSSION

Both microwave heating and frying significantly decreased the moisture of beef and chicken patties (**Table 1**). Microwave heating is not expected to modify the total lipid content of food, whereas frying could (*18*, *23*, *38*). However, other authors have found lipid losses in hamburgers after frying and after cooking with microwaves (*27*). Microwave heating did not increase the lipid percentage, and frying slightly increased it only in chicken. The effects on cholesterol amount were found to be significant, increasing with both types of technologies. In relation to the

Table 4. Student *t* Test for Every Fatty Acid Analyzed between the Two Species (Chicken and Beef) in the Different Types of Samples[a]

	raw	microwaved	fried in 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	*		***
erucic	**	**	***
eicosapentaenoic	NS	NS	NS
docosahexaenoic	NS	NS	**

[a] NS, not significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 5. Fatty Acid Fractions (Grams per 100 g of Product) and Ratios for the Different Analyzed Patties[a]

	beef			chicken		
	raw	micro-waved	fried in olive oil	raw	micro-waved	fried in 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
ω3	0.06a	0.04a	0.08b	0.08a	0.08a	0.09a
ω6	0.64b	0.45a	0.43a	1.20a	1.20a	1.31b
ω6/ω3	10.67b	11.25b	5.37a	15a	15a	14.56a

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

type of meat, no significant differences were found for raw samples or for cooked samples in fat percentages.

Despite the absence of significant differences in the total lipid percentages between species and between applied technologies, some interesting qualitative differences were found in the fatty acid profiles. In chicken patties no changes were observed with microwave heating 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 microwave treatment only eight fatty acids did not change, including oleic and eicosapentaenoic (EPA) acids. Linoleic, linolenic, and docosahexaenoic (DHA) acids decreased. Frying caused greater modifications in the fatty acid profile with only four 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. By comparing the fatty acid profiles for beef and chicken patties, it can be observed that significant differences were found for most of them (**Table 4**). In other works different fatty acid profiles were observed for raw patties depending on the type of meat (*28*, *29*). Chicken patties, 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 patties than in chicken ones. No significant differences were found for EPA and DHA except for fried chicken patties, which showed lower DHA amounts than fried beef ones. Therefore, it can be concluded that differences in the fatty acid profiles of raw patties as a consequence of the different types of meat, are maintained in cooked patties.

The observed changes as a consequence of the cooking process cannot be considered quantitatively relevant by taking into account the total amounts of the different fatty acid fractions in raw and cooked samples (**Table 5**). From a quantitative point of view, total amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (grams per 100 g of product) were hardly affected, as also reported by Rodriguez-Estrada et al. (*27*), although some statistical differences were detected. The use of microwave heating did not modify the ω6/ω3 ratio in any case. It decreased the unsaturated/saturated fatty acids (U/S) in beef patties, whereas no modification was observed in chicken patties. In fried samples the U/S ratio showed increases that, especially in chicken patties, could be considered as beneficial from a nutritional point of view (increase from 1.64 to 2.11). The ω6/ω3 ratio, which is considered to be too high in today's diet (*40*), showed a decrease in beef patties with frying (from 10.67 to 5.37).

In relation to the COP analysis (**Table 6**), in raw beef patties only 7-ketocholesterol was detected; 7β-hydroxycholesterol was

Table 6. Cholesterol Oxide Content Found in Each Type of Patty (Micrograms per Gram of Fat)[a]

	beef			chicken			LS		
cholesterol oxide	raw	micro-waved	fried in olive oil	raw	micro-waved	fried in olive oil	R	M	F
7α-hydroxycholesterol	NDa	4.58 ± 0.32c	0.83 ± 0.13b	NDa	12.67 ± 1.42c	2.93 ± 0.09b		***	***
7β-hydroxycholesterol	NDa	3.68 ± 0.18b	0.86 ± 0.07a	2.14 ± 0.13a	6.43 ± 0.37b	2.25 ± 0.12a	NS	***	***
7-ketocholesterol	2.31 ± 0.26b	3.44 ± 0.03c	0.95 ± 0.1a	1.87 ± 0.21a	4 ± 0.53c	2.53 ± 0.38b	*	NS	**
α-epoxycholesterol	NDa	0.56 ± 0.01b	NDa	NDa	1.53 ± 0.13c	0.65 ± 0.04b		***	***
cholestanetriol	NDa	NDa	0.77 ± 0.1b	NDa	NDa	2.39 ± 0.15b			***
25- hydroxycholesterol	ND	traces	ND	NDa	NDa	NDa			
total COPs	2.31a	12.26c	3.41b	4.01a	24.63c	10.75b	***	***	***
total COPs (ppm in patties)	0.23a	1.23c	0.36b	0.40a	2.63c	1.23b	***	***	***
% of cholesterol oxidation	0.04	0.18	0.05	0.06	0.36	0.16			

[a] 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 in olive oil). NS, not significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. ND, not detected.

also detected in raw chicken patties. 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 the progression of carotid atherosclerosis (*44*). The percentages of 7-ketocholesterol were 100 and 46.53%, in raw patties 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 >50% of the oxidation products in raw samples of veal, beef, pork, and chicken tissues. Quantitatively, 7-ketocholesterol increased significantly in beef patties with heating only when microwaving was used as the cooking method. In the case of chicken, frying also caused a significant increase of 7-ketocholesterol, reaching significantly higher amounts in fried chicken than in fried beef patties ($p < 0.01$). Rodriguez-Estrada et al. (*27*), analyzing 7-ketocholesterol in raw hamburgers, found much higher 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 microwave heating. However, most of the research has observed an increase in total COPs, including 7-ketocholesterol, when meats are cooked (*30*, *46*−*48*). Echarte et al. (*18*) found that 7-ketocholesterol increased its level with frying 8−12 times and constituted >65% of total COPs in raw and cooked samples.

7β-Hydroxycholesterol was, quantitatively, the second compound in microwave-treated beef and chicken patties. Larkeson et al. (*30*) found in raw patties not only 7β-hydroxycholesterol and 7-ketocholesterol but also 7α-hydroxycholesterol and 5,6α-epoxycholesterol, reaching a total COP 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 of the COPs analyzed except for fried beef hamburgers. 25-Hydroxycholesterol has been found as one of the most cytotoxic COPs 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, its level being significantly higher in chicken samples ($p < 0.001$). As beef samples show cholestanetriol but no α-epoxide was detected, the cholestanetriol might be synthesized from β-epoxycholesterol, which was not analyzed in this work.

Significant differences were found for total COPs among the cooking methods and types of meat. Total COP amounts expressed in parts per million on product (patties) did not reach 3 ppm in any case. These data are similar to values obtained by Grau et al. (*50*) in cooked chicken meat (3.26 ppm) and those found in other works for raw beef meat (0.5−3.4 ppm) (*51*, *52*); however, they are lower than for fried pork loin (~9 ppm) (*18*). In a comparison of beef and chicken patties, the latter showed in both raw and cooked samples the highest total COP amounts and also higher increments of COPs than beef patties with cooking. This 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 microwave heating. In the rest of the samples, every COP showed a higher amount in chicken than in beef patties.

Conchillo et al. (*54*), analyzing the effect of grilling and roasting on cholesterol oxidation in chicken breast meat, found that total COPs increased 4−4.5-fold with cooking. In the analyzed patties the increases were 5.3−6.1-fold with microwave heating and 1.5−2.6-fold with frying. Microwaved chicken patties showed a percentage of oxidation of 0.36%, the remaining values being <0.2%, considered low by Lercker and Rodríguez-Estrada (*31*). All of these data pointed out that microwave heating caused the highest cholesterol oxidation.

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