

## Effect of feeding fat sources on the quality and composition of lipids of precooked ready-to-eat fried chicken patties

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### Abstract

The effect of feeding fat sources on the quality and composition of lipids of raw meat and precooked ready-to-eat fried chicken patties was studied. Two homogeneous groups of broilers were fed with animal fats and vegetable oils, respectively. A traditional technology (flash-frying and humid steam-convection oven cooking) was employed to produce the patties. Lipid hydrolysis (total fatty acids, free fatty acids and diacylglycerols) and oxidation (peroxide value (POV) and cholesterol oxidation products (COPs)) were evaluated in the initial, intermediate and final products. Lipid hydrolysis and oxidation were more intense in ground raw meat obtained with animal and vegetable fat integration, respectively. However, these differences tended to decrease along the technological process, due to the addition of other ingredients and the oil absorption. Although flash-frying and humid steam-convection oven cooking promoted lipid degradation, the overall quality of the precooked chicken cutlets was acceptable (low POV and COPS values) and greatly depended on the quality of the raw meat and the frying oil.

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### 1. Introduction

Over the past few years, the consumption of poultry meat has become very popular due to their nutritional characteristics. In fact, chicken meat supplies high protein (around 20 g/100 g raw meat without skin) and low fat intakes (around 5 g/100 g raw meat without skin), respectively. Moreover, chicken lipids are characterized by relatively high levels of unsaturated fatty acids (especially polyunsaturated fatty acids), which are considered as a positive and healthy aspect by consumers.

In recent years, there has been an increased attention towards the manipulation of the meat lipid amount and composition (Wood et al., 2003), in order to obtain spe-

cific nutritional properties. One of the major objectives is to lower the content of saturated fatty acids, since they have been associated with several diseases, in particular cancer and coronary heart disease (Reddy, 1995; Soyland & Drevon, 1993). Lipid composition of poultry meat is generally manipulated by using a selected feeding with particular nutritional characteristics. In fact, the ratio of unsaturated to saturated fatty acids in meat should be increased, so as to achieve positive health effects (Soyland & Drevon, 1993).

However, a higher unsaturation index in meat and meat products may affect their oxidative stability, since the unsaturated fatty acids are more prone to oxidation (Bou et al., 2001; Bou, Guardiola, Tres, Barroeta, & Codony, 2004; Eder et al., 2005; Grau, Codony, Grimpa, Baucells, & Guardiola, 2001; López-Bote, Gray, Gomaa, & Flegal, 1998a, 1998b; O'Neill, Galvin, Morrissey, & Buckley, 1998).

In fact, several studies have been carried out on the effect of the supplementation of feeding with different

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antioxidants and dietary fat sources on the composition and sensory quality of chicken meat (Bou et al., 2001; Bou et al., 2004; Eder et al., 2005; Grau et al., 2001; López-Bote et al., 1998a, 1998b; Maraschiello, Esteve, Regueiro, & García, 1998; O'Neill et al., 1998). In general, it has been demonstrated that feeding supplementation with antioxidants is highly effective in protecting raw, processed, cooked, and stored meat from lipid oxidation.

In addition, the release of new packaging formats for meat products and the diffusion of prepacked or pre-cooked ready-to-eat food based on poultry meat (Pszczola, 2002), has further enhanced the intake of poultry meat. This is particularly true for pre-cooked chicken cutlets with and without addition of other ingredients, such as mozzarella cheese and spinach. These products are prepared by fast deep-frying followed by baking. The success of these food products is mainly related to the rapid and easy cooking. However, the various technological steps could lead to several chemical degradations of the meat components, such as lipid hydrolysis and oxidation. In fact, it has been largely demonstrated how the common culinary steps (mincing, heating, packaging, storage) can seriously affect the lipid pattern, increasing the level of lipid hydrolysis and oxidation (Conchillo, Ansorena, & Astiasarán, 2003, 2004, 2005; Penazzi, 1997; Rodriguez-Estrada, Penazzi, Caboni, Bertacco, & Lercker, 1997).

Lipid oxidation is one of the most important reasons of deterioration in poultry products, because it generates undesirable odors and flavors and limits their shelf-life and commercial stability. As aforementioned, meat oxidation is related to the content of natural antioxidants and the polyunsaturation degree of fatty acids (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998; Nam, Du, Jo, & Ahn, 2001). Therefore, variations of the natural lipid pattern of meat may affect its susceptibility and resistance to the oxidation process. In addition, some lipid oxidation products (e.g. cholesterol oxidation products) can be involved in lipid metabolism, various chronic and degenerative diseases (such as cancer, aging and human atherosclerosis) and disturbance of cell functionality (Garcia-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002; Schroefer, 2000).

The aim of this work was to evaluate the effect of feeding fat sources on the quality and composition of lipids of raw meat and pre-cooked ready-to-eat fried chicken patties. Two homogeneous groups of broilers were fed with animal fats and vegetable oils, respectively. A traditional technology (flash-frying plus humid steam-convection oven cooking) was employed to process broilers meat to obtain ready-to-eat meat-based patties. The effect of lipid integration on the stability and composition of the lipid fraction was evaluated in the initial, intermediate and final products, by determining several parameters of lipid hydrolysis (total fatty acids, free fatty acids and diacylglycerols) and oxidation (peroxide value and cholesterol oxidation products (COPs)).

## 2. Materials and methods

### 2.1. Samples

Two homogeneous groups of male Ross 508 broilers were reared under commercial conditions in two separate houses (20,000 animals/house) and fed ad libitum with two commercial diets for growing broilers. These diets were formulated so as to have the same energy density, protein and fat contents, but different lipid integration. In fact, one group (A) was fed with a diet containing animal fats (cattle tallow and pork lard), whereas the other one (V) was given a feeding prepared with vegetable oils (sunflower and soybean oils). When the broilers were 54 days-old, they were slaughtered at a commercial abattoir and, then, processed after a 6-day holding period. Breasts and legs without skin were cut and used as raw materials.

All the analyzed samples (raw meat, uncooked patties, and cooked patties) were prepared in a pilot plant. Fig. 1 reports the flow diagram of the meat processing. The

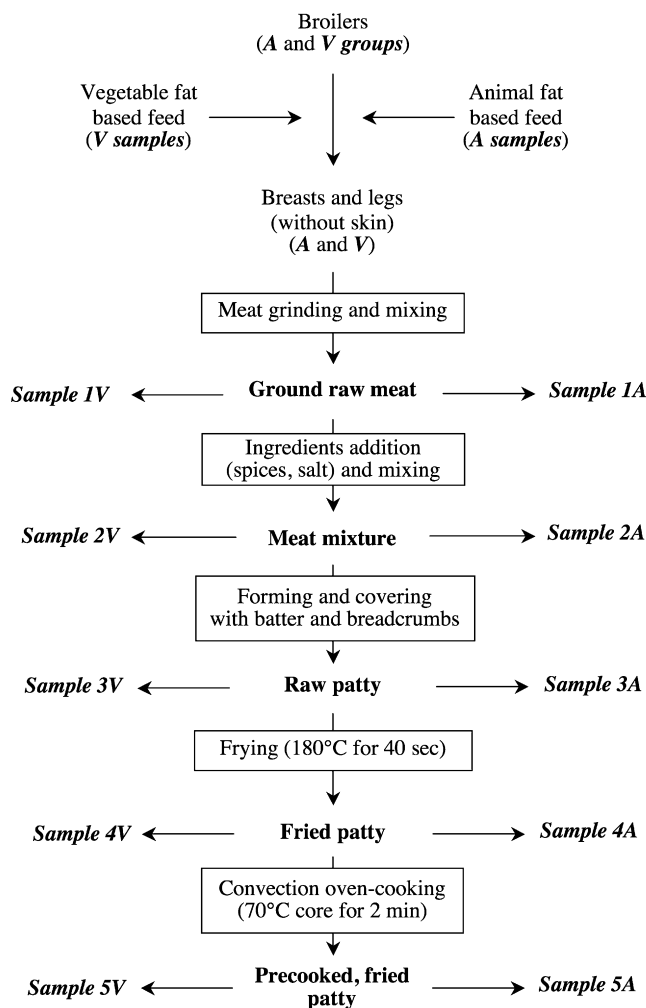


Fig. 1. Pre-cooked chicken cutlet production diagram and sample description.

samples analyzed were ground raw meat/animal and vegetable fats (1A and 1V), meat mixture/animal and vegetable fats (2A and 2V), raw patties/animal and vegetable fats (3A and 3V), fried patties/animal and vegetable fats (4A and 4V), precooked patties/animal and vegetable fats (5A and 5V). This traditional technology is a multiphase process, where the meat is first ground and then added with different ingredients to form the patties. The latter are then coated with a starch-protein batter, breaded, flash-fried at 180 °C/40 s in a rapeseed oil (0% erucic acid) bath and finally cooked in a humid steam-convection oven (core temperature = 70 °C/2 min).

The samples were stored at –40 °C until analyses.

## 2.2. Reagents, solvents and standard

Analytical grade chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

The commercial standards of COPs supplied by Sigma–Aldrich are listed as follows: cholest-5-en-3 $\beta$ ,19-diol (19-hydroxycholesterol) (used as internal standard for COPs quantification), 5 $\alpha$ -cholestan-3 $\beta$ -ol (dihydrocholesterol) (used as internal standard for cholesterol quantification), cholest-5-en-3 $\beta$ -ol (cholesterol), cholest-5-en-3 $\beta$ -ol-7-one (7-KC), 5 $\alpha$ ,6 $\alpha$ -epoxy-cholestan-3 $\beta$ -ol ( $\alpha$ -EC), 5 $\beta$ ,6 $\beta$ -epoxy-cholestan-3 $\beta$ -ol ( $\beta$ -EC), cholestan-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (triol), cholest-5-en-3 $\beta$ ,7 $\alpha$ -diol (7 $\alpha$ -HC), cholest-5-en-3 $\beta$ ,7 $\beta$ -diol (7 $\beta$ -HC), cholest-5-en-3 $\beta$ ,20 $\alpha$ -diol (20-HC) and cholest-5-en-3 $\beta$ ,25-diol (25-HC).

Commercial standards of diacylglycerols (dimyristin, dipalmitin, distearin, diolein) were purchased from Sigma–Aldrich, whereas a standard mixture of fatty acid methyl esters (GLC 463) was supplied by Nu-Chek (Elysian, MN, USA).

NH<sub>2</sub> and silica solid-phase extraction (SPE) cartridges (500-mg stationary phase/3-ml Strata cartridges) were purchased from Phenomenex (Torrance, CA, USA).

## 2.3. Lipid extraction

Lipid extraction was performed according to a modified version (Boselli, Velasco, Caboni, & Lercker, 2001) of the method by Folch, Lees, and Sloan-Stanley (1957). The solvent volumes were 2.5 times larger than those reported by Boselli et al. (2001). Different initial sample weight was used, according to the fat content; in fact, 90 and 40 g were utilized for raw (1–3) and cooked samples (4–5), respectively. The frozen samples were minced, weighed and homogenized with 500 ml of a chloroform:methanol solution (1:1, v/v) in a glass bottle with screw-cap. Successively, the bottle was placed at 60 °C for 20 min before adding 250 ml of chloroform. After 3-min homogenization, the content of the bottle was filtered through filter paper. The filtrate was mixed thoroughly with a 1 M KCl solution and left overnight at 4 °C, in order to obtain the phase separation. The lower phase was collected and dried with a vacuum evaporator. The fat content was determined gravi-

metrically. Three replicates of the lipid extraction procedure were performed per sample.

## 2.4. Spectrophotometric determination of peroxide value (POV)

The peroxide value was determined in 50 mg of lipid extract, as suggested by Shantha and Decker (1994). This method is based on the ability of peroxides to oxidize ferrous ions to ferric ions. Ammonium thiocyanate reacts with ferric ions, resulting in a coloured complex that can be measured spectrophotometrically. The peroxide value was evaluated at 500 nm with a double beam UV/Visible spectrophotometer (UV–Vis mod. UV-1601, Shimadzu, Co. Kyoto, Japan) and POV was calculated from the absorbance. For the quantitative determination of POV, a Fe(III) standard calibration curve was used with a concentration range of 0.1–5  $\mu$ g/ml ( $r^2 = 0.999$ ). Peroxide value was expressed as meq O<sub>2</sub>/kg fat. Three replicates were run per sample.

## 2.5. Determination of total cholesterol and cholesterol oxidation products (COPs)

A 250-mg lipid fraction of the Folch extract was added with known amounts of internal standards (12.5  $\mu$ g of 19-hydroxycholesterol and 1.02 mg of dihydrocholesterol, for the determination of COPs and total cholesterol, respectively). Subsequently, the sample was added with 10 ml of 1 N KOH solution in methanol, in order to perform a saponification at room temperature for 18 h (Sander, Addis, Park, & Smith, 1989). For the extraction of the unsaponifiable matter, 10 ml of water and 10 ml of diethyl ether were added to the samples, which were then shaken and the diethyl ether fraction was then separated; the extraction with 10 ml of diethyl ether was repeated twice. The three shares of diethyl ether were joined, added with 5 ml of a 0.5 N KOH solution and extracted.

For the determination of total cholesterol (sum of free and esterified), 1/10 of the unsaponifiable matter was subjected to silylation (Sweeley, Bentley, Makita, & Wells, 1963), dried under nitrogen stream and dissolved in 100  $\mu$ l of *n*-hexane. One microliter of the silylated solution was injected into a gas chromatograph (Autosystem XL GC, Perkin–Elmer Instruments, Norwalk, CT, USA), equipped with a split–splitless injector and a flame ionization detector. A fused-silica capillary column (30 m  $\times$  0.25 mm i.d.  $\times$  0.1  $\mu$ m film thickness) coated with 65% diphenyl-polysiloxane–35% dimethyl-polysiloxane (Rtx 65TG, Restek, Bellefonte, PA, USA), was used. The injector and detector temperatures were both set at 325 °C. The oven temperature was programmed from 230 to 260 °C at 2.5 °C/min and from 260 to 290 °C at 1 °C/min. Helium was used as carrier gas at a flow rate of 1.25 ml/min (pressure, 114 kPa); the split ratio was 1:32.

Regarding the determination of COPs, the remaining 9/10 of the unsaponifiable matter were purified by NH<sub>2</sub>

SPE (Rose-Sallin, Hugget, Bosset, Tabacchi, & Fay, 1995). COPs were eluted with acetone. The purified fraction was then silylated, dried under nitrogen stream and dissolved in 100  $\mu$ l of *n*-hexane. One microliter of the silylated COPs was injected into GC under the same conditions as reported for the determination of total cholesterol.

Peak identification of cholesterol and COPs was carried out by comparing the peak retention times with those of the commercial standards and by spiking with a small amount of standard. The response factors of COPs were evaluated with respect to the 19-hydroxycholesterol.

All GC data were stored and processed by the Turbochrom Navigator software (Ver. 6.1.1.0.0:K20) (Perkin-Elmer, Norwalk, USA).

### 2.6. Gas-chromatographic determination of total fatty acid

Total fatty acids were transmethylated according to Frega and Bocci (2001). About 50 mg of lipid extract were dissolved in 1 ml of *n*-hexane and then added with 1 ml of 2 N KOH solution in methanol. The mixture was vigorously shaken with a vortex for 2 min, then added with 1 ml of *n*-hexane and further shaken for 2 min. A known amount of internal standard solution (1.01 mg of tridecanoic acid methyl ester) was added to 1 ml of the organic mixture and diluted 10 times with *n*-hexane.

One microliter of the transmethylated solution was injected into a gas chromatograph (Autosystem XL GC, Perkin-Elmer Instruments, Norwalk, CT, USA), equipped with a split-splitless injector and a flame ionization detector. A fused silica capillary column (50 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness) (CPSil 88, Varian Inc., Palo Alto, CA, USA) coated with 100% cyanopropyl-polysiloxane, was used. The injector and detector temperatures were set at 220 °C and 230 °C, respectively. The oven temperature was kept at 160 °C for 1 min, then programmed from 160 °C to 190 °C at 3 °C/min, from 190 °C to 230 °C at 2 °C/min and, finally, kept at 230 °C for 15 min. Helium was used as carrier gas at a flow rate of 1.0 ml/min (pressure, 120 kPa); the split ratio was 1:20.

The peak identification was carried out by comparing the peak retention times with those of the FAMES standard mixture. The internal standard was used for the quantification of fatty acids. The GC response factor of each fatty acid was calculated by using the internal standard (C13:0). The results were expressed as g fatty acid/100 g total fatty acids (%).

### 2.7. Gas-chromatographic determination of free fatty acids (FFA)

Free fatty acids were purified by NH<sub>2</sub> SPE according to Parisi (2001). An aliquot of 25 mg of lipid matter, dissolved in 200  $\mu$ l of chloroform:isopropanol (2:1, v/v), was placed onto a NH<sub>2</sub> SPE cartridge previously loaded with Na<sub>2</sub>SO<sub>4</sub> and conditioned with 3 ml of *n*-hexane. The cartridge was eluted with 6 ml of chloroform-isopropanol (2:1, v/v)

and then with 10 ml of 2% acetic acid in diethyl ether (v/v). The latter fraction was evaporated under nitrogen flow, methylated with 100  $\mu$ l of diazomethane (Fieser & Fieser, 1967), added with a known amount of internal standard (0.05 mg of tridecanoic acid methyl ester) and diluted with 200  $\mu$ l of *n*-hexane. The methylated FFA were then injected in gas chromatography under the same analytical conditions as reported for the determination of total fatty acids.

### 2.8. Gas-chromatographic determination of diacylglycerols

Diacylglycerols were purified by silica SPE as suggested by Bortolomeazzi, Frega, and Lercker (1990). A known amount of internal standard (0.11 mg of squalane) was added to the diacylglycerols SPE fraction and silylated according to Sweeley et al. (1963).

One microliter of the silylated solution was injected into a gas chromatograph (Autosystem XL GC, Perkin-Elmer Instruments, Norwalk, CT, USA), equipped with a split-splitless injector and a flame ionization detector. A fused-silica capillary column (30 m  $\times$  0.25 mm i.d.  $\times$  0.1  $\mu$ m film thickness) coated with 65% diphenyl-polysiloxane-35% dimethyl-polysiloxane (Rtx 65TG, Restek, Bellefonte, PA, USA), was used. The injector and detector temperatures were set at 350 °C. The oven temperature was programmed from 175 to 350 ° at 3 °C/min and kept at 350 °C for 20 min. Helium was used as carrier gas at a flow rate of 1.25 ml/min (pressure, 114 kPa); the split ratio was 1:32.

### 2.9. Statistical analysis

The data are reported as mean values of three replicates ( $n = 3$ ) of each analytical determination, unless otherwise stated. Mean and standard deviation data of the different analytical parameters determined in each sample, are shown in Tables 1–3. One-way analysis of variance (ANOVA) was performed, in order to study the influence of the fat supplementation and the technological steps on several quality parameters (% lipid, total cholesterol, free cholesterol, total fatty acids, free fatty acids, diacylglycerols, POV, COPs). Tukey's honest significant multiple comparison was carried out, in order to determine statistical differences among samples ( $p < 0.05$ ). Statistical analysis of the data was performed by Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA).

## 3. Results and discussion

### 3.1. Effect of feeding fat sources on the lipid and cholesterol content

Table 1 shows that the amount of lipid found in the "A" ground raw meat sample was significantly higher ( $p < 0.05$ ) than the "V" one. In fact, the fat content of 1A (6.0%) was about twice as much that of the 1V (3.2%). It might be possible that this was due to the different level of metabolizable

Table 1

Lipid (% w/w), cholesterol (mg/100 g sample), free fatty acids (FFA, mg/100 g sample), total COPs (mg/kg sample), COPs-to-cholesterol ratio (COPs/chol, %), and peroxide value (POV, meq O<sub>2</sub>/kg fat) of the two types of chicken meat samples, obtained with animal and vegetable fat feeding integration (expressed as means ± SD, *n* = 3)

Sample	Lipid (% w/w)	Free fatty acids (mg/100 g sample)	Total cholesterol (mg/100 g sample)	Tot COPs (mg/kg sample)	COPs/chol (%)	Peroxide value (meq O <sub>2</sub> /kg fat)
1A	6.0 ± 0.1 <sup>a1</sup>	49.6 ± 10.9 <sup>a1</sup>	55.7 ± 0.4 <sup>a1</sup>	1.1 ± 0.1 <sup>a1</sup>	0.2 ± 0.0 <sup>a1</sup>	21.5 ± 2.6 <sup>a1</sup>
1V	3.2 ± 0.1 <sup>b1</sup>	19.4 ± 0.5 <sup>b1</sup>	36.0 ± 2.0 <sup>b1</sup>	0.7 ± 0.0 <sup>b1</sup>	0.2 ± 0.0 <sup>a1</sup>	26.4 ± 0.2 <sup>b1</sup>
2A	2.9 ± 0.1 <sup>a2</sup>	28.7 ± 3.6 <sup>a2</sup>	27.4 ± 1.7 <sup>a2</sup>	0.2 ± 0.0 <sup>a2</sup>	0.1 ± 0.0 <sup>a1</sup>	2.8 ± 1.1 <sup>a2</sup>
2V	2.8 ± 0.2 <sup>a2</sup>	22.1 ± 3.9 <sup>a1</sup>	26.0 ± 0.8 <sup>a2</sup>	0.3 ± 0.2 <sup>a1</sup>	0.1 ± 0.0 <sup>a1</sup>	2.4 ± 0.6 <sup>a2</sup>
3A	2.5 ± 0.0 <sup>a3</sup>	14.6 ± 0.9 <sup>a3</sup>	20.6 ± 1.4 <sup>a3</sup>	1.8 ± 0.1 <sup>a3</sup>	0.9 ± 0.1 <sup>a2</sup>	5.5 ± 0.8 <sup>a3</sup>
3V	2.7 ± 0.1 <sup>a2</sup>	23.1 ± 5.7 <sup>a1</sup>	22.3 ± 1.0 <sup>a3</sup>	1.8 ± 0.1 <sup>a2</sup>	0.8 ± 0.0 <sup>a2</sup>	10.8 ± 2.8 <sup>b3</sup>
4A	12.2 ± 0.5 <sup>a4</sup>	9.8 ± 3.0 <sup>a3</sup>	25.2 ± 0.7 <sup>a2</sup>	3.0 ± 1.0 <sup>a4</sup>	1.2 ± 0.4 <sup>a2</sup>	13.8 ± 1.5 <sup>a4</sup>
4V	11.2 ± 0.4 <sup>a3</sup>	20.3 ± 12.3 <sup>a1</sup>	21.7 ± 3.0 <sup>a3</sup>	2.8 ± 0.9 <sup>a3</sup>	1.5 ± 0.6 <sup>a2</sup>	13.8 ± 0.4 <sup>a3</sup>
5A	11.5 ± 0.1 <sup>a5</sup>	14.2 ± 3.7 <sup>a3</sup>	33.9 ± 2.0 <sup>a4</sup>	1.0 ± 0.1 <sup>a1</sup>	0.3 ± 0.0 <sup>a1</sup>	10.8 ± 0.8 <sup>a4</sup>
5V	12.4 ± 0.2 <sup>a3</sup>	21.2 ± 2.0 <sup>b1</sup>	30.7 ± 4.1 <sup>a12</sup>	1.1 ± 0.1 <sup>a1</sup>	0.3 ± 0.0 <sup>a1</sup>	12.0 ± 1.5 <sup>a3</sup>

Samples: 1A, ground raw meat/animal fat; 1V, ground raw meat/vegetable fat; 2A, meat mixture/animal fat; 2V, meat mixture/vegetable fat; 3A, raw patties/animal fat; 3V, raw patties/vegetable fat; 4A, fried patties/animal fat; 4V, fried patties/vegetable fat; 5A, precooked patties/animal fat; 5V, precooked patties/vegetable fat.

Different letters denote significant differences between the fat feeding integration (A and V) for the same sample (1A vs. 1V, 2A vs. 2V, 3A vs. 3V, 4A vs. 4V, 5A vs. 5V), according to Tukey's test (*p* < 0.05). Different numbers denote significant differences among samples (from 1 to 5) for the same fat feeding integration (A or V), according to Tukey's test (*p* < 0.05).

energy and digestibility of the two lipid integrations (Wiseman, Salvador, & Craigon, 1991), even though the two diets were isocaloric. Moreover, the conversion index is significantly higher for diets based on cattle tallow and other animal fat sources, rather than sunflower oil-based diets. In fact, when broilers are fed with vegetable oils-based diets, rich in polyunsaturated fatty acids, they usually lead to a lower accumulation of lipids with respect to animal fats-based diets, which contain larger amounts of saturated or monounsaturated fatty acids (Sanz, Flores, & López-Bote, 2000).

On the other hand, during the processing steps, the differences between the fat content of V and A samples tended to decrease (see Table 1). The fat content decreased in 2 and 3 (A and V) samples, due to a dilution effect of the addition of spices and salt, the starch-protein batter and breadcrumbs. Finally, the lipid amount drastically increased (*p* < 0.05) in 4 and 5 (A and V) samples, due to the oil absorption by patties during flash-frying. In fact, the amount of the oil absorbed by chicken cutlet can vary between 6% and 8% of their weight (Penazzi, 1996).

Table 1 also shows the amount of cholesterol found in the analyzed samples. The concentration of cholesterol of the ground raw meat was similar to that reported in literature (Garcia, Casal, Margaria, & Pensel, 1995). The amount of cholesterol of the 1A sample was significantly higher (*p* < 0.05) than that of the 1V (55.7 and 36.0 mg/100 g sample, respectively), due to the type of fat used as integration. However, no significant differences between the cholesterol concentration of the A and V samples were observed during the processing steps, despite the fact that a small amount of cholesterol might have migrated from the meat product to the frying oil (Penazzi, 1997). It is important to consider that these values can be affected by the

amount of fresh oil used for the replenishment of the frying oil.

On the other hand, along the patties preparation process, the amount of cholesterol decreased due to the dilution effect caused by the addition of the other ingredients.

However, if total cholesterol is expressed as mg/100 g sample (data not shown), it significantly increased after the humid steam-convection oven cooking, probably due to the dehydration effect of this thermal process.

It must be noticed that phytosterols (mainly campesterol and β-sitosterol) were detected in both raw meat samples (about 0.1 mg/100 g sample). The content of phytosterols drastically increased (about 4 mg/100 g sample) after flash-frying, due to vegetable oil absorption from the frying bath.

The content of free cholesterol (not esterified) was evaluated in the ground raw meat and in the final cutlets. As expected, the content of free cholesterol was significantly higher (*p* < 0.05) for animal-based diet meat samples rather than the vegetable one (4.4 ± 1.9 and 1.3 ± 0.3 mg/100 g sample for 1A and 1V, respectively), whereas this difference tended to disappear in the final samples (16.4 ± 2.0 and 17.2 ± 1.8 mg/100 g sample for 5A and 5V, respectively); this might be due to the dilution effect of the added ingredients, being similar to the trend observed for total cholesterol.

Lipolysis can break down the ester bond of esterified cholesterol and lead to an increase of free cholesterol. In fact, the free cholesterol-to-total cholesterol ratio drastically increased (*p* < 0.05) along the cutlet technological process (7.9 ± 3.5% and 3.5 ± 0.9% for 1A and 1V, respectively; 48.2 ± 4.3% and 56.2 ± 1.7% for 5A and 5V, respectively), confirming a strong lipolytic effect of frying and heating thermal treatments here applied.

**Table 2**  
Total fatty acids composition (the main fatty acids are expressed as % of total fatty acids; means  $\pm$  SD,  $n = 3$ ) of the two types of samples, obtained with animal and vegetable fat feeding integration

Sample	C12:0 lauric	C14:0 myristic	C16:0 palmitic	C16:1 $n-7$ palmitoleic	C18:0 stearic	C18:1 $n-9$ oleic	C18:1 $n-7$ <i>cis</i> -vaccenic	C18:2 $n-6$ linoleic	C18:3 $n-3$ $\alpha$ -linolenic	C20:4 $n-6$ arachidonic	C20:22 $n-3$ PUFA
1A	0.1 $\pm$ 0.0 <sup>a1</sup>	1.4 $\pm$ 0.1 <sup>a1</sup>	23.6 $\pm$ 0.3 <sup>a1</sup>	5.0 $\pm$ 0.1 <sup>a1</sup>	7.8 $\pm$ 0.1 <sup>a1</sup>	39.0 $\pm$ 0.3 <sup>a1</sup>	2.5 $\pm$ 0.0 <sup>a12</sup>	15.4 $\pm$ 0.1 <sup>a1</sup>	1.7 $\pm$ 0.0 <sup>a1</sup>	1.2 $\pm$ 0.1 <sup>a1</sup>	0.4 $\pm$ 0.1 <sup>a1</sup>
1V	0.1 $\pm$ 0.0 <sup>b1</sup>	1.1 $\pm$ 0.1 <sup>b1</sup>	21.3 $\pm$ 1.2 <sup>b1</sup>	3.8 $\pm$ 0.2 <sup>b1</sup>	7.9 $\pm$ 1.3 <sup>a1</sup>	32.4 $\pm$ 2.1 <sup>b1</sup>	2.2 $\pm$ 0.1 <sup>b1</sup>	24.8 $\pm$ 4.9 <sup>b12</sup>	2.4 $\pm$ 0.2 <sup>b1</sup>	1.9 $\pm$ 0.2 <sup>b1</sup>	0.6 $\pm$ 0.0 <sup>b12</sup>
2A	0.1 $\pm$ 0.0 <sup>a2</sup>	1.3 $\pm$ 0.0 <sup>a1</sup>	23.9 $\pm$ 0.7 <sup>a1</sup>	4.8 $\pm$ 0.0 <sup>a2</sup>	7.8 $\pm$ 0.0 <sup>a1</sup>	37.4 $\pm$ 0.4 <sup>a2</sup>	2.6 $\pm$ 0.0 <sup>a1</sup>	16.8 $\pm$ 0.1 <sup>a2</sup>	1.8 $\pm$ 0.0 <sup>a2</sup>	1.6 $\pm$ 0.0 <sup>a2</sup>	0.3 $\pm$ 0.1 <sup>a2</sup>
2V	0.1 $\pm$ 0.0 <sup>b2</sup>	1.1 $\pm$ 0.1 <sup>b1</sup>	22.9 $\pm$ 1.0 <sup>a1</sup>	4.0 $\pm$ 0.1 <sup>b1</sup>	7.4 $\pm$ 0.2 <sup>b1</sup>	33.7 $\pm$ 0.6 <sup>b1</sup>	2.2 $\pm$ 0.1 <sup>b1</sup>	22.3 $\pm$ 0.4 <sup>b1</sup>	2.5 $\pm$ 0.1 <sup>b1</sup>	1.7 $\pm$ 0.1 <sup>a1</sup>	0.6 $\pm$ 0.0 <sup>b1</sup>
3A	0.1 $\pm$ 0.0 <sup>a1</sup>	1.2 $\pm$ 0.1 <sup>a2</sup>	22.0 $\pm$ 0.1 <sup>a2</sup>	3.9 $\pm$ 0.0 <sup>a3</sup>	7.2 $\pm$ 0.0 <sup>a2</sup>	34.8 $\pm$ 0.1 <sup>a3</sup>	2.3 $\pm$ 0.0 <sup>a2</sup>	23.9 $\pm$ 0.2 <sup>a3</sup>	2.1 $\pm$ 0.0 <sup>a3</sup>	1.3 $\pm$ 0.0 <sup>a1</sup>	0.5 $\pm$ 0.0 <sup>a1</sup>
3V	0.1 $\pm$ 0.0 <sup>b1</sup>	0.9 $\pm$ 0.0 <sup>b2</sup>	20.7 $\pm$ 0.5 <sup>b1</sup>	2.8 $\pm$ 0.7 <sup>b2</sup>	6.9 $\pm$ 0.2 <sup>b1</sup>	31.5 $\pm$ 0.3 <sup>b1</sup>	2.0 $\pm$ 0.0 <sup>b1</sup>	29.5 $\pm$ 1.0 <sup>b2</sup>	2.7 $\pm$ 0.1 <sup>b1</sup>	1.4 $\pm$ 0.1 <sup>b2</sup>	0.5 $\pm$ 0.0 <sup>a2</sup>
4A	TR	0.2 $\pm$ 0.0 <sup>a3</sup>	7.6 $\pm$ 0.0 <sup>a3</sup>	0.8 $\pm$ 0.0 <sup>a4</sup>	2.8 $\pm$ 0.0 <sup>a3</sup>	57.4 $\pm$ 0.1 <sup>a4</sup>	3.4 $\pm$ 0.1 <sup>a3</sup>	20.7 $\pm$ 0.0 <sup>a4</sup>	6.9 $\pm$ 0.0 <sup>a4</sup>	0.2 $\pm$ 0.0 <sup>a3</sup>	TR
4V	TR	0.2 $\pm$ 0.0 <sup>a3</sup>	7.9 $\pm$ 0.2 <sup>a2</sup>	0.8 $\pm$ 0.0 <sup>a3</sup>	2.9 $\pm$ 0.0 <sup>a2</sup>	55.6 $\pm$ 0.1 <sup>b2</sup>	3.3 $\pm$ 0.1 <sup>a2</sup>	21.4 $\pm$ 0.0 <sup>b1</sup>	6.8 $\pm$ 0.2 <sup>a2</sup>	0.0 $\pm$ 0.0 <sup>b3</sup>	TR
5A	TR	0.3 $\pm$ 0.1 <sup>a3</sup>	7.8 $\pm$ 0.1 <sup>a3</sup>	0.8 $\pm$ 0.0 <sup>a4</sup>	2.8 $\pm$ 0.1 <sup>a3</sup>	57.2 $\pm$ 0.1 <sup>a4</sup>	3.6 $\pm$ 0.2 <sup>a3</sup>	20.4 $\pm$ 0.1 <sup>a4</sup>	6.6 $\pm$ 0.1 <sup>a5</sup>	0.2 $\pm$ 0.0 <sup>a3</sup>	TR
5V	TR	0.3 $\pm$ 0.0 <sup>a4</sup>	7.7 $\pm$ 0.2 <sup>a2</sup>	0.7 $\pm$ 0.0 <sup>b3</sup>	2.8 $\pm$ 0.0 <sup>a2</sup>	55.9 $\pm$ 0.1 <sup>b2</sup>	3.3 $\pm$ 0.1 <sup>a2</sup>	21.1 $\pm$ 0.1 <sup>b1</sup>	6.7 $\pm$ 0.1 <sup>a2</sup>	0.2 $\pm$ 0.1 <sup>a3</sup>	TR

Samples: 1A, ground raw meat/animal fat; 1V, ground raw meat/vegetable fat; 2A, meat mixture/vegetable fat; 2V, meat mixture/animal fat; 3A, raw patties/animal fat; 3V, raw patties/vegetable fat; 4A, fried patties/animal fat; 4V, fried patties/vegetable fat; 5A, precooked patties/animal fat; 5V, precooked patties/vegetable fat; TR, traces. Different letters denote significant differences between the fat feeding integration (A and V) for the same sample (1 vs. 1, 2 vs. 2, and so on), according to Tukey's test ( $p < 0.05$ ). Different numbers denote significant differences among samples (from 1 to 5) for the same fat feeding integration (A or V), according to Tukey's test ( $p < 0.05$ ).

### 3.2. Effect of feeding fat sources on the total fatty acid composition

Table 2 reports the total fatty acid composition of the samples analyzed. As already reported in literature (Bou et al., 2001; Gray & Crackel, 1992; Lin et al., 1989; O'Neill et al., 1998), the fat sources used as integration for diets can effectively affect the fatty acid composition of mono-gastric's lipid tissues. In fact, the 1V sample contained significant higher and lower amounts of linoleic and oleic acids, respectively, than the 1A sample (see Table 2). As reported in literature (Gray & Crackel, 1992), the most abundant FA in both types of samples was oleic acid, followed by palmitic, linoleic and stearic acids, which represented about the 90% of total fatty acids.

Table 3 displays the content of the different fatty acid classes (according their unsaturation degree), the unsaturated fatty acids/saturated fatty acids (U/S) ratio, the amount of  $n-6$  and  $n-3$  and their ratio ( $n-6/n-3$ ). The U/S ratio of the 1V sample was significantly higher than that of the 1A, confirming that meat from broilers fed with the vegetable oils-based diet accumulated higher levels of polyunsaturated fatty acids, which are more susceptible to oxidation. The 1V and 1A SFA, MUFA, and PUFA contributions were significantly different from each other, where 1A SFA > 1V SFA, 1A MUFA > 1V MUFA (high levels of oleic and palmitic acid for 1A sample), and 1A PUFA < 1V PUFA (high levels of linoleic acid).

Similar trends are reported by Bou et al. (2001), who found that cooked dark meat from broilers fed with sunflower oil increased its linoleic acid content (40.67%, expressed as area normalization) with respect to broilers fed with beef tallow (15.82%). In addition, they observed that cooked dark meat from broilers fed with sunflower oil had a PUFA content (43.47%) significantly higher than that broilers fed with beef tallow (17.85%), while the SFA and MUFA contents were significantly lower. Moreover, Grau et al. (2001) determined that raw meat from chickens fed with beef tallow had 34.41% SFA, 47.29% MUFA, and 18.34% PUFA (% expressed as compensated area normalization), whereas those fed with sunflower oil showed 22.62% SFA, 34.96% MUFA, and 42.47% PUFA.

The fatty acid composition of samples 2 and 3 (both A and V) was similar to that of corresponding raw meats, which may be due to the small lipid contribution of the added ingredients (spices, salt, breadcrumbs), as suggested by the lipid content shown in Table 1. For samples 1, 2 and 3, oleic, palmitic, linoleic, and stearic acids contributed for about 90% of total fatty acids. In addition, the SFA, MUFA and PUFA percentages of samples 2 and 3 followed the same behavior as those of samples 1, where A SFA > V SFA, A MUFA > V MUFA, and A PUFA < V PUFA (all significant at  $p < 0.05$ ). Moreover, the U/S ratio of the 2V and 3V samples was significantly higher than that of A samples, as observed for raw meats.

After the flash-frying step, the U/S ratio markedly increased ( $p < 0.05$ ), due to the incorporation of unsatu-

Table 3

Saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA),  $n - 3$  fatty acids,  $n - 6$  fatty acids amounts (expressed as % of total fatty acids), unsaturated/saturated (U/S) and  $n - 6/n - 3$  ratios of the two types of samples, obtained with animal and vegetable fat feeding integration (values are expressed as means  $\pm$  SD,  $n = 3$ )

Sample	SFA	MUFA	PUFA	$n - 6$	$n - 3$	$n - 6/n - 3$	U/S
1A	33.7 $\pm$ 0.3 <sup>a1</sup>	47.2 $\pm$ 0.2 <sup>a1</sup>	19.1 $\pm$ 0.2 <sup>a1</sup>	17.0 $\pm$ 0.2 <sup>a1</sup>	2.1 $\pm$ 0.0 <sup>a1</sup>	8.0 $\pm$ 0.2 <sup>a1</sup>	2.0 $\pm$ 0.0 <sup>a1</sup>
1V	30.6 $\pm$ 2.2 <sup>b1</sup>	39.1 $\pm$ 2.5 <sup>b1</sup>	30.3 $\pm$ 4.6 <sup>b12</sup>	27.3 $\pm$ 4.7 <sup>b1</sup>	3.0 $\pm$ 0.1 <sup>b1</sup>	9.2 $\pm$ 2.0 <sup>a1</sup>	2.3 $\pm$ 0.2 <sup>b1</sup>
2A	33.6 $\pm$ 0.8 <sup>a1</sup>	45.4 $\pm$ 0.4 <sup>a2</sup>	21.0 $\pm$ 0.4 <sup>a2</sup>	18.9 $\pm$ 0.3 <sup>a2</sup>	2.1 $\pm$ 0.1 <sup>a1</sup>	9.0 $\pm$ 0.3 <sup>a2</sup>	2.0 $\pm$ 0.1 <sup>a1</sup>
2V	31.7 $\pm$ 1.2 <sup>b1</sup>	40.6 $\pm$ 0.6 <sup>b1</sup>	27.7 $\pm$ 0.6 <sup>b1</sup>	24.6 $\pm$ 0.5 <sup>b1</sup>	3.1 $\pm$ 0.1 <sup>b1</sup>	7.9 $\pm$ 0.0 <sup>b2</sup>	2.2 $\pm$ 0.1 <sup>b1</sup>
3A	30.7 $\pm$ 0.2 <sup>a2</sup>	41.4 $\pm$ 0.1 <sup>a3</sup>	27.9 $\pm$ 0.2 <sup>a3</sup>	25.3 $\pm$ 0.2 <sup>a3</sup>	2.5 $\pm$ 0.0 <sup>a2</sup>	9.9 $\pm$ 0.2 <sup>a3</sup>	2.3 $\pm$ 0.0 <sup>a2</sup>
3V	28.9 $\pm$ 0.7 <sup>b1</sup>	36.7 $\pm$ 0.3 <sup>b1</sup>	34.4 $\pm$ 1.0 <sup>b2</sup>	31.2 $\pm$ 0.9 <sup>b2</sup>	3.1 $\pm$ 0.1 <sup>b1</sup>	10.0 $\pm$ 0.4 <sup>a3</sup>	2.4 $\pm$ 0.1 <sup>b1</sup>
4A	10.8 $\pm$ 0.1 <sup>a3</sup>	61.5 $\pm$ 0.0 <sup>a4</sup>	27.7 $\pm$ 0.0 <sup>a3</sup>	20.9 $\pm$ 0.0 <sup>a4</sup>	6.9 $\pm$ 0.0 <sup>a3</sup>	3.0 $\pm$ 0.0 <sup>a4</sup>	8.3 $\pm$ 0.1 <sup>a3</sup>
4V	11.7 $\pm$ 0.2 <sup>b2</sup>	59.7 $\pm$ 0.2 <sup>b2</sup>	28.6 $\pm$ 0.1 <sup>b1</sup>	21.8 $\pm$ 0.0 <sup>a3</sup>	6.8 $\pm$ 0.2 <sup>a2</sup>	3.2 $\pm$ 0.1 <sup>b4</sup>	7.6 $\pm$ 0.2 <sup>b2</sup>
5A	10.9 $\pm$ 0.1 <sup>a3</sup>	61.6 $\pm$ 0.2 <sup>a4</sup>	27.6 $\pm$ 0.1 <sup>a3</sup>	20.9 $\pm$ 0.1 <sup>a4</sup>	6.6 $\pm$ 0.1 <sup>a4</sup>	3.2 $\pm$ 0.0 <sup>a4</sup>	8.2 $\pm$ 0.1 <sup>a3</sup>
5V	11.6 $\pm$ 0.3 <sup>b2</sup>	60.0 $\pm$ 0.1 <sup>b2</sup>	28.4 $\pm$ 0.2 <sup>b1</sup>	21.7 $\pm$ 0.1 <sup>b3</sup>	6.7 $\pm$ 0.1 <sup>a2</sup>	3.3 $\pm$ 0.0 <sup>a4</sup>	7.6 $\pm$ 0.2 <sup>b2</sup>

Samples: 1A, ground raw meat/animal fat; 1V, ground raw meat/vegetable fat; 2A, meat mixture/animal fat; 2V, meat mixture/vegetable fat; 3A, raw patties/animal fat; 3V, raw patties/vegetable fat; 4A, fried patties/animal fat; 4V, fried patties/vegetable fat; 5A, precooked patties/animal fat; 5V, precooked patties/vegetable fat. Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; U/S, unsaturated fatty acids/saturated fatty acids ratio.

Different letters denote significant differences between the fat feeding integration (A and V) for the same sample (1 vs. 1, 2 vs. 2, and so on), according to Tukey's test ( $p < 0.05$ ). Different numbers denote significant differences among samples (from 1 to 5) for the same fat feeding integration (A or V), according to Tukey's test ( $p < 0.05$ ).

rated fatty acids from the oil bath to the patties. In fact, both the amount of oleic acid and linolenic acid increased, whereas the levels of palmitic acid and stearic acid decreased (see Table 2). Thus, the SFA and MUFA amounts of 4 samples, A and V, were significantly lower and higher than those of 3 (A and V) samples ( $p < 0.05$ ), respectively. The decrease on the PUFA percentage from samples 3 to 4 corresponds to the marked reduction in linoleic acid, which is not compensated by the raise on linolenic acid due to frying; this difference is significant for vegetable samples only.

However, the flash-frying step tended to reduce the differences between the A and V samples. Moreover, after the flash-frying step, the SFA amount of the vegetable samples was unexpectedly higher than that of animal ones; the MUFA and the PUFA of A samples were, as for the previous samples, significantly higher and lower than those of V samples, respectively. As a result, the U/S ratio of the 4A and 5A samples became significantly higher than those of 4V and 5V (Table 3).

The changes on the fatty acid profile of chicken patties after frying, are controlled by a mass equilibrium exchange between the fatty acids present in the meat and those of the frying oil (rapeseed oil). Therefore, the composition of the frying bath plays a significant role on the fatty acid pattern of cooked patties. In fact, the quality of frying oil can influence the total fatty acid composition of the precooked cutlets (Penazzi, 1997), masking the initial differences found in the cutlets obtained with different feeding sources.

Finally, the oven-cooking step did not lead to significant differences of the fatty acid composition, keeping the same significant differences already observed between animal and vegetable samples.

$n - 3$  and  $n - 6$  fatty acids were significantly higher in 1, 2 and 3 vegetable samples as compared to the animal ones, as reported in literature (Bou et al., 2001; Grau et al., 2001). However, these initial differences were masked after flash-frying, becoming almost identical. Regarding the processing effect on the amount of  $n - 3$  fatty acids, it is evident that oil absorption during flash-frying led to a noticeable increase of 2–3%, which is a positive feature from nutritional standpoint. This difference is directly related to the linolenic acid content. On the contrary, the  $n - 6$  amount decreased after flash-frying (between 5% and 10%) due to the drop of the linoleic acid content.

The importance of a low dietary  $n - 6/n - 3$  ratio is well known, due to the prothrombotic and proaggregatory state induced by a high intake of  $n - 6$ , as well as to the anti-inflammatory, antithrombotic and hypolipidemic properties of  $n - 3$  fatty acids (Simopolous, 1999, 2002).

The  $n - 6/n - 3$  ratio of V and A samples did not present significant differences. However, the influence of the cooking steps on this parameter is clearly evinced in Table 3. In fact, after flash-frying, the  $n - 6/n - 3$  significantly decreased in consideration to the large absorption of linoleic acid from the oil bath (rapeseed oil).

Since the recommended Italian  $n - 6/n - 3$  ratio is 4 (LARN, 1996), both vegetable and animal chicken patties present a good  $n - 6/n - 3$  ratio, considering that chicken meat is not an important food source of  $n - 3$  fatty acid in the diet.

### 3.3. Effect of feeding fat sources on the free fatty acids and diacylglycerols

Table 1 shows that the amount of free fatty acids found in the 1A sample was significantly higher ( $p < 0.05$ ) than in

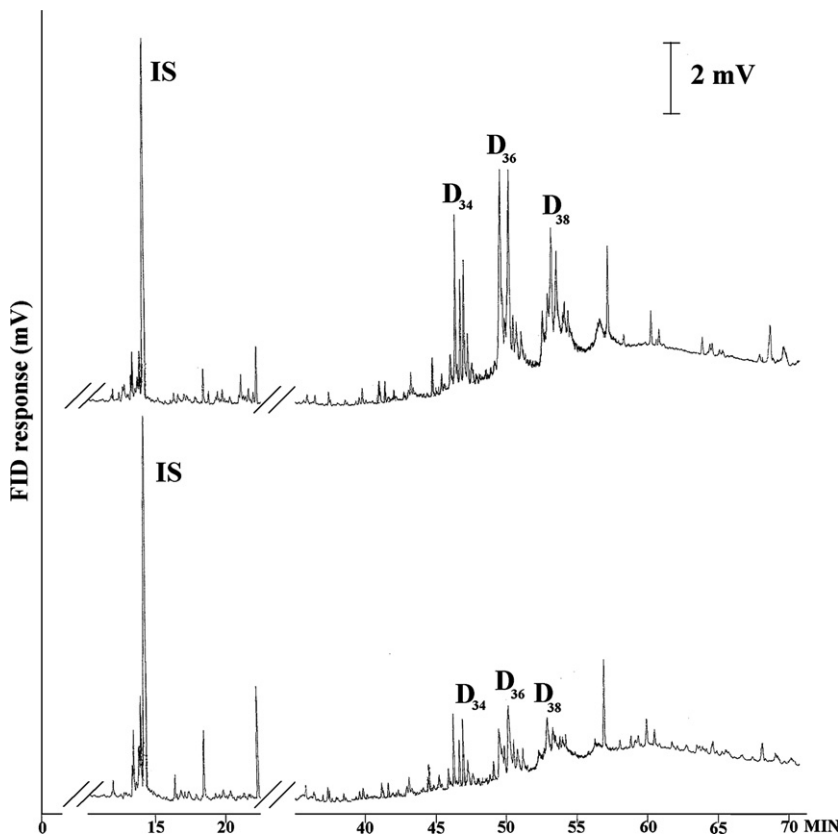


Fig. 2. GC traces of the diacylglycerols present in ground raw chicken meat obtained with animal and vegetable fat feeding integration (1A, upper trace; 1V, lower trace). Abbreviations: D<sub>34</sub>, C16–C18 diacylglycerols; D<sub>36</sub>, C18–C18 diacylglycerols; D<sub>38</sub>, C18–C20 diacylglycerols; IS, internal standard (squalane). For analytical conditions, please refer to Section 2.

the 1V (49.6 and 19.4 g/100 g sample for 1A and 1V, respectively). This could be attributed to the higher affinity of lipases for the animal fat sources with respect to the vegetal ones (Elliot, Drackley, Beaulieu, Aldrich, & Merchen, 1999). However, during the processing steps, the differences between the free fatty acid content of V and A samples tended to decrease due to the addition of other ingredients and to the process itself, which became more complex thus masking the initial differences. Despite the lipolysis phenomena, the amount of free fatty acids in the final pre-cooked patties obtained with an animal diet, was lower than the initial level found in the raw meat, which might be due to the migration of free fatty acids into the frying bath and/or combination with another molecules (Rodríguez-Estrada et al., 1997). On the contrary, the amount of FFA of the vegetable samples was rather stable during the technological process (around the value of 20 mg/100 g sample), which apparently did not affect the FFA content of vegetable chicken patties. The different behaviors between the A and V samples might be partly due to the fact that lipid oxidation is favored rather than lipolysis, because of the higher fatty acid unsaturation degree of V samples. However, the entity of the difference cannot fully explained by the latter statement, as can be observed in the oxidation results (see Section 3.4).

Another parameter to evaluate lipolysis is the content of diacylglycerols. This determination was only performed in

the initial and final samples, assuming that the evolution of these compounds is similar to that of free fatty acids. Fig. 2 shows the GC traces of the diacylglycerols present in ground raw chicken meat obtained with animal and vegetable fat feeding integration. The D<sub>34</sub> (C16–C18 diacylglycerols), D<sub>36</sub> (C18–C18 diacylglycerols), and D<sub>38</sub> (C18–C20 diacylglycerols) series were particularly characteristic of the analyzed samples.

As reported for free fatty acids, the amount of diacylglycerols found in A samples was significantly higher ( $p < 0.05$ ) than the V samples ( $0.03 \pm 0.00$  and  $0.01 \pm 0.00$  g/100 g sample for 1A and 1V, respectively). This result can also be attributed to the higher affinity of lipases for the animal fat sources with respect to the vegetal ones (Elliot et al., 1999). No significant differences were found between the diacylglycerol content of the two types of final pre-cooked patties ( $0.17 \pm 0.02$  and  $0.16 \pm 0.01$  g/100 g sample for 5A and 5V, respectively), which might be due to the dilution effect of the added ingredients, as well as to the masking effect of this complex processing system. In this case, however, the effect of the thermal treatments on lipolysis was evident, because the amount of diacylglycerols in the final products was about 6–10 times higher than the initial level detected in the raw meat. Nevertheless, it must be noted that most diacylglycerols may come from the frying oil (Penazzi, 1996), considering the actual amount of oil absorbed by the patties during frying.



In fact, Penazzi (1996) reported that the diacylglycerol content found in precooked chicken cutlets is directly related to that of the frying oil.

#### 3.4. Effect of feeding fat sources on the peroxide value and extent of the cholesterol oxidation of the meat samples

Table 1 shows that the peroxide value found in the “V” ground raw meat sample (26.4 meq O<sub>2</sub>/kg fat) was significantly higher ( $p < 0.05$ ) than in the “A” one (21.5 meq O<sub>2</sub>/kg fat). This result can be explained considering that the broilers fed with the vegetable oils-based diet accumulate higher levels of polyunsaturated fatty acids, which are highly prone to oxidation (Gray & Crackel, 1992). Moreover, the relatively elevated peroxide values found in the raw meat could be due to grinding and mixing, which lead to an increase of the exposed surface and the incorporation of oxygen into the medium, thus promoting oxidation (Gray, Gomaa, & Beckley, 1996; Rodriguez-Estrada et al., 1997). In addition, such level of primary lipid oxidation may be due to the holding period to which the meat is usually subjected (Boselli et al., 2005; Rodriguez-Estrada et al., 1997). The peroxide value can be used as a useful index to establish the freshness of lipid matrices, because peroxides represent the primary products of the lipid oxidation.

In the meat mixture samples (2A and 2V), the peroxide value strongly decreased (2.8 and 2.4 meq O<sub>2</sub>/kg fat, respectively), due to the dilution effect of the ingredients, the conversion of peroxides into secondary oxidation products and/or interactions among peroxides and other constituents. Before the cooking steps, the peroxide value increased (10.8 and 5.5 meq O<sub>2</sub>/kg fat, for 3V and 3A, respectively) and reached high levels after flash-frying (13.8 and 13.8 meq O<sub>2</sub>/kg fat, for 4A and 4V, respectively), due to the pro-oxidative action of the thermal process. The peroxide value of the 5V sample was slightly higher than that of the 5A one, but not significantly different. The humid steam-convection oven cooking probably contributed to the conversion of the peroxides into secondary oxidation products. It must be noted that the overall trend of the peroxide value along the cutlet production line reflects the classical sinusoidal behavior of the primary oxidation products.

Table 1 also reports the total amount of COPs found in the analyzed samples. Since the NH<sub>2</sub>-SPE purification is unable to completely remove all sterols from the unsaponifiable fraction, a GC capillary column coated with 65% diphenyl-polysiloxane–35% dimethyl-polysiloxane was utilized, so as to avoid overlapping between  $\beta$ -EC and campesterol. As reported in literature (Boselli et al., 2005; Conchillo et al., 2003; Conchillo, Ansorena, & Astiasarán, 2005; Maraschiello et al., 1998; Osada, Hoshina, Nakamura, & Sugano, 2000), the main COPs found were 7-KC,  $\beta$ -EC, 7 $\beta$ -HC, 7 $\alpha$ -HC and  $\alpha$ -EC. 7-KC accounted for 27–71% of total COPs. The sample 1V had a significantly lower ( $p < 0.05$ ) concentration of COPs than that of 1A, exhibiting an opposite behavior to that found for the peroxide value. Assuming that the level of cholesterol of the animal

fat fed was higher than that of the vegetable one, the type of fat used as integration and its corresponding cholesterol content may have affected the initial concentration of COPs in the meat. However, along the cutlet production line, the COPs content followed the same trend for both V and A samples and no significant differences were detected among samples obtained with different fat integration. On the other hand, a bell-shaped behavior of COPs was observed during processing, reaching a maximum level after flash-frying. In fact, recent studies have demonstrated that broiler muscle increased its COPs concentrations after heating (Grau et al., 2001; Maraschiello et al., 1998). However, a marked decrease was detected after oven-cooking, which may be due to COPs thermal decomposition or COPs combination with other molecules, such as Maillard reaction products and proteins (Rodriguez-Estrada et al., 1997). In fact, Maillard reaction products are generated during frying and oven-cooking; these compounds are well-known for the antioxidant properties, which stabilize food products during storage (Alfawaz, Smith, & Jeon, 1994; Smith & Alfawaz, 1995). Penazzi (1996) reported a similar trend for free 7-KC in pre-cooked chicken cutlets. In any case, the total COPs content of the final precooked product was about 1 mg/kg of sample, which does not represent an appreciable risk from the toxicological standpoint, since the minimum amount of 7-KC equivalent to a minimum biological activity of the other COPs (about one-fifth of 7-KC) (Caboni, Zullo, Lercker, & Capella, 1989) is equal to 10<sup>-7</sup> molar basis (Sevanian, 1991).

No significant correlations among total COPs, 7-KC and POV were found, because cholesterol oxidation products are more correlated to TBARs (Conchillo et al., 2003, 2005; Grau et al., 2001), which is used for the evaluation of secondary lipid oxidation.

Other workers have reported similar levels of COPs in chicken products. Conchillo et al. (2003) recorded very low values of COPs in raw chicken meat (2.88  $\mu$ g/g fat), but grilling and roasting led to a 4–7 times increase in COPs levels; in this case the most abundant COPs were 7-KC,  $\beta$ -EC, and 7 $\beta$ -HC. Eder et al. (2005) found that B-ring cholesterol oxides (7-KC,  $\beta$ -EC, 7 $\beta$ -HC, and 7 $\alpha$ -HC) were quantitatively the most important COPs in raw and oven-heated broiler meat. They found that the dietary fat integration strongly affected the level of COPs in raw and heated meat. In fact, they demonstrated how dietary fats rich in PUFA (linseed oil with respect to palm oil) can cause a dramatic increase in COPs concentration in broiler muscle, particularly during heating; this increase in COPs levels is more evident when dietary vitamin E integration is low.

Grau et al. (2001) found that total COPs content in raw and cooked frozen dark chicken meat slightly depended on the dietary fats (beef tallow, sunflower oil, oxidized sunflower oil and linseed oil); the most abundant COPs were, in this case, 7 $\beta$ -HC,  $\alpha$ -EC,  $\beta$ -EC, and 7-KC.

Regarding the COPs/cholesterol ratio (Table 1), it followed the same trend for both V and A samples and no significant differences were detected among samples

obtained with different fat integration. However, the extent of cholesterol oxidation after flash-frying was about 5–10 times higher than the level found in the raw meat and the raw patties, which evinces the pro-oxidant effect of frying and heating treatments (Grau et al., 2001; Maraschiello et al., 1998). Oven-cooking did not lead to a further increase on the extent of cholesterol oxidation, which may be due to COPs thermal decomposition or COPs combination with other molecules, as aforementioned. In fact, the COPs/cholesterol ratio of both types of precooked chicken cutlets is similar to values reported in literature for cooked meat products (0.5%) (Rodriguez-Estrada et al., 1997). Moreover, Grau et al. (2001) found a cholesterol oxidation degree of about 0.15% in raw frozen dark chicken meat, whereas Conchillo et al. (2005) recorded values between 0.01 and 1.4 mg COP/g cholesterol in frozen raw and cooked (grilled and roasted) chicken meat, respectively.

Since the amount of oil absorbed by chicken cutlets can reach up to 8% of their weight, the quality of the precooked cutlets will greatly depend on the quality of the frying oil. Phytosterols present in the frying oil can oxidize in a similar way to cholesterol and give rise to oxidation products with still uncertain biological effects (Adcox, Boyd, Oehrl, Allen, & Fenner, 2001; Maguire, Konoplyannikov, Ford, Maguire, & O'Brien, 2003), so it would be important to perform further studies to evaluate their presence in these food products.

#### 4. Conclusions

The effect of feeding fat sources on the quality and composition of lipids of raw meat and precooked ready-to-eat fried chicken patties, was studied. Two homogeneous groups of broilers were fed with animal fats and vegetable oils, respectively. A traditional technology (flash-frying plus humid steam-convection oven cooking) was employed to process broilers meat to obtain ready-to-eat meat-based patties. Lipid hydrolysis and oxidation was evaluated in the initial, intermediate and final products. The results confirm that different fat sources can effectively influence the stability, quality and composition of poultry meat lipids. Lipid hydrolysis and oxidation were more intense in ground raw meat obtained with animal and vegetable fat integration, respectively. Lipid oxidation was influenced by the unsaturation degree of the fat source. However, these differences tended to decrease along the technological process, due to the addition of other ingredients and to the oil absorption. Although flash-frying and humid steam-convection oven cooking promoted lipid degradation, the overall quality of the precooked chicken cutlets is acceptable from the oxidation standpoint, displaying low levels of both peroxides and COPs. However, it must be pointed out that part of the oxidation products may have decomposed or combined with other molecules, such as Maillard reaction products and proteins, during oven-cooking. In addition, the raw meat used for the patties preparation

already presented an advanced degree of oxidation, regardless of the type of fat integration; the holding period and grinding may have greatly affected the meat oxidative status.

Considering that the amount of oil absorbed by chicken cutlets can reach up to 8% of their weight, the quality of the precooked cutlets depend to a great extent on the quality of the frying oil. Since oil phytosterols can oxidize in a similar way to cholesterol, it would be important to perform further studies to evaluate the presence of phytosterol oxidation products in these food products, as well as to monitor the stability of the lipid fraction during the shelf-life of the final products.

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#### References

- Adcox, C., Boyd, L., Oehrl, L., Allen, J., & Fenner, G. (2001). Comparative effects of phytosterol oxides and cholesterol oxides in cultured macrophage-derived cell lines. *Journal of Agricultural and Food Chemistry*, *49*(4), 2090–2095.
- Alfawaz, M., Smith, J. S., & Jeon, I. J. (1994). Maillard reaction-products as antioxidants in precooked ground-beef. *Food Chemistry*, *51*(3), 311–318.
- Bortolomeazzi, R., Frega, N., & Lercker, G. (1990). Rapid determination of free and esterified sterols using prepacked silica columns. *Italian Journal of Food Science*, *2*, 265–268.
- Boselli, E., Caboni, M. F., Rodriguez-Estrada, M. T., Gallina Toschi, T., Daniel, M., & Lercker, G. (2005). Photooxidation of cholesterol and lipids of turkey meat during storage under commercial retail conditions. *Food Chemistry*, *91*(4), 705–713.
- Boselli, E., Velazco, V., Caboni, M. F., & Lercker, G. (2001). Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. *Journal of Chromatography A*, *917*(1–2), 239–244.
- Bou, R., Guardiola, F., Grau, A., Grimpa, S., Manich, A., Barroeta, A. C., et al. (2001). Influence of dietary source,  $\alpha$ -tocopherol, and ascorbic acid supplementation on sensory quality of dark chicken meat. *Poultry Science*, *80*(6), 800–807.
- Bou, R., Guardiola, F., Tres, A., Barroeta, A. C., & Codony, R. (2004). Effect of dietary fish oil,  $\alpha$ -tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poultry Science*, *83*(2), 282–292.
- Caboni, M. F., Zullo, C., Lercker, G., & Capella, P. (1989). Cholesterol and oxisterols determination in food lipids. In Actes du Congrès International “Chevreul” pour l’Etude des Corps Gras (Première Congrès Eurolipid), Angers, 6–9 Juin.
- Conchillo, A., Ansorena, D., & Astiasarán, I. (2003). Combined effect of cooking (grilling and roasting) and chilling storage (with and without air) on lipid and cholesterol oxidation in chicken breast. *Journal of Food Protection*, *66*(6), 840–846.
- Conchillo, A., Ansorena, D., & Astiasarán, I. (2004). The effect of cooking and storage on the fatty acid profile of chicken breast. *European Journal of Lipid Science and Technology*, *106*(5), 301–306.

- Conchillo, A., Ansorena, D., & Astiasarán, I. (2005). Intensity of lipid oxidation and formation of cholesterol oxidation products during frozen storage of raw and cooked chicken. *Journal of the Science of Food and Agriculture*, 85(1), 141–146.
- Eder, K., Grünthal, G., Kluge, H., Hirche, F., Spilke, J., & Brandsch, C. (2005). Concentrations of cholesterol oxidation products in raw, heat-processed and frozen-stored meat of broiler chickens fed diets differing in the type of fat and vitamin E concentrations. *British Journal of Nutrition*, 93(5), 633–643.
- Elliot, J. P., Drackley, J. K., Beaulieu, A. D., Aldrich, C. G., & Merchen, N. R. (1999). Effects of saturation and esterification of fat sources on site and extent of digestion in steers: digestion of fatty acids, triglycerides, and energy. *Journal of Animal Science*, 77(7), 1919–1929.
- Fieser, L. F., & Fieser, M. (1967). *Reagents for organic chemistry* (pp. 191–192). NY: Wiley.
- Folch, J., Lees, M., & Sloan-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1), 497–509.
- Frega, N., & Bocci, F. (2001). *L'analisi rapida dell'olio di oliva* (Vol. 3, p. 28). Laboratorio 2000, Italy.
- Garcia, P. T., Casal, J. J., Margaria, C. A., & Pensel, N. A. (1995). Cholesterol content in different meats. In *Proceedings of the 41st international congress of meat science and technology* (A21, pp. 54–55). S. Antonio: American Meat Science Association.
- Garcia-Cruset, S., Carpenter, K. L., Codony, R., & Guardiola, F. (2002). Cholesterol oxidation products and atherosclerosis. In F. Guardiola, P. C. Dutta, R. Codony, & G. Savage (Eds.), *Cholesterol and phytosterol oxidation products: Analysis, occurrence, and biological effects* (pp. 241–277). Champaign: Springer.
- Grau, A., Codony, R., Grimpa, S., Baucells, M. D., & Guardiola, F. (2001). Cholesterol oxidation in frozen dark chicken meat: influence of dietary fat source, and  $\alpha$ -tocopherol and ascorbic acid supplementation. *Meat Science*, 57(2), 197–208.
- Gray, J. I., & Crackel, R. L. (1992). Oxidative flavour changes in meats: their origin and prevention. In D. A. Ledward, D. E. Johnston, & M. K. Knight (Eds.), *The chemistry of muscle-based foods* (pp. 145–168). Cambridge, UK: The Royal Society of Chemistry.
- Gray, J. I., Goma, E. A., & Beckley, D. J. (1996). Oxidative quality and shelf life of meats. *Meat Science*, 43(Suppl. 1), S111–S123.
- LARN. (1996 edition). Available from <<http://www.sinu.it/>>.
- Lin, C. F., Gray, J. I., Asghar, A., Buckley, D. J., Booren, A. M., & Flegal, C. J. (1989). Effects of dietary oils and  $\alpha$ -tocopherol supplementation on lipid composition and stability of broiler meat. *Journal of Food Science*, 54(6), 1457–1460.
- López-Bote, C. J., Gray, J. I., Goma, E. A., & Flegal, C. J. (1998a). Effect of dietary oat administration on lipid stability in broiler meat. *British Poultry Science*, 39(1), 57–61.
- López-Bote, C. J., Gray, J. I., Goma, E. A., & Flegal, C. J. (1998b). Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. *British Poultry Science*, 39(2), 235–240.
- Maguire, L., Konoplyannikov, M., Ford, A., Maguire, A. R., & O'Brien, N. (2003). Comparison of the cytotoxic effects of  $\beta$ -sitosterol oxides and a cholesterol oxide, 7 $\beta$ -hydroxycholesterol, in cultured mammalian cells. *British Journal of Nutrition*, 90(4), 767–775.
- Maraschiello, C., Esteve, E., Regueiro, J., & García, A. (1998). Cholesterol oxidation in meat from chickens fed  $\alpha$ -tocopherol- and  $\beta$ -carotene-supplemented diets with different unsaturation grades. *Lipids*, 33(7), 705–713.
- Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49(Suppl. 1), S73–S86.
- 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(4), 431–435.
- O'Neill, L. M., Galvin, K., Morrissey, P. A., & Buckley, D. J. (1998). Comparison of effects of dietary olive oil, tallow and vitamin E on the quality of broiler meat and meat products. *British Poultry Science*, 39(3), 365–371.
- Osada, K. (2002). Cholesterol oxidation products: Other biological effects. In F. Guardiola, P. C. Dutta, R. Codony, & G. Savage (Eds.), *Cholesterol and phytosterol oxidation products: Analysis, occurrence, and biological effects* (pp. 278–318). Champaign: AOCS Press.
- 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 Agriculture and Food Chemistry*, 48(9), 3823–3829.
- Parisi, E. (2001). *Valutazione dello stato di ossidazione di prosciutti crudi e di insaccati non tradizionali*. B.Sc. Thesis on Agricultural Sciences and Technologies, Università di Bologna, Italy.
- Penazzi, G. (1996). Industrial cooking of chicken cutlets: evaluation of lipid quality related to technology. In *Proceedings of 1st workshop on the developments in the Italian Ph.D. research in food biotechnology* (pp. 64–67). Viterbo, IT: Università della Tuscia.
- Penazzi, G. (1997). *Composizione e modificazioni della frazione lipidica nella cottura industriale di carni avicole impanate*. Ph.D. Thesis on Food Biotechnology, Università degli Studi di Firenze, Italy.
- Pszczola, D. E. (2002). Beefing up innovations for meat and poultry ingredients. *Food Technology*, 56, 54–66.
- Reddy, B. S. (1995). Nutritional factors and colon cancer. *Critical Reviews in Food Science and Nutrition*, 35(3), 175–190.
- Rodriguez-Estrada, M. T., Penazzi, G., Caboni, M. F., Bertacco, G., & Lercker, G. (1997). Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Science*, 45(3), 365–375.
- Rose-Sallin, C., Hugget, A. C., Bosset, J. O., Tabacchi, R., & Fay, L. B. (1995). Quantification of cholesterol oxidation products in milk powders using [ $^3$ H] cholesterol to monitor cholesterol autoxidation artefacts. *Journal of Agricultural and Food Chemistry*, 43, 935–941.
- 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.
- Sanz, M., Flores, A., & López-Bote, C. J. (2000). The metabolic use of calories from dietary fat in broilers is affected by fatty acid saturation. *British Poultry Science*, 41(1), 61–68.
- Schroepfer, G. J. Jr., (2000). Oxysterols: modulation of cholesterol metabolism and other processes. *Physiology Reviews*, 80(1), 361–554.
- Sevanian, A. (1991). Personal communication at the international symposium “Biological free radical oxidations and antioxidants”, Udine, July 1–4, Ursini, F., & Cadenas, E. (Eds.), Padova, Italy: CLEUP University Publisher.
- Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC International*, 77(2), 421–424.
- Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition*, 70(Suppl.), 560S–569S.
- Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy*, 56(8), 356–379.
- Smith, J. S., & Alfawaz, M. (1995). Antioxidative activity of Maillard reaction-products in cooked ground-beef sensory and TBA values. *Journal of Food Science*, 60(1), 234–239.
- Soyland, E., & Drevon, C. A. (1993). The effect of very long-chain  $\omega$ -3 fatty acids on immune-related skin disease. *European Journal of Clinical Nutrition*, 47(6), 381–388.
- Sweeley, C. C., Bentley, R., Makita, M., & Wells, W. W. (1963). Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *Journal of the American Chemical Society*, 85, 2497–2507.
- Wiseman, J., Salvador, F., & Craigon, J. (1991). Prediction of the apparent metabolizable energy content of fats fed to broiler chickens. *Poultry Science*, 70, 1527–1533.
- Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., et al. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, 66(1), 21–32.