

Full Length Research Paper

Effects of sodium lactate and lactic acid on chemical, microbiological and sensory characteristics of marinated chicken

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This study was undertaken to evaluate the chemical changes, microbiological effects and sensory attributes of marinated chicken thighs treated by lactic acid (LA) at different concentrations (0.2, 0.3, 0.5, 0.6, 0.8 and 1%) and sodium lactate (SL) at 1, 1.5, 2, 2.5 and 3%, stored at 4°C. The results reveal that these additives were efficient ($P < 0.05$) against the proliferation of various spoilage microorganisms; including aerobic, psychrotrophic populations, *Pseudomonas* spp., *Enterobacteriaceae*, *Staphylococcus aureus* and *Salmonella* spp. The general order of antibacterial activity of the different additives used was; LA > SL. Chemical analysis revealed a reduction in the pH value and also in the total volatile bases nitrogen contents in treated thigh. Overall, the findings demonstrate that the addition of 1% LA in marinated chicken can delay the proliferation of spoilage microorganisms and the appearance of undesirable chemical. This LA concentration improves the sensory attributes and extends the shelf life of the product during refrigerated storage. The LA additive have strong potential and promising properties that can, therefore, open new pathways and opportunities for the poultry industrial production for using efficient, safe, and cost-effective additives.

Key words: Marinated chicken, sodium lactate, Lactic acid, Microbial quality, Sensory evaluation.

INTRODUCTION

Marination originated in the Mediterranean region (“mare” is Latin for “sea”), is a traditional process in which meat is immersed in a solution that extends its shelf life and imparts a specific flavour characteristic (Lemos et al., 1999). More recently, marination has been used in meat and poultry to improve tenderness, juiciness, flavour, colour and cooking yield (Yang and Chen, 1993). Marination consists of soaking a food in various foodstuffs and flavourings (Lemos et al., 1999; Guerrero-Legarreta and Hui, 2010). It's is a method of reducing aging time required for meat tenderization (Guerrero-

Legarreta and Hui, 2010).

Marination of poultry meat has been an active topic of research because of its potential to extend the versatility of processed products (Guerrero-Legarreta and Hui, 2010). In industry, marinades are based on weak acids (acetic, lactic, citric acid...), generally supplemented with NaCl (Goli et al., 2007; Lemos et al., 1999; Yusop et al., 2010). The most required functions of poultry meat marination are the extension of shelf life and the improvement of the microbiological culinary and technological quality (taste, tenderness, water retention and mass yield) (Okolocha and Ellerbroek, 2005). Marinade ingredients such as salt, phosphates, acids, tenderizers, sugar, seasonings, and flavouring have been reported to have various functions when applied to chicken.

Two preservatives agents are generally utilised: sodium lactate (SL) and lactic acid (LA). Sallem (2007) concluded that the use of aqueous solution of SL (2.5%) was efficient against the proliferation of various

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Abbreviations: LA, Lactic acid; SL, sodium lactate.

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categories of spoilage microorganisms, as it delayed lipid oxidation and extended the shelf life of the product during refrigerated storage of marinated salmon slices. SL is frequently added to meat and poultry products; it is recommended as a flavour enhancer in cooked meat and poultry products and as a pH-control agent (McKee, 2007). Poultry meat oxidation seems to be related to the development of a characteristic termed warmed-over flavour (WOF), which is an issue in further processed and ready-to-eat poultry products. SL has been investigated for the reduction of WOF in poultry but did not fully prevent other off-flavour development (McKee, 2007). SL, used in poultry preparation, is an antioxidant reinforcing approved to retard the oxidative rancidity and subsequently, protect flavour and colour. SL reduces water activity as well as inhibiting bacterial growth, particularly *Lactobacilli*.

LA has been shown to have some antibacterial effect on the major spoilage organisms and to be effective in reducing microbial counts on poultry (Okolocha and Ellerbroek, 2005). LA was used in poultry meat marination to improve the water-holding capacity and tenderness of poultry muscle. Various investigators have shown that LA used as food acidulants can inhibit the growth or accelerate the inactivation of the food borne pathogen *Listeria monocytogenes* (Goli et al., 2011; Guerrero-Legarreta and Hui, 2010). A maximum LA concentration of 1% can inhibit or inactivate *L. monocytogenes*, even at neutral pH (Anang et al., 2010). Some authors concluded that the inactivation rate depends not only on the environmental pH but also on the type and concentration of the used acid (Guerrero-Legarreta and Hui, 2010).

Considering the promising properties of additives and the growing interest in the use of efficient, safe and low-cost natural substrates, this study postulates that LA and SL which are naturally abundant and available, may represent an efficient additive. These two agents can replace the commonly used expensive preservatives and can improve the cost-effectiveness of the overall production process, hence, the final end product.

Accordingly, this study focused on the added value of LA and SL to poultry meat. Several concentrations of these additives were investigated in terms of their effects on chemical changes, microbiological and sensory attributes of poultry meat.

MATERIALS AND METHODS

Raw material

Chickens having no trauma tear or other abnormalities (badly evisceration fracture, presence of feathers) were collected from a local firm. Meat samples were vacuum-packaged, cold-stored and used immediately or shortly after evisceration; samples (size = 1.2 kg) undergoing a churn pickling in automatic. Various ingredients were added during the preparation of the commercial marinade (water at 82%, salt at 7%, spices at 1%, oils at 7.5% and lemon at 2.5%) which was used as the control (pH 4.2). All marinades were prepared the day before marinating and held at 4°C until required.

Action parameters of the churn were maintained constant. The choice of these parameters has been optimized and pre-established by the manufacturer: air pressure, 90%; scraper speed, 6 trs/min; action time, 11 min and temperature of 0°C.

Chemical composition of the chicken meat

Moisture content of samples was determined by air drying in an air oven following AOAC method 950.46 (AOAC, 2000). Protein content was determined using Kjeldahl nitrogen as described in AOAC method 928.08 (AOAC, 2000). Ether extraction as described in AOAC method 954.02 was used to determine total crude fat content (AOAC, 2000). Ash content was determined using AOAC method 930.30 (AOAC, 2000).

Influence of additives on marinades

To study the effect of additives on marinated poultry meat, various SL concentrations (1 to 3%, v/v) and LA (0.2 to 1%, v/v) were investigated.

Cooking

Marinated samples were subsequently dry cooked at 170°C in a convection oven for 15 min to an internal temperature of 75°C measured by an internal temperature probe. All test samples were cooked at the same time and segregated to prevent any mixing.

Chemical analyses

pH

10 g of thigh muscle were homogenized in 50 ml of distilled water. The pH was measured, on individual raw fillets after marinating at 20°C using an MP 220 pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) and adjusted to 7.0.

Total volatile basic nitrogen (TVBN)

To determine total volatile basic nitrogen (TVBN), samples (10 g) were mixed with 100 ml distilled water and washed into a distillation flask with 100 ml distilled water after which 2 g of magnesium oxide and an antifoaming agent were added. The mixture was distilled using the micro-Kjeldahl distillation apparatus. Distillate was collected for 25 min into 25 ml 4% boric acid with 5 drops of Tashero indicator. The solution was titrated using (0.1 M) HCl to calculate the total VBN in the sample in terms of mg VBN/100 g (Pearson, 1976).

Microbiological analyses

10 g of chicken thigh samples were weighed into a sterile stomacher bag and added to 90 ml of 0.1% peptone and 0.8 % NaCl. The pH was adjusted to 7.2. The mixture was then macerated for 2 min in a stomacher. 1 ml of the homogenate was serially diluted in aseptic conditions and used for enumeration of microorganisms (AFNOR, 2004).

Aerobic plate count

Aerobic plate counts (APC) were determined by inoculating 0.1 ml

of the sample homogenate, at selected dilutions, onto triplicate sterile plates of pre-poured and dried Standard Method Agar using the surface spread technique, then the plates were incubated for 48 h at 35°C (APHA, 1992).

Psychrotrophic count

Psychrotrophic counts were determined as described above for APC except that the plates were incubated at 7°C for 10 days (Cousin et al., 1992).

Pseudomonas count

Pseudomonas were enumerated on *Pseudomonas* agar base (CM 559; Oxoid) supplemented with cetrimide, fucidin, and cephaloridine (CFC) supplements providing a selective isolation medium for *Pseudomonas* spp. Colonies were counted after 2-days of aerobically incubation at 25°C.

Enterobacteriaceae count

Enterobacteriaceae counts were enumerated by the pour plating method on violet red bile glucose agar. The plates were overlaid with a virgin layer of the same growth medium before aerobically incubation at 37°C for 24 h.

Staphylococcus aureus

Surviving population of *S. aureus* is determined by standard plating methods (Lindsay and Von Holy, 1999). At each sampling time, colonies of *Staphylococcus* were selected, Gram-stained and observed for catalase and oxidase reactions in order to confirm its presence (Ingham et al., 2006). Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g). All counts were performed in duplicate.

Salmonella

Chicken thighs were sampled aseptically by excising 25 cm² from the surface areas. A sterile filter paper (5 x 5 cm) was used to outline the area. Filter paper and skin were homogenized for 2 min in 250 ml of sterile buffered peptone water (Oxoid CM 509) incubated at 37°C for 24 h. After incubation in aerobic conditions, 1 ml of green enrichment cultures were transferred to 10 ml of Tetrathionate Broth (Oxoid CM 29) and incubated at 42°C for 24 h (AL-Rajab et al., 1986).

These enrichment cultures were streaked on xylose lysine desoxycholate (XLD) (Oxoid CM469) and on brilliant green agar (BGA) (Oxoid CM329). The plates were incubated at 35°C for 24 h. Colonies, red with black centers (On XLD) and red colonies surrounded by bright red (on BGA) were picked off the plates and subcultured to triple sugar from agar (Merck No. 3915), Lysine Decarboxylase Broth (Oxoid CM 308) and urea agar base (Oxoid CM53). The slants were incubated at 35°C for 24 h (AL-Rajab et al., 1986).

Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/25g). All counts were performed in duplicate.

Sensory evaluation

Thirty consumers are invited to perform sensory properties of the

marinade using 11 added preservatives: (0.2, 0.3, 0.5, 0.6, 0.8 and 1%) of LA and (1, 1.5, 2, 2.5 and 3%) of SL. Each person has to mention levels of colour (golden brown or pale), aroma, texture (toughness or juiciness) and flavour (sourness or sweetness). For sensory evaluation, samples are presented after storage period in a separate area where distractions, noises and odors are minimized. Panelists were presented with one treatment at a time (consisting of 1 cm cube of thigh) and the treatment order was completely randomized for each panelist. Once the consumers received the samples, they were asked to evaluate the product. A tasting note belonging to a 10-point hedonic scale with 1 = dislike extremely and 10 = like extremely was given by each consumer.

Statistical analysis

All measurements were made in triplicate for each sample. Results were expressed as means ± standard deviations. Data were subjected to analysis of variance (ANOVA) using the general linear models procedure of the statistical analysis system software of SAS Institute (SAS, 1990). Differences among the mean values of the various treatments and storage periods were determined by the least significant difference (LSD) test, and the significance was defined either at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of the chicken meat

The composition study of the chicken thigh showed that they are characterized by a high protein percentage (19.25%) and high lipid content (6.88%). These observations can be related to the chicken race and feed.

Chemical quality

In order to establish deterioration indices of chicken thigh quality during storage at 4°C, many chemical methods have been investigated. Chemical tests usually measured the amounts of breakdown products resulting from enzymatic, bacterial and oxidative activities.

In this study, the chemical quality indicators used to determine the chemical changes in marinated chicken thigh were pH value and TVBN contents.

Changes in pH value

Tables 1 and 2 indicate the pH evolution in thighs treated with various additives (SL and LA) during storage at 4°C. The initial pH value of the control and the SL added samples was above 6.0, whereas marinated samples added with LA showed a decrease in their initial pH reaching 5.81 for samples added with 1% LA (Table 2).

Furthermore, the determination of pH values for all treatments during 15 days, showed an increase in pH value especially for the control samples which become 6.87 at the end of the storage period, while treatment with LA at 1% concentration showed the lower pH value (6.06).

Table 1. pH evolution in thighs treated with SL using storage at 4 °C

Treatment	Day of storage at 4 °C					
	0	3	6	10	13	15
Control	6.08±0.01 ^a	6.18±0.03 ^b	6.27±0.01 ^a	6.52±0.03 ^b	6.69±0.01 ^a	6.87±0.0 ^b
1% SL	6.07±0.01 ^a	6.15±0.02 ^a	6.22±0.01 ^a	6.33±0.01 ^a	6.40±0.01 ^b	6.50±0.0 ^c
1.5 % SL	6.06±0.03 ^b	6.14±0.04 ^c	6.18±0.01 ^a	6.29±0.01 ^b	6.37±0.01 ^a	6.50±0.01 ^a
2 % SL	6.05±0.04 ^c	6.11±0.02 ^a	6.15±0.03 ^b	6.27±0.01 ^a	6.31±0.01 ^b	6.44±0.04 ^c
2.5 % SL	6.03±0.01 ^a	6.09±0.01 ^a	6.14±0.04 ^c	6.19±0.02 ^b	6.28±0.01 ^b	6.39±0.02 ^b
3 % SL	6.01±0.03 ^b	6.08±0.03 ^b	6.11±0.02 ^b	6.20±0.01 ^a	6.24±0.03 ^c	6.33±0.04 ^c

a–c: Averages with different letters in the same column are different (P<0.05).

Table 2. pH evolution in thighs treated with LA during storage at 4 °C.

Treatment	Day of storage at 4 °C					
	0	3	6	10	13	15
Control	6.08±0.01 ^a	6.18±0.03 ^b	6.27±0.01 ^a	6.52±0.03 ^b	6.69±0.01 ^a	6.87±0.02 ^b
0.2% LA	6.03±0.02 ^b	6.10±0.01 ^a	6.16±0.02 ^b	6.21±0.01 ^a	6.32±0.03 ^c	6.40±0.01 ^a
0.3% LA	5.99±0.03 ^b	6.05±0.01 ^a	6.11±0.02 ^a	6.14±0.03 ^c	6.22±0.02 ^a	6.29±0.03 ^c
0.5% LA	5.91±0.03 ^c	5.95±0.03 ^c	5.98±0.02 ^b	6.02±0.02	6.09±0.02 ^b	6.13±0.03 ^c
0.6% LA	5.88±0.04 ^c	5.93±0.02 ^b	5.97±0.03 ^c	6.00±0.02 ^b	6.06±0.01 ^a	6.11±0.01 ^a
0.8% LA	5.85±0.02 ^b	5.90±0.04 ^c	5.97±0.02 ^b	6.01±0.03 ^c	6.05±0.03 ^c	6.09±0.03 ^c
1 % LA	5.81±0.01 ^a	5.86±0.03 ^c	5.97±0.02 ^a	5.99±0.01 ^a	6.03±0.02 ^a	6.06±0.04 ^c

a–c: Averages with different letters in the same column are different (P<0.05).

According to Gonzalez-Fandos et al. (2009), the buffering capacity of the acid system seems to be sufficient to maintain a low pH of the meat; these observations are in accordance with our results. Moreover, the marinades with the lower pH values could be the result of muscle protein denaturation (Alvarado and Sams, 2003) which also agree with our findings. Acidic marinades are known to be involved in several functioning factors, including; weakening structures due to swelling meat, increasing proteolysis by catharsis and also increasing conversion of collagen to gelatin at low pH during cooking (Goli et al., 2011).

No significant difference in the pH value was detected throughout the storage period of marinated chicken thighs between 1% and 3% SL solutions. Indeed, SL is a multifunctional ingredient and was found to have various applications in meat products. Zhu et al. in 2009 demonstrated that SL helped to maintain stable pH during storage periods and that this observation was possible due to its buffering capability.

Total volatile bases nitrogen

Total volatile bases nitrogen (TVBN) is a traditional chemical method widely used for the evaluation of spoilage degree in seafood. TVBN can be used as a quality indicator for poultry products and it is associated

with the amino acid decarboxylase activity of microorganisms during storage. Evolution in TVBN value during storage is shown in Figures 1 and 2.

For marinated chickens, TVBN should not exceed 60 mg/100 g chicken meat (NF V 01-003, 2004). The initial TVBN in marinated chicken thigh was 4.5 mg/100 g chicken meat but after the seventh days of storage the TVBN value becomes higher than the standard limit.

For the samples added with SL, maximal allowed levels of TVBN are reached at the 8th, 9th and 11th days for the samples (1, 2 and 3% SL), respectively. For the samples added with LA, maximal allowed level of TVBN are reached at the 9th, 13th and 16th days for the samples (0.2, 0.5 and 1% SL), respectively. These results indicate the significant effect of SL and LA in the reduction of chemical changes in marinated chicken.

Microbiological evaluation

Treatment with SL

The chemical formula of sodium lactate is CH₃-CHOH-COONa. SL has been used for several years in the meat industry because of its ability to increase flavour, shelf-life and microbiological safety of these products.

Tables 3 and 4 show the mean log reductions on aerobic plate count (APC), Psychrotrophic count,

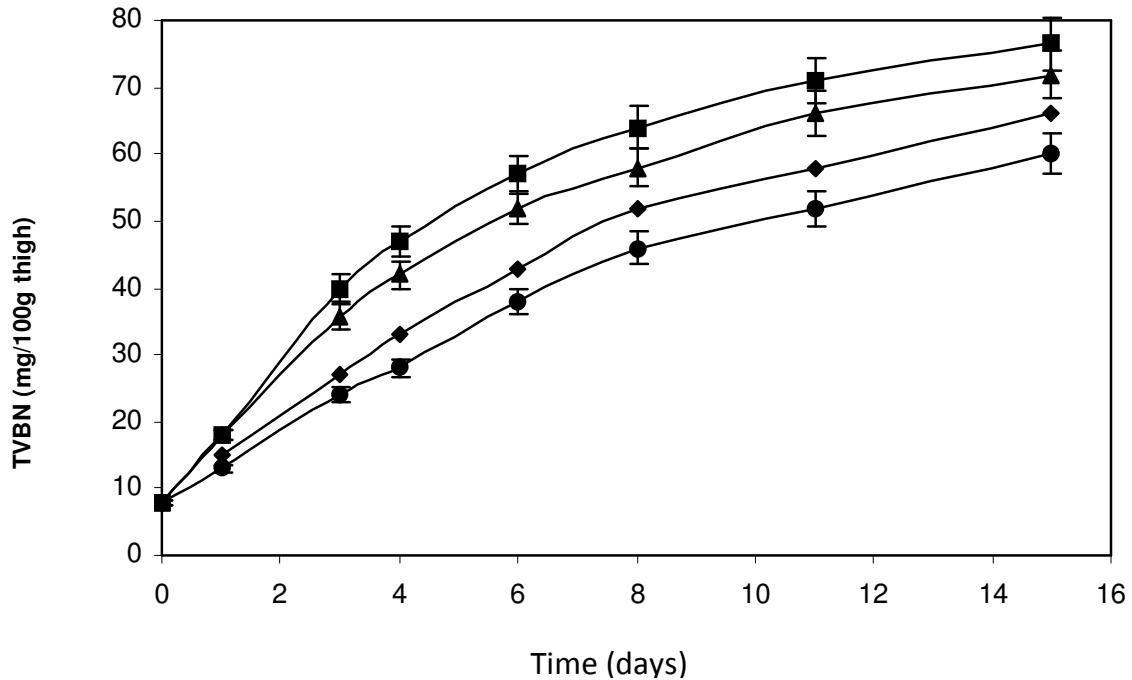


Figure 1. Changes in the total volatile bases nitrogen (TVBN) content in marinated thigh with SL stored at 4°C. P < 0.05; Control (■), 1% SL (▲), 2% SL (◆) and 3% SL (●).

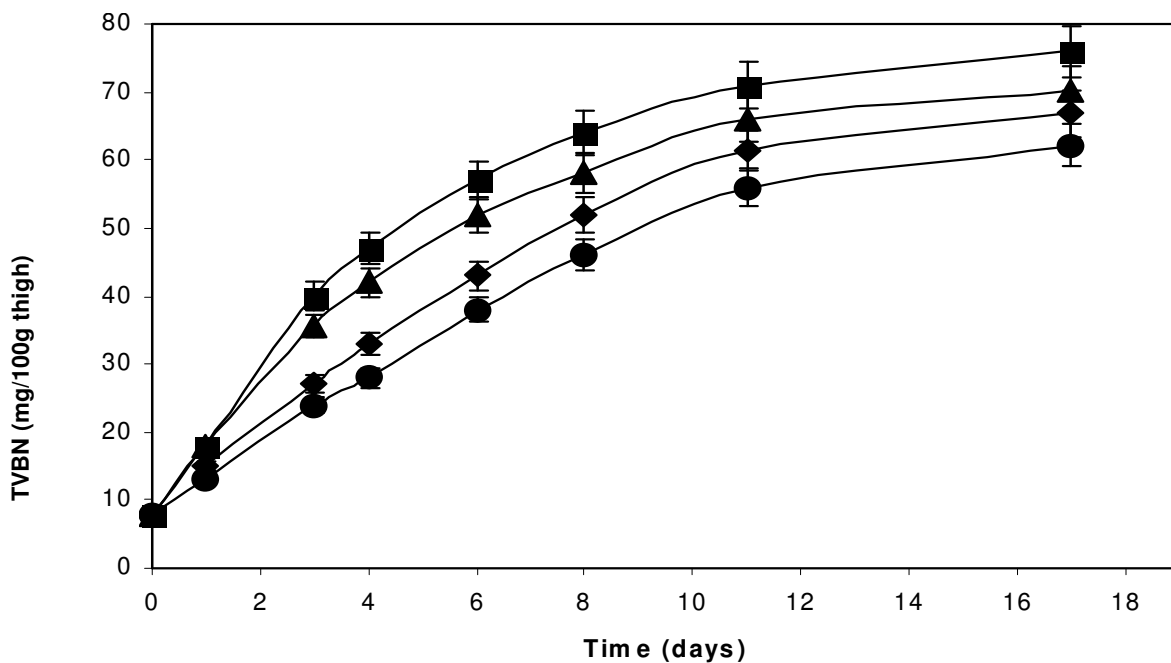


Figure 2. Changes in the total volatile bases nitrogen (TVBN) content in marinated thigh with LA stored at 4°C. P < 0.05; control (■), 0.2 % LA (▲), 0.6% LA (◆) and 1% LA (●).

Pseudomonas counts and *S. aureus* counts following treatments with 0.1, 1.5, 2, 2.5 and 3% of SL.

Aerobic plate count: Initial populations of APC in non treated samples were around (3.83 log CFU/g). The initial

APC (log CFU/g) in marinated thighs did not show a significant change. In fact, it was about 3.51 in treated samples with 3% SL indicating that dipping marinated chicken in different treatment solutions of SL did not result in drastic reduction of the initial APC (only 0.1-0.3

Table 3. Effect of the treatment with various concentrations of SL on the growth of aerobic bacteria and psychrotrophic count on chicken thighs stored at 4 °C.

Treatment	Day of storage at 4 °C					
	0	3	6	9	12	15
Aerobic plate count						
Control	3.83±0.38 ^a	5.04±0.47 ^a	6.86±0.27 ^c	7.52±0.22 ^a	8.2±0.35 ^b	9.42±0.33 ^a
1%SL	3.79±0.45 ^a	4.63±0.32 ^c	5.20±0.32 ^a	6.50±0.33 ^b	7.91±0.27 ^b	8.10±0.22 ^a
1.5%SL	3.65±0.24 ^b	4.54±0.43 ^b	5.0±0.22 ^c	6.30±0.31 ^b	7.42±0.33 ^a	7.92±0.29 ^b
2%SL	3.62±0.25 ^c	4.46±0.21 ^b	4.61±0.12 ^b	6.22±0.42 ^c	7.15±0.22 ^c	7.82±0.34 ^c
2.5%SL	3.54±0.32 ^a	4.0±0.15 ^c	4.46±0.21 ^b	5.91±0.21 ^a	6.93±0.41 ^b	7.55±0.11 ^b
3%SL	3.51±0.19 ^c	3.9±0.11 ^a	4.22±0.13 ^a	5.72±0.22 ^b	6.62±0.21 ^b	7.31±0.17 ^a
Psychrotrophic count						
Control	3.30±0.25 ^a	4.42±0.32 ^a	6.02±0.11 ^b	6.71±0.17 ^b	7.0±0.22 ^b	8.21±0.21 ^a
1%SL	3.13±0.32 ^b	4.11±0.31 ^b	5.83±0.19 ^a	6.52±0.11 ^c	6.72±0.19	6.92±0.22 ^b
1.5%SL	3.03±0.25 ^a	3.82±0.22 ^b	5.35±0.32 ^b	6.00±0.14 ^c	6.43±0.13 ^c	6.83±0.31 ^c
2%SL	3.0±0.29 ^c	3.51±0.27 ^c	4.93±0.34 ^b	5.53±0.21 ^a	5.98±0.31 ^c	6.32±0.18 ^c
2.5%SL	3.0±0.31 ^b	3.22±0.25 ^c	4.84±0.23 ^c	5.21±0.19 ^b	5.77±0.29 ^c	6.03±0.19 ^a
3%SL	2.91±0.25 ^c	3.00±0.19 ^a	4.72±0.17 ^b	5.01±0.23 ^c	5.44±0.13 ^a	5.74±0.17 ^a

a-c: Averages with different letters in the same column are different (P<0.05).

Table 4. Effect of the treatment with various concentrations of SL on the growth of *Pseudomonas* count and *Staphylococcus aureus* on chicken thighs stored at 4 °C.

Treatment	Day of storage at 4 °C					
	0	3	6	9	12	15
<i>Pseudomonas</i> count						
Control	2.62±0.7 ^a	3.0±0.28 ^b	3.41±0.1 ^a	4.06±0.5 ^a	5.52±0. ^c	6.00±0.6 ^a
1%SL	2.28±0.2 ^b	2.87±0.2 ^c	3.18±0.2 ^a	3.76±0.5 ^b	5.12±0. ^a	5.89±0.25 ^a
1.5%SL	2.21±0.5 ^a	2.76±0.5 ^c	3.08±0.2 ^c	3.77±0.5 ^b 3.43	5.08±0. ^b	5.72±0.3 ^c
2%SL	2.00±0.7 ^c	2.53±0.0 ^b	2.88±0.1 ^a	±0.9 ^c	4.79±0. ^b	5.54±0.19 ^b
2.5%SL	1.93±0.5 ^c	2.42±0.3 ^a	2.76±0.1 ^c	3.23±0.7 ^a	4.57±0. ^c	4.93±0.18 ^b
3%SL	1.81±0.1 ^b	2.10±0.8 ^b	2.62±0.1 ^b	3.00±0.9 ^b	3.91±0. ^a	4.42±0.21 ^c
<i>S. aureus</i>						
Control	1.12±0.20 ^b	1.81±0.27 ^a	2.31±0.32 ^a	3.02±0.34 ^b	3.30±0.45 ^c	3.61±0.45 ^c
1%SL	< 1	1.45±0.22 ^b	1.74±0.30 ^b	1.96±0.27 ^a	2.15±0.33 ^a	2.32±0.42 ^b
1.5%SL	< 1	1.34±0.22 ^c	1.54±0.27 ^a	1.74±0.23 ^c	1.75±0.30 ^a	2.03±0.38 ^b
2%SL	< 1	1.12±0.20 ^a	1.33±0.28 ^b	1.52±0.22 ^c	1.63±0.27 ^c	1.85±0.30 ^b
2.5%SL	< 1	< 1	1.12±0.19 ^a	1.33±0.19 ^b	1.42±0.19 ^b	1.63±0.28 ^a
3%SL	< 1	< 1	< 1	1.10±0.13 ^a	1.32±0.16 ^a	1.52±0.20 ^a

a-c: Averages with different letters in the same column are different (P<0.05).

log CFU/ g). By the 9th day of storage, APC in marinated chicken for (2, 2.5 and 3%) treatments were below 5.7 log CFU/g, while that of the control attained a value of 7.5 exceeding thus, the maximal recommended limit of 5.7 log CFU/g for APC in marinated chicken (AFNOR, 2004).

Indeed, with a concentration of 3% SL, the shelf life of treated thigh could reach 9, whereas for the control, it was between 5 and 6 days. Consequently, a decrease in

microbial counts from the use of SL can have positive implications for shelf-life and food safety.

Psychrotrophic bacteria and *Pseudomonas* count: The Gram-negative psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored marinated chicken. The initial psychrotrophic bacterial count (PTC) in marinated thighs

used for this study was 3.3 log CFU/g. The initial PTC was reduced after the marinating process. In fact, the reduction rates of 0.2-logs and 0.4-logs were achieved for marinated thighs with 1 and 3% of SL.

Since the predominant psychrotrophic organisms found on chicken are typically *Pseudomonas* (Charles et al., 2006), the population trends obtained for *Pseudomonas* were similar to those of total psychrotrophic counts.

As mentioned, the marinated samples stored at 4°C were spoiled after about 4 days of storage. At this time, the *Pseudomonas* and total psychrotrophic populations were approximately 5.10^5 and the products had off-odours characteristic of spoiled poultry products. The increased shelf life obtained by treatment with SL can be explained by the strong inhibition of H₂S-producing bacteria, such as *Pseudomonas* spp. (Lin et al., 2004), resulting in lower levels of hydrogen sulphide and other sulphur-containing spoilage.

Enterobacteriaceae counts: The *Enterobacteriaceae* family is a group of bacteria used to assess the general hygiene status of marinated chicken and was found to be members of the microbial association implicated in the spoilage of a food product during refrigerated storage.

By the end of the storage (day 15), all *Enterobacteriaceae* counts ($P < 0.05$) of treated samples with 1, 1.5, 2, 2.5 and 3% of SL are reduced compared to those untreated, reaching values of 2.51, 2.35, 1.91, 1.75 and 1.74 log CFU/g, respectively. These results are interesting since all counts are lower than the maximal recommended limit (3.7 log CFU/g).

Staphylococcus aureus count: Illness due to *S. aureus* is caused by enterotoxins which are preformed in food. Only some *S. aureus* contain enterotoxin genes and therefore have the potential to cause food poisoning (HPA, 2009). The growth of *S. aureus* was slower than that of the other microbial groups, starting by less than 2 log CFU/g and never exceeding 4 log CFU/g in the control samples. By the end of the 15th day, the marinated chicken treated with SL revealed significant ($P < 0.05$) lower *S. aureus* counts: for the control, the limit is exceeded (2.7 log CFU/g) by the seventh day of storage, whereas for the treated samples this limit is not attained after 15 days of storage. In addition, it should be noted that *Salmonella* spp. is totally absent from samples treated with SL and that 3% (v/v) of SL, extended the shelf life of marinated poultry by 3 days.

In conclusion, different concentrations of SL can be used as safe organic preservatives for marinated chicken under refrigerated storage. In addition, SL delayed the growth of food spoilage bacteria. In fact, it was shown to have antibacterial activities against various food-borne pathogens including *Staphylococcus aureus*, *Escherichia coli* (Lee et al., 2002), as well as *Salmonella* sp. Furthermore, SL is widely available, economic and generally 'recognized-as-safe' (Mc William Leitch and

Stewart, 2002).

Treatment with LA

Several organic acids have been used as decontaminating agents for poultry and meat, such as LA. The chemical formula of lactic acid is CH₃-CHOH-COOH. In this part, we studied microbiological evolution of samples treated with 0.2, 0.3, 0.5, 0.6, 0.8 and 1% (v/v) of LA (Tables 5 and 6).

Aerobic plate count: On the 11th day of storage, treatment with LA inhibited the APC growth. In fact, the APC counts were reduced by about 1 log and 2 log CFU/g, respectively with 0.2 and 1% LA, compared to the control samples. Chicken thighs treated with 1% LA have the highest shelf life (11 days) whereas the latter is between 5 and 6 days for control samples. In addition, treatment with 0.8% LA caused an increase in shelf life for about 9 days.

The comparison between SL and LA treatment showed that they have the same effect on shelf life when concentrated at 3 and 0.8%, respectively. Indeed, the use of 1% LA or 3% SL increased the shelf life by two days. These results are very interesting since, firstly, important reduction in the shelf life of treated thighs was reached and secondly, the lactic acid was able to give the same results as the sodium lactate but at lower concentration.

Psychrotrophic bacteria and Pseudomonas count: The Gram-negative *Pseudomonas* spp. seemed to be the most vulnerable group to the treatment with LA (Gerez et al., 2009).

The initial PTC and *Pseudomonas* count are widely reduced after the marinating process. For PTC, reduction rates of 0.3 and 0.5-logs were achieved for marinated thighs with 0.2 and 1% of LA. For *Pseudomonas* count, reduction rates of 0.7 and 1.4 logs were achieved for marinated thighs with 0.2 and 1% of LA. These results show that treatment with LA (1%) significantly ($P < 0.05$) inhibited PTC and *Pseudomonas* count on marinated thighs.

The increased shelf life obtained by treatment with LA can be explained by the strong inhibition of H₂S-producing bacteria (Table 5 and 6), such as *Pseudomonas* spp., resulting in lower levels of hydrogen sulphide and other sulphur-containing spoilage compounds (Gonzalez-Fandos et al., 2009). This is supported by the fact that total psychrotrophic plate counts were identical.

Enterobacteriaceae counts: The initial *Enterobacteriaceae* counts were reduced after the marinating process with LA treatments. Reduction rates of 0.4 and 0.7-logs were achieved for marinated thighs with 0.2 and 1% LA. This result showed that treatment

Table 5. Effect of the treatment with various concentrations of LA on the growth of aerobic bacteria and psychrotrophic count on chicken thighs stored at 4 °C

Treatment	Day of storage at 4 °C					
	0	3	6	9	12	15
Aerobic plate count						
Control	3.83±0.38 ^a	5.04±0.47 ^a	6.86±0.27 ^c	7.52±0.22 ^a	8.2±0.35 ^b	9.42±0.33 ^a
0.2 % LA	3.79±0.35 ^b	4.62±0.46 ^b	5.20±0.23 ^a	6.50±0.20 ^a	7.88±0.33 ^b	8.10±0.26 ^a
0.4 % LA	3.65±0.30 ^c	4.50±0.34 ^a	4.95±0.26 ^b	6.29±0.19 ^b	7.4±0.30 ^a	7.90±0.29 ^c
0.6 % LA	3.62±0.31 ^b	4.35±0.37 ^c	4.56±0.18 ^c	6.20±0.22 ^c	7.06±0.29 ^b	7.80±0.32 ^a
0.8 % LA	3.54±0.25 ^b	3.98±0.29 ^b	4.40±0.17 ^c	5.75±0.18 ^a	6.90±0.18 ^a	7.52±0.22 ^b
1 % LA	3.51±0.27 ^a	3.89±0.19 ^b	4.20±0.15 ^b	5.36±0.15 ^c	5.9±0.11 ^c	6.93±0.18 ^b
Psychrotrophic count						
Control	3.30±0.25 ^a	4.40±0.32 ^a	6.00±0.11 ^b	6.71±0.17 ^b	7.0±0.22 ^b	8.21±0.21 ^a
0.2 % LA	3.13±0.24 ^a	3.92±0.22 ^b	5.65±0.15 ^a	5.91±0.21 ^a	6.34±0.19 ^c	6.56±0.23 ^a
0.4 % LA	3.03±0.15 ^b	3.65±0.20 ^a	5.15±0.13 ^c	5.63±0.23 ^b	6.00±0.23 ^b	6.00±0.19 ^b
0.6 % LA	3.0±0.14 ^c	3.33±0.16 ^c	4.82±0.15 ^a	5.43±0.18 ^a	5.85±0.18 ^a	5.91±0.17 ^b
0.8 % LA	3.0±0.15 ^c	3.00±0.11 ^b	4.61±0.19 ^a	5.00±0.15 ^c	5.54±0.15 ^c	5.71±0.17 ^a
1 % LA	2.91±0.11 ^a	2.92±0.11 ^b	4.53±0.11 ^b	4.83±0.16 ^a	5.22±0.15 ^b	5.42±0.18 ^c

a–c: Averages with different letters in the same column are different (P<0.05).

Table 6. Effect of the treatment with various concentrations of LA on the growth of *Pseudomonas* count and *Staphylococcus aureus* on chicken thighs stored at 4 °C.

Treatment	Day of storage at 4 °C					
	0	3	6	9	12	15
<i>Pseudomonas</i> count						
Control	2.62±0.37 ^a	3.0±0.28 ^b	3.41±0.19 ^a	4.06±0.45 ^a	5.52±0.34 ^c	6.00±0.26 ^a
0.2 % LA	1.92±0.35 ^b	2.47±0.2 ^b	2.86±0.20 ^a	3.32±0.42 ^a	4.79±0.34 ^a	5.54±0.25 ^c
0.4 % LA	1.82±0.28 ^b	2.32±0.2 ^a	2.66±0.15 ^a	3.30±0.39 ^b	4.66±0.22 ^c	5.42±0.22 ^a
0.6 % LA	1.64±0.16 ^a	2.11±0.2 ^c	2.43±0.14 ^c	3.06±0.27 ^b	4.41±0.27 ^c	5.10±0.20 ^b
0.8 % LA	1.51±0.11 ^b	2.00±0.15 ^b	2.36±0.16 ^c	2.81±0.17 ^b	4.11±0.23 ^b	4.54±0.17 ^c
1 % LA	1.44±0.12 ^c	1.71±0.14 ^b	2.21±0.11 ^a	2.64±0.19 ^c	3.60±0.13 ^a	4.00±0.11 ^a
<i>S. aureus</i>						
Control	1.12±0.20 ^b	1.81±0.27 ^a	2.31±0.32 ^a	3.02±0.34 ^b	3.30±0.45 ^c	3.61±0.45 ^c
0.2 % LA	< 1	1.16±0.19 ^a	1.41±0.29 ^a	1.65±0.32 ^b	1.75±0.35 ^c	2.02±0.34 ^b
0.4 % LA	< 1	< 1	1.22±0.27 ^b	1.44±0.27 ^a	1.43±0.30 ^b	1.81±0.25 ^a
0.6 % LA	< 1	< 1	< 1	1.23±0.18 ^c	1.31±0.28 ^a	1.62±0.27 ^c
0.8 % LA	< 1	< 1	< 1	< 1	1.22±0.21 ^b	1.53±0.18 ^b
1 % LA	< 1	< 1	< 1	< 1	< 1	1.20±0.11 ^b

a–c: Averages with different letters in the same column are different (P<0.05).

with LA (1%) significantly (P<0.05) inhibited *Enterobacteriaceae* counts on marinated thighs (data not shown).

By the end of the storage (day 15), *Enterobacteriaceae* counts treated samples with LA are reduced compared to those treated with SL, concluding that *Enterobacteriaceae* counts are more inhibited by the LA

at 1% (1.31 log CFU/g) than by SL at 3% (1.74 log CFU/g) (data not shown).

***Staphylococcus aureus* count:** By the end of storage period (day 15), the marinated samples treated with LA revealed significant (P < 0.05) lower *S. aureus* counts. Compared with sample treated with SL (3%), sample treated with LA showed a lower *S. aureus* count (1.2 log

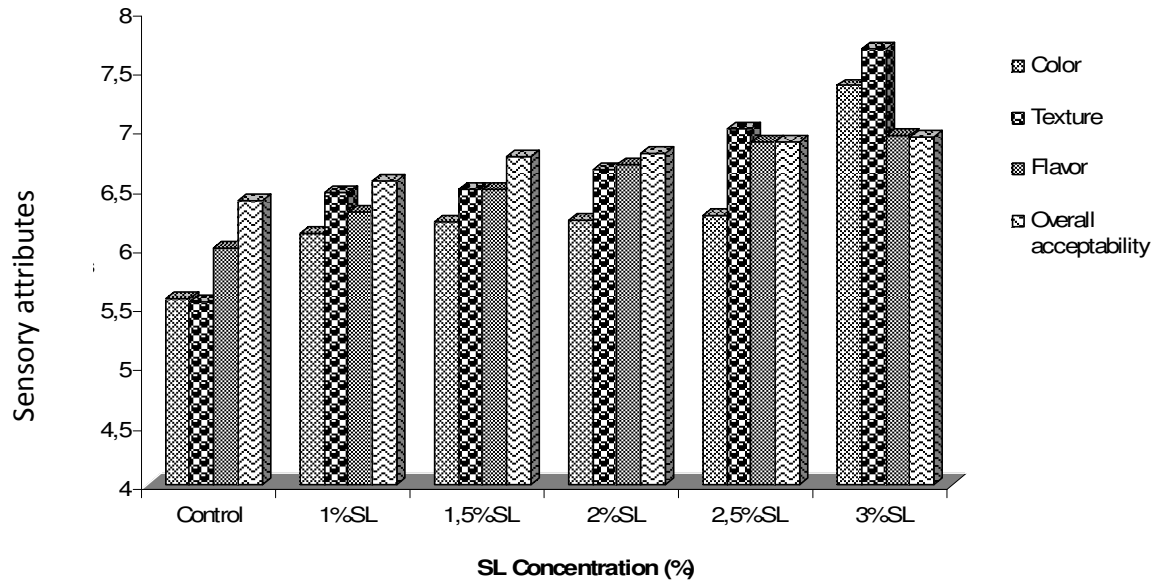


Figure 3. Mean sensory attribute scores of marinated chicken thigh treated with SL

CFU/g). It has been suggested that the inhibitory effect of LA on bacteria is mostly due to the reduction in the environmental pH (Koutsoumanis et al., 2006); this result are in accordance with our study since samples treated with LA at 1% showed lower pH compared to other samples and a remarkable reduction in micro-organisms counts.

LA has been reported to improve shelf-life by decreasing microbial loads (Sawyer et al., 2008) on meat products: treatment with LA at 1% increased the shelf life until 11 days at 4°C. On the basis of the microbiological analyses reported in this study, the general order of antibacterial activity of the different preservatives used for treatment of marinated chicken was: LA > SL.

Sensory evaluation

Sensory evaluation is the most popular way of assessing the freshness of marinated thighs. It is fast, simple, and provides immediate quality information. Colour, texture and flavour of marinated thighs treated with SL and LA samples are shown in Figures 3 and 4.

Significant differences ($P < 0.05$) in the overall acceptability scores were detected between marinated samples treated with LA before storage but not significant differences for samples treated with SL. Sensory scores of both samples treated with SL and treated with LA were in the typical categories of colour, texture and flavour and no off-odours or off-flavours are detected in any treatment. No differences were found, in the sensory attributes analyzed, between 1, 1.5, 2, 2.5 and 3%.

For the entire results, marinated sample treated with 1% LA received the highest overall acceptability score (7.94), followed by 0.8% LA marinated samples (7.38)

and 3% SL (6.94). A significant difference ($P < 0.05$) was detected, for the overall acceptability, between marinated samples, while no differences were detected between the two preservative treatments.

All of the analyzed samples were considered as acceptable during sensory analysis. Increase in populations of bacteria, as well as in chemical indicators (pH and TVBN) coincided with the sensory scores detected by the sensory panel.

Conclusion

This study concludes that treatment with LA and SL can delay the microbial growth, reduce the chemical changes and improve or maintain the sensory attributes. 1% LA is the best concentration allowing the extending of the shelf life of the product during refrigerated storage. Therefore, marinating with LA can be used as a safe method for preservation of poultry products.

The findings presented in this study are promising; in fact the industrial poultry production can be extended and maximized through the use of these low cost additives. For this reason, further studies are currently under way in our laboratory to render the treatment of these additives suitable for future poultry meat production and to explore their potential application for other compounds conservation.

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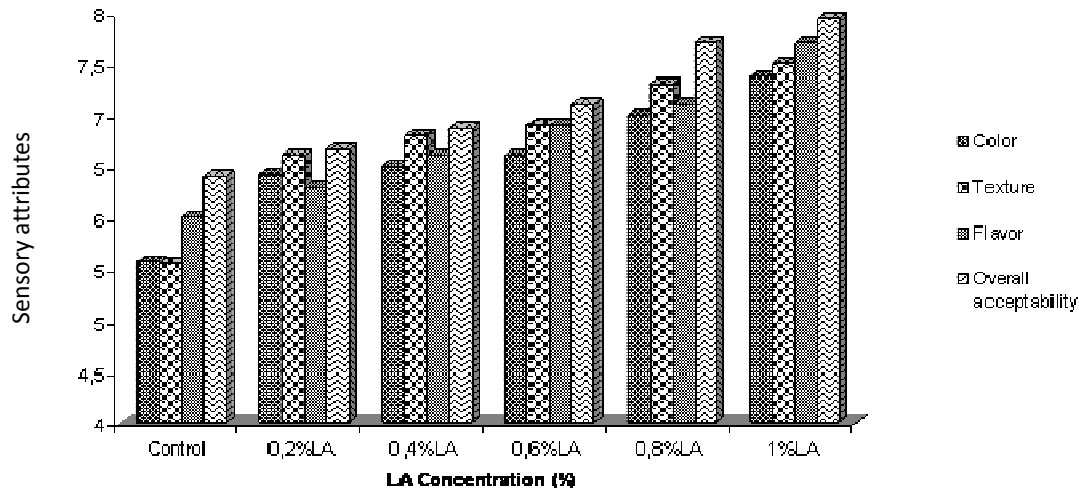


Figure 4. Mean sensory attribute scores of marinated chicken thigh treated with LA.

industry for purchasing poultry meat.

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