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PREVENTIVE EFFECTS OF MANGO KERNEL, OLIVE AND CORIANDER LEAVES EXTRACTS ON THE QUALITY LOSSES OF THE REFRIGERATED GROUND BEEF PATTIES

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Abstract

With the increased demand for high quality, convenience, safety, fresh appearance and an extended shelf life in fresh meat products, the present study aimed to investigate the effect of incorporation mango seed kernel (MSK), olive leaves (OL) and coriander leaves (CL) ethanolic extracts (2%), on the quality characteristics and shelf-life extension of raw ground beef patties during 12 days of storage at 4±1°C under aerobic packaging. The optimum concentrations of MSKE, OLE and CLE were established and added to ground beef patties. Beef patties samples were refrigerated at 4±1°C to be periodically examined for their sensory quality, physicochemical parameters and bacteriological status. The Results indicated that as the time of cold storage progressed, the overall mean scores of physicochemical and microbiological parameters were increased, while sensory scores and color parameters were decreased ($p<0.05$) irrespective of treatment. The obtained results showed that individually addition of tested antioxidants and antimicrobial compounds significantly ($p<0.05$) affected color parameters, lipid stability, microbiological loads, protein degradation and appeal to consumers as compared to control samples during the entire refrigerated storage period. MSKE provided the highest significant ($p<0.05$) antioxidant and antimicrobial properties followed by OLE then CLE during cold storage. This study demonstrates the potential use of Egyptian plants (MSKE and OLE) to improve the microbial quality, retard lipid oxidation, maintain the good color and quality indices and extended the shelf-life of treated beef patties by 2-6 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, OLE, MSKE, CLE, Quality attributes, Shelf-life.

Introduction

Meat and meat products are rich sources of nutrients, providing good quality animal proteins, essential amino acids, fatty acids, minerals and vitamins particularly B-complex (Singh *et al*., 2014). However, these muscle foods also provide favorable growth condition for spoilage and common food-borne pathogens microorganisms, which give rise to quality and economic loss (Sánchez-Ortega *et al.,* 2014). Extremely perishable muscle foods (MF) are also very much susceptible to spoilage due to chemical and enzymatic activities. 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 (Devatkal *et al*., 2014). Moreover, oxidative processes (apart from microbial spoilage) in meat and meat products are the main cause of their quality loss in the term of texture, color, flavor, nutritive value and safety (Folowo *et al.,* 2014).

In recent years, the application of suitable plant extracts possessing both antioxidant and antimicrobial activities is a major concern for increasing consumer safety and quality, extending shelf--life and preventing economic loss (Gahruie *et al.,* 2017). Among the plants rich in antioxidant and antimicrobial compounds are OLE, CLE and MSKE.

Olive leaf (*Olea europaea* L.) is the by-product of the olive oil industry, which can be used in the food industry for improving the nutritional value and functionality. Leaves from olive tree, are rich in biophenols (BPs), such as oleuropein, verbascoside, ligostroside, tyrosol,

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demethyoleuropein or hydroxytyrosol (Ivanov *et al.,* 2018). These compounds have shown several biological activities such as antioxidant and antimicrobial (Ayoub *et al.,* 2019 and AlShaal *et al.,* 2019). Olive leave extract has been shown to have a variety of bioactive properties including; antioxidant, antibacterial, antiviral, anticancer and cardio protective properties (Hukerdi *et al.,* 2018).

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world. Mango seed kernels (MSK) are rich sources of phenolic compounds and flavonoids such as gallic acid, ellagic, pyrogallol, chlorogenic, catachin, mangiferin, protocatechuic, cinnamic, catechol. It also contained myricetin caffeine, coumaric, sinapic acid, erulic acid, salicylic, kaempferol, quercetin and tannin, which showed potent tyrosinase inhibitor, antioxidant activity and chelating activity (Abdel-Aty *et al.,* 2018 and Melo *et al.,* 2019). MSKE showed good antibacterial activity against pathogenic bacteria (Ahmed, 2015; Bernal-Mercado *et al.,* 2018 and Raju *et al.,* 2019). MSKE is a suitable by-product that could represent a valuable input into functional foods production.

Coriander (*Coriandrum sativum* L.), commonly known as Cilantro, is a herb widely used as spice, or in folk medicine, in the pharmacy and food industries (Ashika *et al.,* 2018). Coriander leaves is a good source of polyphenols and phytochemical due to its high antioxidant activity (Hihat *et al.,* 2017 and Jangra *et al.,* 2018). Coriander leaves extract showed good antibacterial activity against pathogenic bacteria (Farah *et al.,* 2015; Agrawal *et al.,* 2018). HPLC analysis revealed that camphor, rutin, apigenin, luteolin, quercetin, catechin, chlorogenic acid, caffeic acid, ferulic acid and gallic acid were the main components of coriander leaves (Ahmed *et al.,* 2018 and Ashika *et al.,* 2018).

Ground beef patties are increasing in popularity and have extensively developed in the world food market. MSKE, OLE and CLE are classified as GRAS ingredients in ready-to-cook/eat meat based products. Although, several studies have demonstrated the beneficial effects of these natural plant organs, their effects on microbiological safety, chemical indices, sensory properties, lipid oxidation and color preservation of perishable foods have not been clearly investigated. Accordingly, the aim of this study is to consider the antioxidant and antimicrobial activities of MSKE, OLE and CLE (2%) to determine the freshness quality and shelf-life (sensorial, microbiological and chemical attributes) of raw ground beef patties during refrigerated storage at 4±1C°C for 12 days. Proximate composition of raw fresh patties paste and powdered MSKE, OLE and CLE were also included.

Materials and Methods

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, bromocresol 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 used were of analytical grade or the highest grade available and were obtained either from ADWIC or El-Gomhouria Companies, Cairo (Egypt). Potato starch, corn flour, and bread crust powder were purchased from local market at Dokki, Giza. Egypt.

Plant source

Fine-quality fresh green olive leaves (*Olea europaea* L.) were collected during March 2018, from the west farm of Faculty of Agriculture, Cairo University, Giza, Egypt. A ripe mango seed as by-products (waste) was collected after mango pulp processing of Zebdia variety (*Mangifera indica* L.), during the summer season of 2018, from local fruit processing units (Farghly), Giza, Egypt. The kernels were removed manually from the seeds for further extraction. Fresh coriander (*Coriandrum sativum*) leaves were collected from local market in Egypt. All the plant samples Fig. 1 were kept in polyethylene bags at 4±1°C until extraction.

Plants extraction

Olive leaves; coriander leaves and mango seed kernels were cleaned from extraneous matter and properly washed then dried in hot air-oven for 24 h at 40°C. The dried leaves and kernels were milled with grinder into a powdery form and kept separately in a closed dark glass bottle and stored at 4°C until further analysis.

According to the extraction method of El Anany (2015), one hundred gram of OL, CL and MSK powder were extracted overnight with 1000 ml of 80% ethanol solution in a shaking incubator (100 rpm) at room temperature. Then the extracts were centrifuged at 3500 rpm for 15 min. The supernatants were filtered through a Whatman No.1 filter paper, and the residue re-extracted and filtered then extract solutions were concentrated to dryness in a rotary evaporator (Eyela, Rikakikai, Tokyo, Japan), at 40°C and complete the drying of extract in

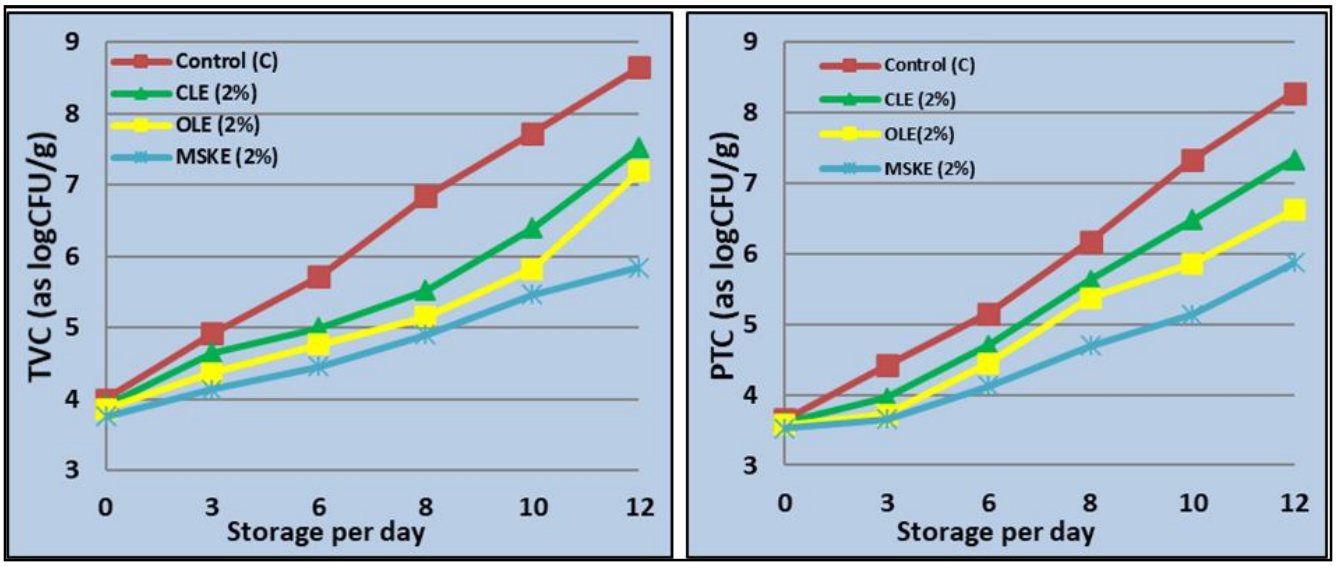

Fig. 1, 2: TVC: Total Viable Count – PTC: psychrotrophic count – during cold storage for 12 days. OLE: Olive Leaves Extract, CLE: coriander leaves extract, MSKE: mango seed kernel extract.

oven overnight at 40°C to form powder, which was stored separately at -20°C until further use. The extraction yield of each sample was calculated and reported as a percentage (g d wt. extract/100 g d wt. sample).

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 patty on the day of purchase. After thawing, they were cut in small cubes and minced twice (first minced through a 6 mm "coarse grinding".

Beef patties were prepared without seasonings using a simple traditional formulation: 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 a 3-mm holes "fine grinding" to ensure uniform distribution of the ingredients. The homogenized meat mixture obtained was divided into four 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.

Beef patties treatments

Each of the previous batches was mixed separately for 8 min with the appropriate antioxidants and antimicrobials, MSKE, OLE and CLE were added to the raw minced beef and mixed thoroughly; 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 three treatments contained MSKE, OLE and CLE (2%). The concentrations of above extracts; which are sensorially acceptable and also effective against bacterial load, were based on our primary studies, and also showed the best antioxidative and antimicrobial effect in the studies of Bouarab Chibane *et al.,* (2017) and Salem *et al.,* (2018) 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 (Lee and Song, 2015). The patties were packed separately in polyethylene bags (with two patties in each bag), then labeled and stored at 4±1°C for 12 days. Five bags of each group were withdrawn at regular intervals over storage period (0, 3rd, 6th, 8th, 10th and 12th day) for chemical, microbiological, sensorial and Hunter color values evaluations. The chemical and microbiological determinations were made on finely ground samples. Averages of three replicates were considered.

Optimization the conditions of using MSKE, OLE and CLE 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. MSKE, OLE and CLE were used at 0, 1 and 2%; mixed with the minced beef and formed into patties

(50±3g) using a Petri dish. Beef patties samples were package 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 at the initial day (0 day) 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 (1995). 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 (2007). A thiobarbituric acid reactive substance (TBARS) was determined according to the method proposed by Kilinc *et al.,* (2007). TBARS content was expressed as mg of malondialdehyde (MDA)/kg 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.,* (2009).

Bacterial enumeration

Twenty five grams of beef patty samples were aseptically excised from beef patties 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 patty 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 (Tajik *et al.,* 2014). 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 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 of AMSA (1995). The whole four 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 a HUNTERLAB Colorimeter (Hunter Associates Laboratory Inc., Reston, USA), calibrated to black and white standards. Lightness (L^*), redness (a^*), and yellowness (b^*) were measured on three 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 (M±SD) from triplicate determinations. Analysis of variance (ANOVA) was performed to compare the effect of MSKE, OLE or CLE treatments on beef patties quality. Significant differences were defined as $P<0.05$; according to PC-STAT, (1985).

Results and Discussion

Proximate Analysis

The proximate analysis is important from many aspects including nutritional value, processing and preservation. So, the proximate composition of MSKE, OLE, CLE and raw fresh meat mixture (paste) used for the preparation of beef patties were determined and presented in Table 1. Mean values for protein, fat, ash, moisture and carbohydrates were as follows: 18.65, 17.28, 1.18, 60.42 and 2.47%, respectively; In good agreement with the previous findings reported by other authors (Boruzi and Nour, 2019) 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

Table 1: Proximate composition of beef patty; MSKE, OLE and CLE (on fresh weight basis).

Component	%Moisture	%Protein	%Fat	%Ash	%Carb.
Beef Patty	60.42±0.26^{b}	18.65±0.16^{a}	17.28±0.12^{a}	1.18±0.08^{d}	2.47±0.14^{d}
MSKE	7.65±0.13^{d}	8.50±0.14^{b}	11.14±0.15^{b}	2.35±0.12^{b}	70.36±0.10^{a}
OLE	43.18±0.32^{c}	4.36±0.16^{c}	5.84±0.11^{c}	5.27±0.14^{a}	41.35±0.13^{b}
CLE	86.71±0.10^{a}	4.05±0.12^{d}	0.95±0.18^{d}	1.90±0.17^{c}	6.39±0.15^{c}

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].

attributes of formulated muscle origin passed patties.

Table 1 also reveals that MSKE showed the highest protein, fat and carbohydrates contents, while OLE exhibited higher content of protein, fat, ash and carbohydrate than CLE. Coriander leaves extract had the highest moisture and the lowest protein, fat, ash and carbohydrates as compared to the other natural extract. The proximate composition is within the normal limits for the species and is in agreement with those found in the plant extract literatures (AlShaal *et al.,* 2019 and Das *et al.,* 2019). However, slight differences in proximate composition may be due to the differences in the season, geographical location, weather, storage conditions, species and variety.

Sensory scores changes

Table 2 represents the mean score values for appearance, odor and overall acceptability of all examined beef patties samples. Results of table 2 reveal that no, significant ($P<0.05$) differences in the sensory scores were detected between raw control and treated beef patties samples at the beginning of storage (0 time). 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 2, indicating that MSKE, OLE and CLE are potent preservatives having better function, which enhanced the pleasant odor, lipid and microbial safety; essential for maintaining the sensory attributes of meat products.

Table 2 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 (Gahruie *et al.,* 2017; Zahid *et al.,* 2018; Salem *et al.,* 2018; Martínez *et al.,* 2019 and Boruzi and Nour, 2019). As shown in table 2, decline of sensory attributes begin after the third day of storage with marked reduction of odor, appearance and overall acceptability values in the control patties samples at the 4th day and rejection scores after the six day. In contrast, addition of MSKE, OLE and CLE to beef patty formulation exhibited higher scores ($P<0.05$) with no negative effect on organoleptic characteristics during cold storage, whereas MSKE showed the highest ($P<0.05$) appearance, odor, and overall

Table 2: Sensory scores changes of raw beef patties during refrigerated storage at 4±1°C for 12 days.

Beef Patty	Sensorycriteria	Cold storage per day					
		0	3	6	8	10	12
Control (C)	Appear.Scores	9.00± 0.13^{a}	8.21 ± 0.28^{C}	6.85± 0.14^{d}	4.76± 0.10^{d}	3.14± 0.25^{d}	2.16± 0.15^{d}
CLE (2%)		8.84 ± 0.11^{a}	8.52 ± 0.17^{b}	7.74± 0.25^{c}	5.84± 0.15^{c}	4.45± 0.11^{c}	3.14± 0.24^{c}
OLE (2%)		9.00 ± 0.21^{a}	8.76 ± 0.11^{a}	8.13 ± 0.12^{b}	7.16 ± 0.36^{b}	5.11 ± 0.28^{b}	4.78± 0.18^{b}
MSKE (2%)		8.92 ± 0.16^{a}	8.82 ± 0.16^{a}	8.46 ± 0.34^{a}	7.51± 0.14^{a}	6.38 ± 0.17^{a}	5.17 ± 0.27^{a}
Control (C)	OdorScores	8.75 ± 0.32^{a}	7.46± 0.35^{c}	5.72 ± 0.24^{d}	4.14± 0.12^{d}	3.86± 0.17^{d}	2.78± 0.21^{d}
CLE (2%)		8.86± 0.13^{a}	8.10 ± 0.11^{b}	7.45 ± 0.32^{c}	5.42 ± 0.25^{c}	4.32± 0.25^{c}	3.46± 0.32^{c}
OLE (2%)		8.70 ± 0.17^{a}	8.37 ± 0.24^{a}	7.84 ± 0.18^{b}	6.17 ± 0.15^{b}	5.00 ± 0.14^{b}	4.18± 0.26^{b}
MSKE (2%)		9.00 ± 0.25^{a}	8.52 ± 0.10^{a}	8.00 ± 0.25^{a}	7.38 ± 0.24^{a}	6.59 ± 0.32^{a}	5.10 ± 0.12^{a}
Control (C)	OverallAccept.	8.87 ± 0.23^{a}	7.83 ± 0.31^{c}	6.28 ± 0.19^{d}	4.45± 0.11^{d}	3.50± 0.21^{d}	2.47± 0.18^{d}
CLE (2%)		8.85 ± 0.12^{a}	8.48 ± 0.14^{ab}	7.59 ± 0.28^{c}	5.63 ± 0.20^{c}	4.38± 0.18^{c}	3.30± 0.28^{c}
OLE (2%)		8.85 ± 0.19^{a}	8.56 ± 0.17^{a}	7.98± 0.15^{b}	6.66± 0.26^{b}	5.05 ± 0.21^{b}	4.48± 0.22^{b}
MSKE (2%)		8.96 ± 0.21^{a}	8.67± 0.13^{a}	8.23 ± 0.30^{a}	7.44 ± 0.19^{a}	6.48 ± 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 ($P<0.05$).

MSKE: mango seed kernel extract, OLE: Olive leaves extract, CLE: coriander leaves extract (2%).

acceptability values for 12 days at 4±1°C; followed by OLE was accepted after 10 days, meanwhile, CLE exhibited acceptability after 8 days.

Decreases in sensory quality were assessed from the 3rd days of storage in samples stored at 4±1°C. Thereafter, almost all the samples with added extracts were evaluated more highly (P<0.05) than the control samples. Similar results have been reported in muscle origin foods (Marangoni *et al.,* 2017; Khemakhem *et al.,* 2018; Shalaby *et al.,* 2018; Salem *et al.,* 2018 and Martínez *et al.,* 2019). ANOVA tests indicated that the use of extracts (MSKE, OLE or CLE) had a significant effect on the sensory characteristics investigated. The longer the period of storage, the greater the differences in appearance and odor scores of samples containing MSKE, OLE or CLE as compared to control samples. On advancement of storage period, the overall acceptability scores of sensory attributes were sharply decreased (P<0.05) irrespective of treatment, as a result of microbial spoilage, oxidation of lipid and degradation of protein in the meat.

The results table 2 clearly indicated that the discoloration, sliminess and off-odors were observed in the control patty samples which became unfit for sale after day 6 of storage, while CLE, OLE and MSKE treated patty samples were just beginning to show only slight color change, putrid odors and sliminess after days 8, 10 and 12, respectively. Similar extension trend of meat shelf-life was achieved during refrigerated storage by other authors (Gahruie *et al.,* 2017; Zahid *et al.,* 2018; and Boruzi and Nour, 2019), who indicated that natural extracts have strong antioxidant and antimicrobial activities.

Microbiological Analysis

Microbiological evaluation, together with chemical indices, has been used extensively to assess the quality and shelf-life of meat products (Gram and Huss, 1996). The antimicrobial activity of MSKE, OLE and CLE in beef patty samples stored at 4±1°C for 12 days, are shown in Fig. 1-3. Initial contamination of the beef patty in terms of total viable count (TVC) of bacteria in control, CLE, OLE and MSKE treated patty samples was 4.00±0.15, 3.91±0.10, 3.86±0.12 and 3.75±0.18 log cfu/g, respectively. The statistical analysis of TVC revealed significant difference (P>0.05) between examined patty samples at zero day of 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 meat product, as defined by the Egyptian Standard, after the six day for the control samples, indicating a shelf life of about 6 days.

Compared with the control, all other treatments significantly reduced the initial TVC with the increase in the storage time (P<0.05), and caused inhibition of the growth of bacteria in patty samples and extension of the shelf-life under storage at 4±1°C. The use of the MSKE was the most effective, achieving the greatest initial reduction and TVC reached the limit of 6 log cfu/g after 12 days storage period resulting in a shelf-life of 12 days as compared to the control samples. With regard to OLE resulted in a shelf-life of 10 days as compared to control samples. In the contrast CLE extended the shelf-life to 8 days. Similar extension periods have been reported in meat products treated with natural extracts (Abdeldaiem *et al.,* 2017; Khemakhem *et al.,* 2018; Salem *et al.,* 2018; Shalaby *et al.,* 2018 and Martínez *et al.,* 2019).

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 natural extracts pretreatment groups Fig. 1, 2. At storage day 0, the initial Psychrotrophic bacteria in beef patty samples Fig. 2 were 3.65 ± 0.13, 3.61 ± 0.21, 3.57 ± 0.16 and 3.52 ± 0.15 log cfu/g in control, CLE, OLE and MSKE treated patty treated samples, respectively. The changes in psychrotrophic count were approximately similar to those of TVC, with control also being the highest followed by samples treated with CLE, OLE and much lower counts was detected in samples treated with MSKE.

ANOVA indicated that significant differences were detected in the Gram-negative psychrotrophic count between samples treated with natural extracts and those of controls. Also MSKE has prolonged the beef patty shelf-life to 12 days followed by OLE which prolong the shelf-life to 10 days, then CLE which increase the shelf-life to 8 days, compared with the control (6 days). The results indicate that MSKE, OLE and CLE had antimicrobial activity which helps for prolonging expiry of beef patty, leading to safer meat product (Martínez *et al.,* 2019; Przygonski *and Wojtowicz* 2019 and Zhu *et al.,* 2019). Generally, antibacterial effect of all extracts may be due to phenolic content which affected bacterial cell through changes in its membrane, changes in the surface charge of cells to less negative values; alteration in the cytoplasmic membrane permeability (Agrawal *et al.,* 2018).

Regarding Enterobacteriaceae Fig. 3, considered as

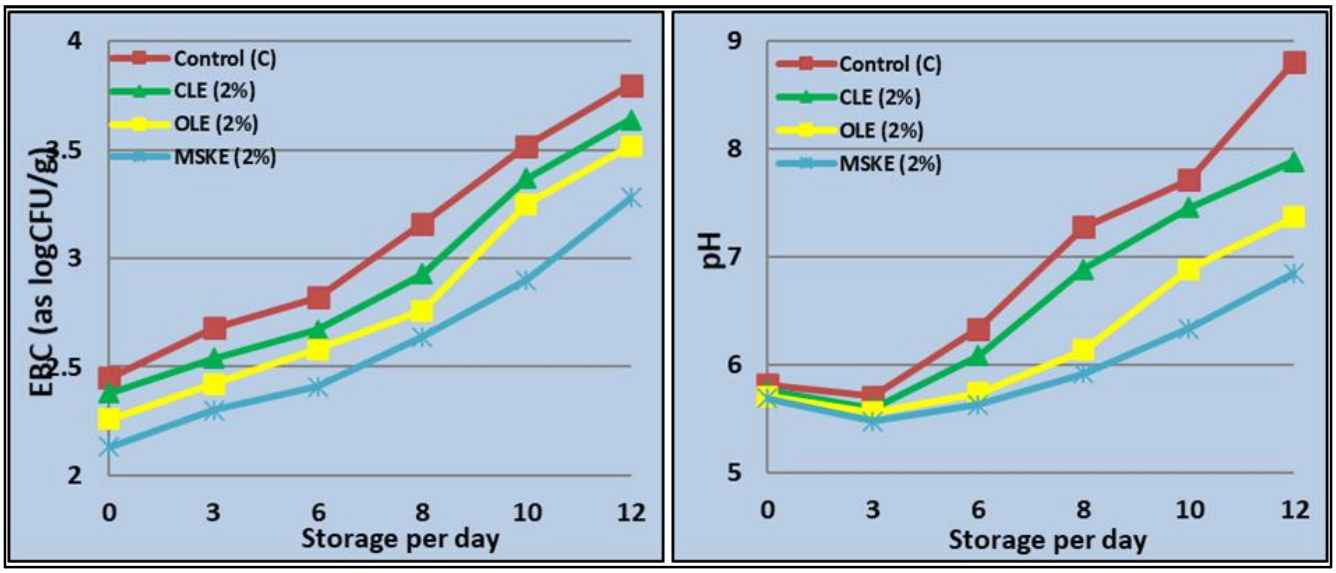

Fig. 3, 4: Enterobacteriaceae count (EBC), pH value of beef patties during refrigerated storage for 12 days. OLE: Olive Leaves Extract, CLE: coriander leaves extract, MSKE: mango seed kernel extract.

a hygiene indicator (Gram and Huss, 1996), the initial counts were 2.45 ± 0.12, 2.38 ± 0.14, 2.26 ± 0.15 and 2.13 ± 0.10 log cfu/g (indicative of good quality beef patty) in control, CLE, OLE and MSKE treated patty samples at zero day of refrigeration storage, respectively. During chilling storage, these counts were reached 2.82 ± 0.11 after 6 days in control samples and slightly increased for patty samples treated by CLE, OLE or MSKE after 8, 10 and 12 days, respectively. Compared to control, Enterobacteriaceae grew in CLE, OLE and MSKE samples at a slower rate and never exceeding 10^3 CFU/g. At the end of the storage period (day 12), treated patty samples exhibited much lower (P<0.05) counts as compared with control samples. These results are in accordance with those of (Abdeldaiem *et al.,* 2017; Khemakhem *et al.,* 2018; Shalaby *et al.,* 2018 and Martínez *et al.,* 2019). These findings could be attributed to antibacterial impacts of ethanolic extracts of CLE, OLE and MSKE.

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 Fig. 4-6.

pH value: pH is the most important technological properties that alters pigment, lipid stability, microbial growth and food spoilage (Zahid *et al.,* 2018). Fig. 4 shows that no significant differences (P<0.05) in the initial pH values of all beef patties, the values were almost similar (ranging from 5.69 to 5.82), which might be due to the buffering capacity of meat. Initial (day 0) means pH values in control (C) patties was 5.82 and are consistent with results reported for meat patties (Gahruie *et al.,* 2017; Bouarab Chibane *et al.,* 2017; Zahid *et al.,* 2018 and Salem *et al.,* 2018). For the samples pretreated with CLE, OLE and MSKE the slight decrease in pH (5.76, 5.71 and 5.69) was observed. Our results Fig. 4 confirmed the findings of other authors (Abdeldaiem *et al.,* 2017; Khemakhem *et al.,* 2018; Shalaby *et al.,* 2018 and Martínez *et al.,* 2019), in their frameworks on meat products treated with natural extracts.

Fig. 4 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 to end day of storage. Conversely, MSKE treated patties had the lowest and the most stable pH values (less than 7.1), due to their antibacterial property that delay the formation of basic nitrogen compounds. Except control samples, there were no significant differences (P<0.05) 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 (Gram and Huss, 1996). Similar increasing trend in pH values was observed in refrigerated meat products by other authors (Abdeldaiem *et al.,* 2017; Khemakhem *et al.,* 2018; Shalaby *et al.,* 2018 and Martínez *et al.,* 2019), who worked in meat products treated with natural extracts.

Total volatile base nitrogen (TVB-N): 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 (Parvaneh, 2007). At the beginning of the storage period, TVB-N levels were found to be 10.17, 9.83, 9.45 and 9.18 mgN/100 g meat for control (C), CLE-, OLE- and MSKE-treated beef patties, respectively Fig. 5. These values are indicative of good quality raw material used in this assay and they are in good agreement with literature data (Shalaby *et al.,* 2018; Martínez *et al.,* 2019 and Przygonski *and Wojtowicz* 2019). TVB-N levels progressively increased ($P < 0.05$) with time of storage at 4±1°C for all treatments until the end of 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 (Gram and Huss, 1996). On the other hand, the lower TVBN for treated samples may be due to the effectiveness of natural extracts used in this investigation on microorganisms (Abdeldaiem *et al.,* 2017; Khemakhem *et al.,* 2018; Shalaby *et al.,* 2018 and Martínez *et al.,* 2019).

As shown in Fig. 5 OLE treatment exhibited significantly lower ($P < 0.05$) TVB-N values than CLE-treated patties, in contrast MSKE treated patties had the lowest and acceptable TVB-N values to end day of storage (12th day), due to their antibacterial property on delaying microbial growth and protein decomposition that delay the formation of basic nitrogen compounds (Parvaneh, 2007). 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 (EOS, 2005). Accordingly, control beef patty samples still acceptable with regard to TVBN index for six days in comparison to 8 days for coriander leaves extract (CLE), 10 days for olive leaves extract (OLE) -treated patties; whereas MSKE exceeded the shelf-life to 12 days under the same conditions. TVB-N values in the present study confirmed the results of microbiological and sensory analyses table 2 and Fig. 1-3 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 in determination of lipid oxidation (Kilinc *et al.*, 2007). Fig. 6 reveals no significant differences ($P<0.05$) were observed among treated and control samples at the beginning of refrigerated storage (0 day), which indicates that oxidative deterioration of beef patty occurred during storage time. From the third 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. Similar trend of TBARS changes was observed by other works (Abdeldaiem *et al.,* 2017; Khemakhem *et al.,* 2018; Shalaby *et al.,* 2018 and Martínez *et al.,* 2019). Results also indicate that, TBARS values of control

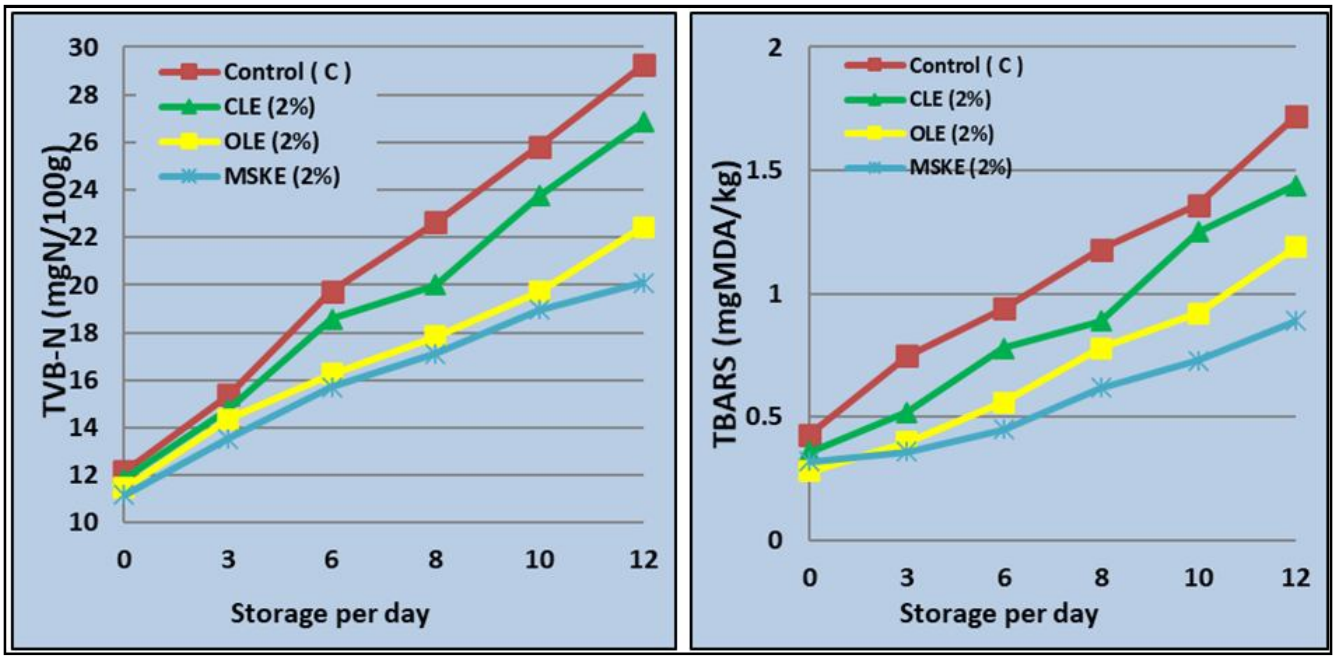

Fig. 5, 6: Total volatile basic nitrogen (TVB-N, as mg N/100g beef), Thiobarbituric acid reactive substances (TBARS, as mg. MDA/kg beef) of beef patties during refrigerated storage for 12 days. OLE: Olive Leaves Extract, CLE: coriander leaves extract, MSKE: mango seed kernel extract.

group have reached to the acceptable limit (< 0.9 mgMDA/kg), as required by the Egyptian Organization Standards for meat products (EOS, 2005) at the 6th of storage. While, CLE, OLE and MSKE- treated patty exhibited antioxidant activity over the 8, 10 and 12 days, respectively.

It is clear from the results in Fig. 6 that, OLE-treated patties exhibited significant higher ($P<0.05$) protection against lipid oxidation than did CLE. Moreover, the lowest TBARS values were recorded in samples containing MSKE till the end of storage time (12th day). Such findings may be due to the antioxidant property of CLE, OLE and MSKE on delaying the formation of secondary oxidation products (Ashika *et al.,* 2018; Ayoub *et al.,* 2019 and Melo *et al.,* 2019). 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 (Chatli and Joseph, 2014).

On the other hand, the phenolic compounds of OLE such as aromatic compounds, phenolic acids, flavonoids, oleuropein, verbascoside, rutin, tyrosol, hydroxytyrosol; and hydrolysable tannins, gallic acid, ellagic, pyrogallol, chlorogenic, catachin, mangiferin, protocatechuic, cinnamic, catechol (Ivanov *et al.,* 2018, Ayoub *et al.,* 2019). MSKE also contained myricetin caffeine, coumaric, sinapic acid, erulic acid, salicylic, kaempfero, quercetin and tannin (Abdel-Aty *et al.,* 2018 and Melo *et al.,* 2019), while CLE revealed that camphor, rutin, apigenin, luteolin, quercetin, catechin, chlorogenic acid, caffeic acid, ferulic acid and gallic acid were the main phenolic components (Ahmed *et al.,* 2018 and Ashika *et al*., 2018). All these components might be involved in the inhibition of lipid oxidation, because compounds 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. These activities make the CLE, OLE and MSKE a possible replacement for synthetic antioxidants. Generally, the obtained data Fig. 4-6 support each other and was confirmed by the microbiological and sensorial evaluations tables 2 and Fig. 1-3.

Color Loss During Refrigerated Storage

First decision about food before purchasing or consuming is up to its appearance and odor (Rogers *et al.,* 2014). The average L*, a* and b* values for all beef patty samples as affected by addition of CLE, OLE and MSKE during 12 days of cold storage are given in Table 3. ANOVA revealed significant differences between control and treated samples for all color parameters during storage. The results in table 3 also indicated that, over storage time a* values of all patty samples decreased, indicating that samples were becoming less red or brown due to metmyoglabin formation. Lightness (L) values steadily decreased as expected until day 12. The yellowness (b*) values followed similar trend increasing to day 12. The protective effects of CLE, OLE and MSKE against color loss during storage were obvious in the present study.

In general, the color stabilizing effects of CLE, OLE and MSKE may be the result of its ability to chelate transition metals involved in free radical generation and/ or free radical scavenging, thereby delaying the oxidation of oxymyoglobin to metmyoglobin. In contrast, control

Table 3: Color parameter (L*, a* and b* Values) loss of beef patties during storage at 4±1° for 12 days.

PattyTreatment	ColorProfile	Z-Day	3	6	8	10	12
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}
CLE 2%		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}
OLE 2%		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}
MSKE 2%		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}
CLE 2%		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}
OLE 2%		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}	10.13±0.15^{b}
MSKE 2%		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}
CLE 2%		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}
OLE 2%		15.52± 0.15^{d}	16.18±0.14^{d}	16.75±0.16^{d}	17.22±0.10^{d}	17.65±0.21^{d}	18.00±0.12^{d}
MSKE 2%		15.23± 0.19^{c}	15.75± 0.11^{c}	16.37± 0.12^{c}	16.80± 0.16^{c}	17.14± 0.13^{c}	17.68±0.10^{c}

Color parameters lightness (L*), redness (a*) and yellowness (b*) Values are given as mean ± S.D. from triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly ($P<0.05$). CLE: coriander Leaves Extract (2%) – OLE: Olive leaves extract (2%), MSKE: Mango seed kernel extracts (2%).

samples showed however, poorer color stability initially and during refrigerated storage. Microbial spoilage, the decomposition of pigments as a result of 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 (Gahruie *et al.,* 2017; Bouarab Chibane *et al.,* 2017 and Zahid *et al.,* 2018).

As shown in table 3, L*-values of beef patties significantly decreased (indicating graying) during storage regardless of treatments, with a higher rate in control samples as compared to treated samples. Moreover, olive leaves extract (OLE) treatment exhibited a greater significant ($P<0.05$) effect in preventing changes of L*-value than coriander leaves extract (CLE)-treated and control patty samples, but mango seed kernel extract (MSKE) showed the strongest effect on L*-value than other treatments: the beef patty samples incorporated with MSKE 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 is considered more acceptable by the consumer than the darker (dull) one because it can be considered old or spoiled (Gahruie *et al.,* 2017; Bouarab Chibane *et al.,* 2017 and Zahid *et al.,* 2018).

The redness of meat is an important component of visual appeal to consumers, data depicted in table 3 also revealed that, redness (a* value) was increased ($P<0.05$) after addition of CLE, OLE and MSKE; olive leaves extract (OLE) containing patties showed significantly ($P< 0.05$) higher a* values compared to coriander leaves extract (CLE) or untreated control samples throughout the storage. The best effect was again obtained from the mango seed kernel extract (MSKE), which exhibits the lowest deterioration effect on Hunter a* values. The results in table 3 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. Decreasing a* values with increasing storage time were reported in meat products during cold storage (Gahruie *et al.,* 2017; Bouarab Chibane *et al.,* 2017 and Zahid *et al.,* 2018). The antioxidant activities of CLE, OLE and MSKE 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”; control beef patties samples exhibited higher ($P< 0.05$) yellowness than treated patty samples with CLE, OLE and MSKE, and remained higher at the end of the storage period indicating increased yellowness of those patties. The treatment of ground beef with CLE, OLE and MSKE could lower the b* values of patties samples kept under aerobic conditions. This suggested the ability of these extracts in inhibiting lipid oxidation. However, b* values increased in patty sample during refrigerated storage which was thought to be related with brown oxidation pigments (met-heame proteins “metmyoglobin”). Beef patties treated with MSKE had the lowest b* values, whereas the highest b* value was found in CLE and OLE treated patties table 3. Similar observations have been also made by other authors (Lorenzo *et al.,* 2014; Gahruie *et al.,* 2017; Bouarab Chibane *et al.,* 2017 and Zahid *et al.,* 2018). The results of the current study showed that high antioxidant activity of natural extracts (CLE, OLE and MSKE) can be potentially used to develop natural color stabilizers, particularly for controlling * and * values in refrigerated beef patty.

Conclusion

Color changes, lipid oxidation and microbial contamination are serious concern for meat producers and consumers. Inclusion of coriander leaves extract (CLE), olive leaves extract (OLE) and mango seed kernel extract (MSKE), 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 (2-6 days over that of control) of minced beef patties during refrigerated storage at 4±1°C. Moreover, the natural antioxidant and antimicrobial compounds are superior against meat quality deterioration during 12 days of cold storage. Application of olive leaves extract (OLE) and mango seed kernel extract (MSKE) 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 CLE, OLE and MSKE 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 2-6 days over that of control (6 days). Therefore, they could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the meat industry.

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