

Effect of dietary olive leaves on the lipid and protein oxidation and bacterial safety of chicken hamburgers during frozen storage

da Silva, S. L., Marangoni, C., Brum, D. S., Vendruscolo, R. G., Silva, M. S., de Moura, H. C., Rampelotto, C., Wagner, R., de Menezes, C. R., Barin, J. S., Campagnol, P. C. B., *Cichoski, A. J.

Universidade Federal de Santa Maria, CEP 97105-900 Santa Maria, Rio Grande do Sul, Brazil

Article history

Received: 7 December 2016
Received in revised form:
15 December 2016
Accepted: 16 December 2016

Abstract

In total, 2520 chickens were randomly allocated into 36 pens with 70 chickens per pen. One group that served as the control was fed a basal diet, while the others were fed diets supplemented with olive leaves at 5 or 10 g/kg. At 42 days of age, chickens were slaughtered and the breast was used in the manufacture of chicken hamburgers. The lipid and protein oxidation and bacterial count of chicken hamburgers were assessed. The results showed that the incorporation of olive leaves in chicken diets decreased ($P < 0.05$) the lipid and protein oxidation of chicken hamburgers during frozen storage. Dietary olive leaves at 10 g/kg were more effective ($P < 0.05$) in inhibiting lipid and protein oxidation. Diet supplementation with olive leaves resulted in lower ($P < 0.05$) aerobic mesophilic and psychrophilic bacteria, *Staphylococcus* spp. and *Enterobacteriaceae* counts over the entire storage period.

Keywords

Olive leaves
Chicken meat
Hamburger
Natural antioxidant
Natural antimicrobial

© All Rights Reserved

Introduction

Chicken meat is important in human nutrition for its natural content of health-promoting compounds and nutritive value. In the near future, demand for chicken-derived products with enriched and valuable nutritional components is expected to increase. These new chicken meat products must be not only high in nutritional value and health enhancing, but also safe, tasty and convenient for consumption (Stangierski and Lesnierowski, 2015).

Chicken meat contains a low amount of total fat and cholesterol compared with other sources of meat (Cagdas and Kumcuoglu, 2015). Further, chicken fat contains 25–30% polyunsaturated fatty acids as well as approximately 40% monounsaturated fatty acids (Stangierski and Lesnierowski, 2015). These high levels of unsaturated fatty acids in muscle membranes increase the predisposition to lipid oxidation (Engberg *et al.*, 1996). This reaction jeopardizes sensory quality and decreases the shelf life of meat and meat products. Lipid oxidation also produces free radicals, which are associated with mutagenesis, carcinogenesis and aging (Yagi, 1987). Chicken meat contains high amounts of proteins (18–25%), which can also be affected by oxidative reactions (Stangierski and Lesnierowski, 2015).

The impact of protein oxidation on meat quality is

still the subject of multiple studies but it is generally accepted that this reaction is commonly linked to a loss of nutritional value and a decrease in muscle protein functionality, leading to increasing water losses, weaker protein gels or less stable emulsions (Estévez, 2011). Moreover, the texture and color deterioration of meat has been related to the protein oxidation phenomenon (Estévez *et al.*, 2005). In addition to lipid and protein oxidation, microbial growth is another primary factor of chicken meat spoilage (Lerasle *et al.*, 2014). Consequently, there is a need to increase the antimicrobial and antioxidant capacity of muscles that can be achieved by the supplementation of the diet with phytochemicals. This approach is in general more effective than the direct addition of preservatives because the compound is deposited where it is most needed (Govaris *et al.*, 2004).

Olive leaves (*Olea europaea*) are agricultural by-products from the pruning and shaking of olive trees for fruit harvest, and they represent around 10% of the total weight of collected material arriving at the olive oil press. Olive leaves contain up to 129 g kg⁻¹ dry matter of crude protein, with high levels of arginine, leucine and valine but low levels of tyrosine and cysteine (Martín-García and Molina-Alcaide, 2008). The nutritive value of olive leaves is greater when they are fed fresh, although dried leaves may

*Corresponding author.

Email: cjoale@gmail.com

Tel: +55-55-3220-8254; Fax: +55-55-3220-8254

also be incorporated into the diet (Delgado-Pertíñez *et al.*, 1998).

Olive leaves contain up to 100 g kg⁻¹ of phenolic compounds such as oleuropein, hydroxytyrosol and tocopherol (Savournin *et al.*, 2001). Bisignano *et al.* (1999), Markín *et al.* (2003) and Sudjana *et al.* (2009) demonstrated the antimicrobial and antioxidant activity of these phenolic compounds in vitro. Moreover, Bisignano *et al.* (1999) also reported the antimicrobial properties of other non-phenolic components of olive leaves such as aldehydes. The supplementation of olive leaves in feed improves the oxidative and microbial stability of pork meat (Paiva-Martins *et al.*, 2009; Botsoglou *et al.*, 2012) and turkey breast fillets (Botsoglou *et al.*, 2010). However, little information has been published concerning the quality during frozen storage of meat products processed from chickens fed supplemental olive leaves. Therefore, in this study, we evaluated the lipid and protein oxidation and microbial safety of hamburgers from chickens fed with supplemental olive leaves.

Material and Methods

Animals and diets

The animal protocol was approved by the Animal Care and Ethics Committee of the Cooperative Central Aurora Alimentos. A total of 2520 one-day-old female broiler chickens (Cobb genetic line) obtained from a commercial hatchery (Aurora Alimentos S. A, Xanxeré, Brazil) were randomly allocated into 36 pens (3 m length × 2 m width) with 12 replicates per treatment and 70 chickens per pen. The chicken groups were assigned to three dietary treatments: control diet without olive leaves (T1); control diet plus 5 g of olive leaves kg⁻¹ (T2); and control diet plus 10 g of olive leaves kg⁻¹ (T3). The control basal diet (Table 1) was formulated to be adequate in all nutrients (National Research Council 1994) and was provided in four phases: pre-initial (PI: 1 to 12 days), initial (I: 13 to 21 days), growth (G: 22 to 32 days) and final (F: 33 to 42 days). Olive leaves were provided in these four phases.

Olive leaves of Ascolano variety were harvested in Chapecó (Santa Catarina State, Brazil; latitude -27°05'47''S and longitude 52°37'06''W, altitude 674 m) in the second week of February (summer) of 2012, from six-year-old trees. Leaves were collected from different parts of several trees in order to minimize the effect of sun exposure and differences related to different maturation stages. After drying in an oven with air circulation (45±5°C, 48 h), the leaves were ground in a rotor mill (Marconi, MA-340) and

Table 1. Ingredients and nutrient composition of experimental control diet (g kg⁻¹ as fed basis)

	PI*	I	G	F
Corn grain	558	636	662.667	690.667
Soybean meal	315.342	238.977	189.403	155.675
Poultry offal meal	68	58	56	49.333
Hydrolyzed poultry feather meal	-	10	25.333	34.667
Soybean oil	28	-	40	-
Poultry fat	-	30	-	45.333
Calcereous	7.5	7	6.667	6.333
Sodium chloride	4.4	4.6	4.267	4.133
L-Lysine HCL 50%	6.7	6.8	7.667	7.733
Methionine MHA	4.6	3.6	3.4	2.867
Choline chloride 75%	0.45	0.4	0.4	0.367
Anti-mycotoxin	2	-	-	-
L-Threonine	2.078	1.793	1.767	1.572
Rovabio Excel AP xilase + B-glucanase®	0.05	0.05	0.05	0.05
Ronozyme ProAct®	0.2	0.2	0.2	0.2
Ethoxyquin	0.15	0.15	0.15	0.15
Ronozyme® HiPhos (M)	0.02	0.02	0.02	0.02
Lincomycin 44%	0.01	0.01	0.01	-
Anticoccidial	0.5	0.5	0.5	-
Premix**	2	1.9	1.5	0.9

Note: * The diet was provided in four phases: pre-initial (PI: 1 to 12 days), initial (I: 13 to 21 days), growth (G: 22 to 32 days) and final (F: 33 to 42 days). **Premix: vitamin A (4.275 IU), vitamin D₃ (1.395 IU), vitamin E (22.5 mg), vitamin K₃ (1.35 mg), vitamin B₁ (1.35 mg), vitamin B₂ (3.6 mg), vitamin B₆ (1.8 mg), vitamin B₁₂ (9.9 µg), pantothenic acid (9 mg), niacin (18 mg), folic acid (900 mg), biotin (76.5 µg), Cu (7.2 mg), Fe (27 mg), Mn (27 mg), I (450 mg), Zn (45 mg) and Se (180 mg).

the powder was stored at -20°C and protected from light until use. Diets in mash form and water were offered ad libitum through the 42-day feeding trial. Mortality was lower than 1%.

Formulation and preparation of chicken hamburgers

At 42 days of age, chickens were slaughtered, processed and eviscerated at a local slaughterhouse at Chapecó, Santa Catarina State (Brazil). For each of the three diets, 100 kg of hand-deboned breast was obtained to be used in the manufacture of chicken hamburgers. The chicken breast meat was frozen at -18°C for 120 days prior to use. The frozen meat materials were thawed at 5°C for 24 h prior to manufacture.

Three replications were performed on separate days. The chicken breast meat was ground in a 3 mm plate grinder (Model PJ22, Jamar, Brazil). Ten-kilogram batches of the appropriate amounts of each formulation (Table 2) were mixed using a paddle mixer (Model MJ135, Jamar, Brazil) until a homogenous mix was obtained (about 5 min) and then processed into chicken hamburgers (1.5 cm thick and 10 cm diameter) by using a metal shaper. Hamburgers were placed on plastic trays, over wrapped with high-density polyethylene film (7.5 µm thickness, with oxygen transmission rates > 15 cm³/m².day and water vapor transmission rates < 8 g/m².day) and stored at -18°C for 120 days.

Table 2. Formulation of chicken hamburger

Ingredients	(%)
Chicken breast meat	88
Ice	6.2
Salt	1.3
Extruded soy protein	4
Sodium tripolyphosphate	0.15
Sodium erythorbate	0.05
Garlic powder	0.2
Onion powder	0.1
TOTAL	100

Proximate composition

The moisture, protein and ash content were determined according to the Association of Official Analytical Chemists (AOAC, 2005). The moisture content was determined by drying in an oven at 105°C ± 2°C; the nitrogen content was determined by the Kjeldahl method; the protein content was estimated by multiplying the nitrogen content by 6.25; and the ash content was determined by incineration in a muffle at 550°C. Lipids were extracted from the samples using the Bligh and Dyer (1959) method and used both for lipid quantification and for the determination of conjugated dienes and peroxide values. All determinations were performed in triplicate. The proximate composition was determined in the chicken breast meat and in the hamburgers at 30 days of storage.

pH and aw

The determination of pH was performed by direct insertion of a pH meter (MA 130, Mettler Toledo Indústria e Comércio Ltda, SP, Brazil) equipped with a penetration electrode (LoT406-M6-DXK-S7/25). Water activity (a_w) was measured by using a Decagon Aqualab instrument (Decagon Devices Inc., Pullman, USA). All determinations were performed in triplicate. pH and aw were determined in the chicken breast meat (0 days) and in the hamburgers (after 30, 60, 90 and 120 days of storage).

Color determination

Color determination was performed with a HunterLab Colorimeter (ColorQuest II, Hunter Associates Laboratory Inc., Virginia, USA) using a 10-mm port size, illuminant D65 and a 100 standard observer. The color variables were measured at four points on the central part of the cut surface of three slices of the samples. CIELAB L^* , a^* and b^* values were determined as indicators of lightness, redness and yellowness, respectively. Color instrumental was determined in the chicken breast meat (0 days) and in the hamburgers (after 30, 60, 90 and 120 days of storage).

Lipid oxidation measurement

Lipid oxidation during frozen storage was evaluated by measuring conjugated dienes and peroxide values for primary oxidation, and thiobarbituric acid-reactive substances (TBARS) for secondary oxidation. All analyses were performed in triplicate. Lipid oxidation was determined in the chicken breast meat (0 days) and in the hamburgers (after 30, 60, 90 and 120 days of storage).

Conjugated dienes

Conjugated diene concentrations were determined according to the Recknagel and Glende (1984) method. The extracted fat (0.01 g) was dissolved in 3 ml of cyclohexane and absorbance was measured at 232 nm using a UV spectrophotometer (Model Cirrus 8051, Femto, Brazil). Conjugated dienes were expressed as Abs/mg fat/ml cyclohexane.

Peroxide value

Peroxide value was determined according to the method of American Oil Chemists Society (AOCS, 2003). To 0.05 g of fat extracted, 50 µl of 30% ammonium thiocyanate and 50 µl of 20 mM ferrous chloride solution in 3.5% HCl were added and mixed thoroughly. After 3 min, the absorbance of the colored solution was measured at 500 nm using a JENWAY 6300 spectrophotometer (Jenway, Staffordshire, UK). Peroxide value was expressed as milliequivalents of peroxides per kg of lipid.

TBARS (Thiobarbituric acid reactive substances)

A TBARS assay was performed as described by Raharjo *et al.* (1992) and modified by Wang *et al.* (2002) by monitoring the interference of sugar in the reaction. Ten grams of the sample were mixed with 36 ml of trichloroacetic acid (TCA 50 g l⁻¹) and 1 ml of BHT 0.15%. Samples were homogenized for 1 min at 3000 rpm with an Ultra-Turrax (ULTRA 380, Raker Solutions, Brazil) and then filtered through Whatman N°1 filter paper. One milliliter was mixed with 1 ml of thiobarbituric acid (TBA) solution (0.02 mol l⁻¹) and maintained at 40°C for 80 min in a heated bath (DL-SOLOD, Deleo, Brazil). The solution was cooled in a cold water bath (10°C) for 10 min and absorbance was measured at 532 nm using a JENWAY 6300 spectrophotometer (Jenway, Staffordshire, UK). TBARS values were calculated from a standard curve of malondialdehyde (MDA) prepared by the acidification of 1, 1, 3, 3-tetraethoxypropane and the results were expressed as milligrams of MDA per kilogram of meat.

Table 3. Physicochemical characteristics of raw meat and hamburgers from chickens fed with supplemental olive leaves

	Raw chicken breast meat			Days	Hamburger		
	B0	B5	B10		H0	H5	H10
pH	5.9 ^a ± 0.16 [*]	5.8 ^a ± 0.07	5.9 ^a ± 0.09	30	6.4 ^{As} ± 0.01 ^{**}	6.2 ^{As} ± 0.02	6.2 ^{As} ± 0.03
				60	6.3 ^{As} ± 0.03	6.2 ^{As} ± 0.03	6.2 ^{As} ± 0.01
				90	6.4 ^{As} ± 0.03	6.2 ^{As} ± 0.02	6.1 ^{As} ± 0.04
				120	6.1 ^{As} ± 0.05	6.0 ^{As} ± 0.00	6.0 ^{As} ± 0.03
a _w	0.99 ^a ± 0.006	0.99 ^a ± 0.003	0.98 ^a ± 0.001	30	0.97 ^{As} ± 0.006	0.98 ^{As} ± 0.005	0.98 ^{As} ± 0.002
				60	0.98 ^{As} ± 0.001	0.98 ^{As} ± 0.004	0.98 ^{As} ± 0.004
				90	0.98 ^{As} ± 0.002	0.98 ^{As} ± 0.005	0.98 ^{As} ± 0.002
				120	0.98 ^{As} ± 0.005	0.98 ^{As} ± 0.001	0.98 ^{As} ± 0.002
L*	49.29 ^a ± 1.06	48.21 ^a ± 1.98	50.11 ^a ± 0.55	30	54.96 ^{As} ± 2.14	57.57 ^{As} ± 1.82	57.68 ^{As} ± 0.87
				60	56.87 ^{As} ± 0.20	56.81 ^{As} ± 2.49	58.55 ^{As} ± 0.12
				90	58.43 ^{As} ± 0.54	58.16 ^{As} ± 0.73	59.06 ^{As} ± 0.12
				120	50.61 ^{Bs} ± 1.12	53.62 ^{Bs} ± 1.00	52.33 ^{Bs} ± 0.91
a*	5.55 ^a ± 0.54	5.09 ^a ± 0.16	5.40 ^a ± 0.29	30	7.37 ^{As} ± 0.26	7.94 ^{As} ± 0.13	7.87 ^{As} ± 0.21
				60	6.92 ^{Bs} ± 0.23	7.14 ^{As} ± 0.12	6.90 ^{As} ± 0.05
				90	8.13 ^{As} ± 0.45	7.84 ^{Bs} ± 0.83	7.01 ^{As} ± 0.18
				120	6.66 ^{Cs} ± 0.02	6.53 ^{Cs} ± 0.05	6.29 ^{Bs} ± 0.03
b*	5.43 ^a ± 1.28	5.31 ^a ± 2.22	4.90 ^a ± 2.78	30	20.96 ^{As} ± 0.22	21.33 ^{As} ± 1.41	21.49 ^{As} ± 0.70
				60	21.80 ^{As} ± 0.79	21.99 ^{As} ± 0.36	21.33 ^{As} ± 0.20
				90	17.31 ^{As} ± 0.49	17.47 ^{As} ± 0.93	19.44 ^{As} ± 0.38
				120	14.81 ^{Bs} ± 0.24	16.38 ^{Bs} ± 0.37	16.02 ^{Bs} ± 0.27

*Values represent the mean (± standard deviation). **Averages within the same line followed by the same lowercase letters did not show any significant difference ($P > 0.05$) by Tukey's test. Averages within the same column followed by the same upper case did not show any significant difference ($P > 0.05$) by Tukey's test. Experimental treatments: B0 and H0: control diet without olive leave; B5 and H5: control diet plus 5 g of olive leave kg⁻¹ of diet, and B10 and H10: control diet plus 10 g of olive leave kg⁻¹ of diet.

Protein oxidation measurement

Protein carbonyl content was determined in the chicken breast meat (0 days) and in the hamburgers (after 30, 60, 90 and 120 days of storage) by derivatization with 2,4-dinitrophenylhydrazine (DNPH) as described by Levine *et al.* (1990) with slight modifications. The concentration of protein was measured at 280 nm in the HCl control using bovine serum albumin in 6 M guanidine as standard. Carbonyl concentration in the treated sample was measured with 2,4-DNPH incorporated on the basis of a molar absorption coefficient of 21 nM⁻¹.cm⁻¹ at 370 nm of protein hydrazones. The results were expressed as nmol of carbonyl/mg of protein.

Microbiological analysis

The microbiological characteristics were evaluated according to the methodology described by Downes and Ito (2001). For that, a 25 g aliquot was homogenized with 225 mL 0.1% peptone water (Oxoid Unipath Ltd, Basingstoke, Hampshire, UK) and serial decimal dilutions were performed. The total aerobic mesophilic and psychophilic counts were quantified on Plate Count Agar (Merk, Darmstadt, Germany) after incubation at 36°C for 48 h and at 7°C for 7 days, respectively. Staphylococcus spp. was quantified in Baird-Parker Agar (Merk, Darmstadt, Germany) at 36°C for 48 h; total Enterobacteriaceae count was quantified in Violet Red Bile Glucose Agar (VRBGA) (Merk, Darmstadt, Germany) at 36°C for 24 h. The microbiological characteristics were determined in the chicken breast meat (0 days) and in the hamburgers (after 30, 60, 90 and 120 days of

storage). For all microbial counts, the results were expressed as log cfu g⁻¹.

Statistical analysis

The data were assessed by analysis of variance (one-way ANOVA), and means were compared using Tukey's test, with a significance level of 5% ($P \leq 0.05$) using the Statistica v.8 statistical package (Statsoft, Inc., Tulsa, OK, USA).

Results and Discussion

Physicochemical analysis

The proximate composition was not different ($P > 0.05$) for raw meat and chicken hamburgers processed from chicken supplemented with olive leaves compared with the control (data not shown). The moisture, protein, fat and ash content were 70.22–72.07%, 22.17–22.72%, 1.28–1.79% and 1.79–1.84%, respectively.

The physicochemical characteristics of raw meat and hamburgers from chickens fed with supplemental olive leaves are shown in Table 3. Olive leaf enrichments had no significant effect on the pH, a_w, L*, a* and b* values of the raw meat. Over the entire storage period (120 days), the pH and a_w values for chicken hamburgers from chickens supplemented with 5 (H5) and 10 g (H10) of olive leaves were not different ($P > 0.05$) compared with those from the control (H0). During the entire storage, there was no significant difference in the L*, a* and b* values among the hamburgers prepared using breast from chickens supplemented with 5 g of olive leaves kg⁻¹

Table 4. Lipid and protein oxidation of raw meat and hamburgers from chickens fed with supplemental olive leaves

	Raw chicken breast meat			Days	Hamburger		
	B0	B5	B10		H0	H5	H10
Conjugated diene (Abs/mg fat/ml cyclohexane)	0.3448 ^a	0.3547 ^a ± 0.003	0.2935 ^b ± 0.015	30	0.4182 ^{ab} ± 0.03 ^{bc}	0.3241 ^{bc} ± 0.01	0.3013 ^{ab} ± 0.06
				60	0.4224 ^{ab} ± 0.02	0.3122 ^{bc} ± 0.04	0.2813 ^{ab} ± 0.01
				90	0.4856 ^{ab} ± 0.03	0.3477 ^{ab} ± 0.02	0.2885 ^{ac} ± 0.02
				120	0.5167 ^{ab} ± 0.02	0.3960 ^{ab} ± 0.02	0.2982 ^{ac} ± 0.01
Peroxide value (mEq/Kg sample)	0.0744 ^a ± 0.01	0.0651 ^a ± 0.01	0.0436 ^b ± 0.01	30	0.8117 ^{ab} ± 0.0001	0.7329 ^{ab} ± 0.0002	0.4026 ^{bc} ± 0.0002
				60	0.3402 ^{bc} ± 0.0005	0.2746 ^{cd} ± 0.0003	0.2858 ^{cd} ± 0.0003
				90	0.6512 ^{ab} ± 0.0003	0.5679 ^{bc} ± 0.0001	0.4829 ^{ac} ± 0.0001
				120	0.4834 ^{cd} ± 0.0002	0.3154 ^{cd} ± 0.0001	0.3156 ^{cd} ± 0.0002
TBARS (mg MDA/Kg sample)	0.018 ^a ± 0.002	0.018 ^a ± 0.006	0.019 ^a ± 0.002	30	0.023 ^{bc} ± 0.001	0.020 ^{cd} ± 0.001	0.017 ^{bc} ± 0.002
				60	0.024 ^{bc} ± 0.001	0.017 ^{cd} ± 0.002	0.018 ^{bc} ± 0.002
				90	0.024 ^{bc} ± 0.004	0.023 ^{bc} ± 0.003	0.021 ^{bc} ± 0.003
				120	0.117 ^{ab} ± 0.003	0.109 ^{ab} ± 0.003	0.103 ^{ac} ± 0.002
Protein carbonyl content (nmol of carbonyl/mg of protein)	0.602 ^a ± 0.14	0.626 ^a ± 0.01	0.637 ^a ± 0.04	30	2.355 ^{ab} ± 0.27	2.103 ^{bc} ± 0.15	1.825 ^{bc} ± 0.23
				60	3.138 ^{ab} ± 0.29	3.335 ^{ab} ± 0.42	2.290 ^{ab} ± 0.38
				90	2.285 ^{bc} ± 0.23	2.118 ^{bc} ± 0.34	2.109 ^{ab} ± 0.54
				120	1.761 ^{cd} ± 0.34	1.607 ^{cd} ± 0.38	1.590 ^{bc} ± 0.18

*Values represent the mean (± standard deviation). **Averages within the same line followed by the same lowercase letters did not show any significant difference ($P > 0.05$) by Tukey's test. Averages within the same column followed by the same upper case did not show any significant difference ($P > 0.05$) by Tukey's test. Experimental treatments: B0 and H0: control diet without olive leave; B5 and H5: control diet plus 5 g of olive leave kg^{-1} of diet, and B10 and H10: control diet plus 10 g of olive leave kg^{-1} of diet.

(H5) or the control (H0). After 90 days, hamburgers elaborated with breast from chickens supplemented with 10 g of olive leaves kg^{-1} (H10) had significantly lower a^* values than the H0 (control) and H5 (Table 3). The hamburgers presented decreased L^* , a^* and b^* values after 120 days of storage, probably as a consequence of the interaction of pigments with the products of lipid oxidation (Kulkarni *et al.*, 2011).

Lipid and protein oxidation

Olive leaves enrichments had a significant effect on the lipid and protein oxidation of raw meat and hamburgers during frozen storage (Table 4). The formation of conjugated dienes reflects the degree of primary products formed during lipid oxidation and increases in parallel with the production of hydroperoxides (Frankel, 2005). The raw breast meat from chickens supplemented with 10 g of olive leaves kg^{-1} (B10) had significantly lower conjugated diene values than B0 (control) and B5 (Table 4). The highest conjugated diene values were observed in the control (H0) hamburgers ($P < 0.05$) during the entire frozen storage period. For the 90 and 120 days of storage, the conjugated diene values of the hamburgers decreased with an increased percentage of olive leaf supplementation (Table 4). Similar trends have been observed in previous studies of muscle from pigs fed with supplemental olive leaves

(Paiva-Martins *et al.*, 2009).

The determination of peroxide values is useful as an oxidative index for the early stage of lipid oxidation (Ramadan and Mörssel, 2004). The raw breast meat from chickens supplemented with olive leaves (B5 and B10) had significantly lower peroxide values than the control (B0). The hamburgers prepared using breast from chickens supplemented with olive leaves (H5 and H10) had significantly lower peroxide values than the control (H0) during frozen storage as an indication of retarding lipid oxidation (Table 4). The peroxide values of the treatments decreased during the later stages of storage. These decreases may be due to the decomposition of hydroperoxides to form low molecular weight compounds e.g., aldehydes and ketones (Wongwichian *et al.*, 2015).

TBARS values indicate the formation of secondary lipid oxidation products, such as aldehydes and ketones, which are considered to be responsible for the rancid taste in meat and meat products (Gray and Pearson 1987). The TBARS values of the raw breast chicken meat were close to 0.02 mg MDA/kg and no difference was observed among the samples ($P > 0.05$). In the hamburgers, TBARS values increased significantly after 120 days of storage in all treatments due to the oxidation of unsaturated fatty acids (Mendes *et al.*, 2008). However, the TBARS values of the modified hamburgers (H5 and H10)

Table 5. Microbiological characteristics (Log cfu/g) of raw meat and hamburgers from chickens fed with supplemental olive leaves

	Raw chicken breast meat			Days	Hamburger		
	B0	B5	B10		H0	H5	H10
Mesophilic aerobic bacteria	6.60 ^{a±}	3.79 ^{b±}	3.73 ^{b±}	30	7.11 ^{ab±}	6.30 ^{ab±}	5.03 ^{bc±}
	0.33 ^c	0.26	0.20		0.64 ^c	0.01	0.18
				60	7.62 ^{ab±}	7.08 ^{ab±}	5.15 ^{bc±}
					0.54	0.26	0.14
				90	7.99 ^{ab±}	7.07 ^{ab±}	5.38 ^{bc±}
Psychrophilic aerobic bacteria	6.19 ^{a±}	3.33 ^{b±}	2.95 ^{b±}	30	7.08 ^{ab±}	5.21 ^{bc±}	4.60 ^{bc±}
	0.12	0.34	0.43		0.64	0.01	0.18
				60	7.80 ^{ab±}	6.45 ^{bc±}	5.72 ^{bc±}
					0.54	0.26	0.14
				90	7.87 ^{ab±}	6.88 ^{bc±}	5.52 ^{bc±}
Staphylococcus spp.	3.98 ^{a±}	1.95 ^{b±}	1.96 ^{b±}	30	4.84 ^{ab±}	4.37 ^{bc±}	2.49 ^{bc±}
	0.42	0.67	0.53		0.07	0.06	0.12
				60	5.34 ^{ab±}	5.22 ^{ab±}	2.51 ^{bc±}
					0.25	0.21	0.13
				90	5.51 ^{ab±}	4.82 ^{bc±}	3.16 ^{bc±}
Enterobacteriaceae	6.29 ^{a±}	3.14 ^{b±}	2.81 ^{b±}	30	5.70 ^{ab±}	4.86 ^{bc±}	3.31 ^{bc±}
	0.12	0.13	0.24		0.23	0.02	0.11
				60	7.24 ^{ab±}	5.31 ^{bc±}	4.80 ^{bc±}
					0.15	0.02	0.51
				90	7.30 ^{ab±}	6.75 ^{bc±}	5.42 ^{bc±}
					0.42	0.29	0.33
				120	7.67 ^{ab±}	6.95 ^{bc±}	5.52 ^{bc±}
					0.18	0.52	0.39
					7.71 ^{ab±}	7.19 ^{ab±}	6.05 ^{bc±}
					0.05	0.06	0.18

*Values represent the mean (\pm standard deviation). **Averages within the same line followed by the same lowercase letters did not show any significant difference ($P > 0.05$) by Tukey's test. Averages within the same column followed by the same upper case did not show any significant difference ($P > 0.05$) by Tukey's test. Experimental treatments: B0 and H0: control diet without olive leave; B5 and H5: control diet plus 5 g of olive leave kg^{-1} of diet, and B10 and H10: control diet plus 10 g of olive leave kg^{-1} of diet.

were significantly lower ($P < 0.05$) than those of the control (H0) on day 120. These results were in agreement with Botsoglou *et al.* (2010), who found a reduction in TBARS values during refrigerated storage in breast fillets from turkeys fed with supplemental olive leaves.

The formation of carbonyl compounds is one of the most marked changes occurring during the oxidation of proteins (Huang *et al.*, 2014). This reaction may cause deterioration in the flavor, texture and overall quality of muscle foods (Soyer *et al.*, 2010). In this study, the olive leave enrichments did not affect ($P > 0.05$) the carbonyl content in the raw chicken breast meat; however, a significant effect ($P < 0.05$) was observed in the hamburgers during frozen storage (Table 4). After 30 and 60 days, hamburgers prepared using breast from chickens supplemented with 10 g of olive leaves kg^{-1} (H10) had a significantly ($P < 0.05$) lower carbonyl content than H0 (control) and H5 (Table 4). The values of the protein carbonyl compounds observed in this study were lower to those previously reported in chicken muscle (1.5 $\mu\text{mol}/\text{mg}$ protein) (Liu and Xiong, 1996) and turkey breast muscle (1.10–3.41 $\mu\text{mol}/\text{mg}$ protein) (Mercier *et al.*, 1998).

In the present study, the total amount of protein carbonyls increased during the first 2 months of

frozen storage, while a significant decrease was detected by the end of the frozen storage period (4 months). These results are in agreement with Estévez *et al.* (2011), who found a reduction in carbonyl content during frozen storage. The apparent net loss of protein carbonyls at prolonged oxidation rates may be caused by the implication of such compounds as reactants in advanced reactions, as hypothesized by Estévez *et al.* (2011).

In general, the results of this study showed the protective effect of olive leave supplementation against lipid and protein oxidation. These results are in agreement with other authors who have reported a decrease in lipid oxidation in stored turkey breast fillets (Botsoglou *et al.*, 2010) as a consequence of olive leave supplementation in the diet, but this is the first study in which protection against lipid and protein oxidation is shown for meat products. Previous studies demonstrate that olive leaves contain a high content of phenolic compounds, especially oleuropein (El and Karakaya, 2009). Potentially, the decrease of lipid and protein oxidation in breast chicken after diet supplementation with olive leaves is the result of phenolic compounds with antioxidant activity that entered the circulatory system, and distributed and retained in chicken tissues. This antioxidant activity of phenolic compounds is associated with the

hydroxyl group attached to the aromatic ring, which is able to donate electrons with hydrogen atoms and neutralize free radicals. This mechanism blocks the further degradation of more active oxidant forms (Radha Krishnan *et al.*, 2014).

Microbiological analysis

The dietary supplementation of olive leaves significantly affected the microbiological characteristics of raw meat and hamburgers during frozen storage (Table 5). The raw breast meat from chickens supplemented with olive leaves (B5 and B10) had significantly ($P < 0.05$) lower aerobic mesophilic and psychrophilic bacteria, *Staphylococcus* spp. and *Enterobacteriaceae* counts compared with the control (B0). In general, over the entire storage period (120 days), the microbiological counts for hamburgers from chickens supplemented with olive leaves (H5 and H10) were lower ($P > 0.05$) compared with those from the control (H0). Further, the microbiological counts of the hamburgers decreased ($P < 0.05$) with an increased percentage of olive leaf supplementation (Table 5). These results indicate that the dietary supplementation of olive leaves improved the microbiological quality of the raw chicken meat and derived hamburgers. This fact could be attributed to the high quantity of phenolic compounds presented in olive leaves (El and Karakaya, 2009). Similar antimicrobial activity in olive leaves has been reported by previous studies *in vivo* (Botsoglou *et al.*, 2010) and *in vitro* (Bisignano *et al.*, 1999, 2001; Markín *et al.*, 2003; Sudjana *et al.*, 2009).

Conclusion

The results obtained showed that the incorporation of olive leaves in the chicken diet decreased lipid and protein oxidation in the hamburgers during their frozen storage (120 days). The supplementation of olive leaves at the level of 10 g/kg was more effective in delaying lipid and protein oxidation compared with the level of 5 g/kg. The supplemented olive leaves also exerted an inhibitory effect on bacterial growth. Chicken hamburgers processed from chickens fed with 5 g/kg and 10 g/kg supplemental olive leaves had lower content of aerobic mesophilic and psychrophilic bacteria, *Staphylococcus* spp. and *Enterobacteriaceae* during storage. The rate of bacterial inhibition was higher in the hamburgers elaborated with breast from chickens supplemented with 10 g of olive leaves kg⁻¹ compared with the level of 5 g/kg. Therefore, the dietary supplementation of olive leaves can be considered as a promising tool to

produce safer chicken meat products.

References

- American Oil Chemists Society. 2003. Official methods and recommended practices of the American Oil Chemists Society. 4th ed. Champaign: American Oil Chemists Society.
- Association of Official Analytical Chemistry. 2005. Official methods of analysis. 18th ed. Washington: Association of Official Analytical Chemists.
- Bisignano, G., Tomaino, A., Lo Cascio, R., Crisafi, G., Uccella, N. and Saija, A. 1999. On the *In-vitro* Antimicrobial Activity of Oleuropein and Hydroxytyrosol. *Journal of Pharmacy and Pharmacology* 51(8): 971–974.
- Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37(8): 911–917.
- Botsoglou, E., Govaris, A., Ambrosiadis, I. and Fletouris, D. 2012. Lipid and protein oxidation of α -linolenic acid-enriched pork during refrigerated storage as influenced by diet supplementation with olive leaves (*Olea europea* L.) or α -tocopheryl acetate. *Meat Science* 92(4): 525–532.
- Botsoglou, E., Govaris, A., Christaki, E. and Botsoglou, N. 2010. Effect of dietary olive leaves and/or α -tocopheryl acetate supplementation on microbial growth and lipid oxidation of turkey breast fillets during refrigerated storage. *Food Chemistry* 121(1): 17–22.
- Cagdas, E. and Kumcuoglu, S. 2015. Effect of grape seed powder on oxidative stability of precooked chicken nuggets during frozen storage. *Journal of Food Science and Technology* 52(5): 2918–2925.
- Delgado-Pertíñez, M., Chesson, A., Provan, G. J., Garrido, A. and Gómez-Cabrera, A. 1998. Effect of different drying systems for the conservation of olive leaves on their nutritive value for ruminants. *Annales de Zootechnie* 47(2): 141–150.
- Downes, F. P. and Ito, H. 2001. Compendium of methods for the microbiological examination of foods. 4th ed. Washington: American Public Health Association.
- El, S. N. and Karakaya, S. 2009. Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. *Nutrition Reviews* 67(11): 632–638.
- Engberg, R. M., Lauridsen, C., Jensen, S. K. and Jakobsen, K. 1996. Inclusion of oxidized vegetable oil in broiler diets. Its influence on nutrient balance and on the antioxidative status of broilers. *Poultry Science* 75(8): 1003–1011.
- Estévez, M. 2011. Protein carbonyls in meat systems: A review. *Meat Science* 89(3): 259–279.
- Estévez, M., Ventanas, S. and Cava, R. 2005. Protein Oxidation in Frankfurters with Increasing Levels of Added Rosemary Essential Oil: Effect on Color and Texture Deterioration. *Journal of Food Science* 70(7): c427–c432.
- Estévez, M., Ventanas, S., Heinonen, M. and Puolanne, E. 2011. Protein carbonylation and water-holding

- capacity of pork subjected to frozen storage: Effect of muscle type, premincing, and packaging. *Journal of Agricultural and Food Chemistry* 59(10): 5435–5443.
- Frankel, E. N. 2005. *Lipid oxidation*. 2nd ed. Cambridge: Woodhead Publishing Limited.
- Govaris, A., Botsoglou, N., Papageorgiou, G., Botsoglou, E. and Ambrosiadis, I. 2004. Dietary versus post-mortem use of oregano oil and/or α -tocopherol in turkeys to inhibit development of lipid oxidation in meat during refrigerated storage. *International Journal of Food Sciences and Nutrition* 55(2): 115–123.
- Gray, J. I. and Pearson, A. M. 1987. Rancidity and warmed-over flavor. In Pearson, A. M. and Dutson, T. R. (Eds.). *Advances in Meat Research: Restructured Meat and Poultry Products*, p. 221–269. New York: Van Nostrand Reinhold Co.
- Huang, L., Kong, B., Zhao, J., Liu, Q. and Diao, X. 2014. Contributions of Fat Content and Oxidation to the Changes in Physicochemical and Sensory Attributes of Pork Dumpling Filler during Frozen Storage. *Journal of Agricultural and Food Chemistry* 62(27): 6390–6399.
- Kulkarni, S., De Santos, F. A., Kattamuri, S., Rossi, S. J. and Brewer, M. S. 2011. Effect of grape seed extract on oxidative, color and sensory stability of a pre-cooked, frozen, re-heated beef sausage model system. *Meat Science* 88(1): 139–144.
- Lerasle, M., Federighi, M., Simonin, H., Anthoine, V., Rezé, S., Chéret, R. and Guillou, S. 2014. Combined use of modified atmosphere packaging and high pressure to extend the shelf-life of raw poultry sausage. *Innovative Food Science and Emerging Technologies* 23: 54–60.
- Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S. and Stadtman, E. R. 1990. Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology* 186: 464–478.
- Liu, G. and Xiong, Y. L. 1996. Contribution of lipid and protein oxidation to rheological differences between chicken white and red muscle myofibrillar proteins. *Journal of Agricultural and Food Chemistry* 44(3): 779–784.
- Markín, D., Duek, L. and Berdícovsky, I. 2003. In vitro antimicrobial activity of olive leaves. *Mycoses* 46(3-4): 132–136.
- Martín-García, A. I. and Molina-Alcaide, E. 2008. Effect of different drying procedures on the nutritive value of olive (*Olea europaea* var. *europaea*) leaves for ruminants. *Animal Feed Science and Technology* 142(3-4): 317–329.
- Mendes, R., Pestana, C. and Gonçalves, A. 2008. The effects of soluble gas stabilisation on the quality of packed sardine fillets (*Sardina pilchardus*) stored in air, VP and MAP. *International Journal of Food Science and Technology* 43(11): 2000–2009.
- Mercier, Y., Gatellier, P., Viau, M., Remignon, H. and Renner, M. 1998. Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in Turkey meat during storage. *Meat Science* 48(3-4): 301–318.
- National Research Council. 1994. *Nutrient Requirements of Poultry: Ninth Revised Edition*. Washington: The National Academies Press.
- Paiva-Martins, F., Barbosa, S., Pinheiro, V., Mourão, J. L. and Outor-Monteiro, D. 2009. The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. *Meat Science* 82(4): 438–443.
- Radha Krishnan, K., Babuskin, S., Azhagu Saravana Babu, P., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M. and Sukumar, M. 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology* 171: 32–40.
- Raharjo, S., Sofos, J. N. and Schmidt, G. R. 1992. Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C18 method for measuring lipid peroxidation in beef. *Journal of Agricultural and Food Chemistry* 40(11): 2182–2185.
- Ramadan, M. F. and Mörsel, J. T. 2004. Oxidative stability of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils upon stripping. *European Journal of Lipid Science and Technology* 106(1): 35–43.
- Recknagel, R. O. and Glende, E. A. 1984. Spectrophotometric detection of lipid conjugated dienes. *Methods in Enzymology* 105: 331–337.
- Savournin, C., Baghdikian, B., Elias, R., Dargouth-Kesraoui, F., Boukef, K. and Balansard, G. 2001. Rapid high-performance liquid chromatography analysis for the quantitative determination of oleuropein in *Olea europaea* leaves. *Journal of Agricultural and Food Chemistry* 49(2): 618–621.
- Soler-Rivas, C., Espi, J. C. and Wichers, H.J. 2000. Oleuropein and related compounds. *Journal of Science and Food Agriculture* 80: 1013–1023.
- Soyer, A., Özalp, B., Dalmiş, Ü. and Bilgin, V. 2010. Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chemistry* 120(4): 1025–1030.
- Stangierski, J. and Lesnierowski, G. 2015. Nutritional and health-promoting aspects of poultry meat and its processed products. *World's Poultry Science Journal* 71(1): 71–82.
- Sudjana, A. N., D'Orazio, C., Ryan, V., Rasool, N., Ng, J., Islam, N., Riley, T. V. and Hammer, K. A. 2009. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. *International Journal of Antimicrobial Agents* 33(5): 461–463.
- Wang, B., Pace, R. D., Dessai, A. P., Bovell-Benjamin, A. and Phillips, B. 2002. Modified Extraction Method for Determining 2-Thiobarbituric Acid Values in Meat with Increased Specificity and Simplicity. *Food Chemistry and Toxicology* 67(8): 2833–2836.
- Wichers, H. J., Soler-rivas, C. and Espi, J. C. 2000. Oleuropein and related compounds. *Journal of the Science of Food and Agriculture* 80(7): 1013–1023.

- Wongwichian, C., Klomklao, S., Panpipat, W., Benjakul, S. and Chaijan, M. 2015. Interrelationship between myoglobin and lipid oxidations in oxeye scad (*Selar boops*) muscle during iced storage. Food Chemistry 174: 279–285.
- Yagi, K. 1987. Lipid peroxides and human diseases. Chemistry and Physics of Lipids 45(2-4): 337-351.