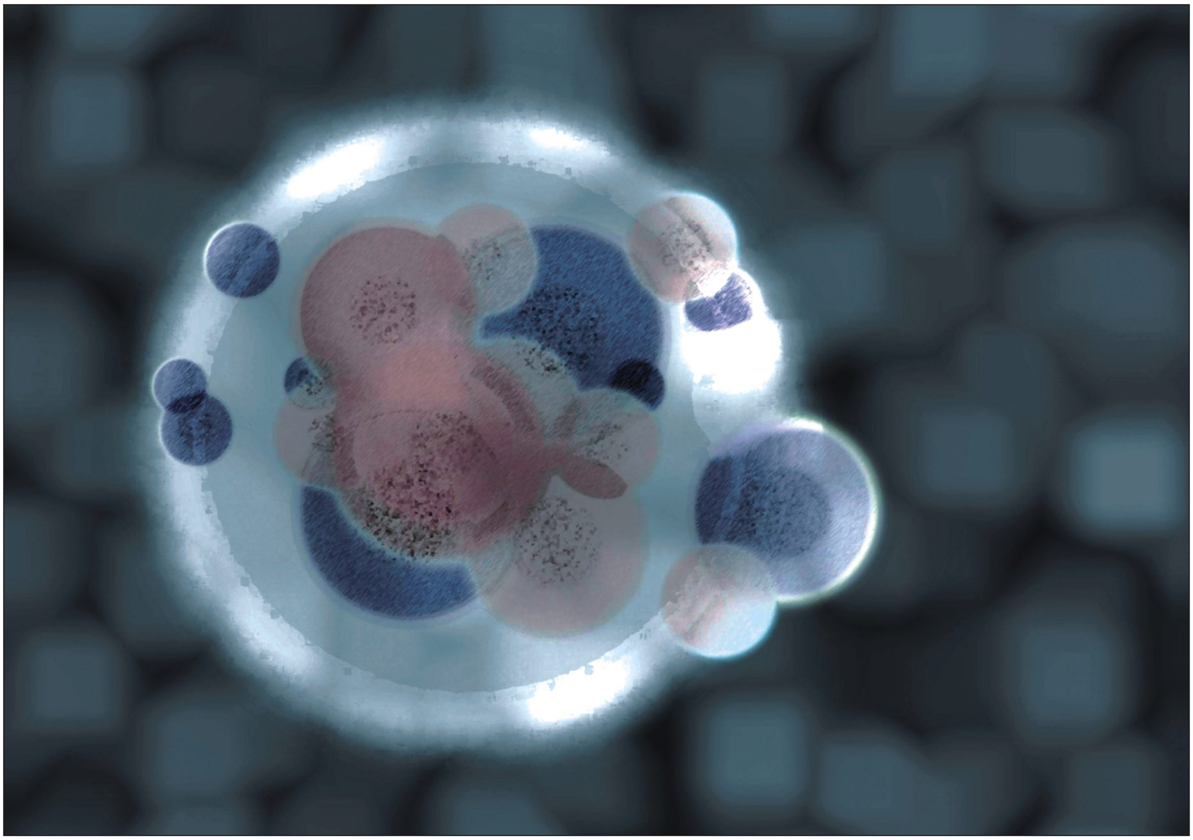

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# Research Article
# Improving the Quality and Shelf-life of Refrigerated Japanese Quail (*Coturnix coturnix japonica*) Carcasses by Oregano/Citrate Dipping

Robiel Kamel Moawad, Hanna Abdelmonem El-Banna, Ola Saleh Saleh Mohamed and Wafaa Aboelsood Ibrahim

Department of Food Science and Technology, National Research Centre, Dokki, Egypt

## Abstract
**Background and Objective:** Consumption of fresh quail meat has become more popular among consumers in recent years, but they are highly perishable. The present article aimed to enhance the quality and shelf-life of fresh quail carcasses through soaking in aqueous solution of cold distilled water (Control; C), Sodium citrate (SC; 2%), Oregano essential oil (OEO; 0.5%) or their combination blend (SC; 2%+OEO; 0.5%; 1:1 v/v) for 10 min, packaged and stored at 4±1°C for 10 days under aerobic conditions. **Materials and Methods:** Freshness tests (pH, total volatile bases nitrogen "TVB-N" as well as thiobarbituric acid reactive substances "TBARS"); Consumer acceptance (color, odor "for raw"-appearance, flavor, texture and overall acceptability "for cooked") and Microbiological evaluation (total viable counts "TVC", psychrotrophic counts "PTC" and enterobacteriaceae counts "EBC") were determined during cold storage. Statistical analysis was done by using one-way analysis of variance (ANOVA). Significant differences were defined as $p<0.05$; according to PC-STAT. **Results:** Significant ($p<0.05$) incremental pattern was observed in TVB-N, TBARS, pH, TVC, PTC and EBC values in all quail samples during subsequent cold storage by different rates, with the control samples always being the highest. Both natural compounds (SC/OEO) dipping significantly ($p<0.05$) reduced lipid oxidation and microbial growth occurring during refrigerated storage. OEO was more significant ($p<0.05$) positively affect than SC. In terms of sensory evaluation, the panelists preferred OEO applied quails in comparison to sodium citrate or control samples. Moreover, quail carcasses soaked in OEO+SC in combination exhibited the lowest TBARS values and microbial counts, the highest acceptance scores and the best shelf-life; possibly due to a synergistic effect. **Conclusion:** Overall, the study revealed that SC+OEO dipping treatment is hereby recommended, since it has been found to keep cold quail carcasses in wholesome state, extended shelf-life, ensure safe consumption for consumers and may offer a promising choice as safe natural preservatives.

Key words: Quail meat, natural preservatives, oregano essential oil, sodium citrate, quality attributes

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Corresponding Author: Robiel Kamel Moawad, Department of Food Science and Technology, National Research Centre, 33 El Bohouth St. Dokki, P.O. Box 12622, Giza, Egypt Tel: 00237386216



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Quail is one of the smallest avian species belonging to the same biological family of chicken. It has been known for centuries as a meat source with no religious restriction against its consumption[1]. Japanese quail (*Coturnix coturnix japonica*) is the most commonly bred for human consumption. The consumption of quail meat increased due to its low price, easy processing with tender and fine-fiber, high protein and iron content, low fat and cholesterol content as compared to red meats[2,3]. The diverse nutrient composition of quail meat makes it an ideal environment for the growth and proliferation of meat spoilage micro-organisms, as well as food-borne pathogens. Therefore, applying adequate preservation technologies is essential to extend the shelf life of perishable quail products, which is a major concern for the poultry industries.

Unfortunately, quail have higher pathogenic and spoilage micro-organisms than most other foods[4,5]. Contamination of quail carcass is possible at any stage of the production process including contamination from feathers plucking and evisceration equipment, washing before storage, as well as from the environment, equipment and operators' hands[6]. Moreover, consumers prefer fresh quail to frozen. Being highly perishable, fresh poultry meat including quail has a limited shelf-life due to their biological composition, autolytic enzymes, lipid oxidation and microbial activities[7,8].

Quail meat is highly perishable with a relatively short shelf life even when it is kept under refrigeration[9]. The initial loss of quail freshness is usually a result of chemical and enzymatic reactions; whereas microbial activity is responsible for slime formation, off-odor production; economic losses and involves public health risks[10]. Moreover, quail lipids is highly susceptible to oxidation due to its large amount of polyunsaturated fatty acids (PUFA); lipid oxidation can accelerate major quality deterioration in term of undesirable flavor, pigment discoloration, toughness, loss of nutritive value and safety of quail products[11].

The application of suitable natural agents possessing both antioxidant and antimicrobial activities during processing could be highly useful for poultry industry[12]. Concerning the safety and toxicity of synthetic antimicrobials and antioxidants, particular interest has been focused on the potential application of natural preservatives as safer additives for destroying disease-causing pathogens, decontaminating, preserving and extending shelf-life of quail carcasses and cuts[13,14].

Food grade sodium citrate (SC) is successfully used as a decontaminant in various food products. It is generally recognized as safe (GRAS) and legally permitted additives by the European Commission, FAO/WHO and FDA, as an antimicrobial agent[15,16]. SC can delay the proliferation of spoilage as well as food-borne pathogens microorganisms, prevent the generation of undesirable chemicals, improve the levels of sensory attributes and extend the shelf life of poultry meat during refrigerated storage[13,17]. Furthermore, sodium citrate (organic salt of citric acid) is widely available, economical and generally functions as pH controllers, humectants, flavor enhancers, lipid oxidation inhibitors and shelf-life extenders of various muscle based foods[18-20]. Thus, there could be advantages to treat fresh quail carcasses with such food additive before refrigeration.

Oregano (*Origanum vulgaris* L.) of the Lamiaceae family is one of the most popular aromatic plants that can be used as a meat preservative[21]. It has an ancient history of nutritional, flavoring, medicinal and traditional usages[22]. Oregano essential oil (OEO) serves as a safe alternative to chemical or synthetic antimicrobials and antioxidants to fight with the food-borne pathogens or spoilage microorganisms, inhibiting lipid oxidation and thus extend shelf-life of muscle origin foods[23-25]. OEO has much attention due to its high content and wide spectrum of phenolic compounds, antimicrobial and antioxidant properties and potential for use in meat and meat products[23,26]. However, the use of OEO is limited by organoleptic criteria[27]. For this reason, it is used at an appropriate concentration or in combination with other natural preservatives[28,29]. Hence, there is an opportunity to enhance safety and quality of refrigerated quail carcasses by using OEO in combination with SC dipping treatments.

Japanese quail (*Coturnix japonica*) is a species with high commercial value and much appreciated by Egyptian consumers; it is generally sold as whole fresh quail. Actually, the effects of oregano essential oil dip treatment in combination of sodium citrate (OEO+SC) on the quality of perishable quail carcasses have not been reported so far. Thus, the objective of the present work is to determine the effect of oregano essential oil (OEO) dip treatment individually or in combination with sodium citrate (SC), as natural preservatives, on the shelf-life extension of fresh quail carcasses stored under refrigeration ($4\pm1°C$) by evaluating certain chemical criteria indices (pH, TVB-N and TBARS), microbiological status (TVC, PTC and EBC) and sensory parameters (color and odor). Chemical composition of raw and organoleptic attributes (appearance, flavor, texture and overall acceptability) of cooked quail meat was also investigated at the beginning of experiments.

## MATERIALS AND METHODS

**Chemicals and supplies:** Plate count agar (PCA), violet red bile glucose agar (VRBGA) and peptone water were from Oxoid (Hampshire, UK). Methyl red, magnesium oxide, 2-thiobarbituric acid, bromocresol green, BHT and TCA were from Sigma-Aldrich (Germany). All other solvents and chemicals used were of analytical grade or the highest grade available and were obtained either from ADWIC or El-Gomhouria Companies, Cairo (Egypt). Food grade Sodium Citrate ($Na_3C_6H_5O_7$; purity 99%; China/made), was purchased from Matrix International Company, Dokki, Egypt. Dried Oregano (*Origanum vulgaris* L.) leaves; grown in Sinai Egypt, were obtained from the Department of Medicine and Aromatic Plants, NRC, Dokki, Egypt and stored in polyethylene bags at 4±1°C until use.

**Preparation of oregano essential oil (OEO):** About 500 g of air-dried Oregano leaves were hydro-distilled in a 2 L flask and 1 L of distilled water was added. A continuous steam distillation extraction was performed for approximately 3 h, until no further increase in the oil was observed. After finishing the distillation process the apparatus was left to be cooled, then Oregano essential oil (OEO) was collected and dried over anhydrous sodium sulphate before held in dark sealed glass vials and stored at 4±1°C until use[28].

**Quail samples:** A total of 60 Japanese quail (*Coturnix japonica*) carcasses of about 220-230 g each were purchased immediately after slaughtering from a local commercial source in Giza Governorate, Egypt in December, 2017 and rapidly transported in coolers containing crushed ice to the Laboratory of Food Industry, Department of Food Science and Technology, National Research Centre. Upon arrival to the laboratory (~90 min after slaughtering), quail carcasses were washed under cold running tap water and divided into four equal batches under complete aseptic conditions.

**Dipping procedure:** Each quail batch was dipped separately for 10 min inside a refrigerator, in pre-cold (4±1°C) aqueous solution of either SC (2%, w/v), OEO (0.5%, v/v), combined application blend (2% SC+0.5% OEO; 1:1v/v), or sterile distilled water (control; C) and gently swirled with a sterile glass rod. Quail to dipping solution ratio was 1:2.5. They were then removed from the solutions and allowed to drain on a stainless wire mesh screen for 3 min. Subsequently, quail carcasses were individually placed in polyethylene bags, labeled and stored at 4±1°C.

The concentrations of 2% SC and 0.5% OEO solutions were chosen in accordance to a preliminary panel test (results not shown) and also to the previous successful pretreatment studies achieved by Kim *et al.*[20], Govaris *et al.*[22], Chouliara *et al.*[24], with the potential to extend the shelf life and improve the quality of perishable muscle origin food. Two bags of each group were withdrawn at each intervals of cold storage (days zero, 3, 6, 8, 9 and 10) for sensorial properties (color and odor), chemical indices (pH, TVB-N and TBARS) and microbiological (TVC, PTC and EBC) analyses. Proximate composition of raw quails and organoleptic attributes (appearance, flavor, texture and overall acceptability) of fresh cooked quail meat were performed at zero time, using another three bags from each quail group. Averages of three replicates were considered.

### Examination techniques

**Compositional and chemical indices determinations:** Proximate composition in terms of moisture, ash, crude lipid and total nitrogen of quail meat were determined according to the methods described in the AOAC[30]. The total volatile basic nitrogen (TVB-N) expressed as mg TVB-N per 100 g quail meat samples was determined according to the method described by Parvaneh[31]. A Thiobarbituric acid reactive substance (TBARS) as mg of malondialdehyde (MDA)/kg quail meat was estimated according to Kilinc *et al.*[32]. For pH determination 10 g of quail meat samples were homogenized in 90 mL distilled water for 1 min in a warring blender and the pH value of the slurry was measured at room temperature using pH meter (JENWAY, 3510; UK). Analyses were conducted in triplicate and all reagents were of analytical grade. Chemical analyses were made on finely ground quail meat (without skin) samples.

**Sensory evaluation of raw and cooked quails:** Color and odor attributes of raw quails were evaluated by modified acceptance test with 10 non-trained panel members of the laboratory staff. Two raw whole quail carcasses from each group were taken at regular intervals and immediately served to the panelists for evaluation using 9-points hedonic scales. Moreover, on day zero only for safety precautions, the same panel members were also asked to state whether the freshly cooked quail samples were acceptable or not. Another three whole quail carcasses from each treatment were cooked in an electric oven (130°C). The method is based on achievement of a temperature of 75°C in the core of the sample (as indicated by a thermocouple) for 15 min[33]. After cooking, each quail meat from both breast and leg muscles was cut into quarters

(~2.0×3.0 cm) and placed in small plastic plates, then labeled and served warm to the panelists at room temperature in random order; water was served for rinsing the mouth between samples. Organoleptic attributes (appearance, flavor, texture and overall acceptability) of cooked quail meat were scored. The 9-points hedonic scales were 1: Dislike extremely, 2: Dislike very much, 3: Dislike moderately, 4: Dislike slightly, 5: Neither like nor dislike, 6: Like slightly, 7: Like moderately, 8: Like very much and 9: Like extremely. A score less than 5 indicate that the quail meat is rejected[34].

**Microbiological analysis:** Twenty five grams of composite quail samples (meat with skin) were aseptically excised from the whole carcass and homogenized in 225 mL of sterile buffered 0.1% peptone water for 3 min. From this homogenate, decimal serial dilutions were made in the same sterile peptone water and used for microbiological analyses of the quail meat samples at appropriate time intervals during refrigerated storage. On each of the predetermined sampling days, 0.1 mL of each dilution was pipetted onto the surface of plate count agar to determine total viable counts (TVC) and psychrotrophic counts (PTC); while enterobacteriaceae counts (EBC) were determined by using violet red bile glucose agar. Then, all plates were prepared in triplicate and incubated for 2 days at 30°C for TVC and EBC and 10 days at 5°C for PTC[35]. After specific incubation periods plates showing 25-250 colonies were counted. The number of colonies were multiplied by the reciprocal of the respective dilution and expressed as log CFU $g^{-1}$.

**Statistical analysis:** Results were expressed as means and standard deviation (Mean±SD) from 10-panelists report and triplicate determinations. Analysis of variance (ANOVA) was performed to compare the effect of OEO and/or SC dipping treatments on quail meat quality. Significant differences were defined as p<0.05; according to PC-STAT[36].

## RESULTS AND DISCUSSION

**Chemical composition of quail meat:** The proximate composition of quail meat was summarized in Table 1, from which it was apparent that breast quail meat showed higher significant (p<0.05) protein and moisture contents and lower fat content than leg quail meat. These results confirmed the findings achieved in quail meat by Genchev *et al.*[1] and Fakolade[3].

Data depicted in Table 1 indicated that fresh raw quail meat was characterized by high proteins and minerals and low fat contents; that encourages the consumption of such product. Variations in proximate composition of quail meat, mainly in lipid and moisture were reported[2,33,37]. Such variations in proximate chemical composition of quail meat was related to nutrition, living area, slaughter age, season, quail size and sex; which may possibly lead to changes in the quality attributes.

Table 1 also revealed that quail muscles contain only very little amounts of carbohydrates which limit the degree of post mortem glycolysis; hence the pH of quail meat remained high (6.2-6.5) in comparison to low average values (pH 5.5) reported for the beef[38]. Moreover, soaking quail carcasses in cold active solutions (OEO, SC or OEO+SC in combination) for 10 min almost had no effect on their proximate chemical composition so it did not register here.

**Sensory properties of cooked quail meat:** Results of Table 2 indicated that all cooked quail samples under investigation

Table 1: Proximate composition of raw fresh quail meat (from breast and leg muscles without skin)

| Chemical constituents (%) | Raw quail breast | | Raw quail leg | |
|---|---|---|---|---|
| | Wet weight | Dry weight | Wet weight | Dry weight |
| Moisture | 73.00±0.74$^a$ | - | 72.26±0.96$^b$ | - |
| Protein | 22.59±0.47$^a$ | 83.67 | 21.83±0.54$^b$ | 78.70 |
| Int. fat | 2.92±0.19$^b$ | 10.81 | 3.87±0.27$^a$ | 13.95 |
| Ash | 1.23±0.11$^b$ | 4.56 | 1.49±0.13$^a$ | 5.37 |
| Carbohydrates | 0.26±0.08$^b$ | 0.96 | 0.55±0.46$^b$ | 1.98 |

Values are given as Mean±SD, from triplicate determinations. Total carbohydrates = 100-(Moisture+Protein+Intramuscular-fat+Ash)

Table 2: Sensory scores of freshly cooked quail meat (of both breast and leg muscles) at zero time

| Dipping treatments | Appearance | Flavor | Texture | Overall-acceptability |
|---|---|---|---|---|
| Control-(C) | 8.68±0.17$^b$ | 7.84±0.14$^c$ | 8.15±0.12$^c$ | 8.22±0.14$^d$ |
| SC (2%) | 8.87±0.13$^a$ | 8.23±0.18$^b$ | 8.93±0.15$^a$ | 8.66±0.17$^c$ |
| OEO (0.5%) | 8.93±0.11$^a$ | 9.00±0.10$^a$ | 8.75±0.17$^b$ | 8.89±0.21$^b$ |
| OEO+SC | 9.00±0.24$^a$ | 9.12±0.16$^a$ | 9.00±0.13$^a$ | 9.04±0.15$^a$ |

All values reflect the mean and standard deviation, (n = 10). A score less than 5 indicate that the quail meat is rejected. Mean values in the same column bearing the same superscript do not differ significantly (p<0.05). OEO: Oregano essential oil (0.5%), SC: Sodium citrate (2%), OEO+SC: Combined blend (1:1 v/v)

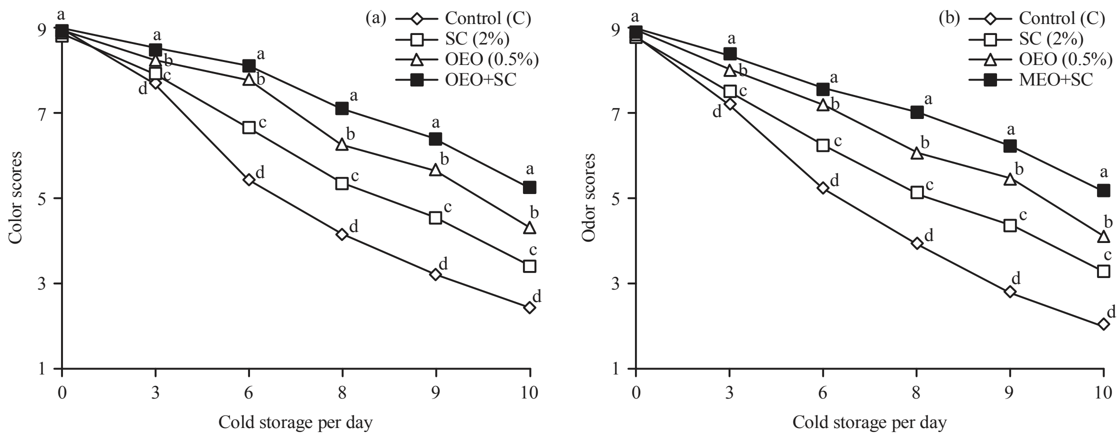


Fig. 1(a-b): Sensory, (a) Color and (b) Odor scores of raw whole quail carcasses during refrigerated storage for10 days

All values reflect the mean and standard deviation, (n = 10). A score less than 5 indicate that the quail meat is rejected. Mean values in the same column bearing the same superscript do not differ significantly (p<0.05). OEO: Oregano essential oil, 0.5%-SC: Sodium citrate, 2%. OEO+SC: OEO, 0.5%+SC, 2%. combined blend (1:1v/v)

were acceptable from the sensory point of view as evident by their high overall acceptability scores. Results also revealed that using 0.5% OEO and/or 2% SC dipping solutions significantly (p<0.05) improved the general sensory attributes of cooked quail samples and never cause any undesired changes in their organoleptic characteristics. Similar results were achieved by Hernandez *et al.*[21] and Pavelkova *et al.*[25]. Moreover, combined OEO+SC dipping treatment exhibited the best scores in all organoleptic attributes of cooked quail; confirming the possible synergistic effect.

Results of Table 2 showed that OEO (0.5%)-treated group was mostly preferred by the panelists in flavor scores (it imparted a sweet flavor for treated quail samples); which reflect the antioxidant, antimicrobial and flavoring properties of OEO in meat and poultry products[22-24,28]. While, 2% SC exhibited more significant (p<0.05) texture scores than other treated quail samples; which could be due to the ability of SC to hold water in cooked quails (humectants). Our results confirmed the findings of Contini *et al.*[18], Morey *et al.*[19], Kim *et al.*[20], Maca *et al.*[39] and Ke *et al.*[40].

**Sensory analysis of raw whole quail carcasses:** The changes in mean color and odor scores of refrigerated quail carcasses were shown in Fig. 1. End shelf life of meat and poultry products was usually determined when spoilage-related sensory attributes such as discoloration and off-odor become strong, caused mainly by lipid oxidation and microbial activity[8]. Quail contains high level of PUFA, which was susceptible to autoxidation causing off-odors and browning of flesh color. The off-odors and browning (metmyoglobin) intensity of the treatment quail groups remained at low levels compared to the control group throughout the storage period (Fig. 1).

Figure 1 revealed that fresh quail carcasses (at zero time) were generally considered to possess very high acceptability with regard to their odor and color attributes; their scores being observed above seven until the third day of storage. However, all sensory scores (Fig. 1) gradually declined during storage with different rates depending on nature of treatments and time of storage, with the control quail samples always having the worst scores. Similar outcomes had been reported in muscle origin products[21,22,40].

Figure 1 also indicated that untreated control quail samples were beginning to spoil and have a rancid odor; they became unfit for sale, based on appearance and odor scores after day 6 of storage period. While by using 2% SC dipping solution; sensory properties of quail samples were significantly (p<0.05) improved and exhibited acceptable scores till 8th day of storage period. Also, quail samples treated with 0.5% OEO, demonstrated higher enhancement of sensory attributes and received an appearance and odor scores higher (p<0.05) than the critical limit score level of five[34], up to 9 days of refrigerated storage.

Importantly, quail samples treated with (OEO+SC) in combination were scored as superior (p<0.05) to the control, SC and OEO treated quails in color and odor scores, as well as they maintained pleasant oregano flavor; consequently extended the shelf-life of the treated quail samples to 10 days (Fig. 1). This could be due to synergistic effect between sodium citrate (SC) and oregano essential oil (OEO). Similar

Table 3: TVC, PTC and EBC (as log CFU $g^{-1}$) of raw quail meat (with skin) during storage for 10 days

| | | Cold storage at 4±1°C per day | | | | | |
|---|---|---|---|---|---|---|---|
| Treatments | Test | 0 | 3 | 6 | 8 | 9 | 10 |
| Control (C) | TVC (log CFU $g^{-1}$) | 4.12±0.16$^a$ | 4.86±0.13$^a$ | 5.62±0.10$^a$ | 6.54±0.15$^a$ | 7.72±0.12$^a$ | 8.25±0.18$^a$ |
| SC (2%) | | 3.97±0.10$^b$ | 4.65±0.17$^b$ | 5.00±0.19$^b$ | 5.64±0.13$^b$ | 6.40±0.17$^b$ | 7.16±0.11$^b$ |
| OEO (0.5%) | | 3.83±0.12$^c$ | 4.40±0.15$^c$ | 4.78±0.12$^c$ | 5.12±0.21$^c$ | 5.80±0.13$^c$ | 6.69±0.17$^c$ |
| OEO+SC | | 3.80±0.18$^c$ | 4.23±0.11$^d$ | 4.45±0.14$^d$ | 4.88±0.10$^d$ | 5.46±0.11$^d$ | 5.92±0.13$^d$ |
| Control (C) | PTC (log CFU $g^{-1}$) | 3.85±0.13$^a$ | 4.41±0.17$^a$ | 5.29±0.12$^a$ | 6.28±0.15$^a$ | 7.00±0.11$^a$ | 7.82±0.16$^a$ |
| SC (2%) | | 3.54±0.21$^b$ | 4.00±0.12$^b$ | 4.65±0.16$^b$ | 5.32±0.11$^b$ | 6.17±0.23$^b$ | 6.94±0.12$^b$ |
| OEO (0.5%) | | 3.41±0.16$^c$ | 3.86±0.21$^c$ | 4.37±0.12$^c$ | 4.83±0.18$^c$ | 5.26±0.14$^c$ | 6.12±0.18$^c$ |
| OEO+SC | | 3.22±0.15$^d$ | 3.65±0.10$^d$ | 4.21±0.14$^d$ | 4.57±0.10$^d$ | 4.90±0.17$^d$ | 5.67±0.21$^d$ |
| Control (C) | EBC (log CFU $g^{-1}$) | 2.13±0.17$^a$ | 2.65±0.12$^a$ | 2.94±0.16$^a$ | 3.26±0.17$^a$ | 3.61±0.17$^a$ | 3.92±0.14$^a$ |
| SC (2%) | | 1.96±0.14$^b$ | 2.14±0.10$^b$ | 2.51±0.11$^b$ | 2.84±0.12$^b$ | 3.13±0.14$^b$ | 3.28±0.11$^b$ |
| OEO (0.5%) | | 1.83±0.21$^c$ | 1.98±0.13$^c$ | 2.28±0.14$^c$ | 2.56±0.18$^c$ | 2.85±0.11$^c$ | 3.10±0.10$^c$ |
| OEO+SC | | 1.70±0.12$^d$ | 1.84±0.15$^d$ | 2.10±0.10$^d$ | 2.39±0.13$^d$ | 2.67±0.12$^d$ | 2.94±0.16$^d$ |

All values reflect the mean and standard deviation (SD) from triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$). TVC: Total viable count, PTC: Psychrotrophic count, EBC: Enterobacteriaceae count, (as log CFU $g^{-1}$), OEO: Oregano essential oil (0.5%), SC: Sodium citrate (2%), OEO+SC: Combined blend (1:1 v/v)

findings have been reported in muscle origin products[19,21], who confirmed strong antioxidant and antimicrobial activities of such natural materials.

**Bacteriological load:** Microbiological analysis, as an important reference index, has been used to evaluate poultry's freshness and shelf-life[29,41]. Microbiological results were presented in Table 3, which revealed that at the beginning of refrigerated storage (day zero), a slight but significant ($p<0.05$) reduction as log (CFU $g^{-1}$) in total viable count (TVC), psychrotrophic count (PTC) and enterobacteriaceae count (EBC) was noticed in treated quail samples when compared with control samples; indicating that using OEO, SC, or their combination (OEO+SC) as dipping solutions caused sudden lethal effect for the tested microorganisms.

Results demonstrated in Table 3 also indicated that TVC and PTC of all quail samples steadily increased as the time of cold storage at 4±1°C progressed, with the control quails always being the highest ($p<0.05$). These results were nearly similar to results obtained by Morey *et al.*[19], Kim *et al.*[20], Hernandez *et al.*[21] and De Barros *et al.*[29], who reported that OEO and/or SC showed significant effect in lowering the microbial count of chicken meat. It is worth mentioning that the Gram-negative psychrotrophic bacteria are very important from the economic point of view; because they are responsible for the most undesirable off-odor, tough texture, discoloration and off-flavor attributes of refrigerated meat and poultry products[7,10,32].

The application of OEO+SC in combination might have synergistic effect on the retardation of bacterial growth during cold stored, leading to safer products. Table 3 also showed that all quail groups exceeded such limit of TVC and PTC at the day when it became unfit for consumption as indicated previously by the sensory results depicted in Fig. 1. Similar results were also reported by Menconi *et al.*[13], Morey *et al.*[19], Kim *et al.*[20], Hernandez *et al.*[21], Boskovic *et al.*[27] and Pinon *et al.*[42] in their frameworks on chicken products pre-treated with OEO or SC and subjected to cold storage.

Enterobacteriaceae bacteria are considered as a hygiene indicator, since this group contains a large number of pathogens[43]. As shown in Table 3 the growth of Enterobacteriaceae (EBC) on quail carcasses was slower than that of TVC, or PTC. However, the increase in cold storage time resulted in significant ($p<0.05$) gradually proliferations in EBC for all quail samples. Table 3 also showed that EBC starting by 2 log CFU $g^{-1}$ (indicative of good quality quail meat) and never exceeding 3 log CFU $g^{-1}$ in the control quail carcasses. At the end of the storage period (day 10), treated quails exhibited much lower ($p<0.05$) counts as compared with control samples which also being the highest. Table 3 further indicated that the Inhibition activity was evident in the order OEO+SC>OEO>SC>control.

Oregano essential oil (OEO) is a rich natural source of bioactive compounds such as carvacrol and thymol; which exhibited strong antimicrobial properties against a wide range of spoilage and pathogenic bacteria[23,26]. The antimicrobial reaction of phenolic compounds was related to the inactivation of cellular enzymes, as well as, weakening of the cell membrane; those results in lethal damage to the bacterial cells and inhibit their growth[29,44]. While, sodium citrate (SC) may delay or inhibit the growth of bacteria by chelating metal ions essential for bacterial proliferation or by decreasing the ionic concentration within the bacterial cell membrane, leading to accumulation of acid within the cell cytoplasm, disruption of the proton motive force and inhibition of substrate transport[45].

Table 4: Quality indices changes of raw quail meat during refrigerated storage at 4±1°C for 10 days

| Soaking treatment | Criteria indices | Cold storage at 4±1°C per day 0 | 3 | 6 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|
| Control (C) | pH value | 6.38±0.12[a] | 6.27±0.10[a] | 6.75±0.11[a] | 7.28±0.10[a] | 7.76±0.11[a] | 8.10±0.16[a] |
| SC (2%) | | 6.04±0.17[c] | 5.95±0.17[c] | 6.23±0.13[c] | 6.48±0.12[c] | 6.70±0.19[c] | 7.00±0.12[c] |
| OEO (0.5%) | | 6.23±0.21[b] | 6.10±0.23[b] | 6.41±0.18[b] | 6.73±0.15[b] | 6.93±0.28[b] | 7.23±0.14[b] |
| OEO+SC | | 6.18±0.10[b] | 6. 13±0.28[b] | 6.35±0.15[b] | 6.62±0.24[b] | 6.85±0.13[b] | 7.14±0.10[b] |
| Control (C) | TVBN (mg N/100 g) | 12.86±0.15[a] | 14.38±0.13[a] | 17.74±0.17[a] | 21.65±0.21[a] | 23.84±0.11[a] | 26.25±0.14[a] |
| SC (2%) | | 12.64±0.12[b] | 13.76±0.16[b] | 15.58±0.13[b] | 18.92±0.10[b] | 22.16±0.10[b] | 23.84±0.12[b] |
| OEO (0.5%) | | 12.38±0.11[c] | 12.65±0.13[c] | 14.00±0.11[c] | 16.14±0.21[c] | 19.34±0.08[c] | 21.72±0.14[c] |
| OEO+SC | | 12.10±0.14[d] | 12.32±0.17[d] | 13.20±0.10[d] | 15.82±0.15[d] | 17.75±0.19[d] | 19.56±0.23[d] |
| Control (C) | TBARS (mg MDA/kg) | 0.48±0.17[a] | 0.66±0.12[a] | 0.84±0.16[a] | 1.18±0.10[a] | 1.40±0.17[a] | 1.72±0.15[a] |
| SC (2%) | | 0.43±0.14[a] | 0.51±0.10[b] | 0.70±0.11[b] | 0.80±0.12[b] | 1.13±0.14[b] | 1.34±0.12[b] |
| OEO (0.5%) | | 0.40±0.15[a] | 0.49±0.13[b] | 0.65±0.14[b] | 0.76±0.18[b] | 0.85±0.21[c] | 1.18±0.10[c] |
| OEO+SC | | 0.45±0.21[a] | 0.50±0.11[b] | 0.58±0.10[b] | 0.71±0.13[b] | 0.80±0.12[c] | 0.89±0.17[d] |

All values reflect the mean and standard deviation (SD from triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly (p<0.05). TVBN: Total volatile basic nitrogen, as mg N/100 g meat. TBARS: Thiobarbituric acid reactive substances, as mg MDA/kg meat, OEO; 0.5%: Oregano essential oil, SC; 2%: Sodium citrate, OEO+SC: Combined blend (1:1v/v)

### Deterioration criteria indices

**pH changes:** pH plays an important role in the technological properties of meat. pH values of quail samples are illustrated in Table 4, from which it is apparent that at the beginning of cold storage the mean pH value of control quail meat samples was 6.38 which is consistent with the results reported for quail meat[2,33]. For quail samples pretreated with oregano oil (OEO), the decrease in pH (6.28) was noticed. Results shown in Table 4 also revealed that sodium citrate (SC) dipping treatment exhibited the lowest pH values (6.04) between quail samples. However, the lowering effect in quail pH values due to dipping treatments confirmed the findings obtained by Kim *et al.*[20], Del Rio *et al.*[46] and Shaltout *et al.*[47] in their researches on meat including poultry pre-treated with essential oil and sodium salt of organic acids.

The data in Table 4 also showed that, the pH values of all quail samples marginally decreased during the first 3 days of storage, then gradually increased (p<0.05) throughout the storage time. However, the decrease in pH values could be explained by the formation of carbonic and lactic acids, while the production of alkaline compounds by the microbial activity is responsible for the increase in pH values thereafter[48]. The sharp (p<0.05) increase in pH values was observed in control samples throughout the storage period. Conversely, quail carcasses soaked in SC gave quiet and constant pH values till the end day of storage; which reflect the buffering and antimicrobial activities for sodium citrate[19,20,39].

Results depicted in Table 4 showed that quail samples soaked in 0.5% OEO solution exhibited moderate and constant pH values up to 9 days of cold storage; confirming strong antimicrobial activity of oregano essential oil, which reduces the accumulation of basic substances[21,23,29]. Moreover, OEO+SC pre-soaking treatment exhibited pH values (7.14) below the critical value at 10th day of refrigerated storage, due to the possible synergistic effect between OEO and SC in preventing microbial deteriorations and extending the shelf-life.

**Protein degradation:** TVB-N was used for the determination of the major parameters of fresh poultry meat[31,35]. Data depicted in Table 4 showed that at day zero of storage period, TVB-N levels were found to be 12.86, 12.64, 12.38 and 12.10 mg N/100 g quail meat for control (C), SC-, OEO- and (OEO+SC)-treated quails; respectively. These results confirmed the findings reported for poultry meat by Kim *et al.*[20], Shaltout *et al.*[47] and Khalafalla *et al.*[49].

Table 4 also revealed that TVB-N levels of quail meat samples steadily increased (p<0.05) with the increase in refrigerated storage time for all quail samples, with the control quails being the highest. A positive relationship was found between the microbiological quality of quails (Table 3) and level of TVB-N formation (Table 4); which was in agreement with[8,49]. Table 4 also indicated that the OEO-treatment was more effective at inhibiting microbial activity and protein decomposition than SC and control treatments. Moreover, the lowest (p<0.05) TVB-N values were noticed for OEO+SC-treated quails to end day of storage (10th day), due to the possible combined synergistic effect that delay the formation of basic nitrogen compounds. Thus, it can be clearly expressed that SC should be applied as combined with OEO application, rather than it's alone application.

The accumulation in TVB-N levels during cold storage may be explained on the basis of deamination of free amino acids, oxidation of amines and degradation of nucleotides by autolytic enzymes and microbial activity[50]. On the other hand, the inhibitor effect observed in TVB-N levels of treated samples confirmed antimicrobial properties of such natural (SC and OEO) preservatives. However, a level above 20 mg N/100 g flesh is usually regarded spoiled of refrigerated poultry meat products ES[51]. According to the results illustrated in Table 4, control quail samples still acceptable with regard to TVBN index for six days in comparison to 8, 9 and 10 days for sodium citrate (SC), oregano essential oil (OEO) and (OEO+SC) combined application; respectively.

**Lipid oxidation:** TBARS value is an index of lipid oxidation measuring malondialdehyde (MDA) content in meat and poultry products[11]. In the present study, TBARS values of control and treated quail groups were presented in Table 4, from which it was cleared that there were no significant differences between all groups ($p<0.05$) at day zero of storage, the amounts of TBARS were almost the same ranging from 0.40-0.48 mg MDA/kg meat; indicating that oxidation of quail lipids occurs during refrigerated storage and the obtained TBARS values reflect good quality meat at the beginning of storage.

From the same given results of Table 4, it was apparent that starting from the third day of cold storage TBARS levels gradually increased in all quail groups throughout the refrigerated storage with different significant ($p<0.05$) rates. However, control quail samples always having the highest TBARS values to the end day of cold storage. In the contrary, quail samples pre-soaked in (OEO+SC) solution gave the lowest TBARS values all over the storage period due to synergistic effect. Such accumulation of TBARS values is in accordance with[20,24,47,49], whose results on oxidation of poultry products are almost parallel to our results.

Results demonstrated in Table 4 also revealed that OEO soaking treatment exhibited more oxidative stability (lower TBARS values) than SC or control (C) treatments. The high efficiencies of OEO are closely related to the high content of bioactive compounds including thymol, carvacrol, cymene and $\gamma$-terpinene with strong antioxidant activities. Moreover, phenolic compounds can inhibit the formation of free radicals through the chelation of transition metal ions, particularly those of iron and copper[23,26]. Sodium citrate (SC) potentially retard the oxidation in poultry products by chelation of pro-oxidant metal ions and inactivation of bacterial enzymatic reaction related with oxidation[18,20,39].

Concerning TBARS value as a spoilage index in chilled poultry products, Egyptian Standards (ES)[51] recommended 0.9 mg MDA $kg^{-1}$ meat as the upper limit of acceptability. According to the obtained data of Table 4; it is obvious that chilled quail carcasses samples exhibited low and acceptable TBARS value (less than 0.9 mg MDA/kg flesh) up to 6 days for control (C) quails, 8 days for sodium citrate (SC)-, 9 days for oregano essential oil (OEO)- and 10 days for OEO+SC- treated quail samples. These results are in agreement with the findings reported by Amani *et al.*[8], Kim *et al.*[20], Shaltout *et al.*[47] and Khalafalla *et al.*[49]. However, TBARS values in the present study confirmed the findings of TVB-N and microbiological examinations (TVC, PTC and EBC).

## CONCLUSION

The current study concluded that dipping of quail carcasses in cold aqueous solutions containing Oregano essential oil (OEO; 0.5%), Sodium citrate (SC; 2%) or their combination (SC; 2%+OEO; 0.5%) are efficient against the proliferation of various categories of food borne pathogens and spoilage causing bacteria, it also retarded lipid oxidation, minimized protein breakdown and extended the shelf-life of the quail carcasses during refrigerated storage at $4\pm1°C$ by 2-4 days more than that of control samples (6 days). Therefore, Oregano essential oil (OEO) and/or Sodium citrate (SC) can be applied as safe natural preservatives for quail carcasses under refrigerated storage.

## SIGNIFICANT STATEMENT

This study discovers the possible synergistic effect of Oregano essential oil (OEO; 0.5%) and sodium citrate (SC; 2%) combination that can be beneficial for successful reduction of lipid oxidation and microbial growth in quail meat stored at $4\pm1°C$. This study will help the poultry industry to maintain good quality characteristics, delay the spoilage and extend the shelf-life of refrigerated quail products and could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the poultry industry.

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