Asian Journal of Scientific Research

ISSN 1992-1454

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OPEN ACCESS Asian Journal of Scientific Research

ISSN 1992-1454
DOI: 10.3923/ajsr.2017.215.226

Research Article
Preventive Effect of Olive Leaves Extract in Combination with Tannic Acid on the Quality Losses of the Refrigerated Ground Beef Patties

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Abstract

Background and Objective: Muscle Origin Foods (MOF) are rich sources of high-quality nutrients but they are also very much susceptible to spoilage due to chemical, microbial and enzymatic activities. These activities can precipitate in major deterioration of the color, sensory attributes and safety of these products. So the present study aims to investigate the effect of incorporating Olive Leaves Extract (OLE; 1%) with or without Tannic Acid (TA; 0.02%) on the quality characteristics and shelf-life extension of raw ground beef patties. **Materials and Methods:** Chemical indices, sensorial properties, microbiological status and color changes were evaluated during the course of 14 days refrigerated storage at 4±1°C under aerobic packaging. Data were subjected to one-way analysis of variance (ANOVA) to compare the effect of OLE and/or TA treatments. **Results:** The obtained results showed that individual addition of OLE and/or TA ($p<0.05$) affected color parameters, lipid stability, microbiological loads and protein degradation. They also increased the appeal to consumers of ground beef patties as compared to control samples. Tannic Acid (TA) exhibited more significant ($p<0.05$) oxidative stability and had a noteworthy effect on the patties' appearance. In contrast, OLE-treated patties showed lower significant ($p<0.05$) microbiological proliferation and odor deterioration as compared to other treatments. In addition, combination of OLE with TA provided the best antioxidative, antimicrobial protection, the highest sensory scores and the longest shelf-live; indicating a possible synergistic effect. **Conclusion:** The results of this study indicate that cold storage time had a significant effect on the quality loss of ground beef patties. Moreover, the tested OLE and/or TA have potential as natural preservatives to reduce microbial load, color degradation, lipid oxidation and extended the shelf life by 3-6 days over that of raw control beef patties (8 days).

Key words: MOF, quality loss, lipid stability, shelf-life, olive leaves extract, tannic acid

Received: April 18, 2017 Accepted: May 11, 2017 Published: June 15, 2017

Citation: Robiel Kamel Moawad, Amir Amin Ibrahim, Nahed Mohamed Abdelmaguid, Wafaa Aboelsood Ibrahim and Abdelaziz Nadir Shehata, 2017. Preventive effect of olive leaves extract in combination with tannic acid on the quality losses of the refrigerated ground beef patties. Asian J. Sci. Res., 10: 215-226.

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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

Muscle Origin Foods (MOF), such as meat burger and patties which are among the most popular and consumed meat products around the world are rich sources of nutrients[1]. They provide good quality animal proteins, essential amino acids, fatty acids, minerals and vitamins, particularly B-complex[2]. MOF also provide favorable growth conditions for spoilage and common food-borne pathogens, which give rise to quality reduction, meat spoilage and economic loss; representing a high risk for consumer health[3,4].

Extremely perishable MOF are also very much susceptible to spoilage due to chemical and enzymatic activities[5]. The breakdown of proteins, fats and carbohydrates of meat results in the development of off-odors, off-flavors and slim formation which make the meat objectionable for human consumption[6]. Moreover, oxidative processes, which occur during raw material storage, processing, heat treatment and further storage of final meat products, can precipitate major deterioration in many quality characteristics such as color, texture, nutritive value and safety of meat products[7]. Thus, the application of suitable agents possessing both antioxidant and antimicrobial activities is a major concern for increasing consumer safety and quality, extending shelf-life and preventing economic loss[8].

Various synthetic chemical preservatives are being used as antioxidant and antimicrobial agents to combat the above-mentioned problems[9]. Since the carcinogenic activity of synthetic preservatives (e.g., nitrate and nitrite compounds, BHT, BHA and TBHQ) has been demonstrated[10,11]. There is at present a growing interest, both in the industry and in the scientific research, for using natural compounds such as herbs, spices, fruits and vegetables extracts or powders and essential oils in food protection[12]. These natural compounds keep food safe for consumers and they have been widely reported as having especially potent antioxidant effects in various meats[13]. They also have a higher consumer acceptability and greater application in extending the shelf- life of food[14,15]. Olive Leaves Extract (OLE) have an ancient history of nutritional, medicinal and traditional usages, is one of the most popular plant extracts that can be used as a meat preservative[16-18].

Olive (Olea Europaea L.) leaves are an abundant by-product (agricultural waste) from olive trees and considered a cheap raw material containing bioactive phenolic compounds comparable to olive oil and fruits but in higher concentrations[18,19]. Olive Leaves Extract (OLE) contains a high level of potentially useful phytochemicals such as oleuropein, verbascoside, rutin, tyrosol, hydroxytyrosol; flavonoids (luteolin, apigenin and quercetin); as well as caffeic acid and tannins[20,21]. Due to the presence of polyphenols, flavonoids and other beneficial compounds in olive leaves, many other health benefits were also mentioned, such as reduction of platelet aggregation, decrease the production of aflatoxin, retard lipid oxidation and microbial growth and also have anti-hypertensive and anti-cancer activities[22,23].

Another natural preservative found in most plant foods (such as food grains, fruits and tea) in different concentrations is tannic acid or tannins, which is a water-soluble polyphenol[24]. The FDA categorized tannic acid as a Generally Recognized As Safe (GRAS) food additive in some food products including meat products[25]. Tannins and their derivatives showed significant antimicrobial activity against several food-borne pathogens[26,27]. Strong evidence indicated that tannic acid has anti-carcinogenic effects[22,28]. Tannic acid effectively inhibited the lipid oxidation and maintained the bright red color in muscle based products through their ability to prevent hydroxyl radical formation, radical-scavenging activity and metal-chelating activity[28,29]. Therefore, tannic acid can be a natural additive with multiple preservative functions in meat and meat products[26,30].

Ground beef patties are typical examples of acceptable fast foods, which are increasing in popularity and have extensively developed in the world food market[31]. Both TA and OLE are classified as GRAS ingredients in ready-to-cook/eat meat based products. Although several studies have demonstrated the beneficial effects of these natural preservatives on microbiological safety, sensory properties, lipid oxidation and color preservation, their effects when applied in combination with the quality of perishable foods have not been clearly investigated. Accordingly, the aim of this study is to consider the antioxidant and antimicrobial activities of Olive Leaves Extract (OLE; 1%), Tannic Acid (TA; 0.02%) and their combination (OLE+TA) to determine the freshness quality and shelf-life (sensorial, microbiological and chemical attributes) of raw ground beef patties formulated with OLE and/or TA prior to refrigerated storage at 4±1°C for 14 days. Proximate composition of raw fresh patties paste and powdered Olive Leaves (POL) were also included.

MATERIALS and METHODS

The laboratory used for the study: The experiment was conducted in the Laboratory of Food Science and Technology, National Research Centre (NRC), Dokki, Egypt.

Chemicals and supplies: Plate Count Agar (PCA) and peptone water were from Oxoid (UK). Methyl red, 2-thiobarbituric acid,

Bromo cresol green, BHT and TCA were from Sigma-Aldrich (Germany). Sodium chloride and magnesium oxide were from El-Nasr Pharm and Chem. Co., (Egypt). All other solvents and chemicals (glacial acetic acid, sulphuric acid, boric acid, hydrochloric acid, methanol, ethanol, etc.,) used were of analytical grade or the highest grade available and were obtained either from ADWIC or El-Gomhouria Companies, Cairo (Egypt). Olive Leaves Extract (OLE) and Tannic Acids (TA) were used in this study as sources of antioxidant and antimicrobial agents. Tannic acid powder containing 90% tannin was obtained from Sigma-Aldrich (USA). Fresh green olive leaves were collected from the west experimental farm at Giza, Egypt, to prepare OLE. Potato starch, corn flour and bread crust powder were purchased from the local market at Dokki, Giza.

Preparation of the Olive Leaves Extract (OLE): The fine-quality fresh green of 'Baladi variety' olive leaves (*Olea Europaea* L.) were collected during the pruning of trees (March 2016), from the West Farm of Faculty of Agriculture, Cairo University, Giza, Egypt. The leaves were cleaned from extraneous matter and properly washed then dried in hot air-oven for 18 h at 40°C. The dried leaves were ground in a blender to form a powder. Thereafter, powdered samples were macerated with 70% ethanol (1:10 w/v) in a closed conical flask for 24 h at room temperature in the dark. The extract centrifuged at 3000 rpm for 10 min at 20°C, the resultant (dark green-brown mixture) was then filtered through Whatman No. 1 filter paper[17,23] and the filtrate was concentrated 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 a final concentration of 0.5, 1 and 2% for primary experimental.

Meat source and patties preparation: About 16.0 kg of beef chuck were obtained 24 h postmortem from El-Moustafa butcher shop at Giza, Egypt and immediately transported in ice box to the laboratory, the external fat, bone and connective tissues were removed from meat samples under aseptic conditions, beef samples were then frozen at -20°C until processing into patties on the next day of purchasing. After thawing, they were cut into small cubes and minced twice (first minced through a 6 mm "coarse grinding").

Beef patties were prepared without seasonings using a simple traditional formulation[31], with a minor modification: 88% minced beef included fat, 4% potato starch, 4% corn flour, 2% bread crust powder and sodium chloride 2% were added as ingredients. These ingredients were mixed together by hand for 5 min using gloved fingers to avoid cross-contamination of minced beef. Afterward, iced water (10%; v/w) was added and, re-minced through 3 mm holes "fine grinding" to ensure uniform distribution of the ingredients. The homogenized meat mixture obtained was divided into 4 batches (3.0 kg each) to prepare the experimental treatments, packed in low-density polyethylene bags and tempered in a freezer for 30 min before treatments[32].

Beef patties treatments: Each of the previous batches was mixed separately for 8 min with the appropriate antioxidants and antimicrobials, first OLE and/or TA was dispersed in about 5 mL of distilled water, added to the raw minced beef and mixed thoroughly[33]; all other meat batches were formulated to contain the same amount of water for uniformity. The first batch was designated as control (C) meat without any extract), while the other 3 treatments contained TA (0.02%), OLE (1%) or TA+OLE (0.02+1%) as combined application. The concentrations of TA and OLE; which are sensorial acceptable and also effective against the bacterial load, were based on the primary studies as indicated below and also showed the best antioxidative and antimicrobial effect in the studies of Maqsood and Benjakul[26] and Baker[23] on minced meat patties.

Storage condition: After completing the mixing, the mixture of each formula was shaped manually into patties (60 patties of approximately 50±3 g weight, 8.5 cm in diameter and 1 cm thickness), using a Petri dish in the laboratory[34]. The patties were packed separately in polyethylene bags (with 2 patties in each bag), then labeled and stored at 4±1°C for 14 days. Five bags of each group were withdrawn at regular intervals over storage period (0, 3rd, 5th, 8th, 11th and 14th day) for chemical, microbiological, sensorial and Hunter color values evaluations. The chemical and microbiological determinations were made on finely ground samples. Averages of 3 replicates were considered.

Optimization of the conditions of using OLE and TA in Beef patties: Initial studies were conducted to obtain the most effective treatments of each natural substance on the sensory and antimicrobial attributes of raw ground beef patties. OLE was used at 0, 0.5, 1 and 2%; while TA was used at 0, 50, 100 and 200 ppm (They were separately dispersed first in about 5 mL of distilled water), mixed with the minced beef and formed into patties (50±3 g) using a Petri dish. Beef

patties samples were packaged individually in polyethylene bags and stored for 8 days in a chilled storage (4±1°C). During this storage, the sensory (appearance and odor) of the raw patties was conducted daily, whereas Total Viable Count (TVC) was conducted on the initial day (0 days) and final days (day 8) of storage. The best concentration of natural substances was determined.

Analytical techniques

Proximate composition and chemical criteria analyses: Proximate composition in terms of moisture, ash, crude lipid and total nitrogen (by applying the Micro-Kjeldahl method) were determined according to the methods described in the AOAC[35]. Carbohydrate content was determined by percentage differences of other contents. The Total Volatile Basic Nitrogen (TVB-N) expressed as mg TVB-N per 100 g beef was measured by steam-distillation according to the method of Parvaneh[36]. A Thiobarbituric Acid Reactive Substance (TBARS) was determined according to the method proposed by Kilinc *et al.*[37]. TBARS content was expressed as mg of malondialdehyde (MDA) kg^{-1} meat. For pH determination 10 g of ground beef patty samples were homogenized in 100 mL distilled water for 1 min in a warring blender and the pH values of the slurry were measured at room temperature using pH meter (JENWAY, 3510; UK) standardized at pH 4 and 7, as described by Ozyurt *et al.*[38].

Bacterial enumeration: Total Viable Count (TVC) and Psychrotrophic Count (PTC) of beef patties samples were determined by Plate Count Agar (PCA). Patty samples were aseptically taken and homogenized with sterile peptone water. After decimal dilutions, 0.1 mL of each dilution was spread on PCA. Then, all plates were prepared in triplicate and incubated for 24 h at 35°C for TVC and 7 days at 7°C for PTC. After specific incubation periods, plates showing 25-250 colonies were counted. The number of colonies was multiplied by the reciprocal of the respective dilution and expressed as log CFU g^{-1} [39].

Sensory assessment of raw beef patties: Modified acceptance test with 10 non-trained panel members of the laboratory staff was carried out using 9-points hedonic scales, following the procedures described by Kassem *et al.*[40]. The whole 4 raw beef patties were taken from each group at regular intervals and immediately packed in small white foam plates, then labeled and served to the panelists at room temperature in random order, to evaluate their appearance (the first impression when looks the product), odor (the intensity of Patty odor) and overall acceptability (it calculated to determine how much a person like or dislike the patties samples through considering the average measures). 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 of 5 was taken as the lower limit of acceptability.

Color measurement: Beef patties samples were allowed to bloom for 30 min prior to color evaluation using an HUNTERLAB Colorimeter (Hunter Associates Laboratory Inc., Reston, USA), calibrated to black and white standards. Lightness (L^*), redness (a^*) and yellowness (b^*) were measured on 2 different spots on each of the patties samples. Three samples from each treatment were analyzed. Results were recorded as the mean of these measurements.

Statistical analysis: Results were expressed as means and standard deviation (Means±SD) from triplicate determinations. Data were subjected to one-way analysis of variance (ANOVA) to compare the effect of OLE and/or TA treatments. Significant differences were defined as $p<0.05$, according to PC-STAT[41], Version I A.

RESULTS AND DISCUSSION

Proximate analysis: The proximate analysis is important from many aspects including nutritional value, processing and preservation. So, the proximate composition of Powdered Olive Leaves (POL) and raw fresh meat mixture (paste) used for the preparation of beef patties were determined and presented in Table 1. Mean values for proximate composition of raw ground beef paste was as follows: protein 18.65±0.46%, fat 17.28±0.32%, ash 1.18±0.08%, moisture 60.42±0.86% and carbohydrate 2.47±0.54%, in good agreement with the previous findings reported by other authors[31,32,42] in their frameworks on ground beef patties. However, formula ingredients, fat percentages as well as processing method affect to a great extent the proximate composition and quality attributes of formulated muscle origin passed patties[31].

Table 1 also reveals that hot-air dried health promoting olive leaves showed 43.18±0.74% moisture, 4.36±0.38% protein, 5.84±0.42 % fat, 5.27±0.25 % ash and 41.35±0.68 % carbohydrate (on a fresh weight basis), respectively. Results in Table 1 also indicate that olive leaves are characterized by moderate protein and fat contents and a high carbohydrates

Table 1: Proximate composition of beef paste and powdered olive leaves 'POL' (on afresh weight basis)

Component	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Beef patty paste	60.42±0.86[a]	18.65±0.46[a]	17.28±0.32[a]	1.18 ±0.08[b]	2.47±0.54[b]
Powdered Olive Leaves (POL)	43.18±0.74[b]	4.36±0.38[b]	5.84±0.42[b]	5.27 ±0.25[a]	41.35±0.68[a]

All values reflect the mean and standard deviation are mean of triplicate determinations, Mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$). [Total carbohydrates = 100-(Moisture+Protein+Intramuscular-fat+Ash]

Table 2: TVC and PTC (as log CFU g^{-1}) of raw beef patties during refrigerated storage at 4±1 °C for 14 days Treatment/Day

		Treatment/Day					
Raw beef patties		0	3	5	8	11	14
Control (C)	TVC	4.00±0.15[a]	4.92±0.13[a]	5.72±0.19[a]	6.84±0.11[a]	7.73±0.15[a]	8.65±0.14[a]
OLE (1%)		3.89±0.10[a]	4.37±0.17[c]	4.76±0.10[c]	5.52±0.13[c]	6.40±0.12[c]	7.52±0.11[c]
TA (0.02%)		3.97±0.12[a]	4.65±0.15[b]	5.00±0.12[b]	5.80±0.16[b]	6.92±0.13[b]	7.89±0.17[b]
OLE+TA		3.85±0.18[a]	4.13±0.11[d]	4.45±0.14[d]	5.18±0.10[d]	5.76±0.16[d]	6.84±0.10[d]
Control (C)	PTC	3.65±0.13[a]	4.42±0.17[a]	5.16±0.12[a]	6.18±0.15[a]	7.34±0.11[a]	8.28±0.16[a]
OLE (1%)		3.57±0.21[a]	3.72±0.12[c]	4.45±0.16[c]	5.24±0.11[c]	6.48±0.23[c]	7.34±0.12[c]
TA (0.02%)		3.61±0.16[a]	3.96±0.21[b]	4.70±0.12[b]	5.63±0.18[b]	6.86±0.14[b]	7.62±0.18[b]
OLE+TA		3.52±0.15[a]	3.65±0.10[c]	4.21±0.14[d]	4.87±0.10[d]	5.90±0.17[d]	6.57±0.23[d]

All values reflect the mean and standard deviation,are mean of triplicate determinations, Mean values in the same column bearing the same superscript do not differ significantly, TVC: Total Viable Count, PTC: Psychrotrophic count, (as logCFU g^{-1}), OLE: Olive Leaves Extract (1%), TA: Tannic Acid (0.02%), OLE+TA: OLE (1%) + TA (0.02%)

and ash contents, which gave the olive leaves importance as a medicinal and food ingredient. It is worth mentioning that, the chemical composition of olive leaves varies according to the origin, weather, storage conditions, moisture content and degree of soil contamination[43,44]. Our present's proximate values (Table 1) are within the normal limits for the species and are consistent with those found in the aromatic and medicinal plants literature for olive leaves[19,44].

Microbiological analysis: Microbiological evaluation, together with chemical indices, has been used extensively to assess the quality and shelf-life of meat products[38,45]. Total Viable Counts "TVC" and psychotrophic count (PTC) of minced beef patty under investigation were evaluated and the mean counts (as log10 CFU g^{-1}) were depicted in Table 2. At the beginning of refrigerated storage (0 days), no significant differences could be established between treated samples and control patties for their TVC and also for PTC, this actually shows that the ingredients used were of good microbiological quality. These results are similar to findings obtained by Aytul[16], Hayes *et al.*[33] and Kassem *et al.*[40].

As may be expected, the increase in storage time produced significant ($p<0.05$) steadily proliferations in TVC and PTC, whatever the treatment conditions. However, the increment noticed for control samples was significantly ($p<0.05$) higher than those found in OLE or TA pretreatment groups (Table 2). The present study also indicates that, at any given time of refrigerated storage, OLE (1%) alone was slightly ($p<0.05$) more effective against microbial growth than TA (0.02%) alone. The initial (day 0) TVC in ground beef patties ranged from 3.85 in patties containing OLE+TA in combination to 4.00 (log10 CFU g^{-1}) in control samples and the development of TVC under aerobic storage condition were similar to the levels reported in the literature[17,23,26,30,31,42].

The relatively high initial TVC numbers in ground beef patties can be attributed to the mincing process, which contributes to the increase of microbial load. Storage time had a significant effect ($p<0.05$) on TVC of raw beef patties in aerobic storage (Table 2), by the day 8 of storage, TVC of control (6.84) was the nearest to the maximally recommended limit of 7 log10 CFU g^{-1} for TVC in raw refrigerated meat[38,39,45] indicating a shelf life of 8 days. While OLE- and TA-treatments significantly ($p<0.05$) delayed the microbial growth and extended the shelf life of the product up to 11 days at which the TVC was 6.40 and 6.92, respectively versus 7.73 log10 CFU g^{-1} and signs of spoilage started to appear for control patties. Results of Table 2 also reveals that at day 14 of storage samples containing combination of OLE with TA had the lowest TVC (6.84 log10 CFU g^{-1}) than the maximal recommended limit, while control samples exhibited the highest count of 8.65 log10 CFU g^{-1}, indicating that such combination is more effective than OLE or TA alone (Table 2). This result might have been due to the synergistic effect of the two adverse factors studied. This finding is consistent with Maqsood and Benjakul[26], Al-Hijazeen *et al.*[30], Hayes *et al.*[33] and Mokhtar *et al.*[42], who reported the similar effect of OLE with TA and/or EO combination on microbial load in ground refrigerated meat products.

The Gram-negative psychrotrophic bacteria (PTB) are the major group responsible for spoilage of refrigerated meat products[5,45]. Table 2 also reveals that at storage day 0, the initial PTC in ground beef samples ranged from

Table 3: Quality indices changes of raw beef patties during refrigerated storage at 4±1 °C for 14 days

		Cold storage day^{-1}					
Beef patty	Criteria indices	0	3	5	8	11	14
Control (C)	pH value	5.76±0.18^{a}	5.68±0.10^{a}	6.04±0.14^{a}	6.28±0.10^{a}	6.52±0.11^{a}	6.89±0.16^{a}
OLE (1%)		5.84±0.14^{a}	5.75±0.17^{a}	5.81±0.13^{b}	5.89±0.12^{b}	5.96±0.19^{b}	6.12±0.12^{b}
TA (0.02%)		5.69±0.21^{a}	5.62±0.23^{a}	5.74±0.18^{b}	5.83±0.15^{b}	5.89±0.28^{b}	5.97±0.14^{b}
OLE+TA		5.71±0.10^{a}	5.66±0.28^{a}	5.70±0.15^{b}	5.78±0.24^{b}	5.83±0.13^{b}	5.89±0.10^{b}
Control (C	TVBN mgN/100g	10.17±0.18^{a}	12.38±0.13^{a}	15.74±0.17^{a}	19.65±0.24^{a}	22.84±0.11^{a}	26.25±0.17^{a}
OLE (1%)		9.45±0.12c	10.76±0.16^{c}	12.58±0.13^{c}	15.92±0.18^{c}	18.76±0.10^{c}	22.84±0.12^{c}
TA (0.02%)		9.83±0.21^{b}	11.10±0.13^{b}	13.25±0.11^{b}	16.14±0.21^{b}	19.34±0.08^{b}	23.42±0.14^{b}
OLE+TA		9.18±0.14^{d}	10.23±0.17^{d}	11.70±0.10^{d}	13.82±0.15^{d}	16.75±0.19^{d}	19.36±0.23^{d}
Control (C	TBARS mgMDA/kg	0.43±0.17^{a}	0.65±0.12^{a}	0.74±0.16^{a}	0.86±0.10^{a}	1.18±0.17^{a}	1.42±0.14^{a}
OLE (1%)		0.36±0.14^{a}	0.42±0.10^{b}	0.51±0.11^{b}	0.62±0.12^{b}	0.83±0.14^{b}	1.14±0.12^{b}
TA (0.02%)		0.28±0.15^{a}	0.39±0.13^{b}	0.45±0.14^{b}	0.56±0.18^{b}	0.78±0.08^{b}	0.98±0.10^{b}
OLE+TA		0.32±0.21^{a}	0.35±0.11^{b}	0.40±0.10^{b}	0.49±0.13^{b}	0.67±0.12^{b}	0.85±0.11^{b}

All values reflect the mean and standard deviation (SD), are mean of triplicate determinations, Mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$), OLE: Olive Leaves Extract (1%), TA: Tannic Acid (0.02%)-OLE+TA: OLE (1%)+TA (0.02%), Total Volatile Basic Nitrogen (TVBN, as mg N/100g beef). Thiobarbituric acid reactive substances (TBARS, as mgMDA kg^{-1} beef)

3.52-3.65 log10 CFU g^{-1} and the changes in PTC were approximately similar to those of TVC, with control also being the highest followed by samples treated with TA, while much lower counts was detected in samples treated with OLE either alone or in combination with TA (Table 2). The psychrotrophic counts (PTC) in beef patty samples were lower than the TVC; this was true for all of the 4 groups analyzed (Table 2). Starting from the 3rd day of cold storage significant differences ($p<0.05$) were detected in PTC between samples treated with OLE either alone or in combination with TA and those of controls, also between samples treated with TA and control samples. In general, the present study indicates that the addition of olive leaf extract (OLE; 1%) and/or TA (0.02%) reduced ($p<0.05$) TVC and PTC in raw beef patties relative to the control during refrigerated storage under aerobic storage conditions (Table 2). These results confirmed the findings obtained by Aytul[16], Ahmed *et al.*[17], Baker[23] and Maqsood and Benjakul[26].

On the basis of the microbiological analyses reported in this study, the general order of antibacterial activity of the different treatments of raw beef patties was (1%OLE+0.02%TA) > 1%OLE alone > 0.02%TA alone > control (no, additives). Olive leaves are a rich natural source of bioactive compounds such as oleuropein, verbascoside, rutin, apigenin, kaempferol, luteolin, caffeic acid, tyrosol and hydroxytyrosol which all display antioxidant and antimicrobial properties[18,20]. The antibacterial activity is possibly due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents and the generation of hydroperoxides from polyphenols[8]. The inhibitory effect of phenolic compounds also could be explained by interaction with enzymes, substrate and metal ion deprivation[46].

The antimicrobial activities of tannins are through the astringent effect of tannins to many microbial enzymes such as cellulase, pectinase and xylanase; their toxicity on the cell membrane of microorganisms; and their binding ability to metals (metal chelator), which reduce the availability of essential metal ions such as iron, which is important for the metabolic activities of microorganisms[24,28].

The quality loss during refrigeration of beef patties: Quality control analyses (pH values, Total Volatile Basic Nitrogen (TVBN) and Thiobarbituric Acid Reactive Substances (TBARS),as an indicator of the degree of raw patties freshness, were performed during the course of refrigerated storage and the mean values as a function of pre-treatments and storage time at 4±1 °C are depicted in Table 3.

pH: pH value is the most important technological properties that alter pigment, lipid stability, microbial growth and food spoilage[46,47]. Table 3 shows that no significant differences in the initial pH values of all beef patties, the values were almost similar (ranging from 5.69-5.84), which might be due to the buffering capacity of meat. Initial (day 0) means pH values in control (C) patties was 5.76 and are consistent with results reported for meat patties[32]. For the samples pretreated with Olive Leaves Extract (OLE; 1%), the slight increase in pH (5.84) was observed. In contrast, Tannic Acid (TA; 0.02%) treatments exhibited slightly lower pH values (5.69) as compared with control samples. Our results (Table 3) confirmed the findings of Aytul[16], Ahmed *et al.*[17] and Al-Hijazeen *et al.*[30] in their frameworks on meat products treated with natural OLE and/or phenolic acid.

Table 3 further shows that the pH values of all samples slightly decreased during the first 3 days of storage, then

gradually increased throughout the storage time. The sharp ($p<0.05$) increase was observed in pH values of control beef patties until the end of storage. Conversely, OLE+TA treated patties had the lowest and the most stable pH values, due to their antibacterial property and combined synergistic effect on delaying microbial growth and protein decomposition that delays the formation of basic nitrogen compounds[28,15]. Except for control samples, there were no significant differences in pH among the different beef patties treatments. However, the decline in pH values could be due to the formation of carbonic and lactic acids, while the increase in pH values thereafter presumably due to the production of volatile bases alkaline compounds; by either endogenous or microbial enzymes[45,48]. The similar increasing trend in pH values was observed in refrigerated meat products by Shan *et al.*[9], Aytul[16], Al-Hijazeen *et al.*[30] and Mokhtar *et al.*[42].

TVB-N: Total Volatile Base Nitrogen, which is mainly composed of ammonia and volatile amines, resulted from degradation of beef patties proteins and non-protein nitrogenous compounds by microbial activity is a widely used meat spoilage indicator[36]. At the beginning of the storage period, TVB-N levels were found to be 10.17, 9.45, 9.83 and 9.18 mgN/100 g meat for control (C), OLE-, TA- and OLE+TA-treated beef patties, respectively. These values are indicative of good quality raw material used in this assay and they are in good agreement with literature data[49]. TVB-N levels progressively increased ($p<0.05$) with the time of storage at $4\pm1^{\circ}C$ for all treatments until the end of the storage period, with a higher rate ($p<0.05$) in control samples than treated samples. However, the increase in TVBN values is related to the activity of spoilage bacteria and endogenous enzymes[45,50]. On the other hand, the lower TVBN for treated samples may be due to the effectiveness of OLE or TA on microorganisms[17,22,23,30,51].

As shown in Table 3 OLE treatment exhibited significantly lower ($p<0.05$) TVB-N values than TA-treated patties, in contrast, OLE+TA treated patties had the lowest and acceptable TVB-N values to end day of storage (14th day), due to their antibacterial property of OLE and/or TA and the possible combined synergistic effect on delaying microbial growth and protein decomposition that delays the formation of basic nitrogen compounds[15,17,30]. Regarding TVBN values as a spoilage index for meat products, a level above 20 mg N/100g flesh is usually regarded spoiled of minced meat products[52]. Accordingly, control beef patty samples still acceptable with regard to TVBN index for eight days in comparison to 11 days for Olive Leaves Extract (OLE)- and Tannic Acid (TA)-treated patties; whereas OLE+TA combination exceeded the shelf-life to 14 days under the same conditions. TVB-N values in the present study confirmed the results of microbiological and sensory analyses and; providing a good index for the assessment of freshness of beef patties.

TBARS: Meat and meat products are susceptible to lipid oxidation during the refrigerated and frozen storage; TBARS-value is a valuable test for the determination of lipid oxidation[37]. Table 3 reveals no significant differences were observed among treated and control samples at the beginning of refrigerated storage (0 days), which indicates that oxidative deterioration of beef patties occurred during storage time. From the 3rd day of storage significant increase ($p<0.05$) of TBARS-values of all examined patties samples during storage time, with the control (C) patties oxidizing most rapidly and to the greatest extent as compared to treated samples. A similar trend of TBARS changes was observed by Kassem *et al.*[40] and Mokhtar *et al.*[42]. Results also indicate that TBARS values of control group have reached the acceptable limit (< 0.9 mg MDA kg^{-1}), as required by the Egyptian Organization Standards for meat products[52] at the 8th day of storage. While OLE/TA exhibited antioxidant activity over the 11-day storage period (Table 3). The results of this study confirmed the findings obtained by Aytul[16], Baker[23] and Al-Hijazeen *et al.*[30].

It is clear from the results in Table 3 that, TA-treated patties exhibited significant higher ($p<0.05$) protection against lipid oxidation than did OLE. Moreover, the lowest TBARS values were recorded in samples containing 1% OLE plus 0.02% TA (OLE+TA) in combination till the end of storage time (14th day). Such findings may be due to the antioxidant property of OLE/TA and the possible combined synergistic effect of delaying the formation of secondary oxidation products[15,30,33]. It is worth to mention that, minced meat patties undergo oxidative changes more quickly as grinding exposes lipid membranes to metal oxidation catalysts, also fine grinding, incorporation of air, haem pigments, metal contact and high temperature during processing contribute to lipid oxidation and microbial deterioration[53].

On the other hand, the phenolic compounds of OLE such as aromatic compounds, phenolic acids, flavonoids, oleuropein, verbascoside, rutin, tyrosol, hydroxytyrosol and hydrolysable tannins, might be involved in the inhibition of lipid oxidation[20,21,22,23]. Tannic Acid (TA) as a phenolic compound can inhibit free radical formation and the propagation of free radical reactions through the chelation of transition metal ions, particularly those of iron and copper[28,29]. These activities make the tannic acid a possible replacement for synthetic antioxidants[26,30]. Generally, the

Table 4: Color parameter (L*, a*and b*Values) loss of raw beef patties during storage at 4±1 °C for 14 days

Beef patty	Color	Z-Day	3	5	8	11	14
Control (C)	L*	46.14±0.12^{d}	44.10±0.15^{d}	43.18± 0.10^{d}	41.78±0.12^{d}	38.65±0.13^{d}	36.28±0.15^{d}
OLE (%)		47.68±0.17^{c}	46.32±0.18^{c}	45.17±0.15^{c}	43.14±0.13^{c}	41.76±0.12^{c}	40.17±0.11^{c}
TA (0.02%)		48.45±0.11^{b}	48.02±0.13^{b}	47.60±0.12^{b}	45.15±0.16^{b}	43.92±0.19^{b}	42.40±0.12^{b}
OLE+TA		49.18±0.21^{a}	48.86±0.11^{a}	48.00±0.17^{a}	46.40±0.14^{a}	45.18±0.11^{a}	43.28±0.10^{a}
Control (C)	a*	12.25±0.14^{d}	11.15±0.10^{d}	10.74±0.14^{d}	8.18±0.13^{d}	7.56±0.17^{d}	6.74±0.17^{d}
OLE (1%)		12.84±0.17^{c}	12.16±0.12^{c}	11.35±0.10^{c}	10.73±0.15^{c}	9.14±0..12^{c}	8.28±0.11^{c}
TA (0.02%)		13.45±0.10^{b}	13.00±0.14^{b}	12.62±0.08^{b}	12.25±0.18^{b}	11.68±0.10^{b}	11.13±0.15^{b}
OLE+TA		13.78±0.13^{a}	13.45±0.21^{a}	13.06±0.13^{a}	12.68±0.14^{a}	12.23±0.15^{a}	11.61±0.12^{a}
Control (C)	b*	17.85±0.12^{a}	18.17±0.10^{a}	18.73±0.14^{a}	19.40±0.15^{a}	20.15±0.10^{a}	20.49±0.14^{a}
OLE (1%)		16.18±0.13^{b}	17.23±0.13^{b}	17.18±0.11^{b}	17.76±0.13^{b}	18.45±0.12^{b}	19.35±0.15^{b}
TA (0,02%)		15.23±0.15^{d}	15.75±0.14^{d}	16.12±0.16^{d}	16.80±0.10^{d}	17.14±0.21^{d}	17.68±0.12^{d}
OLE+TA		15.52±0.19^{c}	16.18±0.11^{c}	16.75±0.12^{c}	17.22±0.16^{c}	17.65± 0.13^{c}	18.00±0.10^{c}

Mean±S.D of lightness (L*), redness (a*) and yellowness (b*) values are given from triplicate determinations, Mean values in the same column bearing the same superscript do not differ significantly, OLE: Olive Leaves Extract (1%) - TA: Tannic Acid (0.02%) -OLE+TA: (OLE; 1% plus TA; 0.02%)

obtained data support each other and was confirmed by the microbiological and sensorial evaluations.

Color loss during refrigerated storage: First decision about food before purchasing or consuming is up to its color; consequently, desirable color must be maintained throughout the storage of meat products[54,55]. The average L*, a* and b* values for all beef patty samples during 14 days of cold storage are given in Table 4. ANOVA revealed significant differences ($p<0.05$) between control and treated samples for all color parameters during storage. The results in Table 4 also indicated that over storage time redness values of all patty samples decreased, indicating that samples were becoming less red or brown due to metmyoglobin formation. Lightness (L) values steadily decreased as expected until day 14, indicating graying of beef patties samples. The yellowness (b*) values followed similar trend increasing until the 14th day. The protective effects of TA and OLE against color loss during storage were obvious in the present study.

In general, the color stabilizing effects of TA and OLE may be the result of its ability to chelate transition metals involved in the free radical generation and/or free radical scavenging, thereby delaying the oxidation of oxymyoglobin to metmyoglobin. In contrast, control samples showed, however, a poorer color stability initially and during refrigerated storage. Microbial spoilage, the decomposition of pigments as a result of the bacterial action and a scape of some pigments (as water soluble protein) were most likely responsible for the overall color loss (graying) effects seen over storage time. The present results confirmed the findings previously obtained[9,26,32,42,44,54,56].

As shown in Table 4, L* values of beef patties significantly ($p<0.05$) decreased (indicating graying) during storage regardless of treatments, with a higher rate in control samples as compared to treated samples. Moreover, Tannic Acid (TA; 0.02%) treatment exhibited a greater significant ($p<0.05$) effect in preventing changes of L*-value than Olive Leaves Extract (OLE; 1%) -treated and control samples but tannic acid in combination with olive leaves extract (TA+OLE) showed the strongest effect on L*-value than other treatments: the beef patty samples incorporated with TA+OLE had the highest L*-value (lighter patties) and the control had the lowest value (darker patties) at any given time of cold storage. The patties with high lightness value are considered more acceptable by the consumer than the darker (dull) one because it can be considered old or spoiled[55]. This is in agreement with the results of Aytul[16], Maqsood and Benjakul[26], Okuda and Ito[28]and Mokhtar *et al.*[42], who found that all ground beef samples treated with tannic acid and/or olive leaves extract or herbal essential oil had higher L*-values (brightness) than control patties.

The redness of meat is an important component of visual appeal to consumers, data depicted in Table 4 also revealed that redness (a* value) was higher ($p<0.05$) after addition of TA and/or OLE; tannic acid containing patties showed significantly ($p<0.05$) higher a* values compared to OLE or untreated control samples throughout the storage. The best synergistic effect was again obtained from the combination of TA with OLE, which exhibits the lowest deterioration effect on Hunter a* values. The results in Table 4 also indicated that over storage time a* values of all patty samples significantly ($p>0.05$) decreased with different rates. The decrease in a* value was in agreement with the oxidation of myoglobin and accumulation of metmyoglobin, which darkens the beef patties[57]. Decreasing redness values with increasing storage time were reported in meat products during cold storage[32,33,42,54]. When meat losses its ability to reduce metmyoglobin to oxymyoglobin, the brown color of

Table5: Sensory scores changes of raw beef patties during refrigerated storage at 4±1°C for 14 days

		Cold storage per day					
Beef patty	Sensory criteria	0	3	5	8	11	14
Control	Appear scores	9.00±0.13^{a}	8.21±0.28^{c}	6.85±0.14^{d}	5.76±0.10^{d}	4.14±0.25^{d}	3.16±0.15^{d}
OLE (1%)		8.84±0.11^{a}	8.52± 0.17^{b}	7.74±0.25^{c}	6.84±0.15^{c}	5.45±0.11^{c}	4.14±0.24^{c}
TA (2%)		9.00±0.21^{a}	8.76±0.11^{a}	8.13±0.12^{b}	7.16±0.36^{b}	6.11±0.28^{b}	4.78±0.18^{b}
OLE+TA		8.92±0.16^{a}	8.82±0.16^{a}	8.46±0.34^{a}	7.51±0.14a	6.38±0.17a	5.17±.27^{a}
Control	Od or	8.75±0.32^{a}	7.46±0.35^{c}	6.72±0.24^{d}	5.14±0.12^{d}	3.86±0.17^{d}	2.78±0.21^{d}
OLE (1%)		8.86±0.13^{a}	8.37±0.11^{a}	7.65±0.32^{b}	6.42±0.25^{b}	5.32±0.25^{b}	4.46±0.32^{b}
TA (2%)		8.70±0.17^{a}	8.10±0.24^{b}	7.41±0.18^{c}	6.17±0.15^{c}	5.00±0.14^{c}	4.18±0.26^{c}
OLE+TA		9.00±0.25^{a}	8.52±0.10^{a}	7.89±0.25^{a}	6.68±0.24^{a}	5.59±0.32^{a}	5.10±0.12^{a}
Control	Overall accept	8.87±0.23^{a}	7.83±0.31^{c}	6.78±0.19^{c}	5.45±0.11^{c}	4.00±0.21^{c}	2.97± 0.18^{c}
OLE (1%)		8.85±0.12^{a}	8.44±0.14^{b}	7.69±0.28^{b}	6.63±0.20^{b}	5.38±0.18^{b}	4.30±0.28^{b}
TA (2%)		8.85±0.19^{a}	8.43±0.17^{b}	7.77±0.15^{b}	6.66±0.26^{b}	5.55±0.21^{b}	4.46± 0.22^{b}
OLE+TA		8.96±0.21^{a}	8.67±0.13^{a}	8.17±0.30^{a}	7.09±0.19^{a}	5.98±0.24^{a}	5.13±0.19^{a}

Appearance, odor and overall acceptability scores reflect the mean and standard deviation, (n = 10), Mean values in the same column bearing the same superscript do not differ significantly, OLE: Olive Leaves Extract (1%)-TA: Tannic Acid (0.02%)-OLE+TA: OLE (1%)+TA (0.02%)

metmyoglobin begins to appear on the surface of beef patties and a* value begins to decrease[32]. The antioxidant activities of OLE and/or TA[20,22,26,29] might reduce metmyglobin formation to some degree, consequently resulted in more stability of a* values and maintain the redness of beef patties for longer ($p>0.05$) period of refrigerated storage.

Regarding Hunter b* parameter defines ''yellowness''; beef patties samples treated with OLE were initially higher ($p<0.05$) than TA or control samples and remained higher at the end of the storage period indicating increased yellowness of those patties, which could be attributed to the natural yellowish color of OLE positively affecting the patty color. These changes may be acceptable to consumers because many spices and herb extracts are traditionally used for seasoning meat products in African countries. The treatment of ground beef with Tannic Acid (TA; 0.02%) could lower the b* values of patties samples kept under aerobic conditions. This suggested the ability of tannic acid in inhibiting lipid oxidation. However, b* values increased during storage, which was thought to be related with brown oxidation pigments "metmyoglobin". Beef patties treated with olive leaves extract had the highest b* values and kept the yellowness value at high levels for all storage time, whereas the lowest b* value was found in tannic acid treated patties (Table 4). Similar observations have been also made by Shan *et al.*[9], Maqsood and Benjakul[26], Mokhtar *et al.*[42] and Sirocchi *et al.*[54].

Sensory scores changes: Meat appearance and odor are important attributes for the consumer evaluation on meat quality[6]. Table 5 represents the mean score values for appearance, odor and overall acceptability of all examined beef patties samples. Results of Table 5 reveal that no, significant differences in the sensory scores were detected between raw control and treated beef patties samples at the beginning of storage (0 times). The present results indicate that these natural functional ingredients can be incorporated into beef patties without having a detrimental effect on product quality producing a healthy meat product. Moreover, during refrigerated storage panel preference for treated samples over stored untreated control (Table 5), indicating that both OLE and TA are potent preservatives having better function, which enhanced the pleasant odor, lipid and microbial safety; essential for maintaining the sensory attributes of meat products.

From Table 5 it is also obvious that the investigated sensorial scores decreased significantly ($p<0.05$) in all patty groups by increasing refrigerated storage time, probably due to microbial effect, oxidation of lipid and degradation of protein in the patties. Similar to our findings, various researches reported significant reductions in the sensory scores during refrigerated storage of different meat products[23,31,40,42]. As shown in Table 5, a decline of sensory attributes begins after the 3rd day of storage with marked reduction of odor, appearance and overall acceptability values in the control patties samples at the 5th day and rejection scores after the 8th day. In contrast, addition of OLE and TA to beef patty formulation exhibited higher scores ($p<0.05$) with no negative effect on organoleptic characteristics during 11 days of storage, whereas OLE+TA in combination showed the highest ($p<0.05$) appearance, odor and overall acceptability values for 14 days at 4±1°C; indicating a possible synergistic effect.

Data depicted in Table 5 also indicate that, starting from the third day of refrigerated storage *o*dor scores were significantly higher ($p<0.05$) in OLE-treated patties than TA treated- samples up to the eleventh day; the use of OLE improved the odor characteristics of beef patties due to its antimicrobial, antioxidant and flavoring properties[15,18]. Similar

results have been reported in the other muscle passed products treated with OLE[16,23]. On the other hand, TA-treated patties rated higher scores ($p<0.05$) for appearance characteristics as compared with OLE-treated and control samples for up to 11 day which confirms its color stability characteristic[26,55]. Our results are in good agreement with the findings obtained by other researchers[26,30], in their frameworks on meat products pretreated with tannic acid. The present results (Table 5) also reveal that no, significant differences in overall acceptability of beef patties samples pre-treated with OLE or TA during the course of refrigerated storage.

CONCLUSION

The meat industry continues to face concerns regarding the hygiene and safety of its products. Color changes, lipid oxidation and microbial contamination are a serious concern for meat producers and consumers. Inclusion of Olive Leaves Extract (OLE; 1%) and Tannic Acid (TA; 0.02%), added individually or in combination in beef patties formulas showed potential in enhancing the color, lipid stability, sensory qualities and microbial status, while maintain the chemical indices and extend the shelf-life (3-6 days over that of control). Moreover, the natural antioxidant and antimicrobial compounds combinations are superior to individual addition against meat quality deterioration during 14 days of cold storage. Application of Olive Leaves Extract and Tannic Acid (OLE+TA) in combination as safe, natural, functional ingredients could have for commercial use instead of synthetic preservatives to improve the safety and quality of beef patties during refrigerated storage.

SIGNIFICANCE STATEMENTS

This study demonstrates the potential use of OLE and/or TA formulations to improve the microbial quality, retard lipid oxidation, maintain the red color and quality indices. According to microbiological, organoleptic and chemical indices analyses it was also shown that the application of such natural components extended the shelf-life of treated beef patties by 3-6 days over that of control (8 days). Therefore, they could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the meat industry.

ACKNOWLEDGMENT

The authors wish to thank Prof Dr. Nabil Fouad Tawfiek, Professor of Dairy Microbiology at the Dairy Science Department, Food Industry and Nutrition division at the National Research Centre, Egypt, for his kind support and assistance for the microbiological analysis.

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