The effect of rosemary extract and lactic acid on the quality of refrigerated broiler fillets

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Abstract The current study investigated the effect of rosemary extract (0.2% RE) and lactic acid (1% LA) on some freshness, bacterial parameters and formation of biogenic amines during refrigeration of broiler fillets. Ninety broiler fillet samples were divided into three groups (each 30). The control group was dipped in sterile distilled water, while the RE and LA groups were dipped in rosemary extract 0.2% (w/v) and lactic acid 1% (w/v), respectively. Sensory evaluation, pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), total *psychrotrophic* and *Enterobacteriaceae* counts were performed at zero time and at 3 days interval until the group were rejected from sensory analysis. Organoleptic scores were unacceptable after the 9th, 12th and 15th day of refrigeration in control, RE and LA groups, respectively. TVB-N was the lowest in LA group (3rd day; 4.36 ± 0.12 , 6th day; 5.62 ± 0.7 and 9th day; 10.13 ± 0.98) when compared with the RE and control groups. Moreover, TBARS was the highest in the control group (3rd day; 0.22 ± 0.06 , 6th day; 0.39 ± 0.07 and 9th day; 0.78 ± 0.09) when compared with the RE and LA groups. LA group showed the lowest number of *psychrotrophic* bacteria during refrigeration period when

compared with the other groups. *Enterobacteriaceae* was detected at zero, 3rd and 6th day in control, RE and LA groups, respectively. This study concluded that dipping in LA 1% or RE 0.2% reduce the *psychrotrophic* and *Enterobacteriaceae* bacteria of broiler fillet and increased the acceptability of refrigerated fillet, decreased the biogenic amines formation and rancidity. Therefore, broiler fillets dipped in RE 0.2% or LA 1% can be used up to 12 or 15 days when refrigerated at 4 °C.

Keywords Broiler fillets · Biogenic amines · Freshness parameters · *Enterobacteriaceae* · Rosemary extract

Introduction

Poultry meat production and consumption rapidly increased worldwide owing to its competitive price, absence of religious obstacles and high content of essential amino acids. Broiler meat is considered an important source for protein due to the shortage of red meat production. Consumers usually prefer fresh fillets marketed at refrigerated temperatures (2–5 °C) more than other broiler meat cuts. Broiler fillet belongs to a perishable food, where microbial growth and lipid oxidation may ensue during the refrigeration storage. Spoilage of fresh broiler fillet is an economic burden to producers and requires investigating new methods to prolong the shelf-life and the overall quality of the fillet, which is the chief problem faced the poultry processing industry (Petrout et al. 2012). The main pursuit of poultry industry is extending the shelf life using a lot of synthetic preservatives, but the consumer needs healthy meals (free from conventional chemical preservatives), therefore the use of natural decontaminant has been proposed (Doulgeraki et al. 2012).

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Lactic acid (LA) is widely used in food products as preservatives, as a consequence of its antibacterial activity, safe substances by the FDA and approved as food additives by the European Commission, FAO/WHO and FDA (Surekha and Reddy 2000), therefore it is widely accepted in decontamination of chicken carcasses (Huffman 2002). The natural preservatives obtained from spices are rich in phenolic compounds such as phenolic acids and flavonoids, which have a wide range of biological activity including antioxidant and anti-bacterial (Botsoglou et al. 2003). Rosemary is an important aromatic spice that used as condiments to enhance the foods sensory quality. In addition to its health benefits, the extract possess a great antioxidant and antimicrobial activity (Wojdyło et al. 2007). Identification of incipient spoilage of broiler fillets depends on some chemical parameters such as: pH, total volatile basic nitrogen (TVBN), biogenic amines (Vinci and Antonelli 2002). The pH of fresh chicken breast was ranged between 6.1 and 6.3, while the lipid oxidation below 0.5 mg MDA/kg did not show oxidative rancidity during the storage period (Petrou et al. 2012). The TVBN was ranged between 12 and 17.63 mg/100 g in chicken breast (Silva and Glória 2002; Patsias et al. 2008), while the upper limit of BAI was 20 mg kg⁻¹ in fresh chicken meat stored aerobically (Ivanov et al. 2015). The Egyptian standard (1090/2005) is considered pH of 5.5–6.4, TVBN of 20 mg/100 g and TBA of 0.9 mg MDA/kg as a maximum acceptable limit for chicken meat. Undesired formation of biogenic amines in chicken product requires both of the availability of precursors amino acid and the presence of the bacteria possessing amino acid decarboxylases (Ivanov et al. 2015). Therefore, the present study was aimed to investigate the effect RE 0.2% and LA 1% on freshness, bacterial parameters and formation of biogenic amines during refrigeration of broiler fillets.

Materials and methods

Sample, RE and LA preparation

One hundred and ten broiler chickens of Avian 48 were reared on a poultry farm at Faculty of Veterinary Medicine, Zagazig University, Egypt. They were housed in one deep litter pen that divided into 5 partitions (22 birds each), bedded with wood shavings. They were fed *ad libitum* standard broilers ration (NRC 1994) without antimicrobial medication. The lighting program was 24 h light with temperature maintained between 25 and 32 °C. The broilers were slaughtered and deboning in a small scale poultry processing plant at 45 days of age with final body weight of 2000 ± 100 g, and the carcasses were chilled at 4 °C for 12 h then the breast muscles manually deboned and filleted

by the aid of skillful person. The samples were preserved in adjusted fridge at 4 ± 1 °C in laboratory of food hygiene for 15 day. Two hundred grams of ground rosemary were mixed with 1000 mL of methanol for 3 h in a stirred vessel and then filtered through Whatman paper (GF/A, 110 mm) (Teruel et al. 2015). The obtained solution was vacuum-distilled at 40 °C in a rotary evaporator and the extracts were kept in sealed bottles refrigerated until use (the final volume of the extract is 40 mL and then lyophilized to 5 g). Also, L-Lactic Acid 90% (Merck, Germany) was used after dilution to 1%.

Treatment of fillet samples

Ninety broiler fillet samples were divided into three groups; the control group, the RE group treated with RE 0.2% (Piskernik et al. 2011) and the LA group treated with LA 1% (Doležalová et al. 2010). The used concentrations produces the desired effects with minimum change in organoleptic character of fillets. The control group was dipped in sterile distilled water, while the second and third groups were dipped in rosemary extract 0.2% (w/v) and 1% lactic acid (w/v), respectively at 4 ± 1 °C for 30 min. After dipping all groups were drained on sterile stainless steel mesh for 30 min at 4 ± 1 °C and then individually packed in polyethylene packs (24 µm thicknesses) and stored at 4 ± 1 °C for subsequent analysis. Sensory evaluation, pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), total *psychrotrophic* count and *Enterobacteriaceae* count were performed at zero time and at 3 days interval until the group were rejected from the sensory analysis. The biogenic amines index was assessed at the 9th day of refrigeration. The biogenic amine index (BAI) is the summation of putrescine, cadaverine, histamine and tyramine which is the most harmful biogenic amines formed in meat near the spoilage point and its limit was 20 mg kg⁻¹ (Balamatsia et al. 2006; Ivanov et al. 2015). The protocol repeated five times as replicates.

Sensory evaluation

Sensory quality (color, appearance, texture and aroma acceptability) of fillet samples was evaluated on a 5-point scale 5—good quality, 4—acceptable quality, 3—medium quality (significant changes in meat color and texture), 2—low quality and 1—very low quality (spoilage stage) by a panel of 15 members of postgraduate students (Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt). Sensory evaluation was performed after heating the muscle samples (60 g each) in 0.6% NaCl solution to mild boiling (one part of muscle/two parts of solution) to an internal temperature of 80 °C.

Muscle samples from each group were cut into three (each sized piece of 2 cm and weighed 20 g) and provided in an unscented plastic dish. Each sample was evaluated independently by all assessors. Panelists were provided with glass of unsalted crackers and water for washing the mouth between samples (Zhuang and Savage 2011). The judgments of each of the 15 panelists were averaged for each fillet sample preceding the statistical analysis (Nasr et al. 2017). The fillet sample with a total sensory evaluation score lower than 3 were evaluated as unacceptable for consumption (Ivanov et al. 2015).

Chemical parameters

The pH value of fillet samples was assayed (PN-ISO 2917, 2001) with a CP-411 pH-meter (Elmetron, Zabrze, Poland). The electrode was calibrated using two buffers of 4 and 7 pH. Five grams of the breast muscle (fillet) were mixed with 45 mL of distilled water was disintegrated twice in a meat grinder and thoroughly mixed to assure sample homogeneity and then the pH was measured. TVB-N was determined (FAO 1986). Ten grams of minced broiler fillet sample were homogenized with 100 mL distilled water and in a blender for 2 min. The sample was washed in distillation flask with further 200 mL of water then 2 g of magnesium oxide and 2 drops of antifoaming agent was added. The mixture was left to boil for 10 min and distilled for about 25 min using the same rate of heating into 25 mL of 2% boric acid solution with few drops of screened methyl red indicator in a 500 mL flask. Heating was stopped followed by the condenser washing down with distilled water. The flask contents and the blank solution (25 mL of 2% boric acid) were titrated using 0.1 N H₂SO₄ (titer) and then TVB-N (mg N/100 g flesh) was calculated using the formula: TVB-N = 14 (titer–blank).

TBARS was determined (Schmedes and Hølmer 1989) through mixing ten grams of broiler fillet with 25 mL of 20% trichloroacetic acid (w/v) and then homogenized in a blender for 30 s. After filtration, 2 mL of the obtained solution were added to 2 mL of 0.02 M aqueous TBA then incubated at room temperature in the dark for 20 h. The absorbance was measured at 532 nm using UV–vis spectrophotometer (model UV-1200, Shimadzu, Japan). TBARS estimates were expressed as mg malondialdehyde (MDA)/kg of broiler fillet sample.

Bacteriological parameters

A fillet sample (25 g) was taken aseptically in a vertical lamina-flow cabinet and then transferred to a stomacher bag; 225 mL of peptone water 0.1% mixture was homogenized and *psychrotrophic* bacteria were counted per gram using plate count agar medium. The plates were inoculated

and incubated at 7 °C for 10 days (ISO 2001). *Enterobacteriaceae* counts were enumerated by the pour plate method on Violet Red Bile Glucose Agar (VRBGA; Difco, Detroit, Michigan, USA). The plates were overlaid with a virgin layer of the same growth medium before incubation at 37 °C for 24 h (ISO 2004).

Biogenic amines contents

The fillet cuts were minced and five grams were added to 20 mL of 0.4 M HClO₄. The samples were homogenized, centrifuged for 5 min at 2500 rpm. The supernatant was collected and diluted to 50 mL with 0.4 M HClO₄. The centrifuged acid extract was derivatised as the following procedure: 200 µL of 2 N NaOH were added to 1 mL of the diluted supernatant then buffered by adding 300 µL of saturated NaHCO₃ solution and then 2 mL of dansylchloride solution (10 mg/mL in acetone). The dansylation reaction continues at room temperature (Ruggieri et al. 1995). One hundred µL of NH₄OH were added after 15 min to finish the reaction and to remove residual dansylchloride. Final volume was adjusted to 5 mL by adding acetonitrile. The collected dansylated solution which was filtered injected into the Liquid chromatograph (Agilent Technologies, Waldbronn, Germany, and Model 1100) and equipped with quaternary pump model G 1311A, ultraviolet detector (Model G 1314A) fixed at 254 nm wavelength, auto sampler (model G1329A VP-ODS) and Shim pack (150 × 4.6 mm) column for biogenic amines separation. Data were incorporated and recorded with a Chemstation Software program. Chromatographic conditions were as described in Table 1 and the r^2 of spermine, spermidine, putrescine, tyramine, histamine, cadaverine, tryptamine and β -phenylethylamine were 0.89, 0.92, 0.95, 0.87, 0.91, 0.88, 0.94 and 0.85, respectively.

Statistical analysis

Microbiological data were transformed to log₁₀ value. General Linear Model was used to check the variations between the different treatments (SAS 2008) using this model:

$$Y_{ijk} = \mu + T_i + R_j + e_{ijk}$$

where: Y_{ijk} is the all investigated parameters, μ is the overall mean, T_i is the effect of i th treatments where $i = 1, 2, 3$, R_j is the effect of j th replicates where $j = 1, 2, 3, 4, 5$ and e_{ijk} is the random residuals. The significant differences among means were assessed with Tukey's honest significant difference. Graphical assessment of the residuals (normality plots) and Shapiro–Wilk tests evaluated the normality assumption of the residuals. All of them demonstrated compatibility with normality assumption

Table 1 Chromatographic conditions of biogenic amines

Time/min.	Flow rate ml/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

with statistically non-significant results of Shapiro–Wilk tests.

Results

The mean organoleptic score (color, texture, appearance and aroma) scores for the control, RE and LA groups were 4.8 ± 0.1 , 4.2 ± 0.1 and 3.9 ± 0.1 , respectively at zero time which decreased by 33.3, 23.80 and 10.25%, respectively after storage of 9 days (Fig. 1a). The pH at zero time was 5.8 ± 0.1 , 5.6 ± 0.1 and 4.9 ± 0.1 for the control, RE and LA, respectively which increased gradually by 11.20, 9.64 and 1.83%, respectively on 9 days of refrigeration (Fig. 1b). The mean value of TVB-N was the same for all fillet groups at zero time. While, it was the lowest in LA

group (4.36 ± 0.1 , 5.62 ± 0.7 and 10.13 ± 0.98) when compared with the RE group (5.25 ± 0.1 , 8.11 ± 0.6 and 13.84 ± 0.8) and the control group (6.41 ± 0.1 , 11.61 ± 0.7 and 17.24 ± 0.9) on the 3rd, 6th and 9th day of refrigeration (Table 2). Moreover, the value of TBARS was similar for all groups at zero time. While, it was the highest the control group (0.22 ± 0.1 , 0.39 ± 0.1 and 0.78 ± 0.1) when compared with RE group (0.19 ± 0.04 , 0.22 ± 0.1 and 0.38 ± 0.1) LA group (0.20 ± 0.1 , 0.27 ± 0.1 and 0.52 ± 0.1) on the 3rd, 6th and 9th day of refrigeration (Table 2).

Psychrotrophic bacteria were detected in all groups at zero time. LA group showed the lowest number of psychrotrophic bacteria during the refrigeration period when compared with the other groups. The reduction counts in psychrotrophic bacteria were 0.7 and 1 log₁₀CFU/g in 6th

Fig. 1 Effect of rosemary extracts 0.2% and lactic acid 1% on organoleptic score (a) and pH (b) of refrigerated broiler fillets at 4 ± 1 °C

Table 2 Effect of rosemary extract (RE) 0.2% and lactic acid (LA) 1% in the formation of TVB-N (mg/100 g) and TBARS (mg/kg) in broiler fillets stored at 4 ± 1 °C (Mean $\pm$ SD)

Group	Refrigeration time											
	Zero time		3rd day		6th day		9th day		12th days		15th days	
	TVB-N ¹	TBARS ²	TVB-N	TBARS	TVB-N	TBARS	TVB-N	TBARS	TVB-N	TBARS	TVB-N	TBARS
Control	2.94 $\pm$ 0.09	0.16 $\pm$ 0.02	6.41 $\pm$ 0.11	0.22 $\pm$ 0.06	11.61 $\pm$ 0.72 ^a	0.39 $\pm$ 0.07 ^a	17.24 $\pm$ 0.87 ^a	0.78 $\pm$ 0.09 ^a	–	–	–	–
RE 0.2%	2.94 $\pm$ 0.09	0.16 $\pm$ 0.02	5.25 $\pm$ 0.14	0.19 $\pm$ 0.04	8.11 $\pm$ 0.64 ^{ab}	0.22 $\pm$ 0.09 ^b	13.84 $\pm$ 0.79 ^b	0.38 $\pm$ 0.07 ^c	18.42 $\pm$ 0.89	0.51 $\pm$ 0.09	–	–
LA 1%	2.94 $\pm$ 0.09	0.16 $\pm$ 0.02	4.36 $\pm$ 0.12	0.20 $\pm$ 0.07	5.62 $\pm$ 0.75 ^b	0.27 $\pm$ 0.08 ^{ab}	10.13 $\pm$ 0.98 ^c	0.52 $\pm$ 0.08 ^b	15.2 $\pm$ 0.83	0.73 $\pm$ 0.08	19.08 $\pm$ 0.74	0.84 $\pm$ 0.10

Means with different superscript letters within the same column were statistically significant ($p < 0.05$)¹TVB-N: Total volatile basic nitrogen²TBARS: Thiobarbituric acid reactive substances

– Not examined as refused from organoleptic examination

day and became 0.8 and 1.26 log₁₀CFU/g in the 9th day of chilling under the effect of rosemary 0.2% and lactic acid 1% dipping for 30 min. *Enterobacteriaceae* were detected at zero time only in the control group, while at the 3rd and 6th days of refrigeration in RE and LA groups, respectively. *Enterobacteriaceae* counts were increased in the same group by time elapsing and the lower count belonged to LA group. The reduction counts in *Enterobacteriaceae* population were 0.28 and 0.92 log₁₀CFU/g in RE and LA treated groups at the 6th day of refrigeration and was 0.52 and 1.09 log₁₀CFU/g at the 9th day of refrigeration, respectively (Table 3).

The biogenic amines on 9th day of refrigeration were 16.32 $\pm$ 1.4, 12.28 $\pm$ 1.3 and 8.48 $\pm$ 1.2 mg kg⁻¹ (putrescine); 8.45 $\pm$ 1.0, 6.21 $\pm$ 1.0 and 4.35 $\pm$ 0.9 mg kg⁻¹ (tyramine); 4.23 $\pm$ 0.8, 2.95 $\pm$ 0.7 and 1.89 $\pm$ 0.6 mg kg⁻¹ (histamine); 2.84 $\pm$ 0.4, 1.79 $\pm$ 0.3 and 1.21 $\pm$ 0.4 mg kg⁻¹ (cadaverine); 0.89 $\pm$ 0.1, 0.67 $\pm$ 0.1 and 0.41 $\pm$ 0.1 mg kg⁻¹ (tryptamine) and 0.19 $\pm$ 0.002, 0.14 $\pm$ 0.01 and 0.11 $\pm$ 0.02 mg kg⁻¹ (β -phenylethylamine) for control, RE and LA groups, respectively (Fig. 2). Moreover, LA 1% has a significant effect on the biogenic amine index (BAI) at the 9th day of refrigeration with a mean value of 15.93 mg kg⁻¹ when compared with the control (31.84 mg kg⁻¹) and RE (23.28 mg kg⁻¹) groups (Fig. 3).

Discussion

Sensory evaluation

There was a significant decrease in the sensory score of the LA group directly after removal from the dipping solution, which was recognized as a side effect of acid treatment due to its bleaching effect (Huffman 2002). Whitening of decontaminated meat coincided with decrease of pH relative to control samples (Lim and Mustapha 2004). Additionally, low pH of the breast muscles had the highest reflectance whiteness than high pH breasts, which had the greatest transmittance into their depth (Swatland 2008). The deviation mainly in color, which became a slightly lighter than the control and RE groups that comparable to the previous results (del Río et al. 2007) who reported a slightly lighter color on the chicken fillet treated with organic acids. On the 9th day of refrigeration the organoleptic score was the lowest at control group and rejected by sensorial evaluation at the 12 day, meanwhile RE and LA groups still accepted (The fillet was defined as unacceptable after development of first off-odor or off-taste). By the end of the refrigeration days the highest organoleptic score was noticed in LA group which is attributed to the protective effects of lactic acid on the

sensorial change and reduce the level of microbial growth that was similar to the finding of others (Smaoui et al. 2011). Moreover, the rosemary enhanced the organoleptic character of chicken meat (Zhang et al. 2016).

Hydrogen ion concentration (pH)

The pH of fillet is considered as an important factor in assessing quality during refrigeration storage. At zero time, comparable findings regarding the control group were obtained by Zhang et al. (2016). However, there was a significant decrease in LA group and the drop in pH of fillet was similar to the finding of Smaoui et al. (2012). At 9th day, pH significantly increased in control group which may be owed to the utilization of amino acids by bacteria, with accumulation of ammonia as end product of amino acid decomposition that resulted in an increase of pH (Valencia et al. 2008). The lower broiler fillet pH dipped in RE that was compatible with Zhang et al. (2016) is attributed to the inhibitory effect of antimicrobial ingredients present in rosemary extract, which limited the growth and proliferation of meat spoilage microorganisms that utilize basic nitrogen compounds. Also, LA has buffering effect in broiler fillet that seems to be sufficient in maintaining a lower pH than other groups. The current finding was comparable with (Anang et al. 2010), who found a lower pH value during the storage period of LA dipped group due to its buffering capability.

Total volatile basic nitrogen (TVB-N)

TVB-N content is considered an imperative marker during evaluation of freshness of meat. TVB-N in chicken meat is mainly ammonia, trimethylamine and dimethylamine that increased with the spoilage caused by bacteria or enzymatic degradation (Urmila et al. 2015). There was a rapid increase of TVB-N with the increase of storage temperature, which was related to enhancement of bacterial growth rate especially *Pseudomonas* spp. (Ghollasi-Mood et al. 2016). The finding of the current study at zero time was similar to Khulal et al. (2017). TVB-N of the control group was in agreement with Silva and Glória (2002), who detected 17.63 ± 2.63 mg/100 g in the 10th day of aerobically stored chicken breast. Also, at the 15th days of refrigeration, the mean value of LA was lower than the finding of (Balamatsia et al. 2007), who detected 54.5 mg/100 g for air-packaged chicken breast fillets after 15 days of storage at 4 °C. The significant decrease in LA group on the 6th and 9th day of storage may be attributed to the potential effect of lactic acid in reduction the proliferation of microorganisms that decomposed the protein.

Thiobarbituric acid reactive substances (TBARS)

The concentration of TBARS is considered as a reliable sign for lipid oxidation in chicken and their products (Fernández et al. 1997). The achievement of RE in reduction of TBARS in the current study came in line with the previous results that showed the rosemary significantly decreased the formation of TBARS in chicken meat (Zhang et al. 2016). RE had the greatest effect on the reduction of the TBARS in comparison to LA as RE directly provides with carnosic acid offering hydrogen from the phenolic groups resulted in the formation of a stable end product and altering free radical chains. Meanwhile, LA indirectly reduces TBARS through the reduction of microbial lipases. Naveena et al. (2013) studied the effect of carnosic acid obtained from dried rosemary in cooked chicken patties during the refrigeration storage and observed a TBARS reduction. The current results declared a significant decrease in the formation of TBARS in RE treated group, which suggested that these antioxidants delayed lipid oxidation during the storage. Natural antioxidants offering hydrogen from the phenolic groups resulted in the formation of a stable end product and altering free radical chains.

Psychrotrophic count

Temperature control is the key role in maintaining the quality of refrigerated fillet throughout the selling period. When the temperature is lowered below the optimum for bacterial growth, multiplication is decreased. However, many of the different food poisoning and food spoilage microorganisms are cold-adapted psychrotrophic bacteria which have the ability to grow at low temperatures, even approaching zero degree (Russell 2002). Psychrotrophic count in the control group of this study was comparable with others (Ivanov et al. 2015). A significant decrease in LA group was obtained at zero time, which was in agreement with Dorn et al. (1989), who found that 15 min contact time was required to achieve the significant decrease in of psychrotrophic bacteria with 1% lactic acid solution, which may be attributed to the ability of undissociated form of lactic acid to penetrate the cytoplasmic membrane, lowering the intracellular pH and disrupt the transmembrane proton motive force (Ray and Sandine 1992). The reduction of psychrotrophic counts seemed to be correlated with pH values in case of lactic acid (Doležalová et al. 2010). On the other hand, a lesser effect of rosemary extract was due to the presence of organic compounds (fat and protein), which decreased the action of active principles in RE. The effect of RE was four times greater in vitro than in chicken meat extract model (Piskernik et al. 2011).

Table 3 Effect of rosemary extract (RE) 0.2% and lactic acid (LA) 1% on *psychrotrophic* (Psy.) and *enterobacteriaceae* (Ent.) count log₁₀CFU/g in broiler fillets stored at 4 ± 1 °C (Mean ± SD)

Group	Zero time				3rd day				6th day				9th day				12th days				15th days			
	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.	Psy.	Ent.
Control	3.6 ± 0.34 ^a	2.3 ± 0.09	4.12 ± 0.38 ^a	2.9 ± 0.10	4.95 ± 0.48 ^a	3.41 ± 0.37 ^a	5.60 ± 0.47 ^a	4.30 ± 0.48 ^a	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
RE 0.2%	3.1 ± 0.29 ^{ab}	< 1	3.51 ± 0.24 ^{ab}	2.5 ± 0.31	4.25 ± 0.34 ^b	3.13 ± 0.35 ^{ab}	4.80 ± 0.54 ^b	3.78 ± 0.41 ^{ab}	5.46 ± 0.35	4.15 ± 0.47	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LA 1%	2.84 ± 0.28 ^b	< 1	3.25 ± 0.31 ^b	< 1	3.95 ± 0.42 ^c	2.49 ± 0.29 ^b	4.34 ± 0.38 ^c	3.21 ± 0.34 ^b	5.13 ± 0.41	3.87 ± 0.37	5.87 ± 0.38	4.12 ± 0.26	—	—	—	—	—	—	—	—	—	—	—	—

Means with different superscript letters within the same column were statistically significant ($p < 0.05$)

— Not examined as refused from organoleptic examination

Enterobacteriaceae count

Enterobacteriaceae is a facultative anaerobic Gram negative bacteria, which is known with its ability for rapid adapting to the surrounding environmental changes. *Enterobacteriaceae* count in control group was lower than the reported count of others (Anang et al. 2010; Zhang et al. 2016). The reduction counts in *Enterobacteriaceae* population was similar to the results of Okolocha and Ellerbroek (2005). Dipping in lactic acid is clearly retarded the growth of *Enterobacteriaceae* in the first 5 days of chilling, and additionally the significant effect occurred on 6th and 9th day of chilling. Lactic acid 1% proved to be superior to rosemary 0.2% regarding the inhibition of *Enterobacteriaceae* populations on broiler fillet after 30 min of dipping. The obtained findings were in agreement with Anang et al. (2010), who found a greater reduction of *Enterobacteriaceae* in plant extracts rich in lactic acid. The antibacterial action of spice extracts is not entirely clear. However, membrane disruption by phenolics and metal chelation by flavonoids are considered to affect the growth of microorganisms. Several researchers have reported that phenolic compounds from different plant had the ability to reduce food-borne pathogens. The predominant compounds in rosemary extract were carnosic acid di-terpenes, volatile oils and phenolic acids, which were responsible for antimicrobial effect (Basaga et al. 1997). The antimicrobial effects of phenolic compounds may include multiple modes of action; a) distract the cell wall, b) disrupt the cytoplasmic membrane, c) leakage of cell content, d) alter fatty acid and phospholipid constituents, e) influence the formation of DNA and RNA, f) inhibit protein translocation (Basaga et al. 1997).

Biogenic amines

The bacteria containing decarboxylase enzyme can serve as an indicator for possible presence of toxic products in meat. The biogenic amines may be formed during the refrigeration preservation (Vinci and Antonelli 2002). The biogenic amines and the spoilage parameter usually detected in chicken meat at the 9th day of refrigeration (Silva and Glória 2002; Balamatsia et al. 2006; Gallas et al. 2010). Spermine and spermidine have an imperative responsibility in cell proliferation and growth, additionally they serve as a source of nitrogen for microorganisms (Bardocz 1995). There were no significant difference in spermine and spermidine at the 9th day of refrigeration in all groups (Fig. 2). The formation of putrescine depends mainly on decarboxylation of amino acid arginine by certain species of *Enterobacteriaceae* (Bover-Cid and Holzapfel 2000). Higher values in stored chicken 20.4 and 26.4 mg kg⁻¹ were previously detected by others (Balamatsia et al. 2006; Gallas et al. 2010). Tyramine produced

Fig. 2 Effect of rosemary extracts 0.2% and lactic acid 1% on biogenic amines in the 9th day of broiler fillets refrigeration at $4 \pm 1^\circ\text{C}$

Fig. 3 Biogenic amine index (Bai) on the 9th day of refrigeration in control, rosemary extract 0.2% and lactic acid 1%

from tyrosine by the action of decarboxylase enzyme group that found in a high value in stored chicken and act as a vasoconstrictor (Gallas et al. 2010).

There have been conflicting findings on the formation of histamine at the 9th day of storage that formed by decarboxylation of histidine. While some authors have found on 9th day of refrigeration, others did not detect (Gallas et al. 2010; Ivanov et al. 2015). However, the majority of studies detected higher values 10.3 and 8.6 mg kg⁻¹ (Silva and Glória 2002; Balamatsia et al. 2006). The formation of cadaverine depends mainly on decarboxylation of amino acid lysine by certain species of *Enterobacteriaceae*. Higher values in stored chicken 8.5 and 4.3 mg kg⁻¹ were detected (Balamatsia et al. 2006; Gallas et al. 2010). Tryptamine (Gallas et al. 2010) and β -phenylethylamine were detected at the 9th day of refrigeration in stored chicken. On contrary, others did not detect β -phenylethylamine in stored chicken (Gallas et al. 2010).

There was a significant difference among the concentrations of putrescine, tyramine, histamine, and cadaverine

in the control, RE and LA groups at the 9th day of chilling. This may be attributed to the growth of bacteria that produced decarboxylase enzyme, in addition to the availability of biogenic amine precursor. The biogenic amine index (BAI) could be used as a helpful tool in evaluating poultry products freshness. BAI is a summation of the putrescine + cadaverine + histamine + tyramine concentrations in poultry meat (Ivanov et al. 2015). Also, they recommended that values higher than 20 mg kg⁻¹ of muscle are proposed as the upper limits for initiation of spoilage in fresh chicken meat stored aerobically. Our results declared that the only accepted group according to the BAI was the LA group.

Conclusion

The results suggested that dipping of broiler fillets in 0.2% rosemary extract and 1% lactic acid improved the organoleptic score during the refrigeration period and

increased the acceptability of broiler fillet up to 12 and 15 days, respectively. Also, lactic acid promoted antibacterial effects on psychrotrophic bacteria and *Enterobacteriaceae* that resulted in the reduction of BAI in broiler fillet. The dipping in 0.2% rosemary extract exceeded the effect of lactic acid on the inhibition of TBARS formation during the days of refrigeration.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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