

IMPACT OF NATURAL AND CHEMICAL AGENTS ON QUALITY AND BIOGENIC AMINE FORMATION OF CHILLED CAMEL MINCED MEAT

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Abstract: Camel minced meat is widely used in the world especially they were used to produce low-cost protein when compared with other animals. Therefore, the current study aimed to assess the effects of chitosan, rosemary essential oils, and sodium lactate on the organoleptic, chemical, and bacteriological quality of camel minced meat during refrigerated storage at 4°C. Twenty kg of camel meat (round meat) were obtained 24 hours after slaughter. The meat was minced and classified into four groups; untreated control and three treated groups (0.2% rosemary oil, 2% sodium lactate and 1% chitosan), which was kept under chilling conditions at 3±1° C and examined at different storage periods (zero-time, 3rd, 5th and 7th days of storage). The organoleptic, chemical, and bacteriological quality of minced meat was assessed. The surface discoloration was noticed on the 3rd day of the control group, but all treated groups retained their normal color especially 1% chitosan group, which maintained the highest value of redness. The sodium lactate group at 7th day of storage had pH, total volatile basic nitrogen (TVB-N mg/ 100 g) and thiobarbituric acid (TBA mg/kg) of 6.13, 14.3 and 1.08, respectively, while they were 6.37, 16.4 and 0.82 of rosemary group and 6.29, 15.64 and 0.72 of chitosan group, respectively. Spermine, spermidine, putrescine and tyramine were detected without significant differences in all examined groups but cadaverine and tryptamine were significantly low in all groups when comparing with the control group. Sodium lactate exhibits a great effect against bacterial growth on the 7th day of chilling by minimizing the bacterial load to be 5.95, 3.44 and 3.21 log₁₀ CFU/g for psychrotrophic, *Enterobacteriaceae* and pseudomonas, respectively with original counts of 6.98, 4.51, and 4.29 log₁₀ CFU/g, respectively. The current study concluded that the quality of camel minced meat could be enhanced by adding rosemary, sodium lactate, and chitosan, but sodium lactate was the best in controlling bacterial proliferation and biogenic amine formation.

Key words: camel minced meat; meat quality; bacterial count; biogenic amines; thiobarbituric acid

Introduction

In the last decade, the world camel's number had increased by 21% (1), especially they are used to produce low-cost protein when compared with other animals that produce red meat as cattle, buffalo, and sheep. From the economic side, camel meat shows low retail and wholesale prices (2).

The large number of camels are reared and bred for meat production in the near Northern and East Africa as well as for export to Egypt, the Gulf States, and Saudi Arabia (3). Camel meat is characterized by higher protein content with lower intramuscular fat than beef (4). Camel minced meat is commonly consumed in Egypt as a popular product which sold at a chilling temperature in retail outlets and butchers' shops. Minced meat is highly

susceptible to deteriorative changes due to several factors: a) original meat contamination, b) mincing increase surface area, leading to rapid decomposition and initiation of rancid flavor (5).

The chilling process alone for minced camel meat is not enough to keep the meat suitable for human consumption for a long time; therefore, it is necessary to find alternative methods with chilling to preserve the camel minced meat for a longer period. The safety and quality of minced meat can be enhanced by reducing the early microbial load, application of hygienic measures, and the addition of decontaminant during processing (6). The efficacy of antimicrobial agents depends on the surrounding product matrix, for example, proteins, carbohydrates, fats, and salts concentrations, as well as the pH of the chilled product (7). Recently, consumers are looking for naturally preserved food, therefore food producers are pursuing to use natural preservatives instead of chemicals to prolong shelf life, improve safety and quality of meat products (8).

Rosemary contains an active ingredient that inhibits a wide variety of spoilage bacteria, including Gram-negative bacteria, and extends the food shelf life (9, 10). Lactates are indeed present in meat after rigor mortis that is considered as a natural preservative for meat at concentrations up to 3 g/100 g by the USDA-Food Safety and Inspection Service (USDA-FSIS). The majority of research revealed that the addition of sodium lactate improves sensory parameters and cooking yield of meat products and minced beef (11).

Chitosan is a multipurpose biopolymer with a wide variety of applications in food processing (12), which is used as a preservative in various meat products (13, 14). Minimizing contamination of meat before mincing and delaying or inhibiting the growth of spoilage microorganisms are major keys for improving camel minced meat shelf life and increasing consumer safety. The objective of this study was to evaluate the effects of chitosan, rosemary essential oils, and sodium lactate on the organoleptic, chemical, and bacteriological quality of camel minced meat during refrigerated storage at 4°C.

Materials and methods

Samples preparation

Twenty kilograms of camel meat (round meat) were collected 24 hours after slaughter (4kg/trial of five replicates). Camel meat was minced and immediately transported to the laboratory of Meat Hygiene, Faculty of Veterinary Medicine Zagazig University, Egypt. Minced meat was divided into four groups. The control group was composed of one kg of minced meat, while the 0.2% rosemary treated group was prepared by adding 2 mL of rosemary oil to 998 g of minced meat. The sodium lactate 2% treated group was prepared by mixing 20 g of sodium lactate with 980 g of minced meat and gently massaged by hand for the homogenous distribution, but the chitosan 1% treated group was prepared by mixing 10 g of chitosan with 990 g of minced meat and gently massaged by hand for the homogenous distribution. Each group was separated into 20 clean identified Ziploc pages and kept on the fridge at $3\pm 1^\circ$ C. Samples of each group were collected immediately after treatment (zero time), 3rd, 5th, and 7th day.

Sensory evaluations

For determining the meat color, a panel of 11 experienced and trained panelists was chosen. Using the modified scale (5 = bright purple-red, 4 = dull purple-red, 3 = slightly brownish red, 2 = mildly brownish red, and 1 = brown), the panelists assessed minced meat for the overall color and worst point color (which describes a cumulative discolored area of at least 2 cm in diameter). On a scale ranging from 7 to 1, percent surface discoloration was measured, where 7 = no discoloration (0%), 6 = mild discoloration (1-20%), 5 = minor discoloration (20-39%), 4 = medium discoloration (40-59%), 3 = moderate discoloration (60-79%), 2 = substantial discoloration (80-95%), 1 = complete discoloration (96-100%). The odor characteristics of minced meat were evaluated as camel meat odor (8 = extremely camel meat like; 7 = very camel meat like; 6 = moderately camel meat-like; 5 = slightly camel meat-like; 4 = slightly non-camel meat-like; 3 = moderately non-camel meat-like; 2 = very non-camel meat-like and 1 = extremely non-camel meat-like) and off-odor (5 = no off-odor; 4 = slight off odor; 3 = small off-odor; 2 = moderate off odor; 1 = extreme off-odor) as described by Hunt *et al.* (15).

Table 1: Chromatographic conditions of biogenic amines

Time/min	Flow rate MI/min	Solvent A% ^a	Solvent B% ^b	Solvent C% ^c
0	1	60	20	20
10	1	20	40	40
15	1	15	35	50
20	1	60	20	20
25	1	60	20	20

^aSolvent A = 0.02 N acetic acid; ^bSolvent B = methanol; ^cSolvent C = acetonitrile.

Instrumental color analysis

The color of camel minced meat was measured by a Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and a light trap given by the manufacturer. The color was represented using the color system of CIE L*, a*, and b* (16). For each sample, a total of three spectral readings were taken. Lightness (L*) dark (0) to light (100), values of redness (a*) red-dish (+) to greenish (-). Yellow (b*) values (+) were determined to be yellowish to (-) bluish (16). Before doing color measurements, the spectrophotometer was standardized against a black and white glass tile.

Chemical parameters

The pH value of camel minced meat was assayed (17) with a CP-411 pH meter (Elmetron, Zabrze, Poland). Using two buffers of 4 and 7 pH, the electrode was calibrated. Five g of camel minced meat was mixed twice in a meat grinder with 45 mL of distilled water and thoroughly mixed to ensure sample homogeneity, and then the pH was calculated. TVB-N was estimated according to FAO (18). Briefly; 10 g of camel minced meat were homogenized and blended for 2 min with 100 mL distilled water then the sample was washed in distillation flask with 200 mL of water followed by addition of two g of magnesium oxide and two drops of an antifoaming agent. The mixture was left to boil for 10 minutes and distilled into 25 mL of 2 percent boric acid solution with a few drops of screened methyl red indicator in a 500 mL flask for around 25 min using the same heating rate. The heating was stopped, followed by washing down the condenser with distilled water. Using 0.1 N H₂SO₄ (titer), the flask contents and the blank solution (25 mL of 2% boric acid) were titrated then TVB-N (mg N/100 g flesh) was

determined using the formula: TVB-N = 14 (titer-blank). TBA was calculated by combining ten g of broiler fillet with 25 mL of 20% trichloroacetic acid (w/v) (19) then homogenized for 30s in a blender. Two mL of the prepared solution was applied to 2 mL of 0.02 mL of aqueous TBA after filtration and then incubated in the dark for 20 h at room temperature. The UV-vis spectrophotometer measured the absorbance at 532 nm (model UV-1200, Shimadzu, Japan). TBARS estimates were expressed in the sample of camel minced meat as mg malondialdehyde (MDA)/kg.

Biogenic amines

Five grams of camel minced meat were homogenized with 20 mL of 0.4 M HClO₄ and centrifuged for 5 min at 2500 rpm. The supernatant was obtained and mixed with 50 mL of 0.4 M HClO₄. The centrifuged acid extract was derivatized as follow: 200 µL of 2 N NaOH was added to 1mL of the diluted supernatant with 300 µL of the saturated NaHCO₃ solution and 2 mL of the dansylchloride solution (10 mg/mL of acetone) were added to the diluted supernatant and then buffered (20). After 15 minutes, 100 µL of NH₄OH was added to complete the reaction and to eliminate the residual dansylchloride.

The final volume was 5 mL after adding acetonitrile. Injected into the Liquid chromatograph, the collected dansylated solution which was purified (Agilent Technologies, Waldbronn, Germany, and Model 1100) fitted with a quaternary pump model G 1311A, a 254 nm wavelength ultraviolet detector (Model G 1314A), an autosampler (Model G1329A VP-ODS) and a Shim pack (150 9 4.6 mm) column for the separation of biogenic amines. With a Chemstation Software program, data were integrated and registered.

As defined in Table 1, the chromatographic conditions were 0.89, 0.92, 0.95, 0.87, 0.91, 0.88, 0.94 and 0.85, respectively, for spermine, spermidine, putrescine, tyramine, histamine, cadaverine, tryptamine and b-phenylethylamine, respectively.

Bacteriological parameters

Twenty-five g of minced meat was transferred to a stomacher bag; 225 mL of 0.1% peptone (OXOID CM1028, UK) water mixture was homogenized and serial dilution obtained up to 10^{-7} . Psychrotrophic bacteria were counted per g using plate count agar medium (OXOID CM0325, UK). For 10 days, the plates were inoculated and incubated at 37 °C (17). *Enterobacteriaceae* was enumerated on Violet Red Bile Glucose Agar (VRBGA; Difco, Detroit, Michigan, USA) by the pour plate method At 37 °C for 24 h. The plates were overlaid with a virgin layer of the same growth medium prior to incubation (21). *Pseudomonas* was counted on *Pseudomonas* Agar Base (CM 559; Oxoid, UK) supplemented with supplements of cetrime, fucidin, and cephaloridine (CFC) (SR 103; Oxoid, Basingstoke, Hampshire, UK) to provide a selective isolation medium for *Pseudomonas* spp. After 2 days of incubation at 25 °C, the colonies were counted (22).

Statistical analysis

The microbiological data was translated to a value of \log_{10} . The variations among groups were tested using the General Linear Model of SAS statistical system Package (23). Kolmogorov–Smirnov test was applied to guarantee the homogeneity and normality of variances among the different study groups. The statistical model was as the following:

$$Y_{ijk} = \mu + G_i + B_j + e_{ijk}$$

where Y_{ijk} showed the studied traits, μ is the overall mean for each trait, G_i is the fixed effect of i th groups with 4 levels where ($i =$ control group, 2 = rosemary oil 0.2 %, 3 = sodium lactate 2% and 4 = chitosan 1%), B_j is the fixed effect of replicates ($j = 1, 2, 3$ and 4) and e_{ijk} is the random residual effect. The difference among means was applied with Tukey's test. Log geometric mean was estimated for microbiological data. The significant was established at $P < 0.05$.

Results

Sensory evaluation

Sensory attributes (surface color, percentage of surface discoloration, camel meat odors, and off-odor) of camel minced meat had no significant changes at zero time. Meanwhile, on the 3rd day of chilling, a significant surface discoloration ($P < 0.05$) was detected in the control group.

By the 5th day, the overall numerical values for the control group significantly decreased ($P < 0.05$) in all sensory parameters with a color scale of 2.6 (slightly to moderately brownish red), 2.75 percentage of surface discoloration (60% to 95% surface discoloration), 4.1 camel meat odor (slightly camel meat like to slightly non-camel meat-like) and 3.8 off orders (slight to small off-odor). On the 7th day of storage, the chitosan 1% treated group had the highest color score of 3.3 with the lowest percentage of surface changes 4.2, and the likeness of camel meat under 5.8. Moreover, the off-odor score was 1.6 ± 0.12 , 3.4 ± 0.26 , 3.00 ± 0.24 , 3.2 ± 0.21 in the control, rosemary 0.2%, sodium lactate 2% and chitosan 1% treated groups, respectively Table 2.

Instrumental color analysis

Data of Table 3 showed the mean values of lightness L^* were higher in rosemary (44.58 and 45.27) and sodium lactate (43.18 and 42.34) than control (40.83 and 39.72) and chitosan (39.54 and 39.41) groups at zero time and 7th days of storage, respectively. The mean values of redness a^* at zero time were in descending manner 30.31, 28.49, 27.43 and 25.12 for rosemary, control, sodium lactate, and chitosan groups, respectively.

Chemical parameters

The results of Table 4 revealed that the mean values of pH were 5.58, 5.56, 5.23 and 5.43 in control, rosemary, sodium lactate and chitosan groups, respectively at zero time. Significant differences between groups were observed ($P < 0.05$) by the end of the storage period. The control group had the highest pH value. Moreover, chitosan had a significant effect on pH. The TVB-N at zero time was 5.88 in all examined groups. On the 3rd day of chilling at 4°C, the TVB-N gradually increased in all groups (Table, 4). The control group significantly had a higher level of TVB-N ($P < 0.05$).

Table 2: Effect of rosemary 0.2%, sodium lactate 2% and chitosan 1% on sensory evaluation of chilled camel minced meat at $3 \pm 1^\circ \text{C}$ (Mean \pm SE)

Storage duration	Criteria	Control	Rosemary 0.2 %	Sodium lactate 2%	Chitosan 1%
Zero time	Color	4.8 \pm 0.32	4.5 \pm 0.41	4.3 \pm 0.56	4.6 \pm 0.25
	SD (%)	6.84 \pm 0.49	6.42 \pm 0.39	6.12 \pm 0.54	6.66 \pm 0.38
	Odor	7.89 \pm 0.14	7.12 \pm 0.34	7.14 \pm 0.24	7.69 \pm 0.28
	Off odor	5	5	5	5
3 rd day	Color	4.3 \pm 0.43	4.4 \pm 0.27	4.2 \pm 0.24	4.5 \pm 0.31
	SD (%)	3.17 \pm 0.38 ^b	5.12 \pm 0.45 ^a	4.8 \pm 0.39 ^a	5.34 \pm 0.52 ^a
	Odor	6.4 \pm 0.35	6.8 \pm 0.26	6.35 \pm 0.39	6.9 \pm 0.43
	Off odor	4.6 \pm 0.36	5	4.8 \pm 0.44	5
5 th day	Color	2.6 \pm 0.18 ^b	3.7 \pm 0.22 ^a	3.1 \pm 0.23 ^{ab}	3.8 \pm 0.16 ^a
	SD (%)	2.75 \pm 0.24 ^b	4.88 \pm 0.36 ^a	3.97 \pm 0.38 ^{ab}	4.95 \pm 0.42 ^a
	Odor	4.1 \pm 0.28 ^b	6.2 \pm 0.37 ^a	5.6 \pm 0.28 ^{ab}	6.4 \pm 0.32 ^a
	Off odor	3.8 \pm 0.19 ^b	4.6 \pm 0.27 ^a	4.2 \pm 0.31 ^{ab}	4.4 \pm 0.29 ^a
7 th day	Color	1.6 \pm 0.11 ^c	3.2 \pm 0.16 ^a	2.5 \pm 0.14 ^b	3.3 \pm 0.12 ^a
	SD (%)	1.32 \pm 0.16 ^c	3.98 \pm 0.27 ^a	3.1 \pm 0.17 ^b	4.2 \pm 0.21 ^a
	Odor	2.44 \pm 0.13 ^c	5.62 \pm 0.37 ^a	4.8 \pm 0.36 ^b	5.8 \pm 0.41 ^a
	Off odor	1.6 \pm 0.12 ^b	3.4 \pm 0.26 ^a	3.00 \pm 0.24 ^{ab}	3.2 \pm 0.21 ^a

Color scale: (5 = bright purplish red, 4 = dull purple red, 3 = slightly brownish red, 2 = moderately brownish red, and 1 = brown). Surface discoloration (SD) %: 7 = no discoloration (0%), 6 = slight discoloration (1-20%), 5 = small discoloration (20-39%), 4 = modest discoloration (40-59%), 3 = moderate discoloration (60-79%), 2 = extensive discoloration (80-95%), 1 = total discoloration (96 -100%). Beef odor: (8 = extremely beef like, 7 = very beef like, 6 = moderately beef like, 5 = slightly beef like, 4 = slightly non-beef like 3 = moderately non-beef like, 2 = very non-beef like and 1 = extremely non-beef like). Off odor: (5 = no off odor, 4 = slight off odor, 3 = small off odor, 2 = moderate off odor, 1 = extreme off odor)

Table 3: Effect of rosemary 0.2%, sodium lactate 2% and Chitosan 1% on instrumental color analysis of chilled camel minced meat at $3 \pm 1^\circ \text{C}$

Storage duration	Groups	L* (lightness)	a* (redness)	b* (yellowness)
Zero time	Control	40.83 \pm 0.43 ^b	28.49 \pm 0.32 ^a	14.14 \pm 0.18 ^b
	Rosemary	44.58 \pm 0.05 ^a	30.31 \pm 0.41 ^a	16.21 \pm 0.22 ^a
	Sodium lactate	43.18 \pm 0.18 ^a	27.43 \pm 0.63 ^{ab}	14.54 \pm 0.41 ^b
	Chitosan	39.54 \pm 0.18 ^b	25.12 \pm 0.14 ^b	16.82 \pm 0.16 ^a
3 rd day	Control	40.22 \pm 0.26 ^b	21.50 \pm 0.25 ^c	13.25 \pm 0.19 ^b
	Rosemary	44.90 \pm 0.12 ^a	28.50 \pm 0.44 ^a	15.41 \pm 0.24 ^a
	Sodium lactate	42.90 \pm 0.15 ^{ab}	21.24 \pm 0.53 ^c	13.47 \pm 0.41 ^{ab}
	Chitosan	39.49 \pm 0.32 ^b	24.35 \pm 0.21 ^b	15.96 \pm 0.32 ^a
5 th day	Control	39.94 \pm 0.15 ^b	17.24 \pm 0.14 ^d	11.26 \pm 0.15 ^c
	Rosemary	45.10 \pm 0.17 ^a	24.60 \pm 0.23 ^b	13.48 \pm 0.19 ^{ab}
	Sodium lactate	42.26 \pm 0.21 ^{ab}	17.21 \pm 0.27 ^d	11.21 \pm 0.29 ^c
	Chitosan	39.45 \pm 0.31 ^b	23.21 \pm 0.12 ^b	14.14 \pm 0.28 ^b
7 th day	Control	39.72 \pm 0.13 ^b	14.10 \pm 0.43 ^{cd}	10.45 \pm 0.38 ^d
	Rosemary	45.27 \pm 0.15 ^a	21.35 \pm 0.21 ^c	12.90 \pm 0.24 ^b
	Sodium lactate	42.34 \pm 0.23 ^{ab}	12.82 \pm 0.19 ^e	10.03 \pm 0.19 ^d
	Chitosan	39.41 \pm 0.26 ^b	22.09 \pm 0.27 ^c	12.95 \pm 0.28 ^b

(^{a,b,c}) different superscript letters in the same column indicate significant differences ($p < 0.05$)

Table 4: Effect of rosemary 0.2%, sodium lactate 2% and chitosan 1% on pH, total volatile basic nitrogen (TVB-N) mg/ 100 g and thiobarbituric acid (TBA mg/kg) of chilled camel minced meat at $3 \pm 1^\circ \text{C}$

	Storage days	Control	Rosemary 0.2%	Sodium lactate 2%	Chitosan 0.1%
PH	Zero time	5.58 \pm 0.41 ^a	5.56 \pm 0.36 ^a	5.23 \pm 0.15 ^b	5.43 \pm 0.26 ^a
	3 rd day	6.18 \pm 0.29 ^a	6.07 \pm 0.12 ^a	5.35 \pm 0.15 ^c	5.82 \pm 0.19 ^b
	5 th day	6.38 \pm 0.32 ^a	6.12 \pm 0.28 ^a	5.92 \pm 0.24 ^b	6.01 \pm 0.23 ^b
	7 th day	6.68 \pm 0.29 ^a	6.37 \pm 0.27 ^b	6.13 \pm 0.18 ^c	6.29 \pm 0.25 ^b
(TVB-N) mg/ 100 g	Zero time	5.88 \pm 0.23			
	3 rd day	8.7 \pm 0.16 ^a	6.8 \pm 0.14 ^a	6.11 \pm 0.10 ^a	6.26 \pm 0.14 ^a
	5 th day	11.8 \pm 0.24 ^a	8.71 \pm 0.19 ^b	6.11 \pm 0.17 ^b	7.13 \pm 0.15 ^b
	7 th day	22.26 \pm 0.94 ^a	16.4 \pm 0.86 ^b	14.3 \pm 0.82 ^b	15.64 \pm 0.78 ^b
TBA mg/kg	Zero time	0.14 \pm 0.02			
	3 rd day	0.4 \pm 0.05 ^a	0.32 \pm 0.02 ^a	0.37 \pm 0.03 ^a	0.27 \pm 0.01 ^b
	5 th day	0.96 \pm 0.06 ^a	0.74 \pm 0.04 ^b	0.89 \pm 0.05 ^b	0.56 \pm 0.03 ^c
	7 th day	1.12 \pm 0.04 ^a	0.82 \pm 0.05 ^b	1.08 \pm 0.03 ^a	0.72 \pm 0.04 ^c

(^{a,b and c}) Means within the same row bearing different small superscript letters are significantly different ($P < 0.05$).

Table 5: Effect of rosemary 0.2%, sodium lactate 2% and chitosan 1% on formation of biogenic amines on 7th day chilling camel minced meat at $3 \pm 1^\circ \text{C}$ (means \pm SE)

Biogenic amine	Control	Rosemary 0.2%	Sodium lactate 2%	Chitosan 0.1%
Spermine	22.82 \pm 1.36 ^a	23.11 \pm 1.38 ^a	22.63 \pm 1.24 ^a	22.84 \pm 1.27 ^a
Spermidine	3.20 \pm 0.54 ^a	3.08 \pm 0.51 ^a	3.39 \pm 0.57 ^a	3.29 \pm 0.53 ^a
Putrescine	1.62 \pm 0.12 ^a	1.51 \pm 0.11 ^a	1.57 \pm 0.11 ^a	1.56 \pm 0.11 ^a
Tyramine	3.17 \pm 0.24 ^a	1.24 \pm 0.13 ^b	1.11 \pm 0.12 ^b	1.21 \pm 0.14 ^b
Histamine	ND	ND	ND	ND
Cadaverine	4.62 \pm 0.37 ^a	2.41 \pm 0.25 ^b	1.91 \pm 0.26 ^b	2.16 \pm 0.23 ^b
Tryptamine	2.32 \pm 0.19 ^a	1.16 \pm 0.16 ^b	1.08 \pm 0.10 ^b	1.26 \pm 0.15 ^b
b-phenylethylamine	ND	ND	ND	ND

(^{a,b,c}) different superscript letters in the same rows indicate significant differences ($P < 0.05$), ND: Not detected

Table 6: Effect of rosemary 0.2%, sodium lactate 2% and chitosan 1% on Psychrotrophic, *Enterobacteriaceae* and *Pseudomonas* count log₁₀CFU/g of chilled camel minced meat at $3 \pm 1^\circ \text{C}$

	Storage days	Control	Rosemary 0.2%	Sodium lactate 2%	Chitosan 0.1%
Psychrotrophic count CFU/g	Zero time	4.62 \pm 0.28 ^a	4.20 \pm 0.26 ^a	4.04 \pm 0.21 ^a	4.15 \pm 0.24 ^a
	3 rd day	5.30 \pm 0.41 ^a	4.98 \pm 0.38 ^a	4.32 \pm 0.36 ^b	4.81 \pm 0.39 ^{ab}
	5 th day	6.30 \pm 0.34 ^a	6.00 \pm 0.32 ^a	5.04 \pm 0.28 ^b	5.78 \pm 0.26 ^b
	7 th day	6.98 \pm 0.45 ^a	6.26 \pm 0.41 ^b	5.95 \pm 0.44 ^c	6.11 \pm 0.39 ^{bc}
<i>Enterobac- teriaceae</i> count CFU/g	Zero time	3.04 \pm 0.28 ^a	2.78 \pm 0.26 ^a	2.6 \pm 0.24 ^a	2.6 \pm 0.25 ^a
	3 rd day	3.34 \pm 0.19 ^a	3.08 \pm 0.18 ^a	2.95 \pm 0.16 ^a	3.04 \pm 0.21 ^a
	5 th day	3.96 \pm 0.25 ^a	3.11 \pm 0.23 ^b	2.86 \pm 0.22 ^c	3.02 \pm 0.26 ^b
	7 th day	4.51 \pm 0.32 ^a	3.85 \pm 0.29 ^b	3.44 \pm 0.29 ^c	3.72 \pm 0.28 ^b
<i>Pseudomo-nas</i> count CFU/g	Zero time	3.10 \pm 0.31 ^a	2.78 \pm 0.28 ^a	2.30 \pm 0.27 ^b	2.48 \pm 0.29 ^a
	3 rd day	3.26 \pm 0.23 ^a	2.75 \pm 0.21 ^b	2.45 \pm 0.29 ^b	2.62 \pm 0.28 ^b
	5 th day	3.86 \pm 0.32 ^a	3.12 \pm 0.29 ^b	2.95 \pm 0.30 ^b	3.06 \pm 0.29 ^b
	7 th day	4.29 \pm 0.62 ^a	3.62 \pm 0.59 ^b	3.21 \pm 0.58 ^c	3.53 \pm 0.54 ^b

(^{a,b and c}) Means within the same row bearing different small superscript letters are significantly different ($p < 0.05$).

The results of Table 4 showed that both rosemary and chitosan groups were in the range of permissible level reported by Egyptian standard 1694 (39) which limited the value of TBA must be not more than 0.9 mg MDA/kg /kg for meat.

There are no significant differences ($P < 0.05$) between examined groups for spermine, spermidine and putrescine (Table 5). On the contrary, significant differences ($P < 0.05$) were obtained in examined groups for tyramine, cadaverine, and tryptamine.

Bacteriological parameters

Psychrotrophic count at zero time in all examined groups was ranged from 4.04 log₁₀ CFU/g in sodium lactate 2% to 4.62 log₁₀ CFU/g in the control group (Table 6). There were significant differences in psychrotrophic count among the examined groups where chitosan showed a clear antimicrobial effect ($P < 0.05$) on the 7th day of storage, while a stronger antimicrobial effect was shown in sodium lactate group. The recorded results of Table 6 showed that *Enterobacteriaceae* count at zero time was 3.04, 2.78, 2.6 and 2.6 log₁₀CFU /g in control, rosemary, sodium lactate and chitosan groups, respectively. The counts of *Enterobacteriaceae* increased directly with progress in the chilling period. Significant differences in the *Enterobacteriaceae* count between the examined groups ($P < 0.05$) on the 5th and 7th days of chilling. There were significant differences in the *Pseudomonas* count among the examined groups ($P < 0.05$) on the 3rd, 5th and 7th days of chilling (Table 6).

Discussion

Meat appearance is the major quality feature influencing the purchasing decision since consumers frequently depend upon fresh meat appearance as a meter of wholesomeness (24). The control group had a significantly ($P < 0.05$) lower in color score, odor likeness with extensive surface discoloration and off odor as shown in Table (2). Generally, the significant effects ($P < 0.05$) of different additives reflected in the organoleptic evaluation of chilled camel minced meat groups as, in a rosemary group, the color score and percentage of surface discoloration in all treated groups attributed to the beneficial effects of natural antioxidants in preventing color

deterioration (25). Sodium lactate stabilizing the color of beef whole-muscle cuts and ground beef as lactate promotes redox stability via direct interactions with myoglobin (26) and indirect interactions with lactate dehydrogenase (27). Off-odor, intensity was the lowest of the rosemary group that may be attributed to the beneficial effects of natural antioxidants in lipid oxidation that change the odor acceptability (25).

The lightness L* was higher in rosemary (44.58 and 45.27) and sodium lactate (43.18 and 42.34) than control (40.83 and 39.72) and chitosan (39.54 and 39.41) groups at zero time and 7th days of storage, respectively (Table 3). Sodium lactate effects on L* were not on the same line with Suman et al. (28) who stated that lactate patties were darker (lower L* values) than their control counterparts. Meanwhile, the effects of rosemary were comparable to the findings of others (8, 29). They found higher L* values of minced beef treated with essential oil when compared with the control group. Chitosan 1% could maintain the redness value of camel minced meat than other additives. Chitosan has a stabilizing effect on the red color of meat. Darmadji and Izumimoto (30) reported higher a* values of minced meat mixed with chitosan after 10 days of storage. The decreasing trend of a* value of sodium lactate group coincided with the findings of Seyfert, et al. (31) who did not detect any readily observable redness of lactate in the ground beef, although it increased metmyoglobin that reduced the activity. Also, a decreasing trend was observed in a* values of the control group, which may be due to the gradual oxidation of myoglobin and accumulation of metmyoglobin during the storage period (32, 33). The mean values of yellowness b* were increased from 14.14 (control) to 16.21 (rosemary), 14.54 (sodium lactate) and 16.82 (chitosan) at zero time. A significant increase of b* in rosemary group was comparable with the findings of others (8, 29). They reported higher b* values of minced beef treated with essential oil as compared to the control group. The b* values gradually reduced with the increased storage period for all groups. Chitosan group was the highest b* value 12.95, which could be attributed to the natural yellowish color of chitosan affecting the minced beef color. Others reported similar observations in pork sausages with added chitosan (34).

The pH value is an important physicochemical characteristic to decide the quality and shelf life of meat. Sallam and Samejima (35) who reported 5.65-5.8 initial pH in ground beef samples at zero time of storage supported the mean values of pH at zero time. There was a significant decrease in pH of the sodium lactate group ($P < 0.05$), which was attributed to the reduction of pH immediately because of adding sodium lactate. On the contrary, Tan and Shelef (36), reported that sodium lactate had no significant effects on the initial pH of ground meat products. Significant differences between groups were observed ($P < 0.05$) by the end of the storage period. The control group had the highest pH value, which may be owed to the utilization of amino acids by bacteria, with the accumulation of ammonia as the product of amino acid decomposition increased pH (37). Chitosan had a significant effect on pH, which attributed to the inhibition of bacterial growth. On contrary, Chounou *et al.* (38) noticed that the addition of chitosan at a concentration of 1% did not affect pH during storage at 4 °C.

The enzymatic action and microbial activity are responsible for protein degradation and formation of total volatile nitrogen. The quantity of TVB-N is a very good chemical indicator to assess the quality and freshness of the meat. The TVB-N gradually increased in all groups (Table 4). All treated groups were in the range of permissible level reported by ES 1694 (39), which limited the content of TVB-N must be not over 20mg/100g. Meanwhile, the control group exceeded the permissible limit. The control group significantly had a higher level of TVB-N ($P < 0.05$) which attributed to the higher and rapid psychrotrophic growth and multiplication in the control group led to degradation of protein and the formation of free amines. Protein, as the main constituent in meat, is uninterruptedly broken down by bacteria and finally produced a diversity of amines, including non-volatiles such as volatile amines and biogenic amines (40).

Degraded consistency of meat during storage due to lipid oxidation is associated with the presence of free radicals contributing to the production of aldehydes responsible for major changes in meat color and rancid flavors (41). Our results proved the efficacy of rosemary and

chitosan groups that were in the range of permissible level reported by Egyptian standard 1694 (39) which limited the value of TBA must be not more than 0.9 mg MDA/kg /kg for meat. A significant reduction in the formation of TBA in the rosemary group is due to the presence of phenolic diterpenes, such as carnosic acid andarnosol. These compounds interact in the free radical chain reaction as hydrogen donors (42). Moreover, the chitosan group contains the lower value of TBA due to the chelation of free iron that is released from meat hemoproteins during heat processing and storage (43). The effect of chitosan on oxidative stability of minced beef was studied by Darmadji and Izumimoto (44) who observed that the addition of chitosan (1%) resulted in a 70% reduction in TBA values of meat after 3 days at 4 °C. Moreover, Chounou *et al.* (38) noticed that the addition of chitosan at a concentration of 1% had a significant effect on the MDA, which is equal to 1.5 and 1 mg MDA/ kg in control and chitosan-treated samples during storage at 4 °C.

The anti-nutritional nitrogenous bases of biogenic amines are generated by the action of free microbial decarboxylases on amino acids. They are commonly found in different concentrations in meat and the products of meat. The importance of public health residues in their high levels of meat and meat products are associated with adverse effects (44). Furthermore, biogenic amine processing offers an indicator of shelf life and the sanitary condition of meat and meat products (46). There are no significant differences ($P < 0.05$) between examined groups for spermine, spermidine and putrescine (Table 5). The results obtained in this analysis are consistent with other studies suggesting that spermine content, higher than spermidine is typically found in foods of animal origin, primarily in muscles, while in foods of plant origin, the opposite is seen. In addition, their production is not restricted by bacterial activity in foods (46). Significant differences ($P < 0.05$) were obtained in examined groups for tyramine, cadaverine and tryptamine, which attributed to the increase in growth and proliferation of *Enterobacteriaceae* and *Pseudomonas*. In meat products, the function of decarboxylase is primarily attributed to *Enterobacteriaceae*, *Pseudomonadaceae*, *Micrococcaceae*, and lactic

bacteria. Many researchers have tried to link the development of biogenic amines in meat and meat products with the behavior of different types of microorganisms (10, 47).

Psychrotrophic count at zero time in all examined groups was ranged from 4.04 log₁₀ CFU/g in sodium lactate 2% to 4.62 log₁₀ CFU/g in the control group (Table 6). The current findings were supported by Sallam and Samejima (35) who reported that the initial psychrotrophic count in ground beef samples was ranged from 4.0 to 4.14 log₁₀ CFU/g at zero storage day. There were significant differences in psychrotrophic count among the examined groups where chitosan showed a clear antimicrobial effect ($P < 0.05$) on the 7th day of storage, while a stronger antimicrobial effect was shown in the sodium lactate group. Important decrease in the sodium lactate group ($P < 0.05$) is due to a decrease in bacterial cytoplasm pH after permeation through the bacterial membranes of the non-dissociated types (48). Since the compounds are primarily present in the aqueous phase, this mass transfer takes place quickly, rendering the compounds very efficient depending on the pH (49). The addition of sodium lactate has been reported to produce a significant reduction in the growth of aerobic plate count in refrigerated ground beef (35, 51) and ground pork (51) as well as in cooked beef products (51). Moreover, the psychrotrophic count in minced meat reduced under the effect of sodium lactate 3% during chilling at 2 °C from 9.98 -7.79 log₁₀ CFU/g (35). Soultos et al. (14) reported a reduction of 0.5 and 1 log CFU/ g in aerobic plate count of fresh sausages after 1 day of storage at 4 °C with the addition of chitosan at a concentration of 0.5 and 1%, respectively. Moreover, Chounou et al. (38) also reported that the addition of chitosan at a concentration of 1% reduced the aerobic plate count of minced beef by 0.4 to 2 log₁₀ CFU /g during storage at 4 °C.

Enterobacteriaceae count was regarded as an indicator of hygiene suggesting good manufacturing practices in the processing plant (52). The recorded results in Table 6 showed that *Enterobacteriaceae* count at zero time was 3.04, 2.78, 2.6 and 2.6 log₁₀ CFU /g in control, rosemary, sodium lactate and chitosan groups, respectively. The counts of *Enterobacteriaceae* increased directly with progress in the chilling period. Significant

differences in the *Enterobacteriaceae* count between the examined groups ($P < 0.05$) on the 5th and 7th days of chilling. Regarding the effect of sodium lactate, the results are in line with Rondinini et al. (53) who reported inhibitory effects of sodium lactate against *Enterobacteriaceae* counts in cooked hams during vacuum-packaged storage for up to 9 weeks at 6 °C, and also with Sallam and Samejima (35) who reported a reduction in *Enterobacteriaceae* population from 7.39 to 5.19 log₁₀ CFU/g during storage of minced beef. Soultos et al. (14) reported a reduction in *Enterobacteriaceae* population of 1.1 log₁₀ CFU/ g after the addition of chitosan 1% in pork sausages after 7 days of storage at 4 °C. The addition of chitosan 1% in minced beef reduced the *Enterobacteriaceae* count by 0.7 log₁₀ CFU/g on day 6 of storage Chounou et al. (38).

Pseudomonas is Gram-negative bacteria, purely aerobic and one of the major spoilage microorganisms in fresh meat, (5). There were significant differences in the *Pseudomonas* count among the examined groups ($P < 0.05$) on the 3rd, 5th and 7th days of chilling. Georgantelis et al. (43) found that adding 1% chitosan to sausages reduced the *pseudomonas* population by 0.8 and 1.1 log₁₀ CFU/g after 5 and 10 days, respectively. The present results were also in general agreement with those of Soultos et al. (14) who stated that the use of chitosan at a concentration of 0.5 and 1% resulted in a reduction of 0.5 and 0.9 log CFU/ g in the *pseudomonas* population in sausages stored at 4 °C. Moreover, the addition of a chitosan 1% in minced beef reduced the *pseudomonas* population by 0.6 log CFU/ g on day 6 of storage (38). In general, the mode of antimicrobial action of chitosan may be due to its association

with membranes and components of the cell wall, resulting in increased membrane permeability and leakage of cell material from the tissue, its ability to attach water and inhibit various enzymes and it absorbs bacterial nutrients and thus inhibits their growth (54). Additionally, the action of essential oil (rosemary) presence of phenolic components, includes disrupting the proton motive force, the cytoplasmic membrane, active transport, flow of electron, and coagulation of cell contents. All these actions are important for coagulation of the cytoplasmic inner cellular

components and destroying the lipid bonds among the protein layers. Also, EOs could increase the cell membrane permeability causing outflow of the necessary intracellular components, in addition to the impairment of cellular respiration and bacterial enzyme system (55-57).

Conclusion

Camel meat produces a low-cost animal protein when compared with other animals that produce red meat as cattle, buffalo, and sheep. From the economic side, camel meat shows low retail and wholesale prices. The quality of camel minced meat could be enhanced by adding rosemary, sodium lactate, and chitosan. The current study revealed that adding sodium lactate to camel minced meat was the best in controlling bacterial proliferation and biogenic amine formation.

Acknowledgments

This research has been funded by the Scientific Research Deanship at University of Ha'il, Saudi Arabia through project number RG-20 200.

All authors contributed equally in conceptualization; data curation; formal analysis; investigation; methodology; resources; validation; visualization; roles/writing - original draft; writing - review & editing. All authors have read and agreed to the published version of the manuscript.

The authors declare no conflict of interest.

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