

Inhibition of Lipid Oxidation of Frozen Chicken Legs by Treatment with Sodium Lactate, Natural Antioxidants and Vacuum-Packaging

Stefan Georgiev Dragoev*

Department of Meat and Fish Technology, University of Food Technologies, Bulgaria

*Corresponding Author: Stefan Georgiev Dragoev, Department of Meat and Fish Technology, University of Food Technologies, 26 Maritza blvd., 4002 Plovdiv, Bulgaria.

Received: April 22, 2015; Published: May 09, 2015

Abstract

The influence of the combined effects of vacuum-packaging and a spray treatment with solutions of sodium lactate, or a spray of a blend of natural antioxidants, containing 36% rosemary extract, 52% dry extract of Japanese pagoda tree (*Sophora japonica*) flower buds, and 12% sodium erythorbate, and the combination of the two solutions together, on lipid oxidation inhibition in frozen chicken legs, 302 - 348 g each, stored for 360 d at -18°C, was studied. Five samples of chicken legs: 1) packaged with no vacuum and without additives; 2) vacuum-packaged without additives; 3) treated with a 1.2% solution of sodium lactate and vacuum-packaged; 4) treated with a 1.2% solution of natural antioxidants and vacuum-packaged; 5) treated with each solution and then vacuum-packaged, were examined. The samples were quickly frozen at a temperature of -40°C ± 1°C until the temperature in the center reached -18°C. They were then stored for 360 d at -18°C. Samples for analysis were taken on 0, 30, 90, 180, 270 and 360 d of storage. The lipid oxidation in the frozen chicken legs with skin, stored at -18°C was most inhibited when vacuum-packaging was applied, preceded by a surface spray treatment with a 1.2% solution of natural antioxidants applied either independently or in combination with a 1.2% solution of sodium lactate.

Keywords: chicken; rosemary; Quercetin; sodium erythorbate; sodium lactate; TBARS

Abbreviations: MDA: malondialdehyde; NS: not significant ($P > 0.05$); POV: peroxide value; TBARS: tiobarbituric acid reactive substances

Introduction

Many factors influence the initiation and development of lipid oxidation in chicken meat [1]. They include: fatty acid composition of the endogenous lipids [2]; the diet influence on the lipids oxidative stability in the skeletal muscles [3-7]; the antioxidant protective system of the skeletal muscles [8]; the presence of multivalent metals [9]; the prooxidative effect of freezing and refrigerated storage [10], among others. The oxygen availability [11], and the method of packaging [12,13] have been discussed as one of the important factors affecting the lipid stability of the chicken lipids.

During the storage of frozen chicken meat, lipid oxidation depends on both enzymatic [14] and non-enzymatic factors [15]. Due to the absence of free water, enzyme activity decreases [16]. Therefore, lipid oxidation of frozen chicken meat primarily depends on non-enzyme catalyzed processes [17,18].

Citation: Stefan Georgiev Dragoev. "Inhibition of Lipid Oxidation of Frozen Chicken Legs by Treatment with Sodium Lactate, Natural Antioxidants and Vacuum-Packaging". *EC Nutrition* 1.4 (2015): 203-216.

There are two major different types of muscle in chicken meat: dark (thighs) and light (breasts). Dark muscle goes through more oxidative changes of muscle lipids than light muscle [19]. This is due to differences in the phospholipid composition, iron content and other differences between the two types of muscles [20]. In chicken meat, the phospholipid fraction is responsible for about 90% of the malondialdehyde (MDA) break-down [21].

Because of its higher content of polyunsaturated fatty acids, chicken meat is more easily oxidized and more prone to rancidity than are beef and pork [1].

The inhibition of the lipid oxidation and preservation of the positive natural properties of the frozen chicken have been achieved by vacuum-packaging of chicken products [11]. According to Ahn, *et al.* [22], vacuum-packaging of thighs and breasts of broilers is not sufficiently effective to stabilize the meat for long term frozen storage. These authors stabilized the lipids by inserting of antioxidants in the poultry finishing diet. In contrast, Park, *et al.* [13] proposed that lipid oxidation development is a function of the specific chicken product, method of packaging and the storage time in the frozen state. Ahn, *et al.* [23] established that the lipid oxidation can be limited when packaged after treatment with a combination of antioxidants. The effective inhibition of lipid oxidation in chicken meat was accomplished with a combined use of fast-freezing with different antioxidants [24], such as phenols - butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), quarternary butylated hydroxyquinone (TBHQ), α -tocopherol, and metal acceptors such as EDTA, citrates, and phosphates. Phenol antioxidants are very effective [25], they can inhibit lipid oxidation at a concentration of 0.2%. Their disadvantage is the fact that they are synthetic substances, some of which (e.g., BHA) have a presumable carcinogenic effect when their concentration is higher than 0.2 g.kg⁻¹ [26]. The introduction of α -tocopherol in poultry feeding systems has been shown to have a stabilizing effect on turkey meat lipids [27]. Polyphosphates also have a stabilizing action on lipids and significantly preserve the positive sensory properties of frozen chicken products [28].

Recently more and more natural antioxidants are been applied in the food industry [6], such as polyphenolic co-pigments [29]. According to Martinez, *et al.* [30] fresh pork sausages can be stabilized by the combined effect of modified atmosphere packaging and addition of antioxidants. The lactic acid and lactates are well known preservatives used by the meat industry [31] and other industries.

The lactates have been successfully used for tenderizing [32] and colour stabilization of beef [33].

One new natural antioxidant, which can be used in meat processing industry is extract of *Sophora japonica* (Chinese: pinyin: huái) [34]. Formerly *Sophora japonica* is one of the 50 fundamental herbs used in traditional Chinese medicine. *Styphnolobium japonicum* (L) Schott, the Pagoda tree (Chinese Scholar, Japanese pagoda tree; syn. *Sophora japonica*), is native to eastern Asia (mainly China), and is a popular ornamental tree in Europe, North America and South Africa [35]. The ovaries, before the flowers open, contain up to 40 % rutin. This is a valuable hypotensive agent [36]. The buds, flowers and pods are used in the treatment of a variety of ailments [36] including internal haemorrhages, poor peripheral circulation, internal worms, etc. [37]. The flower buds are antibacterial, anticholesterolemic, anti-inflammatory, antispasmodic, haemostatic and hypotensive [36-38].

The objective of this experiment is to establish the influence of the combined effects of vacuum-packaging and a spray treatment with sodium lactate solutions, a spray of a blend of natural antioxidants and a combination of the two solutions together on lipid oxidation inhibition in frozen chicken legs stored for 360 d at -18°C.

Materials and Methods

Materials

Raw material: Ross 308 broiler chicks at an average age of 42 days were used in the experiment. One-day-old Ross 308 male broiler chickens were housed in metal battery brooders places in a controlled temperature environment, and were allowed free access to water and feed throughout the growth period (1 to 42 days). The birds were raised to 42 days of age under the usual local conditions, which

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include an open sided building, concrete floor covered with rice hulls as litter material, and a bird density of 12 chickens.m⁻². The chickens had ad libitum access to a corn-soy-bean meal nutritionally adequate feed and water, with a 24h light schedule, alternating spaces of time for stay awake and sleeping, according the animal welfare recommendations [39]. The baseline diet is present in Table 1. The live weight of the broilers was approximately 2.31 kg.

Following the welfare standards of the European Union [39] the stocking density was no more than 30 kg/m² in the production area.

The transportation system employed in the experiment utilizes Anglia Auto flow modules (Wortham Ling, Diss, Norfolk, United Kingdom) that are loaded by forklift onto a trailer unit. The birds were caught by the legs and thrown into crates. The modules span the width of the trailer and contain 12 or 15 crates, each holding 20 to 26 birds. The modules, which are stacked in pairs, were labelled alphabetically and the crates within the modules were numbered. Data loggers were placed in modules during loading and then the transport vehicle was followed to the slaughterhouse where the trailer unit was driven into the live receiving area. Transport distance was up to 50 km. The chickens were sacrificed in a poultry slaughterhouse TER-M Ltd. (Parvomay, Bulgaria), according to the standard practices [40,41]. After the trucks arriving at the slaughterhouse, the modules were immediately removed from the trailer, and chickens were pulled from the crates and shackled upside down by their feet into metal stirrups on an overhead conveyor. The conveyor was carried them into the killing room where their heads were passed through an electrified water-bath stunner. Electric stunning (120-150 mA per bird) was made at 40-60 V (usually above 200 Hz) for 1-3 s. After the birds stunning, a cut was applied to the blood vessels in neck to allow birds to bleed out. The neck cutting was done automatically to one side of the neck only, within 1 min of electrical stunning. The bleed time was approximately 150s. After exsanguinations the carcasses were scalded by submerging in 50-53°C hot water for 60-180s. It was done in a continuous manner employing a multistage scalding bath while the birds were suspended from a moving shackle line. The scalded carcasses were plucked at 49-50°C for 100-120s. Grabber machines are equipped with rubber fingers. They were installed immediately after the scalding process so that carcasses remain warm during feather removal. For this reason a machine was used that pull heads off so that the oesophagus was also removed.

The next technological procedure was evisceration. It was involved the removal of the contents of the body cavity plus the feet and head. The abdomen was opened and the viscera were removed. The feet were removed at the knee joint. After all the contents of the cavity were removed the birds were thoroughly washed inside and out. The washed poultry carcasses were chilled as soon as possible at two stages. Initially, the carcasses were cooled in ice water using an immersion chilling to bring the carcass temperature to about 4-5°C within 30-35 min. For this reason, parallel flow chiller was used. For the next step the spray chilling refrigerator (which includes air and water) was applied. As a chilling medium a cold air (0°C) by the humidity 85-90 %, was used. The average chilling time was 30-40 min while the carcass temperature to about 1-2°C. The chilled carcasses were packed with ice (poultry meat: flake ice = 1:1) in covered stainless steel containers and were hauled to the laboratory of the Department of Meat and Fish Technology in the University of Food Technologies (Plovdiv, Bulgaria). The time for the chilled carcasses transport was 15-20 min. The carcasses (without viscera) were cut and the lab cuts: breast, leg quarters (leg and thigh), wings, and back, were obtained. The chicken legs with skin, with the temperature of 2°C in the center of the cuts, and a net weight of 302-348 g were collected. Chicken legs were separated into five groups. All of the samples (20 chicken legs total in each group), with the exception of the control sample, were vacuum-packaged. The vacuum packaging was made with vacuum-packaging machine Multivac, Model A 300/15 (Multivac Sepp Hagenmüller GmbH & Co. KG, Wolfertschwenden, Germany), using polyethylene/polyamide barrier film Oberfolien, type 406 INC80/500 (Südpack Verpackungen GmbH & Co. KG, Ochsenhausen, Germany). The film was not water and gas (including oxygen) permeable and met the requirements of the Directive 2002/72 EC and Directive 2004/19 EC.

Three groups of chicken legs were spray treated with solutions before vacuum-packaging as follows: first one was sprayed with 1.2% solution of sodium lactate; second one – with 1.2% solution of blend of natural antioxidants, and third one - with mix of 1.2% sodium lactate solution and 1.2% blend of natural antioxidants solution. The manual pulverized (Afrida Peev Ltd., Gabrovo, Bulgaria) was used

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for this reason. The amount of solution used was 0.05 l.kg⁻¹ chicken legs. The chicken legs were drained for 15 min at 0°C in a refrigerator. The drained samples were vacuum-packed. After packaging, the samples were blast frozen as is described below.

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Reagents: 2-Thiobarbituric acid and pyrogallol were purchased from Sigma Chemical Company Ltd. (St. Louis, MO, USA). All other reagents and solvents were procured from Aldrich Chemical Co. (Gillingham, Dorset, UK).

We developed our own antioxidant solutions: 36% rosemary extract (Aromena Ltd, Sofia, Bulgaria), 52% dry extract of Japanese pagoda tree (*Sophora japonicum*) flower buds, prepared in the Biotechnology Department (University of Food Technologies, Plovdiv, Bulgaria) and 12% sodium erythorbate, supplied by F.I.A. Food Ingredients Anthes GmbH (Teising, Germany).

An un-distilled alcohol rosemary extract, with alcohol concentration 28 - 30% (v), was used. It is a liquid that contains some visible solids, has a yellow-brown colour and has a characteristic odour of rosemary. By the data of the producer (Aromena Ltd, Sofia, Bulgaria) the dry matter of the rosemary extract is 60.4 - 69.0 g.kg⁻¹; the flavonoids content is 21.2 - 26.4 g.kg⁻¹; the coefficient of refraction is PD20 = 1.3605 - 1.3650, and the relative density is d₂₀₂₀ = 0.9664 - 0.9845.

A dry extract of Japanese pagoda tree (*Sophora japonicum*) flower buds, at the stage before bursting into bloom, was prepared by the following method of production. The flower buds were dried at 22-25°C for 10 d to final moisture 8 - 10%. The dry mass was ground using a household Braun coffee grinder, Model KSM2-B (Braun GmbH, Kronberg, Germany). The plant powder (20 g) was mixed with 100 ml Fluka Buffer 82585/20 mM sodium citrate solution BioChemika with pH 4.5/ (Sigma-Aldrich Corp., St. Louis, MO, USA), and this mix was macerated for 8h at 60°C [34]. The macerated liquid was triple-extracted with 80% (v) 96 % ethanol [42]. The batches of ethanol were removed by distillation at 70°C and the final solution was dried using a rotary vacuum evaporator Ingos, model RVO 400 (Ingos s.r.o. Laboratoty Instruments Ltd, Praha, Check Republic). The concentration of the flavonol quercetin (a derivative of the glycoside rutin) in the dry extract was 0.533 kg.kg⁻¹ [43]. This extract was suitable for use in the food industry [34].

Staging of the experiments: The experiments were carried out with five samples of chicken legs: 1) Packaged with no vacuum and without additives; 2) Vacuum-packaged without additives; 3) Treated superficially with a 1.2% solution of sodium lactate and vacuum-packaged; 4) Treated superficially with a 1.2% solution of natural antioxidants and vacuum-packaged; and 5) Treated superficially with a 1.2% solution of sodium lactate in combination with a 1.2% solution of natural antioxidants and vacuum-packaged as previously described. After packaging, the samples were shock frozen at a temperature of -40°C until the temperature in the sample centres reached -18°C, measured using an electronic thermometer EBI-2T-F (Ebro Electronic GmbH, Ingolstadt, Germany). They were then stored for 360 d at the same temperature. Changes of pH, POV and TBARS were measured in samples on days 0, 30, 90, 180, 270 and 360 d of storage.

Methods

Samples preparation

Samples were prepared according to standard procedure (EN ISO 3690-2001, 2001). Before analysis, the samples were defrosted in a refrigerator Bosch, Model B 24 IR 70 SRS (Robert Bosch Hausgeräte GmbH, Munchen, Germany) at 2°C for 8h. Immediately before the experiments, portions of uniform weight of the deboned poultry meat (about 4,000 g) were separated. Each group (4,000 g) was mixed thoroughly in an electric blender, Model EB3251S (HKTDC, Xiamen, China) for 10 min while to be obtained the structure mass with to obtain a uniform mince structure.

The final temperature of the samples was 12°C. Every one sample was weighed into 800-g portions. Each ground sample was wrapped in barrier film VerPack, type Colamin V-40 (Apostolidis K. & CO VER PACK, Thessaloniki, Greece). The wrapped samples were put into plastic containers and were stored in a refrigerator no longer than 12h with lids until the time of the test.

The pH determination

Samples were evaluated for pH using a digital pH-meter (MS 2004, Microsyst, Plovdiv, Bulgaria), with a combined electrode contact combined pH-electrode Sensorex S 450 CD (Sensorex, Garden Grove, CA) [44] after homogenizing a 10-g sample in 90 mL distilled water for 30 s, using a homogenizer (Sower, model SAIL-S200 Lab high-speed disperser (Shanghai Sower Mechanical & Electrical Equipment Co. Ltd, Shanghai, China).

The content of hydroperoxides was estimated by determining the POV of the lipids expressed as meqv O₂·kg⁻¹ lipid using the European standard method (EN ISO 3960, 2001).

The secondary products of lipid oxidation were determined with the TBARS procedure. For this purpose, the modified extraction method of Wang, *et al.* [45] was used to determine the lipid oxidation. The rose-pink colour produced by the reaction between MDA and 2-thiobarbituric acid (C₄H₄N₂O₂S) was measured using a spectrophotometer (Helios Gamma, Thermo Spectronic, Madison, WI) at 532 nm. Lipid oxidation was expressed as TBARS (mg MDA.kg⁻¹ sample) [45].

Statistical analysis

Data obtained were processed by established mathematic-statistical methods [46]. The data obtained from nine replications were analyzed as a completely randomized design procedure using the general linear model procedure of the SPSS statistical package program (SPSS, Inc., Chicago, IL). The model included (control, blend of the natural antioxidants, sodium lactate, vacuum-packaged or not) and storage time (0, 30, 90, 180, 270 and 360 d) as main effects, and all their interactions. The differences among means were tested for significance (*P ≤ 0.05) by Duncan's multiple range test. The results of the statistical analyses were shown as mean values ± SE in the tables for the six storage times and five treatments.

Results and Discussion

pH value

The effect of the treatment and storage time on pH, POV and TBARS of chicken legs are shown in Table 1. As can be seen, vacuum-packaging and sodium lactate and/or antioxidant's blend treatment had no significant (P > 0.05) effect on pH, while pH decreased significantly (P < 0.01) during the first 30 days of storage. The pH of chicken legs was the highest at the beginning of storage (P < 0.05). The pH levels of the samples were the highest at the beginning of storage. Any of the examined treatment did not significantly (P < 0.05) affect the pH (Figure 1). The similar results for pH value as the control sample have been demonstrated by other investigators in raw broiler thigh meat [47] and breast meat [48]. Independent of the statistically significant effect of sodium lactate treatment on the slight pH decreases (Figure 1) this had no effect on the changes of POV (Figure 2) and TBARS (Figure 3). Deumier. [49] concluded that the addition of 1-5% sodium lactate induced a light acidification of vacuum-tumbled deboned chicken legs, but alone is unable to induce microbial decontamination of poultry meat.

Ingredients (%)	Starter (1 to 21 days)	Grower (22 to 34 days)	Finisher (34 to 42 days)
Corn	52.22	58.66	68.65
Soymeal 48	37.35	30.67	20.67
Vegetable oil	4.48	4.80	4.80
Limestone	1.07	1.08	1.09
Monocalcium phosphate	1.85	1.67	1.56
Premix vitamin/mineral	0.15	0.15	0.15
Salt	0.46	0.41	0.38
Starch or Arginine	0.50	0.50	0.50
Choline	0.05	0.06	0.07
Lysine	0.14	0.24	0.34
Premix-mixture (vehicle + HMTBA-liquid phase methionine)	1.73	1.76	1.79
Total	100	100	100
Nutrients			
Meal energy (kcal . kg ⁻¹)	3050	3150	3250
Crude protein (%)	22.0	18.5	18.0
Available Phenylalanine (%)	0.40	0.35	0.35
Available Lysine (%)	1.44	1.04	1.04
Available Arginine (%)	1.33	1.14	1.14
Available Methionine + Cysteine (%)	0.60	0.55	0.55
Available Methionine (%)	0.31	0.29	0.29
Available Threonine (%)	0.75	0.65	0.65
Available Tryptophan (%)	0.26	0.20	0.20
Choline (mg . kg ⁻¹)	1500	1400	1300
Chlorine (%)	0.31	0.28	0.26
Calcium (%)	0.90	0.85	0.83
Sodium (%)	0.20	0.18	0.16
Potassium (%)	0.96	0.81	0.76

Table 1: Results of the statistical nutritional levels and basal diet composition of starter, grower and finisher phase. Vitamins and minerals kg⁻¹ Diet: Se: 0, 3 mg; I: 0, 7 mg; Fe: 40 mg; Cu: 10 mg; Zn: 80 mg; Mn: 80 mg; Vitamin A: 8000 UI; Vitamin D₃: 2000 UI; Vitamin E: 30 mg; Vitamin K: 2 mg; Vitamin B₁: 2 mg; Vitamin B₂: 6 mg; Vitamin B₆: 2.5 mg; Vitamin B₁₂: 0, 012 mg; Biotin: 0, 08 mg; Pantothenic acid: 15 mg; Niacin: 35 mg; Folic acid: 1 mg.

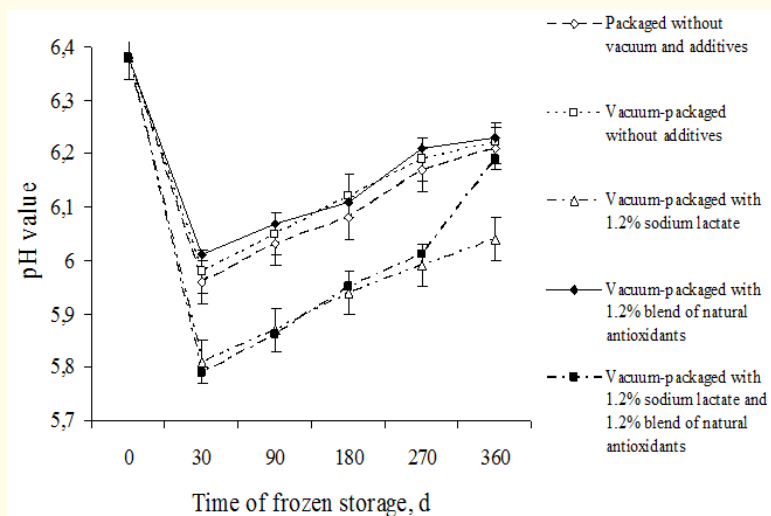


Figure 1: Changes of pH value of five samples chicken legs during 360 d frozen storage at -18°C.

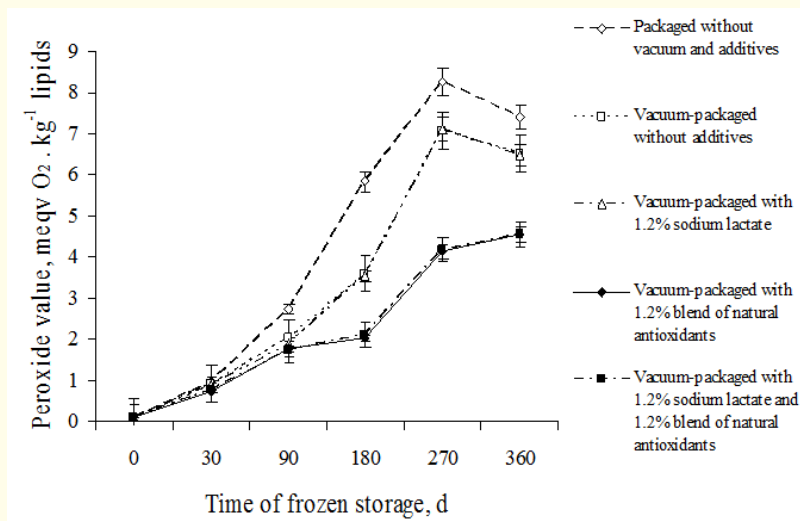


Figure 2: Changes of POV of five samples chicken legs during 360 d frozen storage at -18°C.

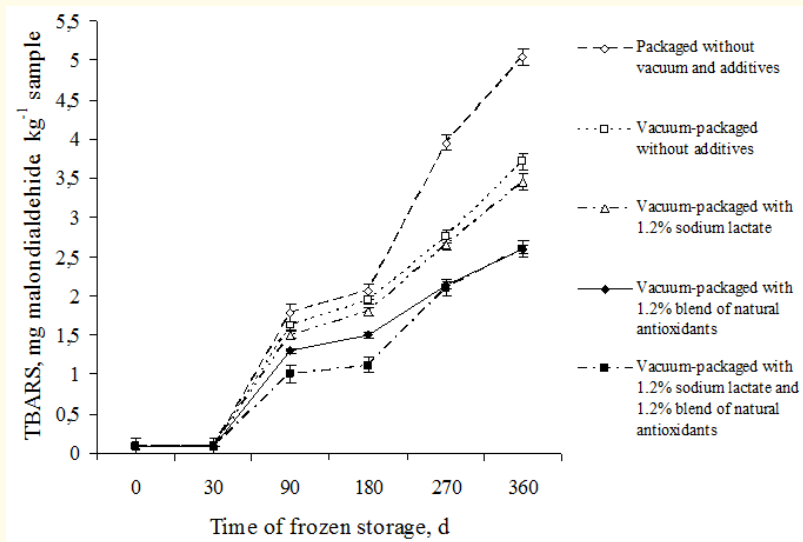


Figure 3: Changes of TBARS value of five samples chicken legs during 360 d frozen storage at -18°C.

Peroxide value

The primary products of lipid oxidation are hydroperoxides, which become peroxides. Therefore, it seemed reasonable to determine the concentration of peroxide in the chicken leg samples to study the extent of oxidation [21]. As indicated in Table 1, both vacuum-packaging combined with additive treatment and storage time had statistically significant ($P < 0.01$) effects on POV. The lowest POV observed was in the sample treated with 1.2% sodium lactate and 1.2% blend of natural antioxidants, which was 2.19 meq $O_2.kg^{-1}$ lipids (Table 2), but was not significantly different from the sample treated with the 1.2% blend of natural antioxidants ($P > 0.05$). The highest POV was observed in the control (Figure 2). The peroxide values of vacuum-packaged samples with 1.2% sodium lactate and without additives were lower than that of the control ($P < 0.05$). The two samples treated with the 1.2% blend of natural antioxidants with or without 1.2% sodium lactate showed the highest antioxidant effects and they achieved, respectively a 46.3 and a 45.8% decrease in hydroperoxides content with respect to control. The reduction of POV in vacuum-packaged samples with and without 1.2% sodium lactate solution was 15.9 and 18.1%, respectively. These results show that a combination of treatments with sodium lactate or natural antioxidants, on the one hand, and vacuum-packaging, on the other, are effective in slowing down lipid oxidation of frozen stored chicken legs. The antioxidant properties of these extracts are related to their phenolic and flavonoid contents. Phenolic antioxidants do not work as oxygen absorbers, rather they prevent formation of lipid free radicals, which react with or absorb oxygen in the autoxidation process, thus delaying the onset of the process of autoxidation in fats or oils [6,26]. No reports have been found in the literature concerning the effects of the addition of blend of plant extracts, especially extract of Japanese pagoda tree (*Sophora japonicum*) flower buds, on chicken meat oxidation. For this reason, the results of this study were compared with the findings of other researchers on rosemary and other plant extracts. Our results were in agreement with Martinez., *et al.* [30] who reported that the combined effect of modified atmosphere packaging and addition of rosemary, ascorbic acid, red beet root and sodium lactate and their mixtures stabilized the lipids of fresh pork sausages, and had significantly ($P < 0.05$) lower peroxide values compared to control.

In all samples of analyzed chicken legs, POV increased steadily until 270 days of storage ($P < 0.05$). After that, the POV of the control samples and two experimental samples, which were not treated with 1.2% solution of natural antioxidants, began to decrease ($P > 0.05$) (Figure 2). This is probably because of the instability of hydroperoxides. It should also be mentioned that hydroperoxides begin to decompose as soon as they are formed. In the first stages of autoxidation, their rate of formation exceeds their rate of decomposition.

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The reverse takes place at the later stage [21]. The maximum acceptability limit of POV in fatty foods was reported as 10 meqv O₂.kg⁻¹ lipids by Dragoev. [50]. The POV of chicken legs did not reach this value in any of the storage months. An interaction ($P < 0.01$) of the treatment and the storage time was observed for POV (Figure 2). According to this figure, control samples, and vacuum-packaged samples treated with 1.2% sodium lactate or without this type of treatment, showed the highest increase. The vacuum-packaged samples treated with the 1.2% blend of natural antioxidants showed the smallest increase. The increases in peroxide value were higher between 0 and 270 d of storage for control samples, and vacuum-packaged samples treated with 1.2% sodium lactate or without this type of treatment, and between 0 and 90 d of storage for the two vacuum-packaged samples treated with the 1.2% blend of natural antioxidants. Except for those two samples, the POV of other samples began to decline after 270 d. This is probably because of the instability of the peroxides, and passing of the induction period of the lipid oxidation process.

TBARS

The TBARS test is one of the most widely used tests for evaluating the extent of lipid oxidation. During the development of lipid peroxidation, the termination of the chain reactions results in the production of aldehydes, ketones and the other degradation components of oxidized fatty acids [50]. The concentration of secondary products of lipid peroxidation, expressed as the quantity of the free MDA, is determined. As shown in Table 1 and Figure 3 treatment and storage time had statistically significant effects ($P < 0.01$) on TBARS values of chicken legs.

The TBARS of the five samples of chicken legs at 30 d of storage at -18°C are an exception. The TBARS of those samples were not significantly different ($P > 0.05$). In the control sample, with no vacuum and without additives, TBARS increased at accelerated rates and on the 270 d of storage at -18°C, exceeded 3 mg MDA.kg⁻¹ sample (Figure 3). As determined by Dragoev [50], values higher than this lead to undesirable sensory changes in the chicken meat. At 360 d the TBARS value exceeded 5 mg MDA.kg⁻¹ sample and it can be expected the frozen chicken meat to develop a rancid taste [50].

When comparing the data from the experimental samples, the sample that was most unstable with regard to lipid oxidation was the one that was vacuum-packaged without the addition of sodium lactate or the solution of antioxidants (Figure 3). Not until the end of the experiment did the TBARS in the sample that was vacuum-packaged without the addition of sodium lactate or the solution of antioxidants exceed 3 mg MDA.kg⁻¹ sample. A similar tendency was observed for the sample that was vacuum-packaged and surface-treated with a 1.2% solution of sodium lactate, only. The combination of vacuum-packaging and sodium lactate treatment effectively inhibited the processes of lipid oxidation of the chicken legs until 270 d (Table 2). The lowest and most significantly different sample in terms of TBARS was the chicken meat sample that was vacuum-packaged and surface-treated with a 1.2% solution of natural antioxidants, either independently, or in combination with a 1.2% solution of sodium lactate (Table 2). Probably, the lactate can play a role as a synergist to the antioxidants, because it is a salt of lactic acid, and has a mobile electron in each molecule [30]. On the other hand, the lactic acid, (and to a certain degree the sodium lactate) is a preservative. The lactic acid and sodium lactate have been used for microbial decontamination of deboned chicken legs [49]. The TBARS of the samples surface-treated with a 1.2% solution of natural antioxidants on 270 and 360 d were significantly the lowest ($P > 0.05$) of the samples (Figure 3). The TBARS values never reached 3 mg MDA.kg⁻¹ sample in both experimental samples treated with natural antioxidants.

Similar to POV, the lowest TBARS value occurred in the vacuum-packaged chicken legs, preceded by spray treatment with a 1.2% solution of natural antioxidants combined with 1.2% solution of sodium lactate (1.17 mg MDA.kg⁻¹ sample) or only with a 1.2% solution of natural antioxidants (1.29 mg MDA.kg⁻¹ sample) ($P > 0.05$), while the highest TBARS value (2.17 mg MDA.kg⁻¹ sample) occurred in the control sample ($P < 0.05$) (Table 1). The antioxidant effectiveness of vacuum-packaging, preceded by spray treatment with a 1.2% solution of sodium lactate or without this type of treatment were lower than that samples processed with the 1.2% solution of natural antioxidants ($P < 0.05$). According to these results, the lipid oxidation in frozen chicken legs with skin, stored at -18°C, is inhibited to the greatest extent when vacuum-packaging is applied, preceded by spray treatment with a 1.2% solution of natural antioxidants. This combined treatment showed the highest antioxidant effects and showed a 41-46% reduction in TBARS value with respect to control (Table 2). This ratio was 26 and 22% when the chicken legs were spray treated with a 1.2% solution of sodium lactate and vacuum-packaged or only packed under vacuum, respectively.

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Effect	pH	POV, meqv O ₂ kg ⁻¹ lipids	TBARS, mg MDA. kg ⁻¹ sample
Treatment (A)	NS	**	**
Packaged without vacuum and additives	6.13 ± 0.26	4.08 ± 0.14 ^a	2.17 ± 0.03 ^a
Vacuum-packaged without additives	6.15 ± 0.26	3.43 ± 0.13 ^b	1.70 ± 0.03 ^b
Vacuum-packaged with 1.2% sodium lactate	6.02 ± 0.26	3.34 ± 0.15 ^b	1.60 ± 0.03 ^c
Vacuum-packaged with 1.2% blend of natural antioxidants	6.16 ± 0.24	2.19 ± 0.14 ^c	1.29 ± 0.03 ^d
Vacuum-packaged with 1.2% lactate and 1.2% blend of antioxidants	6.04 ± 0.26	2.21 ± 0.13 ^c	1.17 ± 0.03 ^e
Storage time (days) (B)	**	**	**
0	6.37 ± 0.23 ^a	0.10 ± 0.03	0.09 ± 0.02 ^e
30	5.91 ± 0.24 ^b	0.73 ± 0.07	0.09 ± 0.03 ^e
90	5.98 ± 0.24 ^b	2.10 ± 0.12	1.45 ± 0.03 ^d
180	6.03 ± 0.26 ^{a^b}	3.40 ± 0.22	1.69 ± 0.03 ^c
270	6.11 ± 0.28 ^{a^b}	6.09 ± 0.18 ^a	2.72 ± 0.03 ^b
360	6.18 ± 0.29 ^a	5.89 ± 0.22 ^a	3.48 ± 0.03 ^a
A x B interaction	NS	**	**

Table 2: Results of the statistical analysis of data for the pH, POV, and TBARS of frozen chicken legs by vacuum-packaging and treatment with sodium lactate and a blend of natural antioxidants during 360 d of storage at -18°C.

¹Any two means in the same column having the same letters in the same section (treatment or storage time) are significantly different.

²***: P < 0.01.*

³NS: not significant (*P > 0.05*).

⁴POV: peroxide value.

⁵TBARS: 2-tiobarbituric acid-reactive substances.

⁶MDA: malondialdehyde.

An interaction (*P < 0.01*) between treatment and storage time noted for TBARS values is shown in Figure 3. According to the figure, there was an increase in TBARS values in both control samples and in experimental samples and this increase was the most rapid after 30 d of storage. Similarly, between 1 and 60 d of storage at -18°C, non-marinated deboned chicken legs had significantly slower TBARS increases than the other groups (II variance - salted with 0.5% NaCl; III variance - marinated with 1.0% KCl, 2.0% K₃C₆H₅O₇·H₂O and 0.4% sugar; and IV variance - marinated with 0.5% NaCl, 0.5% KCl, and 0.5% sugar), and the TBARS increase was 14% [21].

Combs and Regenstein [51] reported the highest increase in TBARS (92% approximately) between 0 and 30 d of frozen storage at -20°C of tissue from chicken. In contrast to our results, Rhee, *et al.* [1] observed increasing TBARS in chicken thighs with no more than 0.1 mg MDA.kg⁻¹ sample during 150 d storage at -20°C.

Similar to our results, Ahn, *et al.* [52] found that the TBARS values of raw turkey meat were significantly dependent on metal chelators, hydroxyl radical scavengers, and enzyme systems. In support of this Sante, *et al.* [27] demonstrated the effect of dietary α-tocopherol supplementation and antioxidant spraying on TBARS development of turkey meat.

The strong antioxidant effects of rosemary powders and extracts on other meat products have been investigated [30]. They examined the lipid stability of fresh pork sausages with addition of rosemary, ascorbic acid, red beet root and sodium lactate and their mixtures, and in addition the combined effect with modified atmosphere packaging (80% O₂ + 20% CO₂ gas mixture). The TBARS of fresh pork sausages processed with the addition of a mixture of rosemary, ascorbic acid, red beet root and sodium lactate were the

Citation: Stefan Georgiev Dragoev. "Inhibition of Lipid Oxidation of Frozen Chicken Legs by Treatment with Sodium Lactate, Natural Antioxidants and Vacuum-Packaging". *EC Nutrition* 1.4 (2015): 203-216.

lowest ($P < 0.05$) in comparison with other samples. Moreover, the effects of various plant powders and extracts on TBARS value have been tested. McCarthy, *et al.* [26] showed the antioxidant potentials of natural food-plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. Marjoram, wild marjoram and caraway showed the highest inhibitory effect on the TBARS value of minced chicken meat during frozen storage [42]. The blend of natural antioxidants used in this research showed antioxidant activities, which have been attributed to the phenolic contents and chemical properties of the plants [34]. The same blend successfully inhibited the ferric hematin pigments, which are the powerful catalysts of lipid oxidation in raw meat and dry-fermented sausages [43].

The lowest POV and TBARS of the chicken legs observed when the samples were spray treated with a solution of natural antioxidants and vacuum-packed after that can be explain by the ability of compounds from the blend of natural antioxidants to inhibit the transfer of electrons from ferric hematin pigments, which are responsible for increasing the rates of free radical formation [43] in poultry meat.

Due to the unsaturated character of the lipids, when lipid oxidation in chicken meat occurs, hydrogen peroxide is formed [17]. Because it serves as a source of hydroxyl radicals, hydrogen peroxide can activate myoglobin and haemoglobin, and an unstable intermediate substance (oxyferyl radical) is formed [18].

We suggest that the course of action of the blend of natural antioxidants is to inhibit the hydrogen peroxide activation of myoglobin and haemoglobin, and by this mechanism to inhibit the initiation of lipid oxidation in the cell membranes. Lipid oxidation of chicken meat stimulated by enzymes was inhibited by quick freezing and low temperature storage. This change deactivated the peroxides formed in the presence of free fatty acids [53].

The enzymes generate and transfer an electron to the superoxyl radicals. This mechanism for lipid oxidation initiation is most probably inhibited by the effect of the vacuum-packaging and treatment with the 1.2% solution of natural antioxidants in combination with the 1.2% solution of sodium lactate. The explanation is that the removal of the oxygen from the packages, and the inhibition of the oxidative enzyme activity is a result of acidification of the medium by lactate [54].

Conclusion

The conclusion was made that the combination of vacuum-packaging and sodium lactate treatment effectively inhibited the processes of lipid oxidation of the chicken legs until 270 d. The combination of vacuum-packaging and surface-treatments with a 1.2% solution of natural antioxidants either independently, or in combination with a 1.2% solution of sodium lactate are effective in slowing down lipid oxidation of frozen stored chicken legs and can be applied with success in the practice.

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Volume 1 Issue 4 May 2015

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