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The Antibacterial Effect of Mango Seed Kernel Powder in Minced Beef During Refrigerated Storage

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Abstract: This study was aimed to investigate the effect of mango seed kernel powder (MSKP) at 1.0, 2.0 and 3.0% not only on the selection of microbial association of minced beef but also to determine any significant difference in microbial metabolites produced from the prevailing bacteria. Results showed that the methanolic MSK extract showed strong antibacterial activity for gram-positive bacteria than gram-negative bacteria in the ratio of minimum inhibitory concentrations ranged between (0.8 - 1.4%), since *St. aureus* was the most sensitive tested microorganisms followed by *B. subtilis*. It could be noticed that minced beef with 3.0% of MSKP had significant ($P \leq 0.05$) higher in moisture content, lipids and the retention of moisture during cold storage at ($4 \pm 1°C$). The minced beef containing 2.0 and 3.0% MSKP had the lowest thiobarbituric acid and total volatile nitrogen content during 15 days of cold storage. Furthermore, the incorporation of MSKP at 3.0% in refrigerated minced beef showed the lowest bacterial count (1.7×10^5) at the end of storage period compared to other treatments and controls. The results indicated that mango seed kernel was an effective inhibitor of microbial growth and caused a pronounced alteration in the physico-chemical properties in refrigerated minced beef.

Key words: Mango seed kernel powder · Antibacterial activity · Refrigerated minced beef

INTRODUCTION

Minced meat is usually a very perishable food so prolonging its shelf life is an important objective for producers. The loss of meat freshness after grinding results mainly from the activity of endogenous and exogenous enzymes, the oxidation of lipids and pigments and bacterial putrefaction [1]. There are many factors influencing meat shelf life such as pH, water content and availability of oxygen that could promote spoilage bacterial growth and oxidative processes during storage [2, 3]. Refrigeration storage is usually the most common preservation method of fresh meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives especially of synthetic origin, are added to meat products. However, consumers increasingly demand use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in last years [4]. The use of natural antimicrobial compounds is important not only in the preservation of food but also safe for human consumption [5]. Bacterial and fungal infections pose a greater threat to health, most notably in immune compromised subjects, hence the need to find natural, cheap and effective antimicrobial agents.

Mango (*Mangifera indica* L. Anacardiaceae) is one of the most important tropical fruits with a global production exceeding 35 million tons and in Egypt, 450 thousand tons of mango fruits were produced in 2009 [6]. A preliminary study showed that the mango seed represents from 20% to 60% of the whole fruit weight, depending on the mango variety and the kernel inside the seed represents from 45% to 75% of the whole seed. In the food processing industry, a considerable quantity of seeds is discarded as waste, after the extraction of mango pulp [7, 8]. The antimicrobial activity of mango kernel extract is certainly related to seven major phenolic compounds assumed to be mainly ellagic acid, gallic acid, m-digallic acid, opigenin-oglucoside, mangiferin, methylgallate and pentagalloyl glucose [9]. The mango kernels contain a variety of phenolic compounds such as flavonols and xanthones and gallotannins. The antibacterial activities of purified gallotannins have been elucidated only recently. Bacteria of the genera Listeria, Bacillus, Clostridia and Staphylococcus were sensitive to gallotannins, but lactic acid bacteria exhibited strong resistance [10-13].

Mango seed kernel extract reduced total bacterial count, inhibited coliforms growth, showed remarkable antimicrobial activity against *E. coli* strain and extended

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the shelf-life of pasteurized cow milk [14]. The crude methanolic extract of mango seed kernel at a concentration of 100 mg/ml was found to have potential antimicrobial activity against MRSA and *E. coli* compared to *V. vulnificus*. Among the rural folks the mango seed kernels are used traditionally as antimicrobials against gastric pathogens especially for the infants [8]. All mango seed extracts showed interesting antibacterial activity against both gram positive and gram negative bacteria as determined by disc diffusion method. The most sensitive pathogenic strain inhibited by all extracts was *Pseudomonas aeruginosa* [15]. They suggest potential applications for practical uses of mango seed extracts as sources of antioxidant and antibacterial agents in foods and pharmaceuticals. Although more studies are needed, the mango seed kernels extract is promising as a natural food additive for extending the shelf-life of a variety of foods products. Accordingly, the objective of the present work was to study the antimicrobial effect of Egyptian mango seed kernel in fresh minced beef during refrigerated storage to improve the quality and extent their shelf life.

MATERIALS AND METHODS

Materials: Ripe mango seeds as by-products (waste) were collected after mango pulp processing from zebda variety during the summer season of 2010 from Al-Qahera Company for Agriculture Industry, Al-Obor, Egypt. Meat and fat was purchased immediately after slaughtering from the round cuts of hind quarter of cow carcass. Soy protein flour was obtained from Agricultural Research Center, Ministry of Agriculture- Giza - Egypt.

Preparation of Mango Seed Kernel Powder: Mango seeds were washed with tap water to remove any pollutant, air dried and the kernels were removed manually from seeds. The kernels were chopped, spread thin in trays and dried at 50 °C using a cross flow drier for 18 h [16]. The dried material was ground in a hammer mill into a powdery form and sieved through a 150 mm sieve.

Preparation of Crude Methanolic Mango Seed Kernel Extracts (MSKE): Mango seed kernel powder was extracted with methanol according to the maceration method of Kaur *et al.* [17] and the extract was filtered by Whatman No.1 filter paper. The filtrate was concentrated in a rotary evaporator at 40°C. The concentrated extract was oven dried at 40°C for 4 days and then diluted with methanol to prepare concentrations from 0.2 to 1.6 % (w/v).

Antibacterial Activity of MSKE: The antibacterial activity of MSKE was individually studied against some pathogenic and food spoilage bacterial strains including three strains of Gram-positive bacteria (*Bacillus subtilis* ATCC 14085, *Bacillus cereus* NRRL-B 3711 and *Staphylococcus aureus* ATCC 6528) and three strains of Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas fluorescens* NRRL 800). These strains were obtained from the Egyptian Microbial Culture Collection (EMCC) at the Microbial Resource Center (Cairo, MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Preparation of Bacterial Inoculates: Bacterial strains were activated by inoculated a loop of the stock culture into 50 ml nutrient broth [18] and incubated at 30 or 37°C for 24 h before the assays in order to reach the stationary phase of growth. Suspensions of the tested microorganisms were counted using plate count method on nutrient agar [18].

Screening of the Antimicrobial Activity: The disc diffusion technique was used as screening method to determine the antimicrobial activity of MSKE against bacterial strains according to Kaur *et al.* [17] with some modifications as follows: About 3 ml of soft nutrient agar (0.75% agar) containing 1.0% inoculum of bacterial strain was layered over 20 ml of hard nutrient agar containing (2.0% agar). Sterilized filter paper discs (10 mm) were soaked in 50 µl of different concentrations 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6% (w/v) of crude methanolic MSKE, where the methanol was included as a negative control. The soaked discs were put in the middle of plates which were contained 1.0% bacterial inoculums and the plates were incubated at 37°C for (*B. subtilis*, *Staph. aureus, E. coli* and *S. typhimurium*) and at 30°C for (*B. cereus* and *Pseud. fluorescens*) for 24 h. The diameter of the zone of inhibition around each of the discs (disc diameter included) was taken as measured of the antimicrobial activity. All tests were performed in triplicates.

Determination of the Minimal Inhibitory Concentrations, Bacteristatic and Bactericidal Effects: The antimicrobial activity of MSKE against both Gram-positive and Gram-negative bacterial strains was examined by detecting of the Minimum Inhibitory Concentrations (MICs). Bacteriostatic and bactericidal concentrations of MSKE were determined as the appropriate volume of MSKE which selected as MICs were added to 100 ml of nutrient broth. The nutrient broth was inoculated with 1.0 ml of a 24 h bacterial culture. Different flasks were incubated at 37

or 30°C with regular shaking for 48 h. Every 4 hours, growth was compared with the control broth visually and through the measurement of the number of Colony Forming Units (cfu/ml) by plate count procedure. Plates were incubated at 37 or 30°C for 24 h and then counted for viable bacteria.

Refrigerated Minced Beef Manufacture: Minced beef samples were prepared using a simple traditional formulation listed in the Egyptian standard [19] as follows: meat and fat tissues were obtained from beef carcasses at 1 h post-slaughter in a local abattoir and transported to the laboratory under refrigerated conditions within 45 min. The outer surface of each muscle was sterilized by immersion in 95% (v/v) ethanol and then burning the residual ethanol on the meat surface, as previously described by Greer and Jones [20]. The inner core of the sterile meat was aseptically cut into pieces of about egg-size and frozen at -18°C for 24 h. The frozen meat and fat were ground by means of a sterile steel meat grinder (autoclaved at 121°C for 15 min) to particles of about a rice grain size. Samples were prepared by blending the following ingredient (beef 70%, fat 20% and soy protein 10%). The effects of the investigated concentrations of MSK powder (1, 2 and 3%) as substitutions of soy protein were studied compared with negative control (without preservatives) and with positive control (with 100 ppm sodium nitrite) on the product quality. The produced mixtures were divided to six batches and each batch was package in polyethylene bags and refrigerated stored at (4±1 °C) for 15 days.

Chemical Composition and pH: Moisture content, ash, fat and protein of the refrigerated samples wered eterminded according to AOAC [21]. The pH values were determined according to the method described by Deferitase *et al.* [22] by using a pH meter (HANNA, 2211-Instrument, USA).

Thiobarbituric Acid Value (TBA): Thiobarbituric acid (TBA)-reactive substances were determined colorimetrically according to the procedure described by Siu and Draper [23]. Refrigerated minced samples (10 g) were homogenized in 25 ml of distilled water for 2 min and then mixed with 25 ml of 10% trichloroacetic acid. The mixture was filtered and then 1 ml of 0.06 M TBA was added to 4-ml aliquots of the filtrate and then boiled for 10 min for color development. Absorbance at 532 nm was measured with a spectrophotometer (U-1900, Hitachi, Tokyo, Japan). The TBA values were calculated by multiplying the absorbance by the factor (7.8) and the result was represented as mg of malonaldhyde per kg sample.

Total Volatile Nitrogen (TVN): Total volatile nitrogen content was performed according to the method of Harold *et al.* [24] by using macrokjeldahl procedure as follows: ten grams of minced beef samples were placed in a 500 ml kjeldahl flask with 300 ml distilled water and 2 g of magnesium oxide and a drop or two of antifoam solution were added, then the mixture was distillated and the ammonia were received in boric acid 4.0% where the formed ammonia borate was titrated by hydrochloric acid solution (0.01 N) in the presence of mixed indicator (bromocrysol green / methyl red) and the results were calculated as mg nitrogen (T.V.N) per 100 g sample.

Water Holding Capacity (WHC) and Plasticity: Water holding capacity and plasticity were evaluated by following the filter press method according to Volovinskaia and MerKoolova [25] as follows: 0.3 g of the refrigerated samples were put above an ashless filter paper (Whatman No. 41) and pressed for 10 minutes using a 1 kg weight. After pressing, two zones were formed on the filter paper; the outer zones resulted from secretion of water from samples and the internal zones resembled the area of pressed meat. Then, the zones were measured by a planimeter (KOIZUMI Digital Planimeter PLACOM KP-92) in cm^2. Results were presented in cm^2 per 0.3 g sample.

Bacterial Count: Raw materials used for manufacture of refrigerated minced beef samples were first microbiologically examined under aseptic condition. Samples were taken immediately after processing and during refrigerated storage. Eleven grams of each sample were weighted and added to 99 ml of sterilized physiological solution and the mixture was blended for 30 sec [26]. Samples were examined for total viable bacterial count, Coliforms, *Staph. aureus* count and detection of *Salmonella sp.*

Statistical Analysis: The experimental data were analyzed using Analysis of Variance (ANOVA) followed by Duncan's multiple range test ($P \leq 0.05$) to determine a significant difference among samples. The data were analyzed according to User's Guide of Statistical Analysis System [27] at the Computer Center of Faculty of Agriculture, Ain Shams University.

RESULTS AND DISSCUSION

Antibacterial Activity of Methanolic Mango Seed Kernel Extracts (MSKE): Antibacterial activity of MSKE against several bacterial species has been recognized and considered as one of the most important properties linked directly to their possible biological applications. The results of the antibacterial effects of MSKE at different

concentrations ranged from 0.2 to 1.6% against six pathogenic and food spoilage bacterial strains and the inhibition zone diameters (mm) are given in Table 1. The control treatment (methanol only) did not show any inhibitory effect on all bacterial strains. Results obtained from disc diffusion method, followed by measurements of minimum inhibitory concentrations (MICs) with MSKE indicated that the MIC values of MSKE ranged between 0.8 - 1.4% (w/v). It could be noticed that *St. aureus* was the most sensitive tested microorganism, with the lowest MIC value 0.8% followed by 1.0% which given by *B. subtilis* in the presence of the crude MSKE. Both of *B. cereus, E. coli, S. typhimurium* and *Pseud. fluorescens* were also sensitive against the MSKE with MICs values of 1.0, 1.2, 1.4, 1.2 and 1.4%, respectively.

Figure 1 illustrated that a significant inhibitory effect of MSKE on the cell viability of tested bacterial strains was occurred. It could be reported that the antibacterial effects on all tested bacterial strains showed bacteriostatic effects, it is also clear from the results that the antibacterial activity increased with increasing the concentrations of MSKE. Similar results were obtained by Kabuki *et al.* [11] and Kaur *et al.* [17]. Generally, MSKE markedly inhibited the growth of most tested bacterial strains; however, the effects differed with the type of bacteria. The results indicated that the effect strengthened as the concentration of extract was increased. MSKE showed strong antibacterial activity for gram-positive bacteria than for gram-negative bacteria in the ratio of MIC ranged between (0.8-1.4%). The most feasible hypothesis of the antibacterial effect is due to the active component was shown to be a polyphenolic-type structure; however, its exact nature still remains to be elucidated. Kabuki *et al.* [11] reported that, the antimicrobial spectrums of ethanolic mango seed kernel extracts were more effective against gram-positive bacteria than gram-negative bacteria. This tendency of tannin could be explained by that the structures of cell envelope,

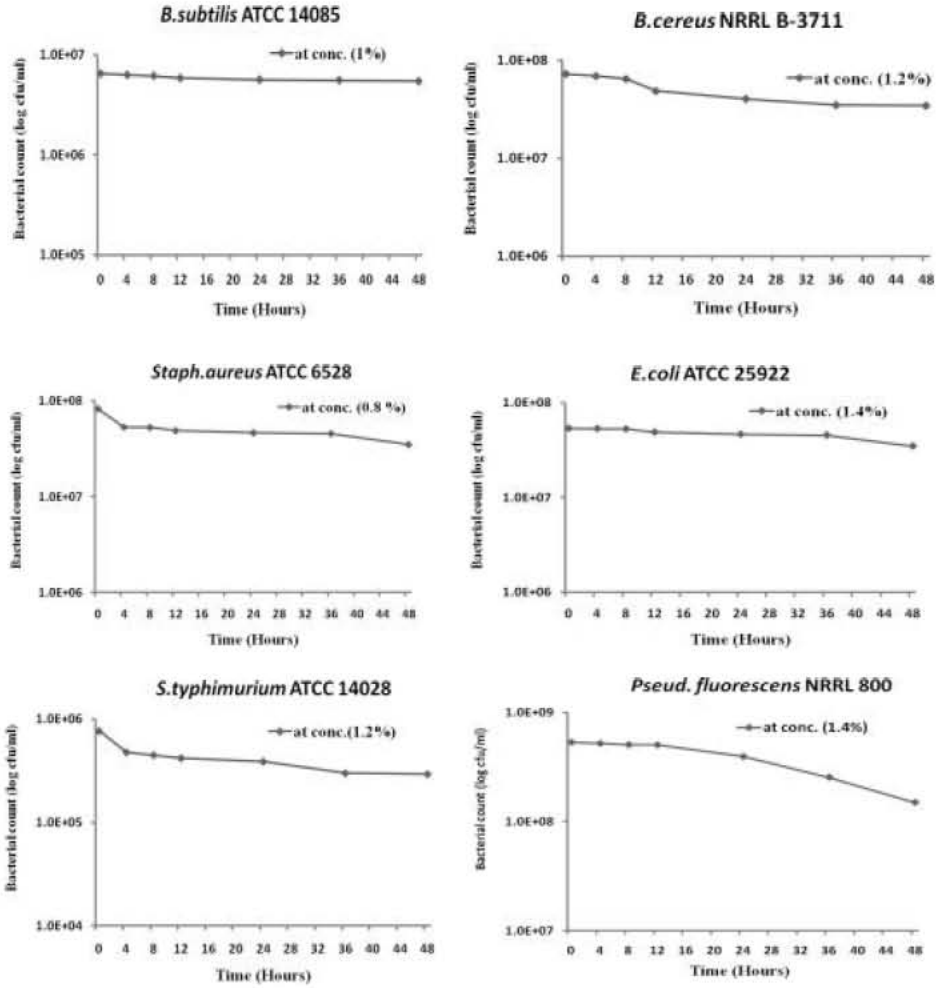

Fig. 1: Effect of different MIC concentrations of MSKE on the viability of tested bacterial strains.

Table 1: Antibacterial activity of MSKE at different concentrations against some food spoilage and pathogenic bacterial strains.

	*Diameter of inhibition zones (mm.)									
	MSKE conc.%									
Bacterial strains	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	Methanol	MIC % (w/v)
B. subtilis	19	21	23	25	26	26	26	28	-	1.0
B. cereus	17	18	19	20	21	22	22	23	-	1.2
St. aureus	18	23	25	28	28	27	27	28	-	0.8
E. coli	0	13	14	16	18	20	25	25	-	1.4
S. typhimurium	15	20	21	22	24	25	25	26	-	1.2
Pseud. fluorescens	-	-	13	18	19	20	25	25	-	1.4

* Including disc diameter (10 mm).
- = no inhibition zone.

Table 2: Chemical composition of minced beef incorporated with different levels of MSKP.

Treatment	Moisture	Ash	Crude protein	Lipids	Carbohydrate
Control (-)	62.86 ± 0.05^{c}	2.48 ± 0.09^{a}	15.85 ± 0.62^{a}	10.54 ± 0.28^{b}	8.27 ± 0.12^{a}
Control (+)	62.93 ± 0.07^{c}	2.56 ± 0.16^{a}	15.87 ± 0.04^{a}	10.49 ± 0.48^{b}	8.15 ± 0.08^{b}
MSKP 1.0 %	62.33 ± 0.11^{d}	2.58 ± 0.04^{a}	15.68 ± 0.18^{a}	11.39 ± 0.36^{a}	8.02 ± 0.24^{c}
MSKP 2.0 %	63.84 ± 0.16^{b}	2.44 ± 0.05^{a}	15.46 ± 0.24^{a}	11.52 ± 0.47^{a}	6.74 ± 0.17^{d}
MSKP 3.0 %	64.78 ± 0.05^{a}	2.51 ± 0.34^{a}	15.34 ± 0.09^{a}	11.69 ± 0.49^{a}	6.40 ± 0.06^{e}

Data are the mean ± SD, n = 3, mean followed by different letters in the same column differs significantly (P = 0.05)
Carbohydrate was calculated by difference.

Table 3: Changes in moisture content of minced beef incorporated with different levels of MSKP during cold storage at (4 ± 1°C).

	Storage time (day)					
Treatment	Zero	3	6	9	12	15
Control (-)	62.86±0.05^{cA}	62.21±0.21^{cAB}	61.89±0.44^{aB}	61.53±0.07^{abBC}	61.08±0.13^{aCD}	60.75±1.20^{aD}
Control (+)	62.93±0.07^{cA}	62.63±1.62^{bcAB}	61.86±1.02^{aABC}	61.64±0.43^{abABC}	61.46±1.06^{aBC}	60.88±0.26^{aC}
MSKP 1.0 %	62.33±0.11^{dA}	61.99±0.53^{cAB}	61.63±2.04^{aABC}	61.25±0.99^{bABC}	60.39±0.66^{aBC}	60.06±1.38^{aC}
MSKP 2.0 %	63.84±0.16^{bA}	63.51±0.29^{abA}	61.93±0.95^{aB}	61.40±0.41^{abBC}	60.52±2.06^{aBC}	60.36±1.62^{aC}
MSKP 3.0 %	64.78±0.05^{aA}	64.14±0.10^{aB}	62.34±1.42^{aC}	61.87±0.11^{aD}	60.93±0.61^{aE}	60.71±0.78^{aF}

Data are the mean ± SD, n = 3, mean followed by different small letters in the same column or different capital letters in the same raw differs significantly (P ≤ 0.05).

including cytoplasmic membrane and cell wall component, are different between gram-positive and gram-negative bacteria. Gram-negative bacteria possess an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. Without outer membrane, the cell wall of gram-positive bacteria can be permeated more easily and tannins can disturb the cytoplasmic membrane, disrupt the proton motive force (PMF), electron flow, active transport and coagulation of cell contents [28]. Kaur *et al.* [17] stated that the crude methanolic extract of mango seed kernel at 100 mg/ml had potential antimicrobial activity against methicillin resistant *S. aureus* and *E . coli* compared to *Vibrio vulnificus*. The antibacterial activity may be due to the phytochemical constituents (especially tannin) of the mango seed kernel [29].

Chemical Composition of Minced Beef Treated with MSKP: Chemical composition of minced beef containing 1, 2 and 3% of MSKP and negative and positive controls are presented in Table 2. It could be noticed that minced beef with 3% of MSKP had significantly (P≤0.05) higher moisture content (64.78%) followed by 63.84% which recorded by adding 2% of MSKP compared to controls. No significant (P≤0.05) difference was found in ash and crud protein between all selected treatments and controls. Results also showed that adding MSKP at any levels to minced beef caused significant (P≤0.05) increase in lipids content ranged from 11.39 to 11.69% compared to negative and positive controls. These results may be due to the high water holding capacity (2.08 g H_2O/g) and lipid content (8.15%) of MSKP [30].

Changes in Moisture Content of Minced Beef with MSKP During Cold Storage: Data in Table 3 show the changes in moisture content of minced beef containing 1, 2 and 3% of MSKP and negative and positive controls during refrigerated storage at (4 ± 1°C) until 15 day. At zero time the highest moisture content was recorded by adding 3%

of MSKP followed by 2% of MSKP with significant ($P \leq 0.05$) increase compared to negative and positive controls. The same trend was found in moisture content after 3 days of refrigerated storage. It could be noticed that beginning from sixth day until the last day of storage, no significant ($P \leq 0.05$) differences were observed between minced beef treated with 1, 2 and 3% of MSKP and controls. Regarding to the effect of storage period on moisture content of selected treatments, it was found gradually significant decrease from zero to 15 day of storage. It was clear that minced beef containing 3% of MSKP had the highest retention of moisture ranged from 64.78 to 60.71% followed by adding 2% of MSKP compared to other treatments and controls.

Changes in pH of Minced Beef with MSKP During Cold Storage: The effect of adding MSKP at 1, 2 and 3% to minced beef during refrigerated storage at (4 ± 1°C) until 15 day on pH values was studied and the results are presented in Table 4. It could be observed that no significant ($P \leq 0.05$) difference was found at zero time in pH between minced beef with adding 1, 2 and 3% of MSKP which ranged from 7.11 to 7.15 and positive control which recorded 7.12. On the other hand, from third day of refrigerated storage positive control had the highest pH values compared to all treatments and negative control. Generally, the pH was slightly significantly ($P \leq 0.05$) decreased during storage for all treatments and controls. The decrease in pH values during cold storage might be attributed to the breakdown of glycogen with the formation of lactic acid. This result is close to that reported by Pawar *et al.* [31].

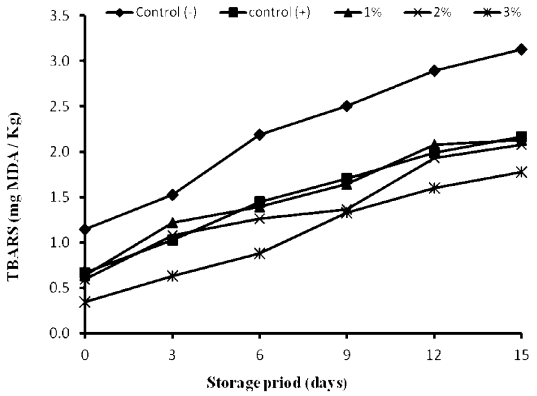

Fig 2: Changes in the TBARS of minced beef incorporated with MSKP during cold storage at (4±1°C).

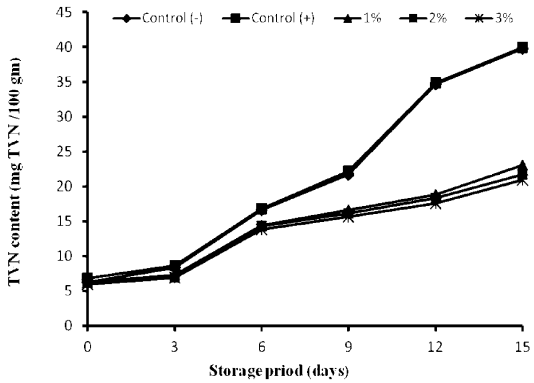

Fig. 3: Changes in the TVN of refrigerated minced beef incorporated with MSKP during cold storage at (4±1°C).

Changes in the Thiobarbituric Acid-Reactive Substance (TBARS) of Minced Beef During Cold Storage: The thiobarbituric acid (TBA) test has been widely used to estimate the extent of lipid oxidation in meat and meat products [32].The condensation between TBARS and malonaldehyde, a product of fatty acid oxidation, is the core of TBA test. The harmful effect of malonaldehyde on human health is mutagenic and carcinogenic agents [33].TBA values (expressed as mg malonaldehyde/kg) of refrigerated minced beef containing different concentrations of MSKP were measured during cold storage (4 ± 1°C) and the results are illustrated by Fig. (2). The results indicated that, during cold storage, TBA values tended to increase significantly and the negative control recorded the higher TBA value after 15 days of storage (3.128 mg malonaldehyde/kg), whereas the positive control and the samples containing different concentration of MSKP powder at 1.0, 2.0 and 3.0% showed the lowest TBA values after 15 days of storage (2.168, 2.129, 2.075 and 1.778 mg malonaldehyde/kg, respectively). From the previous results, it could be seen that, TBA values of minced beef samples containing MSKP exhibited their useful effect on the deterioration reactions happened in beef lipids during cold storage. Finally, it is worth to mention that, TBA values of all treatments were much less than the critical limit (10 mg malonaldehyde/kg) reported by Greene and Cumuze [34].

Changes in Total Volatile Nitrogen (TVN) of Refrigerated Minced Beef: It is well known that, total volatile nitrogen (TVN) content could be widely used as an indicator for protein decomposition caused by microorganisms as well as protein breakdown caused by tissue proteolytic enzymes during storage [35]. The TVN of refrigerated minced beef was determined at several times intervals during cold storage experiment

Table 4: Changes in pH of minced beef incorporated with different levels of MSKP during refrigerated storage at (4 ± 1°C).

	Storage time (day)					
Treatment	Zero	3	6	9	12	15
Control (-)	7.06±0.05^{bA}	6.93±0.06^{abB}	6.41±0.07^{cC}	6.40 ± 0.02^{abC}	6.36 ± 0.04^{bC}	6.33 ± 0.03^{bC}
Control (+)	7.12 ±0.03^{abA}	6.95±0.08^{aB}	6.63±0.05^{aC}	6.53 ± 0.06^{aD}	6.47 ± 0.05^{aD}	6.44 ± 0.07^{aD}
MSKP 1.0 %	7.14±0.02^{aA}	6.96±0.05^{aB}	6.57±0.04^{abC}	6.49 ± 0.11^{abC}	6.38 ± 0.09^{bD}	6.35 ± 0.05^{abD}
MSKP 2.0 %	7.11±0.02^{abA}	6.84±0.07^{abB}	6.54±0.10^{abC}	6.46 ± 0.08^{abC}	6.29 ± 0.02^{cD}	6.24 ± 0.06^{cD}
MSKP 3.0 %	7.15±0.02^{aA}	6.76±0.17^{bB}	6.50±0.03^{bcC}	6.37 ± 0.01^{bC}	6.22 ± 0.05^{cD}	6.19 ± 0.08^{cD}

Data are the mean ± SD, n = 3, mean followed by different small letters in the same column or different capital letters in the same raw differs significantly (P≤0.05).

Table 5: Effect of different concentrations of MSKP on WHC and plasticity as (cm^2/0.3 g) of minced beef during cold storage at (4 ± 1 °C).

	* Water holding capacity (WHC)				
Storage period (days)	Control (-)	Control (+)	1.0% MSKP	2.0% MSKP	3.0% MSKP
0	5.46±0.20^{bE}	5.77±0.26^{abE}	5.49±0.14^{abE}	5.73±0.18^{abE}	5.88±0.25^{aE}
3	5.93±0.29^{aDE}	6.06±0.26^{aE}	5.98±0.39^{aE}	6.17±0.31^{aE}	6.28±0.35^{aE}
6	6.47±0.29^{bD}	6.79±0.39^{abD}	7.32±0.27^{aD}	7.41±0.46^{aD}	7.46±0.44^{aD}
9	8.36±0.26^{aC}	8.50±0.25^{aC}	8.35±0.34^{aC}	8.39±0.32^{aC}	8.42±0.22^{aC}
12	9.26±0.61^{aB}	9.28±0.57^{aB}	9.69±0.39^{aB}	9.75±0.20^{aB}	9.77±0.12^{aB}
15	10.49±0.28^{aA}	10.48±0.50^{aA}	10.69±0.39^{aA}	10.80±0.13^{aA}	10.97±0.34^{aA}
	* Plasticity				
0	5.09±0.19^{aA}	5.07±0.52^{aA}	4.95±0.46^{aA}	5.13±0.31^{aA}	5.48±0.25^{aA}
3	4.68±0.22^{aAB}	4.67±0.28^{aAB}	4.62±0.39^{aAB}	5.07±0.49^{aA}	5.17±0.42^{aAB}
6	4.20±0.41^{bB}	4.15±0.33^{bB}	4.45±0.14^{abABC}	4.72±0.13^{abAB}	4.83±0.39^{aBC}
9	3.45±0.21^{bC}	3.25±0.19^{bC}	4.21±0.21^{aBCD}	4.53±0.37^{aAB}	4.56±0.18^{aC}
12	3.21±0.39^{bCD}	3.04±0.51^{bC}	3.95±0.24^{aCD}	4.25±0.19^{aBC}	4.32±0.40^{aCD}
15	2.74±0.25^{bD}	2.37±0.07^{bD}	3.76±0.37^{aD}	3.85±0.35^{aC}	3.92±0.15^{aD}

Data are the mean ± SD, n = 3, mean followed by different small letters in the same column or different capital letters in the same raw differs significantly (P≤0.05).

Table 6: Effect of cold storage at (4 ± 1°C) for 15 days on total viable bacterial count of minced beef containing different concentrations of MSKP.

	TVBC (cfu /g)				
Storage period (days)	Control (-)	Control (+)	1.0% MSKP	2.0% MSKP	3.0% MSKP
0	6.4 X 10^2	5.8 X 10^2	3.0 X 10^2	2.0 X 10^2	5.3 X 10^2
3	8.2 X 10^4	6.1 X 10^3	7.6 X 10^3	4.2 X 10^3	7.7 X 10^2
6	1.7 X 10^5	8.4 X 10^3	5.7 X 10^4	7.8 X 10^3	2.7 X 10^3
9	4.5 X 10^6	2.6 X 10^4	9.8 X 10^4	6.7 X 10^4	3.2 X 10^4
12	9.1 X 10^6	6.5 X 10^4	2.1 X 10^5	9.7 X 10^4	7.8 X 10^4
15	2.6 X 10^7	1.8 X 10^5	1.3 X 10^6	4.2 X 10^5	1.7 X 10^5

(for 15 days) and the results were expressed in term of mg TVN/100g sample on wet weight basis. Results illustrated in Fig. 3 showed that all samples had closed TVN content at zero time of storage (5.97- 6.77 mg TVN /100 g sample). Furthermore, the obtained data indicated that, TVN content was significantly increased during storage of different samples (20.90- 39.99 mg TVN /100 g sample). Results also revealed that the negative and positive controls had a higher increasing in TVN content which recorded 6.18 and 6.77 mg/100 g, respectively at zero time of cold storage and continuous increased to 39.73 and 39.99 mg/100g, respectively after 15 days. While the corresponding values for the refrigerated sample which prepared with 2.0 and 3.0 % MSKP had a lowest TVN content from the first of cold storage 6.09 and 5.97 mg/100g, respectively, to the end of cold storage period after 15 days which were 21.70 and 20.90 mg/100 g, respectively. The increase in TVN during cold storage of refrigerated minced samples might be attributed to the break-down of nitrogenous substances by microbial activity.

Changes in Water Holding Capacity and Plasticity of Refrigerated Minced Beef: Water holding capacity (WHC) and plasticity as area of released water in cm^2/0.3 g sample of the refrigerated minced beef containing 1.0, 2.0 and 3.0% of MSKP were determined during cold storage at (4 ± 1°C) for 15 days and the results are given in Table 5. It could be noticed that, the WHC of all samples progressively decreased ($P \leq 0.05$) with the increase of outer zones, resulted from secretion of water from samples, throughout the storage period. The progressive significantly ($P \leq 0.05$) decrease in the WHC was observed in all samples, possibly due to the protein denaturation or aggregation, or to the biochemical changes associated with cooling and freezing of meat products. The reduction of WHC values at the end of cold storage could be attributed to the loss of water by evaporation [36]. The plasticity (cm^2/0.3g sample) of all samples under investigation tended to a progressively decrease during cold storage until the termination of storage period (15 day). This might be explained on the basis of denaturation and/or aggregation of protein during cold storage, as well as the decrease in WHC of the studied cold and frozen meat products; and this reduction might be due to the tightening of minced beef structure because of evaporation of water. The decrease of plasticity was clearly pronounced in the positive control followed by negative control at the end of storage period.

Total Viable Bacterial Count: Total viable bacterial count (TVBC) of refrigerated minced beef incorporated with 1.0, 2.0 and 3.0% of MSKP was evaluated during cold storage at (4 ± 1°C) for 15 days and the resultant data are shown in Table 6. The obtained results revealed that all treatments exhibited closely or similar initial TVBC at zero time of cold storage which ranged from 2.0×10^2 to 6.4×10^2 cfu/g. This may be related to the good sanitary conditions followed during minced beef preparation. With the increasing of cold storage period, TVBC of negative control was increased progressively overtime from 6.4×10^2 cfu/g at zero time to 2.6×10^7 after 15 days. On the other hand, the obtained data revealed that other treatments showed slightly increase in TVBC overtime during cold storage. Also it is very important to consider the antimicrobial effect of sodium nitrite and MSKP. It could be observed that MSKP at 3.0% was more effective in extending the shelf life of refrigerated minced beef until 15 day in cold storage which had the lowest TVBC (1.7×10^5) followed by positive control compared to negative control. These results might be due to a variety of phenolic compounds such as flavonols and xanthones and gallotannins in the mango kernels. This observation is agreed with those reported by Puravankara [10], Engels *et al.* [12] and Tian *et al.* [13]. In addition, all investigated treatments were subjected to counting of Coliforms, *St. aureus* counts and *Salmonella* detection tests at zero time and every 3 days during cold storage until 15 days and the results were <10 cfu/ g sample for each Coliforms, *St. aureus* and negative for Salmonella detection test. Engels *et al.* [12] and Tian *et al.* [13] reported that bacteria of the genera Listeria, Bacillus, Clostridia and Staphylococcus were sensitive to gallotannins which consider the main component in MSKP. Generally, no previous publication searches on the application of MSKP as an antimicrobial agent in refrigerated minced meat were found.

CONCLUSION

The results of the current study show that the addition of mango seed kernel powder (MSKP) to minced beef with refrigerated storage significantly increase the moisture content and lipids, while the pH was slightly decreased for all treatments and controls. It could be also concluded that minced beef prepared with 2.0 and 3.0% MSKP had the lowest TBA and TVN content during cold storage period. It could be noticed that *St. aureus* was the most sensitive investigated microorganism with the lowest MIC value 0.8% followed by *B. subtilis* in the presence of the methanolic extract of mango seed kernel. In addition, the antibacterial effects on all tested bacterial strains showed bacteriostatic effects. The present study indicated that MSKP at 3.0% resulted in extension of the shelf life of refrigerated minced beef until 15 day in cold storage. These results reveal potential applications of mango seed kernel powder as good natural source of antibacterial agents in refrigerated minced beef.

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