Title: Use of microwave in chicken breast and application of different storage conditions: consequences on oxidation.

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Abstract

Slices of chicken breast were subjected to microwave heating (750W, 3minutes) and further storage in different conditions (refrigeration at 4ºC and freezing at –18ºC combined with aerobic, vacuum and modified atmosphere packaging). Evaluation of the intensity of the oxidation process was carried out. A 16 fold increment in the amount of cholesterol oxidation products (COP) was found as a consequence of microwave cooking (45.86µg/g lipid after microwave and 2.88µg/g lipid in raw samples). 7-ketocholesterol was the most affected COP by microwave, accounting for a 25% of the total COP. Storage of microwaved samples under aerobic refrigeration led to the highest oxidation status with the following values: peroxide 19.41meqO_2/kg lipid, TBA 0.32 ppm and COP 123.50 µg/g lipid. MAP refrigerated samples showed 50.94µg/g lipid of total COP , an amount slightly higher than in vacuum conditions (46.81 µg/g lipid). Under frozen storage MAP **and vacuum** samples showed the lowest **amounts** of total COP (29.76 µg/g lipid **and 39.28µg/g lipid, respectively**).

Keywords: Cholesterol, COP, modified atmosphere packaging, vacuum, refrigeration, freezing.

Introduction

Microwave treatment is nowadays one of the most usually employed cooking methods both at home and also in restaurants and catering systems, because of its high rate of heat transmission. Also microwave shows advantages like savings in time and energy, cleanness and easiness of use. All these reasons make microwave cooking one of the most attractive cooking methods, and the target of emerging studies in order to evaluate its effect on different foods.

Some studies report that, comparing microwave with conventional heat treatments, the first one induced higher losses of vitamins and unsaturated fatty acids [1, 2] and higher oxidation intensity [3]. Lipid oxidation can affect cholesterol stability, producing cholesterol oxidation products (COP), which are known to have a wide range of adverse biological effects, including cytotoxicity, mutagenesis, carcinogenesis and specially atherogenesis [4, 5, 6]. Intensity of COP formation in foods seems to be affected by heat treatment and by storage conditions [7, 8, 9, 10, 11].

Besides vacuum packaging, modified atmosphere packaging (MAP) is an increasing technology to extend the shelf life of foods by controlling microbial growth and also the oxidation processes that take place during storage. Different results have been obtained depending on the product and atmosphere used. Zanardi et al. [12] found that sliced Milano-type sausages packed in MAP (100% N_2) had higher color and lipid oxidative stability than those vacuum packed. Jiménez et al. [13] found that MAP (70% CO_2 and 30%N_2) extended the shelf-life of raw chicken breasts. On the other hand, MAP packaging did not extend the product shelf life of cooked pork sausages in comparison to vacuum packaging [14]. Other works do not elucidate between vacuum or MAP for the best preservation of the type of food studied [15, 16]. Consequently, it could be of

interest to study whether MAP is a better choice than vacuum packaging for storage of microwaved chicken breast in terms of stability against oxidation.

The objective of this paper was to evaluate the consequences of the use of microwave heating on the intensity of oxidation process and COP formation. Also the consequences of refrigeration and frozen storage and the use of different packaging system of those microwaved foods were studied in order to minimize risks related to oxidation intensity during the storage of microwaved foods.

Material and methods

Sample preparation

8 kg of chicken breasts was obtained directly from a local slaughter house, coming from the same **flock** of animals. They were sliced into 1.5 cm thick portions and randomly distributed into 8 batches of 1 kg each. One batch for analyzing in raw and the other 7 were cooked in microwave oven **(Whirlpool Corporation, Norrköping, Sweden)** at 750W during 3 minutes (final internal temperature ranged 82-88ºC). **Internal temperature of samples was measured with a digital thermometer (51 J/K RS 614-299, Fluke, USA).** One of these batches was analyzed immediately after being subjected to microwave (t=0) and the other 6 were analyzed after being stored in different conditions: combination of refrigeration (6 days at 4ºC) and freezing (3 months at –18ºC) with aerobic storage, vacuum and modified atmosphere packaging (20% CO2 and 80% N2) **(Extendapack 14, Praxair).** The vacuum sealer used was Model VP-1000 (Ramon, Barcelona, Spain). All bags were of polyamide/polyethylene 90 μm (Corsan, Pamplona, Spain). **The film used for packaging has an oxygen transmission rate of 8,3 cc/m2/24h al 23ºC and 0%R.H.**

Chemical analysis

Extraction of lipids was made with a mixture of chloroform/methanol according to the method of Folch et al. [17]. Peroxide value was determined using the official method of AOAC [18]. TBA value was determined according to Tarladgis et al. [19] with modifications by Zisper et al. [20], Tarladgis et al. [21], Pikul et al. [22]. Results are shown in mg malonaldehyde/Kg sample (ppm).

The determination of cholesterol was made by gas chromatography, according to the method described by Kovacs et al. [23]. A Perkin-Elmer Autosystem gas chromatograph equipped with an HP1 column (30 m x 0.25 mm x 0.1 μm) was used.

The oven temperature was 265ºC. The temperature of both the injection port and detector was 285ºC. The sample size was 0.5 μl. 5α-cholestane (Sigma, St. Louis, MO, USA) was used as an internal standard. A Perkin-Elmer Turbochrom programme was used for quantification.

COP analysis was carried out according to Echarte et al. [*24*]. Identification and quantification of cholesterol oxides was performed by gas chromatography-mass spectrometry (GC-MS). A GC Hewlett-Packard 6890 coupled to a 5973 mass selective detector (Wilmington, Delaware, USA) was used. Chromatography conditions are fully described elsewhere [24].

Statistical analysis

Four samples were analyzed for each of the eight different conditions tested (raw; t=0; refrigeration in aerobiosis, vacuum and MAP; freezing in aerobiosis, vacuum and MAP). Each parameter was determined four times for each sample. Mean and standard deviation data for each condition are shown in tables. One-way analysis of variance (ANOVA] with a posteriori Tukey b test was carried out in order to determine statistical differences among samples ($p<0.05$). Data analysis was carried out with a SPSS 11.0 program (SPSS Inc. Chicago, Il, USA).

Results and discussion

Effects of microwave

Microwave cooking of chicken breast caused a slight, **although statistically significant**, increase of TBA and peroxides (table 1). TBA increased from 0.10 (raw samples) to 0.15 ppm (cooked samples) and peroxides value increased from 0 (raw samples) to 2.49 meqO_2/kg lipid (cooked samples). Conchillo et al. [25] analyzing the effect of other culinary technologies over the lipid fraction in the same type of food (chicken breast), found that grilling increased TBA in 0.17 ppm **(from 0.04 to 0.21) and peroxides from 0 to 4.33 meqO_2/kg lipid.** After roasting, that work showed increases of 0.20 ppm for TBA, without changes in peroxides. Pikul et al. [26] analyzing the intensity of lipid oxidation in chicken meat did not find significant differences in **the** level of lipid oxidation products (TBA) between microwaved and conventional oven cooking, although certain secondary fluorescent products were higher in meats cooked by convention oven.

The increase of COP as a consequence of heat treatments has been widely proved [27, 28]. Table 2 shows the amounts of the different COP found in every sample. In raw samples all analyzed compounds were detected, except for α-epoxycholesterol (fig.1). Microwave heating resulted in a 16 fold increase of total COP, reaching 45.86 μg/g lipid, value that meant a 0.1% of cholesterol oxidation. Conchillo et al. [25] reported increases around 4-5 fold of total COP in grilled and roasted chicken breast with regard to raw samples, corresponding to percentages of oxidation of 0.04 and 0.03%, respectively. It could be concluded that the increment of COP in chicken breast was notably higher than when using other cooking methods, despite the lower oxidation increment measured by TBA and peroxides.

When the effect of microwave in COP formation has been compared to other cooking treatments different results have been reported in various foods. Echarte et al. [29] **analyzed** the effect of two cooking processes on cholesterol oxidation in beef and chicken patties **and** concluded that microwave heating caused higher cholesterol oxidation (0.18% in beef and 0.36% in chicken patties) than frying (0.05% in beef and 0.16% in chicken patties). On the contrary, Kim et al. [30] found that pan frying and deep fat frying resulted in a higher cholesterol oxidation intensity than microwave treatment of saury.

Every COP showed significant increments in microwaved samples in comparison to raw matter. The largest increases as a consequence of **microwaves** were found for 7-ketocholesterol, 7β-hydroxycholesterol and β-epoxycholesterol. 7-ketocholesterol has been considered as an useful marker of the total oxidative process [31, 32], the major oxysterol in arterial macrophages [33] and, together with 7β-hydroxycholesterol, the most effective in causing neuroretinal cell death [34]. 7-ketocholesterol was present in a very low amount in raw samples (0.07 μg/g lipid), suffering the greatest increase among all COP with microwave cooking. It was, together with β-epoxycholesterol (11.73 μg/g lipid) one of the most abundant COP in cooked samples, reaching 11.66 μg/g lipid, a 25% of the total COP. Rodriguez-Estrada et al. [35] however found a decrease of 7-ketocholesterol in microwaved-heated beef hamburgers with regard to raw samples. 7β-hydroxycholesterol has been shown to be a good marker of lipid peroxidation in vitro [36], in vivo [37] and a potential predictor of the progression of carotid atherosclerosis [38]. It reached 10.27 μg/g lipid, about 3 fold values obtained with other cooking methods for chicken breast [25].

In microwaved samples, cholestanetriol and 25-hydroxycholesterol were the less abundant COP, although their amounts were statistically different from raw chicken. 25-

hydroxycholesterol has been found as one of the most cytotoxic COP on human hematopoietic progenitor cells [39].

Effect of storage conditions

The effect of different storage conditions over the intensity of the oxidation process in microwaved samples was also studied. A significant increment of the TBA **value was found, only** in aerobically refrigerated microwaved chicken (0.32 ppm), whereas vacuum packaging in refrigeration (0.15 ppm) and aerobic packaging during freezing (0.14 ppm) maintained similar values compared to immediately cooked samples (t=0). According to Kowale et al. [40], the changes in TBA values were more pronounced during refrigeration than during frozen storage, indicating that changes were directly related to the increase in temperature of storage. The obtained values of TBA were below the threshold value for rancidity development in meat (1-2 ppm) reported by Watts [*41*]. Similar results were obtained for peroxides, where the highest amount was found for aerobically refrigerated samples (19.41 meqO_2/Kg lipid), and the rest of conditions did not reach 8 meqO_2/Kg lipid. Concerning the type of packaging, both in refrigeration and freezing conditions, TBA and peroxides showed significantly higher values in vacuum than in MAP. It has also to be pointed out that for TBA freezing gave rise to significant decreases in vacuum and MAP conditions **with regard to t=0**, whereas under refrigeration this decrease was only observed for MAP. Wang et al. [42], detected a greater intensity of oxidation (peroxide and TBA values) in vacuum than in MAP when studying Chinese-style sausages stored at 4ºC. **Our data seemed to confirm that finding, although it has also to be stated that values were all so low that, regarding oxidation, had no relevance in any case.**

Comparing the COP formation during refrigeration and freezing for each packaging modalities (aerobic, vacuum and MAP) it can be stated up that refrigeration gave rise to

the highest COP amount in all cases. The highest difference was found in the aerobic conditions with increments around 10 μg/g lipid in frozen samples and around 77 μg/g lipid in refrigerated samples suffering also the highest rate of cholesterol oxidation (0.18 and 0.23%, respectively). Every analyzed COP increased significantly during refrigeration at aerobic conditions, particularly 7-ketocholesterol and 7β-hydroxycholesterol. A chromatogram of one of those samples is shown in figure 2. Lee et al. [43] found that 7β-hydroxycholesterol was the most abundant COP in irradiated chicken samples aerobically stored. In this work it was also the most abundant compound after aerobic storage under refrigeration, with a 30% of the total COP. In the case of frozen storage 7α-hydroxycholesterol did not change and 25-hydroxycholesterol decreased.

A previous work established that vacuum packaging was particularly efficient in slowing down the oxidation process during frozen storage of grilled and roasted chicken [44]. There are some studies where cholesterol oxidation did not appear to be inhibited by vacuum packaging or frozen storage [40, 45, 46]. This paper confirms the idoneity of vacuum packaging to reduce the COP formation during storage of cooked chicken, both at 4 and -18ºC. In the case of refrigeration, vacuum conditions did not differ from t=0 samples in the total COP amount (46.81 and 45.86 μg/g lipid, respectively) neither in the percentage of oxidation (0.10%). Under frozen conditions even a lower amount of COP was obtained (**39.28** μg/g lipid) **compared with t=0, but the percentages of oxidation did not show significant differences**.

Few studies deal with the preservation of cholesterol oxidation by using MAP. Zanardi et al. [**12**] reported that vacuum packaging was less efficient in controlling the oxidation of cholesterol during the storage of Milano-type sausages than 100% N_2 atmosphere. The level of COP found in MAP refrigerated samples was 50.94 μg/g lipid showing

higher percentage of oxidation than vacuum storage samples. Under frozen conditions MAP samples showed the lowest amount of total COP (29.76 μg/g lipid) **without significant differences in the percentage of oxidation (0.08%) in relation to vacuum samples (0.09%). Also, it has to be noted that both TBA and COP showed lower values in samples under MAP frozen conditions than in t=0. These data are difficult to be explained and they will probably require further studies.**

Results presented in this paper indicate **that vacuum and** MAP are very efficient in slowing down the oxidation intensity and the COP amounts of microwaved chicken breast, **during refrigeration and particularly during freezing conditions, specially in the case of MAP**.

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Table 1. TBA and Peroxides values measured in raw and microwaved chicken breast at t=0 and after 6days at 4ºC (Refrigeration) and 3 months at –18ºC (Freezing) stored under aerobic, vacuum and modified atmosphere conditions. Values are given as mean and standard deviation, between brackets.

	Raw	Microwave						
		t=0	Refrigeration			Freezing		
			Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP
TBA (ppm)	0.10c (0.02)	0.15d (0.01)	0.32e (0.02)	0.15d (0.01)	0.05a (0.01)	0.14d (0.03)	0.11c (0.01)	0.08b (0.01)
Peroxides ($meqO_2$/kg lipid)	0a	2.49b (0.56)	19.41f (0.21)	3.41c (0.51)	0a	7.51e (0.41)	4.60d (0.16)	3.99c (0.05)

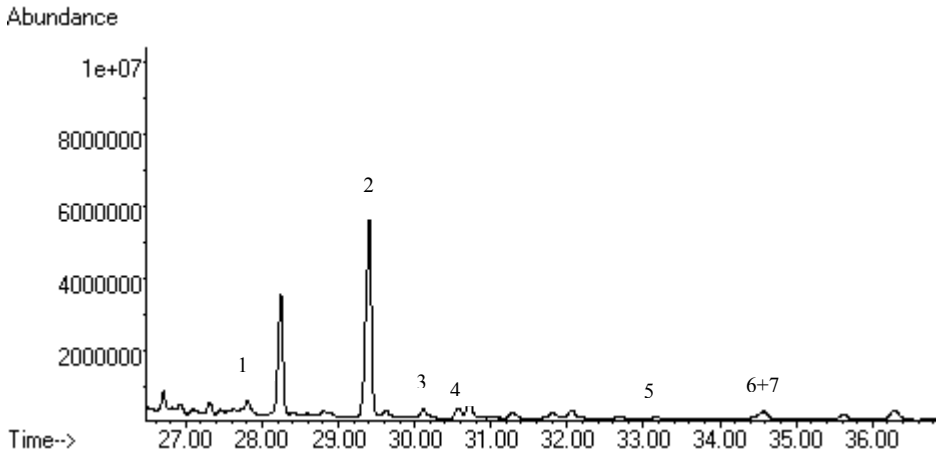
Different letters indicate significant differences among conditions ($p<0.05$).

Table 2. Cholesterol oxidation products (COP) values (μg/g lipid), cholesterol **(mg/g lipid)** and percentage of oxidation in raw and microwaved chicken breast at 0 days and after 6days at 4ºC (Refrigeration) and 3 months at −18ºC (Freezing) stored under aerobic, vacuum and modified atmosphere conditions. Values are given as mean and standard deviation, between brackets.

	Raw	Microwave						
		t=0	Refrigeration			Freezing		
			Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP
7α-hydroxy cholesterol	0.23a (0.02)	8.68c (0.08)	22.82d (1.08)	8.28c (0.38)	7.93c (0.21)	8.38c (0.16)	8.14c (0.4)	3.53b (0.11)
7β-hydroxy cholesterol	0.40a (0.03)	10.27c (0.09)	37.26f (2.74)	12.83de (0.09)	11.56cd (0.21)	13.66e (0.52)	10.38c (0.37)	6.99b (0.12)
β-epoxy cholesterol	1.32a (0.02)	11.73d (0.20)	19.48f (1.42)	10.49c (0.18)	9.38c (0.08)	13.89e (0.45)	7.15b (0.95)	7.78b (0.35)
α-epoxy cholesterol	0a	2.34c (0.08)	6.42e (0.47)	2.66c (0.05)	6.99f (0.44)	3.23d (0.13)	1.72b (0.11)	1.62b (0.09)
Cholestanetriol	0.68a (0.02)	0.83b (0.01)	0.97c (0.02)	0.78b (0.01)	1.26d (0.03)	0.98c (0.07)	0.82b (0.06)	0.93c (0.02)
25-hydroxy cholesterol	0.20a (0.01)	0.35d (0.01)	0.50e (0.04)	0.34d (0.01)	0.36d (0.03)	0.27c (0.02)	0.22ab (0.02)	0.26bc (0.02)
7-keto cholesterol	0.07a (0.01)	11.66cd (0.40)	35.65g (2.76)	11.40cd (0.88)	13.43ef (0.75)	15.05f (0.2)	10.85bc (1.16)	8.66b (0.43)
Total COP	2.88a	45.86d	123.50g	46.81d	50.94e	55.46f	39.28c	29.76b
Cholesterol (mg/g lipid)	47.95cd	47.45d	52.93e	47.36d	43.99cd	31.11a	42.07bc	37.74b
% Oxidation	**0.006a**	**0.097c**	**0.233f**	**0.099c**	**0.116d**	**0.178e**	**0.093bc**	**0.079b**

Different letters indicate significant differences among conditions ($p<0.05$).

Figure 1. Chromatogram of TMS ether COP of raw chicken breast.



1.- 7α-hydroxycholesterol
2.- 19-hydroxycholesterol
3.- 7β-hydroxycholesterol
4.- β-epoxycholesterol
5.- cholestanetriol
6.- 25-hydroxycholesterol
7.- 7-ketocholesterol

Figure 2: Chromatogram of TMS ether COP of microwaved breast chicken storage in aerobic conditions during 6 days in refrigeration (4ºC).