



IMPROVING THE MICROBIAL QUALITY, LIPID STABILITY AND SHELF LIFE OF CHICKEN CARCASSES BY OREGANO AND MULBERRY LEAVES EXTRACTS DIPPING

Robiel K. Moawad*, Nahed M. Abdelmaguid, Ola S. Mohamed and Wafaa H. Emam

Food Technology Department, 33 El-Bohouth St., National Research Centre, Dokki, Egypt.

Abstract

Chicken meat is one of the popular foodstuffs all over the world; they are highly perishable with a limited shelf-life. The present article aims to enhance the quality and shelf-life of fresh chicken carcasses through soaking in aqueous solution of cold distilled water (Control; C), mulberry leaves extract (MLE; 2%), Oregano leaves extract (OLE; 2%) for 15 min. Methodologies: The optimum concentrations of MLE and OLE were established and used for soaking solutions. Raw chicken samples were refrigerated at $4\pm 1^\circ\text{C}$ to be periodically examined for their sensory quality, chemical indices and bacteriological status, until appearance of signs of spoilage. Total phenolic content (TPC), antioxidant activity (DPPH and ABTS) and HPLC analysis of the natural extracts were examined. Results indicated that TFC, DPPH and ABTS were higher in MLE than OLE. The panelists preferred OLE applied chickens in comparison to MLE or control samples. Results also revealed that OLE provided the highest significant ($P<0.05$) antioxidant and antimicrobial properties and the lowest ($P<0.05$) protein degradation (TVB-N) and pH followed by MLE, then control samples during cold storage. Conclusion: This study demonstrates the potential use of MLE and OLE to improve the microbial quality, retard lipid oxidation, maintain the quality indices and extended the shelf-life of treated chicken samples by 2-4 days over that of control (6 days) as confirmed by microbiological, chemical indices and organoleptic analyses, and could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the meat industry.

Key words: Antioxidant, Antimicrobial, MLE, OLE, DPPH, Quality attributes, Shelf-life.

Introduction

Poultry meat is a nutritious food and it is consumed all over the world for its relatively low cost and low fat content. Chicken meat contains high levels of polyunsaturated fatty acids, which make it susceptible to oxidative deterioration during storage. Color, microbial growth and lipid oxidation are important factors for the shelf-life and consumer acceptance of fresh chicken meat (Ding *et al.*, 2015). Lipid oxidation causes deterioration of chicken meat by adversely affecting its color, flavor, sensorial quality, nutritional value and generation of toxic products such as malonaldehyde and cholesterol oxidation products (Garcia-Lomillo *et al.*, 2017). However, chicken meat is highly perishable with a relatively short shelf life even when it is kept under refrigeration. Thus, finding an appropriate natural treatment having antioxidant and antimicrobial activities for its preservation could be highly useful.

Oregano (*Origanum vulgare* L.) is an aromatic herb with a wide distribution throughout the Mediterranean area (Park *et al.*, 2015). Oregano is added to dishes in the form of fresh and/or dried leaves, while water and alcohol extracts of oregano and its essential oil can also be used in food processing (Hac´-Szymanczuk *et al.*, 2019). The results of HPLC analysis of *O. vulgare* are rosmarinic acid, caffeic acid, chlorogenic acid, arbutin, and luteolin-7-*O*-glucoside (Duletiæ-Lauševiæ *et al.*, 2018). The primary reason for numerous studies on the use of this spice in food is its high antioxidant (Park *et al.* 2015; Boroski *et al.*, 2018 and Cao *et al.*, 2019) and antimicrobial (Chishti *et al.*, 2014; Duletiæ-Lauševiæ *et al.*, 2018 and Hac´-Szymanczuk *et al.*, 2019) activity. These properties are mainly attributed to carvacrol and thymol that constitute about 78 to 82% of the total oil (Boroski *et al.*, 2018 and Cao *et al.*, 2019).

Mulberry (*Morus alba*) leaves are traditionally used to feed silkworms since ancient times and recently

*Author for correspondence : E-mail : rk_moawad@hotmail.com

become the most popular herbal medicine. Mulberry Leaves Extract (MLE) have a very powerful antioxidant effect that comes from the polyphenolic compounds such as quercetin, kaempferol, flavonoids and morin (Cui *et al.*, 2019 and Cai *et al.*, 2019). Moreover, chlorogenic acid, caffeic acid, vanillic acid, hydroxybenzoic acid, *p*-coumaric acid, sinapic acid, protocatechuic acid and ferulic acid were considered to be mainly potential antioxidant compounds in mulberry leaves (Flaczyk *et al.*, 2013 and Yu *et al.*, 2018). In addition, mulberry leaves have shown strong antimicrobial activity due to some substances known as kuwanon C, mulberrofuran G, mourin and albanol B (Kostiæ *et al.*, 2013; Salem *et al.*, 2013 and Chen *et al.*, 2019). Thus, the use of MLE is hence attractive for keeping the quality of refrigerated rabbit meat.

Actually, the use of oregano leaves extract (OLE) and/or mulberry leaves extract (MLE) dip treatment to keep the quality of perishable chicken meat has not been reported so far. Thus, the objective of this study was to determine the antioxidant and antimicrobial effects of oregano leaves extract (OLE; 2%) and mulberry leaves extract (MLE; 2%) dipping treatments on the quality and shelf-life of broiler chicken meat stored at $4\pm 1^\circ\text{C}$ up to 10 days by evaluating certain sensorial attributes (appearance and odor), physicochemical criteria (pH, TVB-N and TBARS) and microbiological status (TVC, PTC and EBC). The present study was carried out also in order to evaluate the proximate composition of chicken meat, as well as determination of phenolic composition (HPLC), total phenolic content (TPC), antioxidant activity (DPPH and ABTS assays), of the above extracts.

Materials and Methods

Chemicals and Reagents

Standard of phenols: gallic acid, caffeic acid; caffeine, ellagic acid, chlorogenic acid, syringic acid, ferulic acid, naringenin, propyl gallate, pyro catechol, vanillin, coumaric acid, quercetin, cinnamic acid, catechin and 4,7-Dihydroxyisoflavone, butylated hydroxyl toluene (BHT), DPPH (2, 2-Diphenyl-1-picrylhydrazyl), ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), potassium persulphate acid, Folin-Ciocalteu reagent were obtained from Sigma Chemical Co., Germany. Plate count agar (PCA), violet red bile glucose agar (VRBGA) and peptone water were purchased from Oxoid (Hampshire, UK). Methyl red, magnesium oxide, 2-thiobarbituric acid, bromocresol green, Methyl red, magnesium oxide, BHT and TCA were from Sigma-Aldrich (Germany). Methanol, ethanol, were purchased from El-Nasr Co., Cairo, Egypt. All other solvents and chemicals used were

of analytical grade or the highest grade available.

Plant Materials

The tender top leaves of white mulberry leaves (*Morus alba*) used in the present study were collected from the yard of National Research Centre on Jun, 2019. Fine-quality fresh green oregano leaves (*Origanum vulgare* L.) were purchased during July, 2019, from Saudi Market at Dokki, Giza, Egypt. The tested leaves were cleaned from extraneous matter and properly washed then dried in hot air-oven at $45\pm 1^\circ\text{C}$ for 48 h. The dried leaves were ground in a blender to form fine powder. Thereafter, powdered samples were stored separately in polyethylene bags in a dark and dry place at $4\pm 1^\circ\text{C}$ until extractions.

Preparation of Extracts:

According to the extraction method of El Anany, (2015), powdered samples from the tested materials were macerated with 70% ethanol (1:10 w/v) and 80% methanol (1:10 w/v) in a closed conical flask for 24 h at room temperature in the dark. The extract centrifuged at $3000\times g$ for 10 min at 20°C , the resultant was then filtered through Whatman No. 1 filter paper and the residue re extracted and filtered. The filtrate was concentrated separately in a rotary evaporator (Heidolph Instruments Germany) to remove the solvent at 38°C under reduced pressure. The dried crude extract residue was stored at -20°C until use or reconstituted in sterile distilled water to give final concentration of 2% MLE and 2% OLE ethanolic extract for dipping treatments.

Chicken Meat Samples:

A total of 18 broiler chicken Hubbard breed, with initial body weight around 2kg were purchased a live from a local poultry market in Giza. Chickens were slaughtered and the carcasses were immediately prepared by removing feet, liver, lungs, heart, kidneys and heads. Chicken carcasses were divided Longitudinally into two halves (breast and thigh muscles), then they washed, packaged in polyethylene bags and rapidly transported in coolers containing crushed ice, to the Laboratory of Food Industry, Department of Food Science and Technology, National Research Centre.

Dipping Procedure:

Upon arrival to the laboratory, chicken halves were divided into three groups, re-washed and then they were dipped separately for 15 min inside a refrigerator ($4\pm 1^\circ\text{C}$), in aqueous solution of either OLE (2%, w/v), MLE (2%, w/v), or distilled water (control; C). Chicken halves were immersed once every time in cold tested solutions. Chicken carcass to dipping solution ratio was 1:2.5 w/v.

After dipping treatments chicken halves were removed from the tested solutions and allowed to drain on a stainless wire mesh screen for 3 min.

Packaging, storage and analysis:

Subsequently, after draining chicken halves from each group were individually placed in polyethylene bags, labeled and stored at $4\pm 1^{\circ}\text{C}$. Aliquots from each treated group were subjected to sensorial (appearance and odor), physicochemical (pH, TVB-N and TBARS) and microbiological (TVC, PTC and EBC) assessment, at day zero (within 1 h after dipping treatment), then after 1, 3, 6, 8 and 10 days of storage until the time of spoilage. Proximate composition of raw chicken meat (from breast and thigh muscles) was also investigated. Averages of three replicates were considered.

These extracts (OLE and MLE) were chosen because of their beneficial effects for human health, material availability and for their notable antioxidant and antimicrobial properties. The concentrations of 2% OLE and MLE solutions were chosen in accordance to the previous successful pretreatment studies achieved by Abdeldaiem *et al.*, (2017) and Khaled *et al.*, (2016), with the potential to extend the shelf life and improve the quality of perishable beef and chicken meat.

Chemical Assessments:

Chemical analyses were made on finely ground chicken meat (from breast and thigh muscles) samples. Analyses were conducted in triplicate. Proximate composition in terms of moisture, ash, crude lipid and total nitrogen of chicken meat were determined according to the methods described in the AOAC, (1995). For pH determination 10 g of chicken 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). The total volatile basic nitrogen (TVB-N) expressed as mg TVB-N per 100 g chicken meat samples was determined according to the method described by Parvaneh (2007). A Thiobarbituric acid reactive substance (TBARS) as mg of malondialdehyde (MDA)/kg chicken meat was estimated according to Kilinc (2007).

Determination of total phenolic:

The total phenolic contents of the OLE and MLE were quantified by spectrophotometric (Thermo Fisher Scientific, Genesys, Madison, USA) measurement of the absorbance according to the Folin Ciocalteu, as reported by Bakari *et al.*, (2015).

Antioxidant activity (DPPH and ABTS) free radical

assays:

The antioxidant capacity of OLE and MLE tested using DPPH free radical scavenging was evaluated by the method described by Cheurfa and Allem (2016). Total antioxidant activity of OLE and MLE was measured in vitro with ABTS assay, and this procedure followed the method described by Ben Nejma *et al.*, (2017) with slight modifications. Each method was replicated three times.

HPLC analysis of phenolic compounds:

The high performance liquid chromatography (HPLC) analysis was carried out for OLE and MLE according to Kim *et al.*, (2006). The separation and determination were performed on Agilent 1260 series - C18 column (4.6 mm \times 250 mm i.d., 5 μm). The column was eluted by water (solvent A) and 0.02% tri-floro-acetic acid in acetonitrile (solvent B) at a flow rate of 1 ml/min. The obtained peaks were monitored simultaneously at 280 nm. Commercial phenolic compounds were used as standards.

Microbiological Analysis:

Twenty five grams of chicken meat samples (from breast and thigh muscles) were aseptically excised from chicken halves 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 rabbit 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 (Ozogul and Uçar, 2013). 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 per gram.

Sensory evaluation of raw chicken meat:

Appearance and odor attributes of raw chicken meat were evaluated by modified acceptance test with 10 non-trained panel members of the laboratory staff. Two chicken halves (breast and thigh muscles) from each group were taken at regular intervals, and immediately packed in small white foam plates, then labeled and served to the panelists at room temperature in random order for evaluation their appearance and odor using 9-points

hedonic scales. 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; 9, like extremely. A score less than 5 indicate that the chicken meat is rejected (Mexis *et al.*, 2009).

Statistical Analysis:

Results were expressed as means and standard deviation (M±SD) from triplicate determinations. Analysis of variance (ANOVA) was performed to compare the effect of oregano leaves extract (OLE) and mulberry leaves extract (MLE) dipping treatments on chicken meat quality. Significant differences were defined as P<0.05; according to Rao and Blane (1985) using PC-STAT program.

Results and Discussion

Proximate composition of chicken meat:

Moisture, protein, fat, ash and carbohydrate content of the chicken meat samples from the whole carcass (from breast and thigh muscles) were 73.66 ± 0.65%, 21.47 ± 0.54, 2.83 ± 0.36%, 1.16 ± 0.13% and 0.88 ± 0.06%, (on wet basis), respectively. These values were similar to those reported by other authors for fresh chicken meat from the whole carcass (Souza *et al.*, 2011 and Abdul Rehman *et al.*, 2016). The analysis revealed that fresh raw chicken meat is rich in proteins and low in fat contents; thus encourage the consumption of chicken meat. The proximate composition reported in the different studies (Puvaca *et al.*, 2015 and Jung *et al.*, 2015) showed some degree of differences, especially for the lipid and moisture contents. Such variations in chemical composition of chicken meat is greatly due to many factors, among them breed, age, sex and feeding system (Abdul Rehman *et al.*, 2016).

Extraction yield:

Ethanol extraction yields of oregano leaves (OL) and mulberry leaves (ML) are given in table 1. After extraction, mulberry leaves provided higher yield (13.54%) than oregano leaves extract (OLE; 8.5%). Similar results were achieved by Iqbal *et al.*, (2012); Sungthong *et al.*, (2014); Cui *et al.*, (2019) for mulberry leaves extract

(MLE) yields. Lower extraction yields from oregano leaves were observed by Duletia-Lauševičė *et al.*, (2018) and Chinprahast *et al.*, (2018). The variation in the yields of plant organs might be ascribed to the different availability of extractable components, resulting from the different chemical composition of plants.

Total phenolic content (TPC):

Phenolic compounds are widely distributed in plants and have gained much attention, due to their antioxidant activities and free radical scavenging capacities, which potentially have beneficial implications for health (Lin *et al.*, 2017). *In vitro*, antioxidant activity of the extracts table 1 showed that MLE was too rich with polyphenols in terms of TPC the values was 14.50 (mg gallic/g dw), followed by oregano leaves extract (OLE) the TPC was 12.00 (mg gallic/g dw). These differences in phenolic contents might be due to plant cultivars, geographical location, extraction conditions and used different standard equivalents. The amounts of phenols determined in oregano leaves extract (OLE) in the present study is in good agreement with Chishti *et al.*, (2014), Duletiā-Lauševičė *et al.*, (2018) and Chinprahast *et al.*, (2018). Similarly, the total phenolic content in mulberry leaves extract (MLE) was in line with Lin *et al.*, (2017); Hao *et al.*, (2018); Przygonski and Wojtowicz, (2019).

Antioxidant activity:

Antioxidant properties of different plant extracts and pure compounds can be evaluated using various *in vitro* assays. In this study, 2, 2 -diphenyl-1-picrylhydrazyl (DPPH) and ABTS method were used for evaluating the ability of samples for scavenging free radicals.

DPPH assay:

Scavenging activity was measured by the decrease in absorbance as the DPPH radical received an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule²³ (Cheurfa and Allem, 2016). The scavenging ability of the oregano leaves extract (OLE) and mulberry leaves extract (MLE) samples on DPPH free radical was shown in table 1. The results showed a dose dependent scavenging power. Especially, the scavenging ability of MLE increased from 30.80% to 86.03%, at 400 ppm, indicating that it has

Table 1: Total phenolics content, DPPH%, ABTS% assay and yield %.

Samples	DPPH%				ABTS%				Total phenolic mg GAE/g	Total yield %
	50ppm	100 ppm	200ppm	400 ppm	50 ppm	100 ppm	200 ppm	400 ppm		
Mulberry	30.80	49.21	73.40	86.03	32.10	52.00	76.15	88.00	14.50	13.54
Oregano	38.00	57.90	63.78	84.50	41.50	59.95	66.00	84.50	12.00	8.50
BHT	56.75	74.55	87.40	-	58.45	76.53	90.00	-	-	-

All values reflect the mean and standard deviation are mean of triplicate determinations.

generally better scavenging ability, even than BHT (87.40%).

DPPH of OLE increased from 38.00 to 84.50% at the same concentration. Similar results were achieved by other authors (Chishti *et al.*, 2014; Boroski *et al.*, 2018) for oregano leaves extract. Our results table 1 are in contrast with the study of Iqbal *et al.*, (2012); Sungthong *et al.*, (2014); Hao *et al.*, (2018); Przygonski and Wojtowicz, (2019); Lim and Teo, (2019) for mulberry leaves extract. Usually, higher total phenol contents lead to better DPPH scavenging activity (Hao *et al.*, 2018). As known, polyphenols have a metal chelating potential and their redox properties can be justified by their chemical structure. For this reason, the high polyphenolic content in MLE and OLE may explain the high antioxidant activity.

Scavenging activity of ABTS• + free radical:

The principal objective of this test is to measure the capacity of different substances to scavenge the ABTS.+ radical cation. The results showed a dose dependent scavenging ABTS.+ radical cation. As shown in table 1, the scavenging ability of mulberry leaves extract (MLE) was 88.00 % at 400ppm, which was little than BHT (90.00%) at 200 ppm, indicating that it has generally better scavenging ability. ABTS of oregano leaves extract (OLE) was 84.50% at the same concentration. It is obvious that tested samples are effective to provide their capacity to scavenge the ABTS.+ radical cation. The results of ABTS of MLE are within the previous studies by other authors (Iqbal *et al.*, 2012; Hao *et al.*, (2018); Przygonski and Wojtowicz, (2019) and Kobus-Cisowska *et al.*, 2020), who reported that mulberry leaves extract is a rich source of natural antioxidants.

On the other hand, the results of ABTS of oregano leaves extract (OLE) are consistent to other findings (Chishti *et al.*, 2014; Rostro-Alanis *et al.*, 2019 and Cao *et al.*, 2019). Extracts from MLE and OLE revealed a high significance level ($P < 0.05$) between ABTS• + radical and TPC. The positive and significant correlation between TPC and ABTS antioxidant activity strengthens the results observed in the DPPH scavenging method used in this study. This investigation confirms the hypothesis that an increase in total phenolic compounds will increase the antioxidant activity of extracts.

High performance liquid chromatography (HPLC):

High performance liquid chromatography (HPLC) was used to identify and quantified the phenolic compounds that were present in the studied mulberry leaves methanolic extract, and the results are illustrated in Fig. 1. From which it is apparent that, the components assayed for mulberry leaves methanolic extract (according to their retention times), were as follows: 3.115- Gallic acid (13.51%), 3.528- Chlorogenic (58.04%), 3.936- Catechin (2.66%), 4.141- Caffeine (0.2983%), 5.010- Caffeic (0.3138%), 5.856- Pyro catechol (3.2444%), 8.530- Vanillin (1.0680%), 8.960- Ferulic acid (2.3800%), 9.519- Naringenin (4.1476%), 10.127- Propyl gallate (0.2336%), 10.689- Quercetin (0.4514%) and 11.212- Cinnamic (0.1377%) were positively identified in the present study by HPLC analytical system.

The HPLC chromatogram Fig. 1 also reveal that the dominant phenolic compound was Chlorogenic (58.04%), while the peak produced for Cinnamic (0.1377%) was low which indicated that it was found in very small quantities. Such results are in close agreement with those

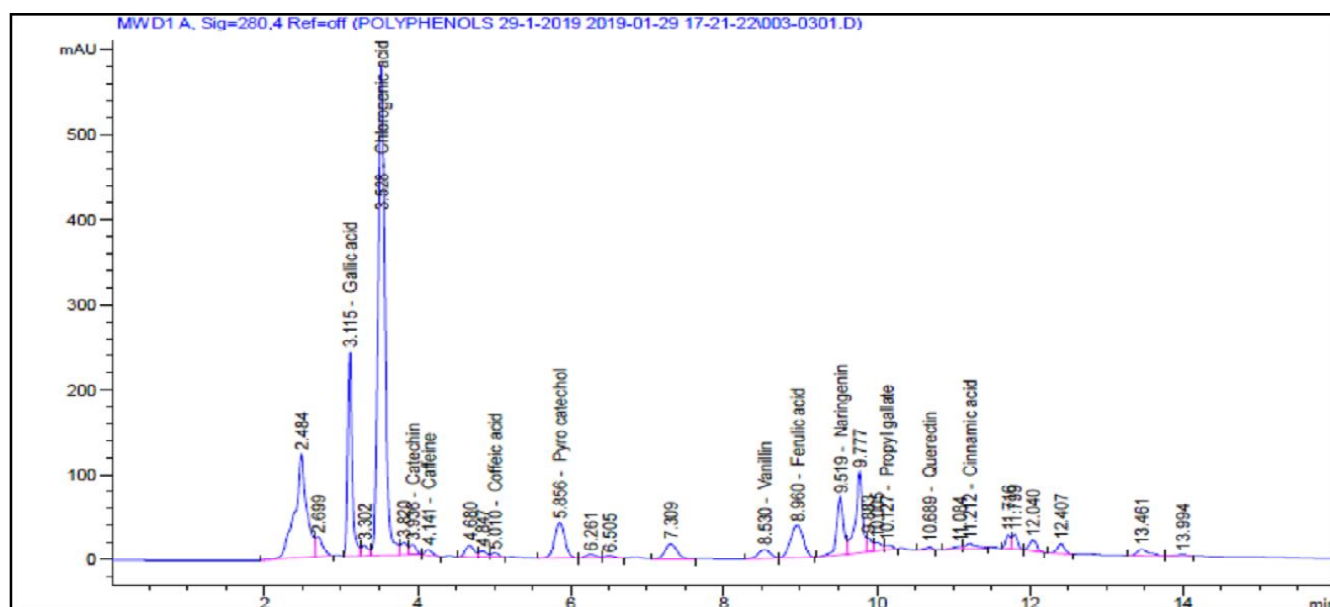


Fig. 1: HPLC chromatogram of mulberry leaves methanolic extract (MLE).

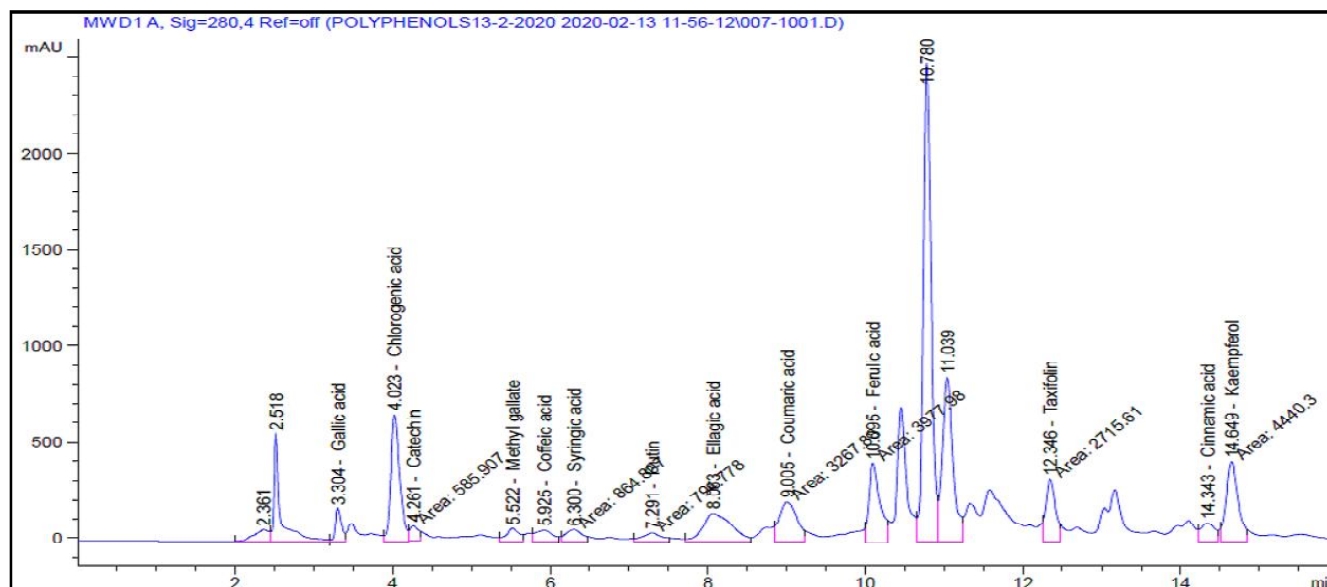


Fig. 2: HPLC chromatogram of oregano leaves methanolic extract (OLE).

reported by other authors (Flaczyk *et al.*, 2013 and Hao *et al.*, 2018).

The components assayed for oregano leaves extract (OLE) (according to their retention times), were as follows: 3.304- Gallic acid (4.415%), 4.023- Chlorogenic acid (20.306%), 4.261- Catechin (5.927%), 5.522- Methyl gallate (0.63%), 5.925- Caffeic acid (1.529%), 6.300- Syringic acid (1.63%), 7.291- Rutin (5.215%), 8.063- Ellagic acid (11.61%), 9.005- Coumaric acid (3.159%), 10.095- Ferulic acid (6.57%), 12.346- Taxifolin (19.91%), 14.343- Cinnamic acid (0.785%), and 14.649- Kaempferol (18.296%) were positively identified in OLE in the present study by HPLC analytical system.

The HPLC chromatogram Fig. 2 also reveal that the dominant phenolic compound in OLE was Chlorogenic acid (20.306%), while the peak produced for Methyl gallate (0.63%) was very low which indicated that it was found in very small quantities. Such results are in close agreement with those reported by other authors (Spiridon *et al.*, 2011 and Duleti -Lau evia  *et al.*, 2018), who found that rosmarinic acid, caffeic acid, chlorogenic acid,

arbutin and luteolin-7-*O*-glucoside were the major phenolic acids and flavonoids in oregano leaves extract (OLE).

Sensorial characteristics changes of raw chicken meat during cold storage:

Chicken meat samples in the course of chilling storage were examined for sensory (appearance and odor) scores, the results is depicted in table 2. The control and treated chicken meat samples showed excellent overall acceptability by panelists at zero day of evaluation, indicating that both natural extracts had no deleterious effect on the sensory attributes. Sensory evaluation of control, mulberry leaves extract (MLE) and oregano leaves extract (OLE) treated chicken meat reached the limits of acceptance after 6, 8 and 10 days of storage, respectively table 2. End of shelf life is usually determined when spoilage-related sensory attributes such as off-odor and flavor becomes strong, caused mainly by microbial origin (Gram and Huss, 1996). Chicken meat contains high level of PUFA, which is susceptible to autooxidation causing off-odors and browning of flesh color. The off-

Table 2: Sensory (appearance and odor) scores of chicken meat during chilling storage.

Treatment/Day		0	1	3	6	8	10
Control (C)	Appear. scores	9.00±0.17 ^a	8.26±0.19 ^c	7.12±0.15 ^c	5.65±0.21 ^c	3.61±0.26 ^c	2.43±0.26 ^c
MLE (2%)		8.84±0.14 ^a	8.50±0.11 ^b	7.65±0.10 ^b	6.34±0.40 ^b	5.28±0.15 ^b	3.42±0.30 ^b
OLE (2%)		8.92±0.13 ^a	8.76±0.14 ^a	8.18±0.17 ^a	7.25±0.35 ^a	6.18±0.36 ^a	5.26±0.15 ^a
Control (C)	Odor scores	8.83±0.15 ^a	8.16±0.30 ^c	6.85±0.53 ^c	5.10±0.54 ^c	3.16±0.19 ^c	2.18±0.28 ^c
MLE (2%)		8.75±0.21 ^a	8.31±0.42 ^b	7.24±0.26 ^b	6.17±0.46 ^b	5.10±0.13 ^b	3.25±0.17 ^b
OLE (2%)		8.90±0.32 ^a	8.52±0.22 ^a	7.68±0.39 ^a	7.00±0.28 ^a	6.08±0.40 ^a	5.14±0.48 ^a

Values represent of 10 panelists (Mean+S.E). A score higher than 5 indicate that the chicken meat is accepted. There is no significant difference ($P>0.05$) between the values having the same superscripts in the same column. MLE: Mulberry leaves extract., OLE: oregano leaves extract.

flavor intensity of the treatment groups remained at low levels compared to the control group until the eights and tens of the storage periods.

Declines in sensory quality were assessed from the 3rd days of storage in samples stored at $4\pm 1^\circ\text{C}$. On advancement of storage period, the overall mean scores of sensory attributes (appearance and odor) were sharply decreased ($P<0.05$) irrespective of treatment, as a result of microbial spoilage, oxidation of lipid and degradation of protein in the chicken meat. The application of mulberry leaves extract (MLE) dipping to the chicken meat stored at $4\pm 1^\circ\text{C}$ led to an improvement in the appearance and odor of the samples, which received higher scores than those of the control ($P<0.05$) until day 8 of storage table 2. Similarly, dipping chicken carcasses in 2% oregano leaves extract (OLE) led to an improvement in the appearance and odor of chicken samples under investigation till the end of chilling course (10 days). MLE and OLE are allowing an extension of 2-4 days for the shelf life. Similar results were obtained from the other studies (Salem *et al.*, 2013; Abdeldaiem *et al.*, 2017; Khaled *et al.*, 2016 and Moawad *et al.*, 2020).

Microbiological count changes

The antimicrobial activity of MLE and OLE in chicken meat samples stored at $4\pm 1^\circ\text{C}$ for 10 days, are shown in table 3. TVC are used as an acceptability index for meat products because of the effect of bacteria in spoilage. The statistical analysis of TVC revealed significant difference ($P>0.05$) between control and extract treated chicken meat samples during refrigeration storage, while the increase in the storage time produce significant proliferations in TVC ($P<0.05$), whatever the treatment conditions. However, TVC reached and exceeded a value of 6 log cfu/g, considered as the upper microbiological limit for good quality chicken meat, as

defined by the Egyptian Standard 2005, after day 6 for the control chicken samples.

The use of the OLE was the most effective, achieving the greatest reduction and TVC reached the limit of 6 log cfu/g after 10 days storage period resulting in a shelf life of 10 days as compared to the control samples (6 days). With regard to MLE resulted in a shelf-life of 8 days as compared to control samples. Similar extension periods have been reported in muscle origin food by other authors (Salem *et al.*, 2013; Khaled *et al.*, 2016; Abdeldaiem *et al.*, 2017; Duletia-Lauševia *et al.*, 2018 and Moawad *et al.*, 2020).

The changes in psychrotrophic count were approximately similar to those of TVC, with control also being the highest followed by samples treated with MLE and much lower counts was detected in samples treated with OLE. The psychrotrophic counts in broiler chicken carcasses were slightly lower than the TVC. This was true for all of the three groups analyzed. As the storage time increased, the PTC steadily increased. The *Enterobacteriaceae* were the most affected organisms, where MLE- and OLE-dipping caused great reduction in their counts in chicken meat. On the contrary, psychrotrophs were the least affected bacteria as the MLE- and OLE-dipping achieved lower mean log reductions in their counts.

Compared to control, Enterobacteriaceae grew in MLE or OLE treated chicken samples at a slower rate and never exceeding 10^3 CFU/g. At the end of the storage period (day 10), treated chicken meat exhibited much lower ($P<0.05$) counts as compared with control samples table 3. These findings could be attributed to antibacterial impacts of ethanolic extracts of MLE and OLE (Chishti *et al.*, 2014; Chen *et al.*, 2019; Rostro-Alanis *et al.*, 2019 and Hac˘-Szymanczuk *et al.*, 2019). Heleno *et al.*,

Table 3: TVC, PTC and EBC (as logCFU/g) of raw chicken meat during cold storage for 10 days.

Treatment/Day		0	1	3	6	8	10
Control (C)	TVC	4.18±0.28 ^a	4.52±0.17 ^a	5.14±0.12 ^a	5.85±0.13 ^a	6.38±0.15 ^a	7.16±0.13 ^a
MLE (2 %)		4.06±0.15 ^b	4.28±0.19 ^b	4.83±0.18 ^b	5.27±0.19 ^b	5.85±0.19 ^b	6.52±0.17 ^b
OLE (2 %)		3.94±0.17 ^b	4.10±0.23 ^c	4.47±0.43 ^c	4.90±0.27 ^c	5.42±0.24 ^c	6.00±0.30 ^c
Control (C)	PTC	3.85±0.12 ^a	3.92±0.14 ^a	4.45±0.18 ^a	5.36±0.35 ^a	6.26±0.13 ^a	6.85±0.14 ^a
MLE (2 %)		3.67±0.19 ^a	3.80±0.25 ^b	4.19±0.27 ^b	4.87±0.12 ^b	5.47±0.17 ^b	6.41±0.27 ^b
OLE (2 %)		3.58±0.23 ^{ab}	3.64±0.16 ^c	4.00±0.12 ^c	4.56±0.16 ^c	5.10±0.24 ^c	5.72±0.42 ^c
Control (C)	EBC	2.76±0.24 ^a	2.93±0.23 ^a	3.14±0.21 ^a	3.27±0.27 ^a	3.58±0.12 ^a	3.82±0.13 ^a
MLE (2 %)		2.69±0.17 ^a	2.78±0.37 ^b	2.96±0.18 ^b	3.11±0.46 ^b	3.34±0.16 ^b	3.56±0.21 ^b
OLE (2 %)		2.52±0.32 ^{ab}	2.63±0.26 ^c	2.78±0.41 ^c	2.90±0.32 ^c	3.15±0.28 ^c	3.32±0.15 ^c

All values reflect the mean and standard deviation are mean of triplicate determinations.

There is no significant difference ($P>0.05$) between the values having the same superscripts in the same column. TVC: Total Viable Count – PTC: psychrotrophic count –EBC: Enterobacteriaceae count. MLE: 2% mulberry leaves extract.; OLE: 2% oregano leaves extract.

(2015) reported that phenolic acids such as protocatechuic, vanillic ferulic and caffeic acids could be used as antimicrobial agents because of the presence of carboxylic acid (COOH), two hydroxyl (OH) groups in *para* and *ortho* positions of the benzene ring and also a methoxyl (OCH₃) group in the *meta* position. In addition, mulberry leaves have shown strong antimicrobial activity due to some substances known as kuwanon C, mulberrofuran G, mourin and albanol B (Kostiæ *et al.*, 2013; Salem *et al.*, 2013 and Chen *et al.*, 2019). On the other hand, antimicrobial activity of OLE are mainly attributed to carvacrol and thymol that constitute about 78 to 82% of the total oil (Boroski *et al.*, 2018 and Cao *et al.*, 2019).

pH changes:

pH values of control and extract treated chicken meat were depicted in table 4, from which it is apparent that dipping chicken carcasses in MLE and OLE solutions reduced the initial pH values of chicken carcasses when compared to control samples, but the difference is not significant ($P < 0.05$). The pH value of all chicken carcass samples slightly decreased during the first 3 days of storage, by more time of refrigeration storage pH values increased in different degrees within untreated and treated chicken meat samples as shown in table 4. This decrease indicates that some fermentation occurs during storage. The last pH values increase might have been due to the liberation of ammonia compounds as a result of enzyme activity or the proteolytic microbial flora present in the raw chicken (Gram and Huss, 1996).

The increase in the storage time, produce significant increase in pH values ($P < 0.05$), whatever the treatment conditions. Soaking chicken carcasses in natural extracts (MLE and OLE) resulted in significant ($P < 0.05$) reduction in pH values when compared with control chicken carcasses during storage. The pH value of

control non-treated chicken was above the acceptable limit (7.1) with objective signs of deterioration after the 6th day of storage. The pH values of chicken carcass soaked in MLE and OLE solutions were higher than the acceptable limit at the 8th and 10th day of storage at 4 ± 1 °C, respectively table 4. These results were in good agreement with previous authors after treatment of different meat products with natural extracts (Salem *et al.*, 2013; Khaled *et al.*, 2016; Abdeldaiem *et al.*, 2017 and Moawad *et al.*, 2020).

However, the lower pH values of extract treated chicken samples reflect protection properties of MLE and OLE against microorganisms, which reduce the accumulation of basic substances (Chishti *et al.*, 2014; Rostro-Alanis *et al.*, 2019 and Hac´Szymanczuk *et al.*, 2019). In this sense, although pH value cannot be considered as an important index to determine chicken meat spoilage, it can be useful as a guideline for quality control of meat and meat products (Abdeldaiem *et al.*, 2017 and Moawad *et al.*, 2020).

Total volatile basic nitrogen (TVB-N):

Protein decomposition is one of the main causes for chicken meat quality deterioration (Parvanch, 2007). The TVB-N values of control and extract treated chicken carcasses are shown in table 4. Chicken carcasses soaked in natural extracts (MLE and OLE) were significantly ($P < 0.05$) lower than those of control chicken samples after dipping and during storage. A sharp rise of TVB-N value was noticed in the control and treated chicken samples during refrigerated storage at 4 ± 1 °C. The TVB-N values of control chicken carcasses reached value 24.18 mg/100g (above the acceptable limit, 20 mg/100g) after the 6th day of storage with the objective signs of spoilage. Chicken carcasses soaked in MLE and OLE solutions revealed TVBN values higher than the

Table 4: Chemical indices of raw chicken meat during cold storage at 4 ± 1 °C for 10 days.

Treatment/Day		0	1	3	6	8	10
Control (C)	pH	6.24±0.17 ^a	6.21±0.32 ^a	6.15±0.14 ^a	6.48±0.42 ^a	7.36±0.13 ^a	7.82±0.12 ^a
MLE (2 %)		6.17±0.23 ^a	6.14±0.18 ^a	6.10±0.25 ^a	6.32±0.21 ^b	6.82±0.27 ^b	7.46±0.16 ^b
OLE (2 %)		6.13±0.15 ^a	6.10±0.12 ^a	6.00±0.16 ^a	6.18±0.11 ^c	6.54±0.22 ^c	6.92±0.28 ^c
Control (C)	TVB-N	11.16±0.13 ^a	13.26±0.18 ^a	16.32±0.15 ^a	19.28±0.16 ^a	24.17±0.18 ^a	28.73±0.19 ^a
MLE (2 %)		10.28±0.21 ^b	11.57±0.32 ^b	14.45±0.23 ^b	17.62±0.21 ^b	20.00±0.15 ^b	24.35±0.14 ^b
OLE (2 %)		9.36±0.17 ^c	10.76±0.12 ^c	12.37±0.11 ^c	15.16±0.14 ^c	17.96±0.36 ^c	19.84±0.25 ^c
Control (C)	TBARS	0.23±0.15 ^a	0.35±0.17 ^a	0.64±0.11 ^a	0.86±0.18 ^a	1.34±0.23 ^a	1.53±0.13 ^a
MLE (2 %)		0.16±0.21 ^a	0.24±0.23 ^a	0.42±0.17 ^b	0.70±0.35 ^b	0.85±0.16 ^b	1.27±0.14 ^b
OLE (2 %)		0.12±0.13 ^a	0.18±0.18 ^b	0.27±0.32 ^c	0.48±0.14 ^c	0.74±0.12 ^c	0.92±0.24 ^c

All values reflect the mean and standard deviation are mean of triplicate determinations.

There is no significant difference ($P > 0.05$) between the values having the same superscripts in the same column. Total volatile basic nitrogen (TVBN, as mg N/100g meat). Thiobarbituric acid reactive substances (TBARS, as mg MDA/kg meat. – MLE: 2 % mulberry leaves extract.; OLE: 2 % oregano leaves extract.

acceptable limit of 20 mg/100g (Egyptian Standards, 2005), at 8th and 10th days of storage, respectively table 4. These results indicated that natural extracts (MLE and OLE) have the ability to protect chicken from protein degradation. This observation was in a good agreement with other authors (Salem *et al.*, 2013; Khaled *et al.*, 2016; Abdeldaiem *et al.*, 2017 and Moawad *et al.*, 2020), who reported that treatment of meat by MLE and OLE were very effective in inhibiting microbial growth, reduced chemical and enzymatic deterioration reactions and prolonged the shelf-life.

The thiobarbituric acid reactive substances (TBARS):

TBARS value is used as an index of meat quality during storage (Kilinc *et al.*, 2007). Data presented in table 4 showed the changes that took place in TBARS values of raw chicken meat samples during refrigerated storage for 10 days. The TBARS values of chicken carcasses soaked in MLE and OLE solutions were significantly ($P < 0.05$) lower than those of control chicken samples after dipping process and during cold storage. The TBARS values of fresh control and treated chicken carcasses were not significantly ($P > 0.05$) different at the beginning of cold storage (zero time), indicating that oxidation of lipid occurred during refrigerated storage. The increase in the storage time, produce significant increase in TBARS values ($P < 0.05$), whatever the treatment conditions. This might be due to auto-oxidation of lipids over a period of low temperature storage. The TBARS values of control chicken samples reached value 1.34 mg/kg (above the acceptable limit, 0.9 mg/kg) after the 6th day of storage with the objective signs of spoilage.

Chicken carcasses dipped in MLE and OLE solutions remained higher than the acceptable limit, 0.9 mg/kg as defined by the Egyptian Standards, 2005 after 8th and 10th days of storage, respectively table 4. These results indicated that mulberry leaves extract (MLE) and oregano leaves extract (OLE) have antioxidant activities in chicken meat (Rostro-Alanis *et al.*, 2019; Cao *et al.*, 2019; Przygonski and Wojtowicz, 2019 and Kobus-Cisowska *et al.*, 2020). The antioxidant activities of these natural extracts have been observed previously in different meat products (Salem *et al.*, 2013; Khaled *et al.*, 2016; Abdeldaiem *et al.*, 2017; Duletia-Lauševia *et al.*, 2018 and Moawad *et al.*, 2020). The antioxidant activity of natural extracts (MLE and OLE) have been attributed to their phenolic compounds which act by terminating the free radical chain reaction by donating hydrogen or electrons to free radicals and converting them to more stable products (Chishti *et al.*, 2014; Lin *et al.*, 2017; Duletia-Lauševia *et al.*, 2018; Chinprahast *et al.*, 2018

and Hao *et al.*, 2018).

Conclusion

The current study indicated that TFC, DPPH and ABTS were higher in MLE than OLE. The results also showed that HPLC chromatogram of MLE contains 12 phenolic compounds Fig. 1 the dominant phenolic compound was Chlorogenic (58.04%), while the peak produced for Cinnamic (0.1377%) was the lowest. In addition, HPLC chromatogram of OLE contains 13 phenolic compounds Fig. 2 the dominant phenolic compound was Chlorogenic acid (20.306%), while the peak produced for Methyl gallate (0.63%) was very low. The results also concluded that soaking chicken carcasses in cold solutions containing mulberry leaves extract (MLE; 2%) or Oregano leaves extract (OLE; 2%) was 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 chicken carcasses during refrigerated storage by 2-4 days more than that of control samples (6 days). Therefore Mulberry leaves extract (MLE) and Oregano leaves extract (OLE) can be applied as safe natural preservatives for chicken carcasses under refrigerated storage at $4 \pm 1^\circ\text{C}$.

Significance Statements

This study demonstrates the potential use of mulberry leaves extract (MLE) and oregano leaves extract (OLE) dipping treatments to improve the microbial quality, retard lipid oxidation, maintain the freshness indices and sensory score of treated chicken carcasses. Regarding the control non-treated samples, all measured parameters were above the acceptable limits after the 6th day of storage with the appearance and odor scores of objective signs of deterioration; therefore, control can be acceptable until the 6th day of refrigerated storage. Chicken samples treated with MLE and OLE revealed values higher than the acceptable limits for pH, TVBN, TBARS and bacterial counts with sensory scores higher than the acceptable limits at the 8th and 10th days of storage, respectively. Therefore, these natural extracts dipping can extend the shelf –life of chicken meat for 2-4 days more than the control (6 days) due to their higher TFC, DPPH and ABTS.

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