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Effects of organic acid treatments combined with modified atmosphere packaging on survival of pathogens and quality parameters of meatballs

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Abstract

The present work was conducted to determine the effects of organic acids (1 and 2% of sodium lactate, 0.5% potassium sorbate, 0.5% sodium citrate, and 1% sodium acetate) combined with ambient air and modified atmosphere packaging (HiOx: 80:20:0/O_2:CO_2:N_2; CO: 0.4:30:69.60/CO:CO_2:N_2) on the quality parameters and shelf-life of meatballs, and to evaluate the survival of *Salmonella* Typhimurium and *Listeria monocytogenes* in inoculated meatballs stored at 4°C for 15 d. Results indicated that the organic acid combinations delayed the microbial growth, and improved the shelf-life of meatballs. Lipid oxidation was retarded with organic acid treatments, and the meatballs in CO-MAP did not exceed the spoilage level during the storage period. A difference of 1 - 2 log and 2 - 3 log units of *S.* Typhimurium and *L. monocytogenes* counts were recorded between the untreated and organic acids treated meatballs, respectively, with effectiveness in HiOx and CO-MAP. Enhancement in colour and textural properties was detected in the meatballs treated with combined organic acids and 2% sodium lactate. Moreover, the overall acceptability of 2% sodium lactate treated meatballs was rated more palatable by the panellists at the end of the storage. In conclusion, organic acid treatments combined with modified atmosphere packaging can maintain the storage properties of meatballs without influencing the sensory characteristics during refrigerated storage.

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Introduction

Meat is indispensable in human nutrition due to its high level of valuable proteins and excellent source of nutrients; this renders it susceptible to contamination by spoilage and pathogenic microorganisms (Djenane *et al*., 2016; Djenane and Roncalés, 2018). Among fresh meat products, ground meat and meatballs are foods with high risk for microbial contamination due to their structural features, thus requiring intervention technologies (Quilo *et al*., 2009). The hygienic quality of ground beef patties depends on the personal hygiene of the producers, the production method, and the quality of spices and other ingredients used in addition to the raw ground meat (Bingol *et al*., 2012; 2014). Improper storage conditions, packaging errors, and insufficient heat applications used in intensively contaminated meat products increase the risk of food poisoning, and affect the public health.

Chilling/cooling and freezing are the most preferred conventional methods for the preservation of meatballs (Ozturk *et al.*, 2017). In addition to these, the use of antimicrobial preservatives in ground meat products is one of the commonly used methods that provide microbial safety and extend the shelf-life of the product, by destroying and preventing the growth of spoilage and pathogenic microorganisms (Pegg and Shahidi, 2000; Theron and Lues, 2007). Among these antimicrobial agents, organic acids and their

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salts are natural compounds that have been extensively used in the meat industry due to their accessibility, non-toxicity, and clear efficiency (Coban, 2020). The use of these acids in meat products has been approved by the FDA due to their generally recognized as safe (GRAS) status (Mir and Masoodi, 2018).

Lactates, acetates, citrates, sorbates, benzoates, propionates, and formates are the organic additive substances used in the food industry to enhance the structural properties of the product, and to slow down the microbial growth. Lactates (L(+)-lactic acid salts) are widely used as antimicrobial agents to ensure microbial safety, prolong shelf-life, and also provide colour stability and minimise lipid oxidation (Kim *et al.*, 2006; Mancini *et al.*, 2009; 2010). Acetates are known as potent antimicrobial agents that are used in meat and meat products, due to the lower pKa (4.76) value of acetic acid, and the fact that a large proportion of acetates remain in undissociated form. The antioxidant effects of acetates improve the colour and lipid oxidation of meat products (Lee *et al*., 2005; Mir and Masoodi, 2018). Citrates are potential antioxidant and antimicrobial preservatives that may improve the appearance, flavour, and shelf-life of meat products (Igwegbe *et al*., 2019). Sorbates, which have been used primarily as an antifungal agent in food, have an antimicrobial activity against many bacteria because of their weak acidity (pKa = 4.76) and undissociated acid form (Stopforth and Kudron, 2020). However, potassium sorbate can cause allergic reaction for some sensitive people, but these allergies are rare in food consumption. Allergies to potassium sorbate are more common with cosmetics and personal care products, where it can cause skin or scalp irritation. It may induce contact allergy and allergic contact dermatitis, especially when used on damaged skin (Dehghana *et al.*, 2018; Dendooven *et al.*, 2021).

In addition to ensuring the safety of food by adding organic acids to the product formulation, modern packaging techniques are preferred because they increase the appeal of the product, and extend its shelf-life.

Modified atmosphere packaging (MAP) is one of the leading modern packaging techniques that are intended to extend the shelf-life and maintain the microbial and sensory quality of meat products (Bingol and Ergun, 2011; Jaspal, 2021). Carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2) are the commonly used gases in MAP (Jaspal, 2021). Furthermore, low levels of carbon monoxide (CO) have been permitted to be used in fresh meat industries in order to maintain the desired properties of meat by stabilising red colour, suppressing microbial growth, preventing oxidation and bone darkening, and increasing tenderness and flavour acceptability (Cornforth and Hunt, 2008; Djenane and Roncalés, 2018).

For this purpose, the aim of the present work was to evaluate the effects of organic acids and MAP on the survival of *Salmonella* Typhimurium and *Listeria monocytogenes*, and the quality parameters of meatballs under cold storage.

Materials and methods

Meatball preparation

The experimental meatball was prepared from grounded veal meat (*musculus longissimus dorsi lumborum*) purchased in a local market in Istanbul, based on the receipt specified by Bingol *et al*. (2018). The meatball dough was divided into two batches for inoculation (five equal sub-groups) and non-inoculation (quality control group) treatments.

For the inoculated samples, 10 mL of bacterial suspension of the *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Listeria monocytogenes* strains were separately added to the meatball dough to obtain 10^6 CFU/mL per kg, and thoroughly mixed and rested for 30 min at 4°C to enable bacterial cell attachment. Then, sodium lactate (NaL) (Merck ME.106522, v/v), potassium sorbate (Sigma-Aldrich 85520, w/v), sodium citrate (Merck ME.106432, w/v), and sodium acetate (Merck ME.106268, w/v) were added alone or in combination, at a maximum concentration of 2%, to the meatball dough in order to obtain the inoculated and non-inoculated experimental groups (NaL_1: 1% sodium lactate; NaL_2: 2% sodium lactate; Sol.A: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium acetate; Sol.B: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium lactate; and control (C): non-additive). The final mixture was formed into round meatballs, each with a radius of 3.5 cm, and a weight of 25 ± 2 g. Thereafter, 12 pieces of inoculated and non-inoculated meatballs were placed in low O_2 permeable (8 - 12 cm^3/m^2/24 h at STP) polyethylene trays, and were heat-sealed with a Ponapack packaging unit (VTK 40 SC, Ponapack, Istanbul, Turkey) using a low O_2 permeable (3 cm^3/m^2/24 h) lidding film (Wrap Film Systems Ltd., Shropshire, UK) for aerobic and modified atmosphere packaging (air: ambient air; HiOx-MAP: 80:20:0/O_2:CO_2:N_2; and CO-MAP: 0.4:30:69.60/CO:CO_2:N_2). Packages of the five sub-groups were immediately stored at 1 ± 1°C for 15 d, and examined at an interval of 3 d. Experimental meatball trials were performed in triplicate on different dates.

Inoculum preparation

Salmonella enterica subsp. *enterica* serovar Typhimurium (ATCC14028) and *Listeria monocytogenes* (ATCC7644) strains were acquired from Microbiologics® (Minnesota, USA). Strains were streaked on tryptone soy agar (Oxoid CM131, UK) plates, and incubated at 35°C overnight. At the end of 24 h, *S.* Typhimurium and *L. monocytogenes* colonies were transferred to tryptone soy broth (Oxoid CM129), and incubated at 37°C for 18 h.

Microbiological analysis

To identify the present microbiota in meatballs before inoculation, 25 g of samples were added to 225 mL of saline peptone water, and decimal dilutions were transferred onto plate count agar (PCA, Oxoid CM0463), and incubated at 30°C for 72 h (ISO 4833; ISO, 2003).

In order to determine whether there were any *Salmonella* spp. and/or *L. monocytogenes* in the meatballs before inoculation, a pre-detection step was conducted. For this purpose, the ISO 6579-1 (ISO, 2017a) and ISO 11290-1 (ISO, 2017b) methods were used, respectively. To determine the *S.* Typhimurium and *L. monocytogenes* counts in inoculated meatball samples, decimal dilutions were inoculated on XLD (Oxoid CM0469) and Hektoen enteric (Oxoid CM0419) agars for *S.* Typhimurium, and to chromogenic *Listeria* agar (Oxoid CM1080) for *L. monocytogenes*. Results were reported as log CFU/g.

To specify the total aerobic bacteria (TAB) counts of non-inoculated meatball samples, appropriate dilutions were spread on PCA, and incubated for 72 h at 30°C (ISO 4833, ISO, 2003) and 10 d at 6.5°C for psychrotrophic microorganisms (PsM) (ISO 17410; ISO, 2019). Lactic acid bacteria (LAB) counts were enumerated on Man, Rogosa, Sharpe agar (Oxoid, CM0361), *Pseudomonas* spp. on CFC-supplemented *Pseudomonas* agar (Oxoid, CM0559 and SR0103), yeast and mould count on DRBC agar (Oxoid, CM0727), and *Enterobacteriaceae* on violet red bile glucose agar (Oxoid, CM0485) following ISO 15214 (ISO, 1998), ISO 13720 (ISO, 2000), ISO 21527-1 (ISO, 2008), and ISO 21528-2 (ISO, 2004) methods, respectively.

Physico-chemical analysis

The pH of non-inoculated meatballs was determined using a pH meter (Hanna HI-9321, Woonsocket, RI) calibrated with pH 4.0 and 7.0 solutions. The water activity (a_w) of the samples was determined using a water activity meter (Decagon AquaLab LITE, USA). The moisture content of meatballs was determined by drying a homogeneous mixture at 105 ± 2°C until obtaining a constant weight (AOAC, 2005). The thiobarbituric acid reactive substances (TBARS) value of the meatballs was determined according to Shrestha and Min (2006) by measuring the absorbance of colour developed at 530 nm using a T80+ UV/Vis spectrometer (PG Instruments Ltd., London, UK). The TBARS value was expressed as mg of malondialdehyde (MDA) per kg, and calculated using Eq. 1:

$$\text{TBARS value} = [(\text{absorbance} - 0.0121)/0.1379] \times [72.06/94] \text{ mg MDA/kg meatball} \quad \text{(Eq. 1)}$$

Colour analysis

The surface colour of the meatballs was measured using a Colour Flex HunterLab Colour Measurement System (Hunter Associates Laboratory Inc., Virginia, USA). Colour coordinates values were recorded at the average of both side areas of four different meatball samples in terms of *L** for lightness, *a** for redness, and *b** for yellowness, using a diffuse illumination (D65 2° observer) with 8 mm viewing aperture, and a 25 mm port size with the specular component excluded (AMSA, 2012).

Metmyoglobin content analysis

The metmyoglobin content (MetMb%) of the meatballs was determined according to Bekhit *et al.* (2003). Samples (5 g) were dissolved in 40 mM cold buffered phosphate solution, and centrifuged at 4,500 *g* for 30 min after 1-h refrigeration storage. Filtered supernatant was measured using a UV spectrophotometer at 572, 565, 545, and 525 nm, and the percentage of MetMb was calculated using Eq. 2:

$$\text{MetMb\%} = [-2.51 \times (A572/A525) + 0.777 \times (A565/A525) + 0.8 \times (A545/A525) + 1.098] \times 100 \quad \text{(Eq. 2)}$$

Texture profile analysis

The texture profile analysis (TPA) of the meatballs was conducted using a texture analyser (Instron Universal Testing Machine model 3343, Instron Ltd., UK) fitted with a compression probe, using a crosshead speed of 80 and 200 mm/min for the first and the second test, and a load cell of 0 - 500 N. Eight different measurements from the both sides of four cylindrical samples (35 mm in diameter and 13.5 mm in height) were recorded to calculate the arithmetic means. Hardness, cohesiveness, gumminess, chewiness, and springiness values were measured for the meatballs (Bourne, 1978).

Sensory analysis

Twelve panellists (aged between 28 to 47 years, five females and seven males) were trained following the ISO 8586 (ISO, 2012) to evaluate the sensory characteristics of the meatballs. Before the sensory analysis, panel members developed the vocabularies of the sensorial attributes in a round-table session using a standardised procedure (ISO 13299; ISO, 2016) in two separate sessions for each of the selected characteristics. Then, an open-discussion session was held to familiarise the panellists with the attributes (tenderness, chewiness, juiciness, off-odour, red colour, flavour intensity, and overall acceptability) and the scale to be used. Each sample was served as raw and warm, encoded with a three-digit number, and the analysis was performed in triplicate in two sessions on days 1, 6, and 12 of cold storage.

The panellists evaluated the intensity observed for each sensory attribute on unstructured line scales, in which 1 referred to “slight” and 9 referred to “extremely strong”. Simultaneously, the overall acceptability of meatballs was assessed using a 5-point descriptive scale, where 1 referred to “dislike extremely” and 5 referred to “like extremely”.

Statistical analysis

The General Linear Model procedure (PROC GLM) of SPSS 16.00 (SPSS Inc., Chicago, IL, USA) was used to determine the effects of organic acids and packaging conditions based on storage time. Mean separations were obtained using Duncan’s multiple range tests ($p < 0.05$), and significant two-way interactions between the main effects were also evaluated. The trial was performed in triplicate, and sensorial data were analysed by ANOVA using Fisher’s least significant difference test (LSD).

Results and discussion

Changes in Salmonella Typhimurium *counts of inoculated meatballs*

S. Typhimurium strains showed an increase over time in each group. Starting with day 3 of storage, a significant difference was observed between the untreated and organic acid treated meatballs. A difference of 1 - 2 log units was recorded between these groups at the end of storage (Figure 1). Meatballs containing 2% sodium lactate performed better than the other meatballs, followed by Sol.B. In air-packed samples, the bacterial count in NaL_2 and Sol.B exhibited an inhibition starting from day 3, and reached a decrease of 0.5 - 0.7 log CFU/g at the end of the storage period. However, in the HiOx and CO-MAP meatballs, the bacterial inhibitions were more effective by a significant decrease of 0.8 - 1.4 log CFU/g ($p < 0.001$), while the counts in the meatballs exposed to other organic acid treatments remained almost the same counts from the beginning of the storage.

Djordjević *et al*. (2018) recorded a significant decrease in *Salmonella* spp. counts of MAP minced meat samples with reductions of about 1.5 log CFU/g in 50% and 30% CO_2 containing packages during 12-d refrigerated storage. Quilo *et al*. (2010) remarked that the addition of 3% potassium lactate to ground beef reduced the *S*. Typhimurium counts to a certain extent through the retail display compared to the untreated samples. Tenderis *et al*. (2020) also stated that lower *S*. Typhimurium levels were noted in cooked ground beef formulated with sodium lactate.

Gram-negative bacteria, including *Salmonella*, are quite sensitive to CO_2 because they interact with cell membrane lipids, causing acidification in the cytoplasm and disordered enzyme synthesis. Moreover, by reducing cell metabolism, they slow down microbial growth and reduce growth in the logarithmic phase (Cornforth and Hunt, 2008; Djordjević *et al*., 2018).

Changes in Listeria monocytogenes counts of inoculated meatballs

The inhibition observed in the *L. monocytogenes* counts in the experimentally contaminated meatballs was found to be 2.1 - 2.6 log CFU/g in the organic acids added to the air-packaged samples at the end of the cold storage, whereas 2 - 3 log CFU/g inhibition was recorded in HiOx and CO

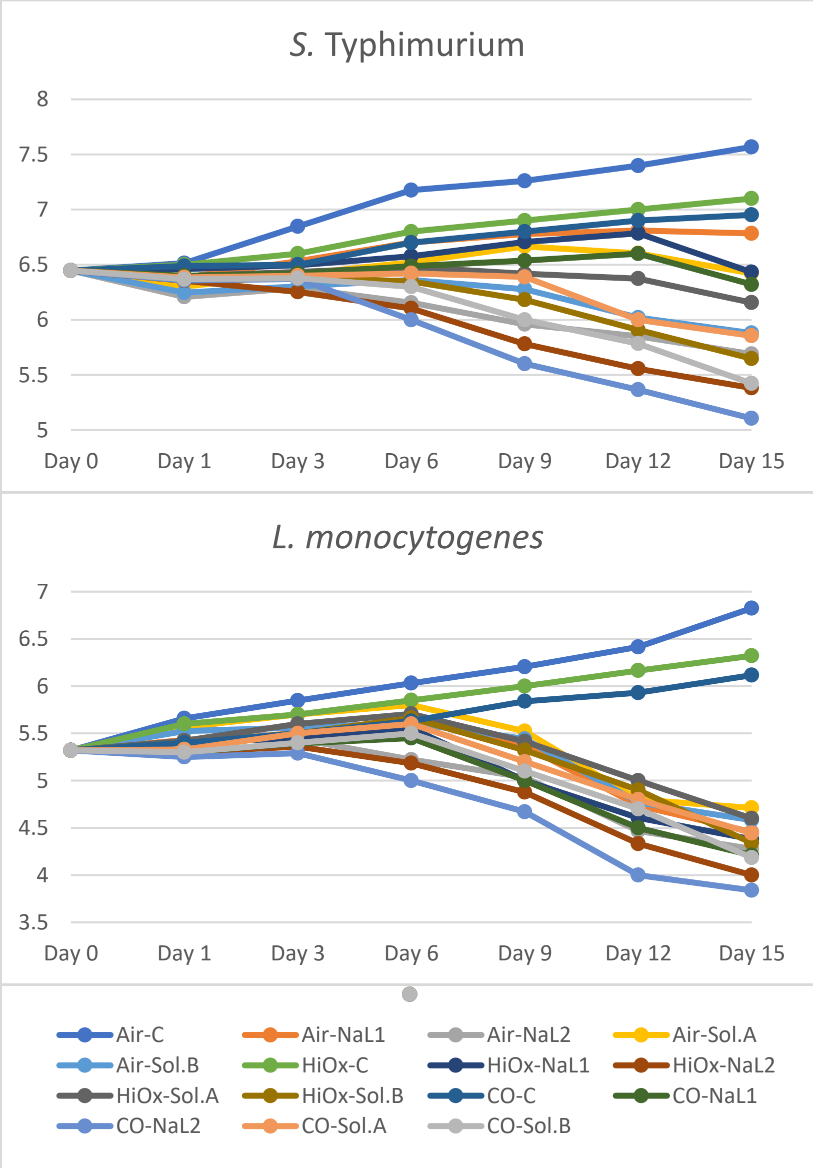

Figure 1. Changes in *S*. Typhimurium and *L. monocytogenes* counts of meatballs stored at 1°C for 15 days (Log CFU/g). C: control; NaL_1: 1% NaL; NaL_2: 2% NaL; Sol.A: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium acetate; Sol.B: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium lactate; HiOx: 80:20:0/O_2:CO_2:N_2; and CO: 0.4:30:69.60/CO:CO_2:N_2.

packaged meatballs (Figure 1). This decrease was significant in MAP samples starting from day 6 till the end of the storage ($p < 0.01$), and NaL_2 treated meatballs showed the highest inhibition during storage. On the other hand, HiOx and CO-MAP resulted in a slight decrease at a level of 0.5 - 0.7 log CFU/g in untreated meatballs compared to air packaged ones.

Apostolidis *et al*. (2008) stated that the inhibiting effect of oregano-cranberry combination with 2% sodium lactate against the growth of *L. monocytogenes* was in the range of 1 log CFU/g, compared to the untreated cooked ground beef. Furthermore, they indicated that 2% sodium lactate addition to the broth at pH 6 resulted in a 1.8 log decrease in *L. monocytogenes* at 4°C. This lower inhibition in ground beef compared to the broth medium could have been due to the proline recovery in meat which helps *L. monocytogenes* to recover from the phytochemical inhibitory activity. Nissen *et al*. (2000) emphasised that the growth of *L. monocytogenes* in ground beef stored in the low CO/high CO_2 combination did not increase as a result of the prolonged shelf-life in modified atmosphere. Similar to this situation, Gonzalez-Fandos *et al*. (2021) stated that *L. monocytogenes* counts in chicken legs packaged in 20% CO_2/80% N_2 and washed with 3.75% lactic acid - 3.75% potassium sorbate significantly decreased compared to untreated MAP samples, and the counts noted after 8-d storage were approximately 2.63 log unit lower than the first ones.

Changes in bacterial counts of non-inoculated meatballs

The changes in the total aerobic bacteria (TAB) counts of non-inoculated meatballs are summarised in Table 1. Untreated meatballs (C) started to deteriorate after day 3 of cold storage, whereas organic acid-treated samples exhibited a longer shelf-life. The meatballs to which 1% sodium lactate was added reached 7 log CFU/g after day 12 of cold storage, while meatballs containing NaL_2, Sol.A, and Sol.B remained at lower counts on the last day of storage in all packaging conditions. CO-MAP provided more than 1 log CFU/g of reduction on day 6 of cold storage, and resulted in significant differences during the storage period ($p < 0.001$). Psychrotrophic bacteria in the untreated meatballs showed higher counts compared to organic acid-added meatballs, particularly, starting with day 6 where an approximately 1 log unit difference between the groups was recorded. During the storage period, the meatballs treated with Sol.A and Sol.B performed better than those with the NaL additions. The packaging conditions also influenced the shelf-life of the meatballs; CO packaging, in particular, showed the best suppression of bacterial growth during the cold storage period. Moreover, the addition of organic acid combinations with CO-MAP was more effective compared to NaL and the untreated groups ($p < 0.001$).

Cetin and Bostan (2002) recorded significant increases in the shelf-life of ready-to-eat meatballs with the addition of 0.5, 1, and 2% sodium lactates. They reported that while the control samples spoiled on day 4, samples containing 0.5 and 1% sodium lactate spoiled on days 6 and 8, respectively. However, meatballs containing 2% sodium lactate showed no marks of spoilage, even on day 10 of storage. Similarly, in the present work, while the untreated meatballs started to deteriorate after day 3, the addition of organic acid extended the shelf-life of the meatballs. Samples containing NaL_1, NaL_2, Sol.A, and Sol.B deteriorated on days 9, 12, and 15, respectively. Sallam and Samejima (2004) reported an extension of 15 - 21 days in the shelf-life of beef minced meat by reducing the counts of aerobic and psychrotrophic microorganisms, lactic acid bacteria, and Enterobactericeae, with an addition of sodium lactate, NaCl, or their mixtures.

The LAB counts in all treatments showed increases during the storage period. However, even though a parallel increase was observed in both untreated and lactate-added meatballs during the storage period, the LAB growth rate slowed down in meatballs treated with Sol.A and Sol.B starting with day 6 ($p < 0.001$). The organic acid combinations added to these two groups led to a 1.5 - 2 log inhibition from the other ones. In addition, the LAB counts of meatballs packaged with CO differed significantly from the meatballs packaged with air and HiOx by an approximate inhibition of 1 log CFU/g on day 9. Then, this decrease regressed to 0.5 log CFU/g till the end of the storage period.

Pseudomonas spp. counts increased in untreated meatballs until day 9, and a relative decrease was observed on the last day of storage (Table 1), while a constant increase was observed in organic acid-added samples during the storage period. Moreover, packaging conditions were significantly effective only between days 3 and 9 ($p < 0.001$).

The Enterobacteriaceae counts showed a slight increase in untreated and NaL-added meatballs until day 6, an increase that continued till day 9 in Sol.A and Sol.B groups; at that point, a decrease was observed until the end of the storage period in all groups (Table 1). Bacterial inhibition was greater in meatballs containing NaL_2 followed by groups to which Sol.B, Sol.A, and NaL_1 had been added, respectively. A similar pattern was detected under the different packaging conditions of meatballs. The addition of organic acid and CO-MAP limited mostly the increase in Enterobacteriaceae counts during storage ($p < 0.05$).

The yeast and mould count of the meatballs increased logarithmically over time during the storage period. Even though this increase was highest in untreated meatballs, samples treated with A and B solutions containing potassium sorbate and sodium citrate showed better results compared to the lactate treated meatballs. More than 1 log CFU/g difference was noted between the untreated and combined organic acids added meatballs from day 9 to the last day of storage (Table 1). Moreover, packaging conditions were significant until day 9 with a maximum 0.7 log difference in CO-MAP than air-packaged samples ($p < 0.001$).

Table 1. Mean values, standard errors, and significant interactions of microbiological parameters of meatballs stored at 1°C for 15 days (log CFU/g).

Characteristic	Application	Group	Day 0	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
TABC	Organic acid treatment	C	4.930	5.423	6.060^{a}	6.548^{a}	6.883^{a}	7.201^{a}	7.571^{a}
		NaL$_1$	4.930	5.301	5.431^{bc}	6.061^{b}	6.483^{b}	6.801^{b}	7.123^{b}
		NaL$_2$	4.930	5.197	5.307^{c}	5.612^{c}	6.044^{c}	6.570^{bc}	6.837^{bc}
		Sol.A	4.930	5.332	5.505^{b}	5.636^{c}	5.794^{d}	6.087^{d}	6.498^{d}
		Sol.B	4.930	5.378	5.536^{b}	5.772^{bc}	5.932^{cd}	6.327^{cd}	6.641^{cd}
		SE	-	0.100	0.043	0.133	0.067	0.100	0.110
		P	-	0.575	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	4.930	5.590^{a}	5.994^{a}	6.360^{a}	6.715^{a}	7.084^{a}	7.510^{a}
		HiOx	4.930	5.360^{b}	5.575^{b}	5.941^{b}	6.284^{b}	6.630^{b}	6.948^{b}
		CO	4.930	5.02^{8c}	5.135^{c}	5.476^{c}	5.682^{c}	6.078^{c}	6.344^{c}
		SE	-	0.077	0.034	0.103	0.052	0.077	0.085
		P	-	0.000	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	-	1.000	0.000	0.209	0.012	0.785	0.994
Psychrotrophic microorganisms	Organic acid treatment	C	5.010	6.006^{a}	6.530^{a}	6.902^{a}	7.177^{a}	7.560^{a}	7.961^{a}
		NaL$_1$	5.010	5.500^{b}	5.971^{bc}	6.214^{b}	6.686^{b}	6.985^{b}	7.447^{b}
		NaL$_2$	5.010	5.339^{b}	5.761^{c}	6.072^{b}	6.418^{bc}	6.679^{c}	7.195^{bc}
		Sol.A	5.010	5.392^{b}	6.020^{b}	6.168^{b}	6.175^{c}	6.411^{d}	6.787^{d}
		Sol.B	5.010	5.512^{b}	6.176^{b}	6.285^{b}	6.258^{c}	6.529^{cd}	6.966^{cd}
		SE	-	0.077	0.083	0.100	0.110	0.067	0.097
		P	-	0.000	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	5.010	5.647^{a}	6.578^{a}	6.779^{a}	6.903^{a}	7.146^{a}	7.828^{a}
		HiOx	5.010	5.700^{a}	6.040^{b}	6.364^{b}	6.721^{a}	7.064^{a}	7.297^{b}
		CO	5.010	5.303^{b}	5.657^{c}	5.841^{c}	6.005^{b}	6.288^{b}	6.688^{c}
		SE	-	0.059	0.065	0.077	0.085	0.052	0.075
		P	-	0.000	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	-	0.067	0.439	0.164	0.821	0.284	0.983
Pseudomonas spp.	Organic acid treatment	C	4.620	5.169	5.619	5.968^{a}	6.186^{a}	6.094^{a}	6.046^{a}
		NaL$_1$	4.620	5.004	5.427	5.650^{b}	5.821^{b}	5.828^{ab}	5.640^{ab}
		NaL$_2$	4.620	4.874	5.174	5.223^{c}	5.355^{d}	5.455^{b}	5.307^{b}
		Sol.A	4.620	4.949	5.434	5.462^{b}	5.542^{c}	5.494^{b}	5.368^{b}
		Sol.B	4.620	4.906	5.457	5.538^{b}	5.656^{c}	5.690^{ab}	5.484^{b}
		SE	-	0.133	0.113	0.080	0.047	0.133	0.147
		P	-	0.561	0.124	0.000	0.000	0.012	0.010
	Packaging condition	Air	4.620	5.094	5.857^{a}	5.856^{a}	5.909^{a}	5.849	5.647
		HiOx	4.620	4.976	5.410^{b}	5.574^{b}	5.772^{b}	5.713	5.411
		CO	4.620	4.871	4.999^{c}	5.275^{c}	5.455^{c}	5.574	5.650
		SE	-	0.103	0.088	0.062	0.036	0.103	0.114
		P	-	0.324	0.000	0.000	0.000	0.186	0.248
	Treatment × Packaging	*P*	-	1.000	0.992	0.839	0.924	1.000	0.992

		C	2.340	2.796^{bc}	4.405^{a}	5.898^{a}	6.659^{a}	7.019^{a}	7.341^{a}
		NaL_1	2.340	3.013^{b}	3.557^{b}	4.284^{b}	6.326^{b}	6.639^{b}	7.113^{ab}
		NaL2	2.340	2.705^{c}	3.400^{b}	3.947^{c}	5.680^{c}	6.375^{c}	6.782^{b}
	Organic acid treatment	Sol.A	2.340	3.286^{a}	3.618^{b}	3.827^{c}	5.178^{d}	5.450^{d}	5.727^{c}
		Sol.B	2.340	3.040^{ab}	3.407^{b}	3.831^{c}	4.988^{d}	5.169^{e}	5.291^{d}
		SE	-	0.087	0.127	0.073	0.100	0.057	0.137
LAB		*P*	-	0.000	0.000	0.000	0.000	0.000	0.000
		Air	2.340	3.407^{a}	4.431^{a}	4.921^{a}	5.893^{a}	6.296^{a}	6.517^{a}
		HiOx	2.340	2.839^{b}	3.414^{b}	4.233^{b}	6.092^{a}	6.341^{a}	6.642^{a}
	Packaging condition	CO	2.340	2.658^{b}	3.187^{b}	3.918^{c}	5.313^{b}	5.755^{b}	6.193^{b}
		SE	-	0.067	0.098	0.057	0.077	0.044	0.106
		P	-	0.000	0.000	0.000	0.000	0.000	0.016
	Treatment × Packaging	*P*	-	0.006	0.043	0.000	0.763	0.001	0.290
		C	3.420	3.899	4.245^{a}	4.393^{a}	4.196^{a}	3.996^{a}	3.676^{a}
		NaL_1	3.420	3.758	3.957^{ab}	4.050^{b}	4.018^{ab}	3.740^{ab}	3.526^{ab}
		NaL_2	3.420	3.586	3.680^{b}	3.830^{c}	3.681^{c}	3.522^{b}	3.358^{b}
	Organic acid treatment	Sol.A	3.420	3.653	3.665^{b}	3.832^{c}	3.866^{bc}	3.717^{ab}	3.539^{ab}
		Sol.B	3.420	3.596	3.555^{b}	3.646^{d}	3.694^{c}	3.540^{b}	3.401^{b}
		SE	-	0.107	0.130	0.040	0.083	0.097	0.060
Enterobactericeae		*P*	-	0.223	0.005	0.000	0.000	0.011	0.007
		Air	3.420	3.998^{a}	4.134^{a}	4.156^{a}	4.018^{a}	3.800^{a}	3.574^{a}
		HiOx	3.420	3.635^{b}	3.773^{b}	4.006^{b}	3.944^{a}	3.768^{a}	3.532^{a}
	Packaging condition	CO	3.420	3.462^{b}	3.555^{b}	3.689^{c}	3.711^{b}	3.542^{b}	3.394^{b}
		SE	-	0.083	0.101	0.031	0.065	0.075	0.046
		P	-	0.000	0.001	0.000	0.006	0.042	0.027
	Treatment × Packaging	*P*	-	0.982	0.998	0.059	0.528	0.996	0.928
		C	3.632	4.121	4.352	4.718^{a}	4.866^{a}	5.140^{a}	5.236^{a}
		NaL_1	3.632	3.979	4.160	4.456^{ab}	4.579^{b}	4.798^{ab}	4.948^{ab}
		NaL_2	3.632	3.944	4.036	4.292^{bc}	4.327^{c}	4.450^{bc}	4.632^{bc}
	Organic acid treatment	Sol.A	3.632	3.838	3.909	3.912^{d}	3.961^{d}	4.038^{c}	4.069^{d}
		Sol.B	3.632	3.888	3.973	4.127^{cd}	4.181^{c}	4.268^{c}	4.254^{cd}
		SE		0.117	0.167	0.100	0.070	0.150	0.133
Yeast and mould		*P*	-	0.504	0.372	0.000	0.000	0.000	0.000
		Air	3.632	4.352^{a}	4.540^{a}	4.610^{a}	4.489^{a}	4.498^{ab}	4.466^{b}
		HiOx	3.632	3.785^{b}	3.913^{b}	4.342^{b}	4.505^{a}	4.742^{a}	4.860^{a}
	Packaging condition	CO	3.632	3.725^{b}	3.805^{b}	3.951^{c}	4.155^{b}	4.376^{b}	4.559^{b}
		SE	-	0.090	0.129	0.077	0.054	0.116	0.103
		P	-	0.000	0.001	0.000	0.000	0.093	0.029
	Treatment × Packaging	*P*	-	1.000	1.000	0.759	0.379	0.785	0.105

C: control; NaL_1: 1% NaL; NaL_2: 2% NaL; Sol.A: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium acetate; Sol.B: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium lactate; HiOx: 80:20:0/O_2:CO_2:N_2; and CO: 0.4:30:69.60/CO:CO_2:N_2. Means within the same row with different lowercase superscripts are significantly different ($p < 0.05$).

Quilo *et al*. (2010) reported that the addition of 3% potassium lactate to ground beef reduced the coliform and aerobic bacterial counts. Similarly, in the present work, there was a significant difference in all bacterial counts between the untreated and the organic acid treated meatballs; approximately 1 - 2 log unit difference was recorded between the untreated (C) and other samples. Mir and Masoodi (2018) indicated that the total bacterial count of meatballs were significantly higher in untreated samples (6.82 CFU/g) compared to 1% (4.56 CFU/g), 2% (3.08 CFU/g), and 3% (2.35 CFU/g) sodium acetate treated meatballs, respectively. Djordjević *et al*. (2018) found significant differences in total aerobic and lactic acid bacterial counts, between vacuum and MA packaged ground meat samples, and stated that the lowest counts were observed in the MA packages containing 50% CO_2. Nissen *et al*. (2000) also indicated that the shelf-life of ground beef was prolonged when stored in low CO (0.4%)/high CO_2 mixture, compared to high O_2 MAP, similar to the results of the present work. Gammariello *et al*. (2015) determined that the shelf-life of ready-to-eat skewer can be extended by approximately 83% when meat pieces were dipped in 40% sodium lactate solution, and packaged under MA with 50%O_2/30%CO_2/20%N_2 gas combinations compared to the untreated air-packaged samples.

Changes in physico-chemical properties of non-inoculated meatballs

Meatballs with initial pH value of 5.85 showed an increase during storage period (Table 2). The pH value was over 6.4 in untreated meatballs after day 3, while in organic acid treated groups, the pH value was over this value after day 9 in 1% sodium lactate added meatballs, after day 12 in NaL_2 added meatballs, and on day 15 in Sol.A and Sol.B, respectively. The increase in organic acid concentration enabled the pH values to remain at lower levels. Packaging conditions also affected the pH values of meatballs by resulting in lower values of CO-MAP during storage ($p < 0.001$). Suman *et al*. (2010) determined that the pH of lactate-treated ground beef patties was 5.79, while that of untreated patties was 5.69, which was in agreement with the present work. Similarly, Djordjević *et al*. (2018) stated that the pH values in all minced meat samples increased during storage periods, starting from the pH of 5.7, and ending to 5.9, both in vacuum and MAP. A significant difference ($p < 0.05$) in pH was observed between the air- and MA-packaged samples starting with a higher initial pH value (5.85) and ending between 6.43 - 6.49 on day 15. Tenderis *et al*. (2020) also demonstrated that the pH values in cooked ground beef ranged between 5.49 - 6.09 and 5.52 - 6.27 at the beginning of storage at 4 and 10°C, respectively, and any significant effect was observed with sodium lactate addition on pH during storage, which was in line with the present work. Besides, Byrne *et al*. (2002) indicated that sodium lactate reduced the pH in beef burger. However, Sallam and Samejima (2004) highlighted the fact that sodium lactate additions to ground beef maintained pH changes at constant level.

The a_w value of the meatballs started to decrease after day 6, which could have been related to the addition of organic acids ($p < 0.05$; Table 2). Meatballs to which Sol.B and NaL_2 were added were the most changed samples at the end of storage, and the packaging conditions were significant after day 9 of cold storage ($p < 0.01$). Tenderis *et al*. (2020) emphasised that the a_w values in cooked ground beef ranged between 0.92 - 0.96 on day 1 of storage at 4 and 10°C, respectively, and highlighted that the samples containing sodium lactate had lowest a_w values as pointed out by María *et al*. (2015) who indicated that sodium lactate had a reducing effect on a_w of the products.

The moisture content of the meatballs decreased during the storage period, and the loss in moisture content observed in meatballs was greater in organic acid-added samples with the values of 49.23, 49.11, 49.42, and 49.33%, respectively, at the end of storage (Table 2). The CO and HiOx packaging differed significantly from the air packaging on the first six days of storage period, and then no significant change was observed until the end of the storage period. The decrease in the moisture content of meatballs during the storage period could have been resulted from the water infiltration observed in meatballs by the prolongation of the storage period, and the effect of organic acids which could bind intracellular water.

Although the TBARS values of the meatballs showed a similar increase until day 3, the values in organic acid-treated groups were found to be lower in the following days (Table 2). As from day 9, values over 1 mg MDA/kg were obtained, indicating spoilage in the meat products in the untreated meatballs. This change has also been found to be in line with the changes in pH and total bacterial counts of the untreated samples. During the storage period,

Table 2. Mean values, standard errors, and significant interactions of physico-chemical parameters of meatballs stored at 1°C for 15 days.

Characteristic	Application	Group	Day 0	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
		C	5.850	6.073[a]	6.360[a]	6.427[a]	6.477[a]	6.623[a]	6.757[a]
		NaL_1	5.850	5.810[b]	6.140[b]	6.343[b]	6.417[b]	6.480[b]	6.590[b]
		NaL_2	5.850	5.750[c]	5.907[c]	6.017[c]	6.163[c]	6.397[c]	6.457[c]
	Organic acid treatment	Sol.A	5.850	5.710[d]	5.753[e]	5.837[e]	6.097[d]	6.200[e]	6.320[e]
		Sol.B	5.850	5.720[d]	5.810[d]	5.937[d]	6.147[c]	6.327[d]	6.420[d]
		SE	-	0.007	0.010	0.003	0.007	0.017	0.003
pH		*P*	NS	0.000	0.000	0.000	0.000	0.000	0.000
		Air	5.850	5.824[a]	6.042[a]	6.180[a]	6.304[a]	6.462[a]	6.602[a]
		HiOx	5.850	5.812[ab]	5.976[b]	6.104[b]	6.256[b]	6.402[b]	6.490[b]
	Packaging condition	CO	5.850	5.802[b]	5.964[b]	6.052[c]	6.220[c]	6.352[c]	6.434[c]
		SE	-	0.005	0.008	0.003	0.005	0.013	0.003
		P	NS	0.019	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	NS	1.000	0.998	0.000	0.819	0.995	0.000
		C	0.970	0.967	0.965	0.963[a]	0.959[a]	0.959[a]	0.959[a]
		NaL_1	0.970	0.966	0.963	0.961[ab]	0.957[ab]	0.955[b]	0.951[b]
		NaL_2	0.970	0.963	0.962	0.960[b]	0.954[b]	0.953[c]	0.948[c]
	Organic acid treatment	Sol.A	0.970	0.964	0.963	0.961[ab]	0.957[ab]	0.954[b]	0.949[c]
		Sol.B	0.970	0.966	0.965	0.960[b]	0.954[b]	0.952[d]	0.946[d]
		SE	-	0.001	0.001	0.001	0.001	0.001	0.001
a_w		*P*	NS	0.076	0.417	0.049	0.009	0.000	0.000
		Air	0.970	0.965	0.964	0.961	0.958[a]	0.957[a]	0.952[a]
		HiOx	0.970	0.965	0.965	0.962	0.956[b]	0.954[b]	0.951[a]
	Packaging condition	CO	0.970	0.964	0.964	0.960	0.954[b]	0.953[c]	0.949[b]
		SE	-	0.001	0.001	0.001	0.001	0.001	0.001
		P	NS	0.510	0.734	0.057	0.003	0.000	0.005
	Treatment × Packaging	*P*	NS	1.000	1.000	0.944	0.850	0.097	0.539
		C	54.000	53.780[a]	53.530[a]	52.447[d]	52.113[bc]	51.690[a]	49.677[a]
		NaL_1	54.000	53.573[bc]	53.063[c]	52.893[b]	52.243[b]	51.237[bc]	49.227[bc]
		NaL_2	54.000	53.483[c]	53.020[c]	52.707[c]	52.033[c]	51.153[c]	49.107[c]
	Organic acid treatment	Sol.A	54.000	53.660[b]	53.513[a]	53.140[a]	52.500[a]	51.360[b]	49.417[b]
		Sol.B	54.000	53.600[b]	53.250[b]	52.977[b]	52.407[a]	51.340[b]	49.330[b]
		SE	-	0.033	0.040	0.300	0.050	0.043	0.070
Moisture content (%)		*P*	NS	0.000	0.000	0.000	0.000	0.000	0.000
		Air	54.000	53.770[a]	53.344[a]	52.842[ab]	52.320	51.336	49.354
		HiOx	54.000	53.570[b]	53.234[b]	52.864[a]	52.248	51.406	49.374
	Packaging condition	CO	54.000	53.518[b]	53.248[b]	52.792[b]	52.210	51.326	49.326
		SE	-	0.026	0.031	0.023	0.039	0.034	0.054
		P	NS	0.000	0.036	0.047	0.142	0.202	0.822
	Treatment × Packaging	*P*	NS	1.000	0.998	0.900	0.623	0.995	1.000

TBARS (mg MDA/kg)	Organic acid treatment	C	0.294	0.497^{a}	0.720^{a}	0.888^{a}	1.112^{a}	1.247^{a}	1.385^{a}
		NaL$_1$	0.294	0.465^{ab}	0.689^{b}	0.754^{b}	0.795^{b}	0.970^{b}	1.144^{b}
		NaL$_2$	0.294	0.453^{ab}	0.647^{c}	0.682^{c}	0.712^{c}	0.814^{c}	1.026^{c}
		Sol.A	0.294	0.436^{b}	0.682^{b}	0.610^{d}	0.661^{c}	0.743^{d}	0.922^{e}
		Sol.B	0.294	0.375^{c}	0.625^{c}	0.672^{c}	0.684^{c}	0.775^{cd}	0.958^{d}
		SE	-	0.017	0.011	0.005	0.020	0.015	0.010
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	0.294	0.430^{b}	0.620^{b}	0.750^{b}	0.826^{b}	0.890^{b}	1.001^{b}
		HiOx	0.294	0.491^{a}	0.797^{a}	0.819^{a}	0.887^{a}	1.087^{a}	1.348^{a}
		CO	0.294	0.415^{b}	0.601^{b}	0.594^{c}	0.665^{c}	0.752^{c}	0.911^{c}
		SE	-	0.013	0.008	0.004	0.015	0.012	0.008
		P	NS	0.001	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	NS	1.000	1.000	1.000	1.000	1.000	0.031
MetMb (%)	Organic acid treatment	C	65	55.969^{e}	56.325^{e}	57.393^{e}	58.579^{e}	58.020^{e}	52.773^{e}
		NaL$_1$	65	66.096^{a}	66.355^{b}	63.776^{c}	61.746^{c}	60.843^{c}	56.215^{c}
		NaL$_2$	65	65.619^{b}	64.120^{d}	61.129^{d}	60.276^{d}	58.932^{d}	55.415^{d}
		Sol.A	65	64.942^{c}	67.080^{a}	68.456^{a}	69.484^{a}	70.908^{a}	71.805^{a}
		Sol.B	65	64.111^{d}	65.893^{c}	66.811^{b}	66.692^{b}	68.091^{b}	70.283^{b}
		SE	-	0.067	0.083	0.067	0.040	0.050	0.033
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	65	67.825^{a}	66.200^{a}	66.172^{a}	65.620^{a}	65.385^{a}	64.628^{a}
		HiOx	65	61.851^{b}	63.331^{b}	63.181^{b}	63.320^{b}	63.613^{b}	60.975^{b}
		CO	65	60.366^{c}	62.333^{c}	61.186^{c}	61.126^{c}	61.079^{c}	58.292^{c}
		SE		0.052	0.065	0.052	0.031	0.039	0.026
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	NS	0.012	0.000	0.000	0.000	0.000	0.000
Colour (*L**)	Organic acid treatment	C	44.580	45.157^{d}	45.583^{d}	45.920^{e}	45.747^{d}	45.270^{d}	45.020^{d}
		NaL$_1$	44.580	45.843^{c}	46.023^{c}	46.520^{d}	46.603^{c}	46.327^{c}	46.213^{c}
		NaL$_2$	44.580	45.883^{c}	46.107^{c}	46.827^{c}	46.890^{b}	46.553^{b}	46.327^{bc}
		Sol.A	44.580	46.050^{b}	46.413^{b}	47.253^{b}	46.980^{b}	46.713^{b}	46.437^{b}
		Sol.B	44.580	46.280^{a}	46.513^{a}	47.540^{a}	47.233^{a}	47.207^{a}	47.090^{a}
		SE		0.033	0.033	0.040	0.067	0.060	0.067
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	44.580	45.394^{c}	45.660^{c}	45.988^{c}	45.938^{c}	46.026^{b}	46.414^{a}
		HiOx	44.580	46.142^{a}	46.516^{a}	47.526^{a}	47.910^{a}	47.148^{a}	46.462^{a}
		CO	44.580	45.992^{b}	46.208^{b}	46.922^{b}	46.224^{b}	46.068^{b}	45.776^{b}
		SE		0.026	0.026	0.031	0.052	0.046	0.052
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	NS	0.000	0.000	0.000	0.048	0.001	0.000
Colour (*a**)	Organic acid treatment	C	7.500	7.777	8.027	8.540^{a}	8.893^{a}	8.737^{a}	8.577^{a}
		NaL$_1$	7.500	7.870	7.833	8.137^{b}	8.620^{b}	8.477^{b}	8.067^{b}
		NaL$_2$	7.500	7.737	7.743	7.973^{bc}	8.240^{c}	8.220^{c}	7.757^{c}
		Sol.A	7.500	7.693	7.517	7.597^{d}	7.817^{d}	7.557^{d}	7.160^{d}

		Sol.B	7.500	7.753	7.633	7.690^{cd}	7.933^{d}	7.667^{d}	7.330^{d}
		SE	-	0.100	0.143	0.110	0.077	0.043	0.100
		P	NS	0.785	0.146	0.000	0.000	0.000	0.000
	Packaging condition	Air	7.500	7.372^{c}	6.902^{b}	6.752^{c}	6.782^{c}	6.538^{c}	5.838^{c}
		HiOx	7.500	7.822^{b}	8.044^{a}	8.476^{b}	8.712^{b}	7.940^{b}	7.270^{b}
		CO	7.500	8.104^{a}	8.306^{a}	8.734^{a}	9.408^{a}	9.916^{a}	10.226^{a}
		SE	-	0.077	0.111	0.085	0.059	0.034	0.077
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	NS	1.000	0.935	0.433	0.000	0.000	0.000
Colour (*b**)	Organic acid treatment	C	16.050	16.617^{a}	16.283^{a}	16.020^{a}	15.637^{a}	15.013^{a}	14.640^{a}
		NaL_1	16.050	15.863^{c}	15.887^{b}	15.590^{bc}	15.013^{c}	14.703^{b}	14.223^{bc}
		NaL_2	16.050	16.110^{b}	15.987^{b}	15.723^{ab}	15.303^{b}	14.813^{b}	14.377^{ab}
		Sol.A	16.050	15.650^{d}	15.480^{c}	15.200^{cd}	14.530^{e}	14.227^{d}	13.953^{c}
		Sol.B	16.050	15.767^{cd}	15.603^{c}	15.393^{d}	14.817^{d}	14.490^{c}	14.053^{c}
		SE	-	0.067	0.077	0.107	0.067	0.040	0.093
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	16.050	16.536^{b}	16.658^{b}	16.440^{b}	15.794^{b}	15.326^{b}	14.854^{b}
		HiOx	16.050	17.484^{a}	17.064^{a}	16.710^{a}	15.996^{a}	15.658^{a}	15.130^{a}
		CO	16.050	13.984^{c}	13.822^{c}	13.606^{c}	13.390^{c}	12.964^{c}	12.764^{c}
		SE	-	0.052	0.059	0.083	0.052	0.031	0.072
		P	NS	0.000	0.000	0.000	0.000	0.000	0.000
	Treatment × Packaging	*P*	NS	0.958	0.196	0.994	0.001	0.002	0.963

C: control; NaL_1: 1% NaL; NaL_2: 2% NaL; Sol.A: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium acetate; Sol.B: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium lactate; HiOx: 80:20:0/O_2:CO_2:N_2; and CO: 0.4:30:69.60/CO:CO_2:N_2. Means within the same row with different lowercase superscripts are significantly different ($p < 0.05$).

the lowest TBA values were found to be Sol.A < Sol.B < NaL_2, respectively. None of the samples in organic acid added groups exceeded the 1 mg MDA/kg value during storage, except in NaL_1 and NaL_2 on the last day of storage.

Packaging conditions also significantly affected the TBARS values of the meatballs ($p < 0.001$). Meatballs in HiOx-MAP were the most oxidised samples compared to the ones packaged with CO and air. As from days 12 and 15, results above 1 mg MDA/kg were observed in the HiOx and air packaged meatballs, respectively, while meatballs in CO-MAP did not exceed the 1 mg MDA/kg during storage period. Similar to this finding, John *et al*. (2004) reported that the rancidity observed in ground beef packaged in HighO_2-MAP were prevented by way of packaging in 0.4% CO.

The use of organic acids as a preservative is one of the preferred alternatives to minimise spoilage. Mir and Masoodi (2018) emphasised that the quality of meatballs can be maintained with the addition of sodium acetate (SA) during storage period. The TBARS value of treated meatballs remained significantly lower than that of untreated ones, and the addition of 3% sodium acetate resulted in the lowest value (0.41 mg MDA/kg) compared to 2% SA (0.65 mg MDA/kg), 1% SA (0.87 mg MDA/kg), and untreated samples (1.45 mg MDA/kg). Likewise, Mancini *et al*. (2010) stated that lactate treatment reduced the formation of lipid oxidation in cooked ground beef patties. Nonetheless, high-O_2 and PVC-packaged patties had more lipid oxidation than patties in CO and vacuum. Quilo *et al*. (2009) indicated that ground beef patties treated with 3% potassium lactate had lower oxidation values during 7-d storage period. In the present work, the lipid oxidation values of the meatballs showed a similar increase until day 3. However, starting on this day, the values were found to be lower in organic acid treated groups. In the untreated meatballs, values above 1 mg MDA/kg were obtained as from day 9, which was a sign of deterioration.

Changes in colour properties of non-inoculated meatballs

An increase was observed in the lightness (L*) values of meatballs with the addition of organic acid, and this effect enhanced in parallel with the increasing storage period (Table 2). The highest increase in L* value was detected in Sol.B treated meatballs, followed by the Sol.A, NaL_2, and NaL_1, respectively ($p < 0.001$). Packaging conditions also affected the lightness of the meatballs; HiOx and CO-MAP samples resulted in higher values than those of air-packaged ones until day 12, while CO-MAP samples were found to have significantly lower L* values than the air- and HiOx-packaged meatballs on day 15.

The redness (a*) values of the meatballs packaged with CO increased continuously during the storage period, reaching approximately two times higher values at the end of the storage period than the air-packaged meatballs. Air-packaged samples showed a decrease over longer storage periods, thus differing significantly from the MAP meatballs ($p < 0.001$). Meanwhile, the a* values of meatballs subjected to HiOx-MAP increased until day 9, and then started to decrease during the last day of cold storage. The redness of meatballs treated with NaL_1 and NaL_2 reached values close to untreated samples until day 6 of storage; even though they seemed to somehow remain constant, they showed a decrease during longer storage periods (Table 2). Meatballs treated with organic acid combinations remained at a lower redness value than other groups during storage period, and differed significantly from the untreated and NaL added meatballs since day 6 ($p < 0.001$).

The yellowness (b*) values of the meatballs decreased during the storage period, and this decrease was greater in meatballs treated with the A and B solutions (Table 2). Meatballs treated with NaL_1 and NaL_2 had closer values to the untreated samples during the storage period, whereas a slight decrease was recorded with NaL addition. Meatballs packaged with CO resulted in lower yellowness values than the HiOx- and air-packaged samples during the storage period. A remarkably higher value of yellowness was seen in HiOx-MAP ($p < 0.001$) contrary to redness value which could be explained with a fading in colour over time because of the high amount of oxygen presence in the package.

Tan and Shelef (2002) investigated the changes in colour in fresh porcine minced meat treated with sodium chloride (1 or 2%), sodium or potassium lactate (2%), or sodium chloride and lactate combinations at 2°C for 15 days, and observed an enhancement in red colour immediately after the addition of sodium chloride and lactates. Similarly, Quilo *et al*. (2010) reported improvement in the colour characteristics of the beef minced meats treated with 3% potassium lactate. In the present work, there was also an increase in the L* values of the meatballs with the addition of organic acids, and it was concluded that this effect showed parallelism with the increasing storage periods. The a* and b* values of the meatballs decreased over time, and this decrease was more prominent in the meatballs treated with solutions A and B.

This positive effect of lactates on product colour can be associated with their minimising effect on lipid oxidation, which may influence the colour stability of beef meat by retarding the myoglobin and lipid oxidation (Mancini *et al*., 2010). Mancini *et al*. (2010) indicated that an improvement in colour stability was obtained with a 2.5% lactate addition to ground beef patties packaged under air, vacuum, and high-oxygen MAP, while no effect was recorded for the redness values of patties in 0.4% CO. On the other hand, cooked patties stored in 0.4% CO and vacuum packages were redder than HiOx and air packaged ones. Quilo *et al*. (2009) observed brighter and darker red colour in beef minced meats treated with 3% potassium lactate. Jayasingh *et al*. (2001) determined that the packaged ground beef containing 0.5% CO would maintain the colour stability for several weeks. The CO concentration in the atmosphere, the exposure time to CO, and the structure of the meat are the main factors that affect the penetration of CO and the depth of the COMb formation in the meat. Likewise, John *et al*. (2004) stated that $HighO_2$-MAP was sufficient to maintain the desirable bright red colour of raw ground beef until day 10, began to darken on day 14, and lost the redness on day 21. However, raw ground beef held in 0.4% CO remained bright red colour during 21 days of storage. Moreover, Jeong and Claus (2011) emphasised that after opening the CO-MAP packaged ground beef, the red colour decreased more slowly than that of the vacuum packaged ground beef. When a CO-packaged product is opened, this discoloration would provide consumers with a visual indication of freshness. Suman *et al*. (2010) expressed that lactate-treated ground beef patties were darker (lower L* values) than untreated ones. The surface redness was greater for lactate patties when stored in PVC, HiOx, and vacuum packages, while the effects of lactate on redness property of ground beef were not evident when packaged in CO. This could have occurred due to the colour-stabilising effect of CO that could mask lactate's effect on surface redness.

While the metmyoglobin changes were lower in untreated and lactate added meatballs, the changes in Sol.A and Sol.B treated samples were observed to be higher (Table 2). This difference was related to the redness and yellowness changes in the meatballs due to the reactions between the ingredients and components in the meatballs and package atmosphere. On the other hand, a higher difference in metmyoglobin contents was recorded in air packaging, while CO and HiOx-MAP somewhat retarded the changes due to undesirable oxygen effect on the products. CO-MAP meatballs resulted in significantly less difference ($p < 0.001$) during the storage period, and protected the desirable visual appearance of the meatballs. Luño *et al*. (2000) stated that the presence of CO and 50% CO_2 extended the shelf-life by inhibiting spoilage bacterial growth, delaying MetMb formation, maintaining the red colour and odour of fresh meat, and slowing down the oxidative reactions. CO concentrations of 0.5 - 0.75% were able to extend the shelf-life of packaged fresh meat by five to ten days at 1°C.

Kim *et al*. (2006) and Mancini *et al*. (2009) asserted that the role of components in meat colour stability is related to increased metmyoglobin-reducing activity as a result of a lactate-dependent rise in lactate dehydrogenase activity. Addition to this, Mancini and Ramanathan (2008) indicated that lactate supports myoglobin redox stability *in vitro*. From this point forth, more myoglobin denaturation was observed in cooked ground beef patties packaged in PVC and high-O_2 compared to vacuum and 0.4% CO, and lactate injection significantly reduced the myoglobin denaturation in high-O_2 packaged patties (Mancini *et al*., 2010).

Changes in textural properties of non-inoculated meatballs

Changes in the texture profile of meatballs during the storage period are given in Table 3. As the storage time progressed, an increase was observed in the hardness, and accordingly the gumminess and chewiness values of untreated meatballs. On the other hand, in meatballs treated with organic acids, a tender product was obtained due to the increase in the water content of the dough and the technological properties of the additives, which provided better chewiness.

The hardness and gumminess of meatballs treated with Sol.A and Sol.B were found to be more tender than untreated samples, followed by the increasing concentration of sodium lactate which made the meatballs juicier. Addition to these, the springiness of meatballs which indicated the chewiness of the product, enhanced significantly by the treatment with combined organic acids ($p < 0.001$). The tenderness of these samples caused them to exhibit a more homogeneous stickiness depending on time.

Packaging conditions also affected the hardness, gumminess, and chewiness properties of the meatball samples. Packaging with a CO and HiOx-modified atmosphere performed better than air packaging, and resulted in more palatable products over a longer storage period.

Walsh *et al*. (2010) stated that the injection of bovine meat with sodium or potassium lactate solutions significantly decreased the WBSF values, and increased sensory tenderness scores, compared to non-injected ones. Likewise, Hoffman *et al*. (2008) indicated that lactates improved the textural properties of meat cuts by obtaining a juicier and tender meat when sodium and potassium lactates were applied to four different beef meat muscles (*biceps femoris, longissimus lumborum, rectus femoris,* and *semitendinosus muscles*).

Sensorial evaluation of non-inoculated meatballs

The colour, odour, texture, taste, and overall acceptability of the meatballs showed similarities with the instrumental analysis of colour and textural characteristics of the samples, and the meatballs treated with organic acids were preferred to untreated meatballs during the storage period (Figure 2).

The tenderness of organic acid-added samples was found to be more tender than untreated meatballs. On the other hand, the juiciness perception was lower in meatballs to which sodium lactate, Sol.A, and Sol.B had been added. This is thought to be due to the fact that organic acids bind water to a certain extent. Likewise, in the organic acid-added samples, the chewiness of the meatballs was easier to swallow, especially in the lactate treated meatballs.

The addition of organic acid caused some paleness in the visual colour of meatballs during storage. While the red colour of the samples was more

Table 3. Mean values, standard errors, and significant interactions of texture profile analyses of meatballs stored at 1°C for 15 days (hardness, cohesiveness, springiness, gumminess, and chewiness).

Characteristic	Application	Group	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
Hardness (N)	Organic acid treatment	C	2.461[a]	3.461[a]	3.517[a]	4.310[a]	4.619[a]	4.473[a]
		NaL_1	2.440[a]	3.451[a]	3.359[b]	3.626[b]	3.502[b]	3.744[b]
		NaL_2	2.376[b]	3.285[b]	3.217[c]	3.442[c]	3.326[c]	3.708[b]
		Sol.A	2.232[c]	3.115[c]	3.037[d]	3.433[cd]	3.280[d]	2.991[c]
		Sol.B	2.180[d]	2.988[d]	2.914[e]	3.386[d]	3.075[e]	2.831[d]
		SE	0.017	0.015	0.012	0.018	0.008	0.017
		P	0.000	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	2.341	3.270	3.216	3.673[a]	3.600[a]	3.649[a]
		HiOx	2.337	3.263	3.208	3.641[ab]	3.574[b]	3.564[b]
		CO	2.335	3.247	3.203	3.604[b]	3.507[c]	3.434[c]
		SE	0.013	0.012	0.009	0.014	0.006	0.013
		P	0.955	0.355	0.598	0.007	0.000	0.000
	Treatment × Packaging	*P*	1.000	0.993	0.986	0.193	0.000	0.015
Cohesiveness	Organic acid treatment	C	0.434	0.434	0.426	0.420	0.427	0.429
		NaL_1	0.443	0.427	0.424	0.413	0.414	0.412
		NaL_2	0.451	0.431	0.425	0.414	0.414	0.411
		Sol.A	0.431	0.423	0.424	0.421	0.415	0.410
		Sol.B	0.447	0.434	0.425	0.415	0.420	0.411
		SE	0.017	0.015	0.012	0.018	0.008	0.017
		P	0.903	0.983	1.000	0.997	0.768	0.925
	Packaging condition	Air	0.442	0.432	0.426	0.418	0.420	0.417
		HiOx	0.441	0.430	0.425	0.416	0.417	0.414
		CO	0.440	0.428	0.424	0.415	0.416	0.413
		SE	0.013	0.012	0.009	0.014	0.006	0.013
		P	0.991	0.979	0.988	0.989	0.918	0.973
	Treatment × Packaging	*P*	1.000	1.000	1.000	1.000	1.000	1.000
Springiness (mm)	Organic acid treatment	C	-2.834[c]	-2.518[b]	-2.675[c]	-2.341[a]	-2.181[a]	-2.022[a]
		NaL_1	-2.170[a]	-2.175[a]	-2.170[a]	-2.531[b]	-2.187[a]	-2.183[b]
		NaL_2	-2.341[b]	-2.174[a]	-2.338[b]	-2.675[c]	-2.249[b]	-2.033[a]
		Sol.A	-3.069[d]	-2.170[a]	-2.687[c]	-2.546[b]	-2.193[a]	-2.368[c]
		Sol.B	-3.071[d]	-2.671[c]	-3.061[d]	-2.682[c]	-2.382[c]	-2.677[d]
		SE	0.017	0.015	0.012	0.018	0.008	0.017
		P	0.000	0.000	0.000	0.000	0.000	0.000
	Packaging condition	Air	-2.667[a]	-2.333	-2.566[a]	-2.533	-2.213[a]	-2.233
		HiOx	-2.709[b]	-2.344	-2.594[b]	-2.563	-2.248[b]	-2.272
		CO	-2.716[b]	-2.349	-2.598[b]	-2.569	-2.254[b]	-2.265
		SE	0.013	0.012	0.009	0.014	0.006	0.013
		P	0.024	0.645	0.038	0.173	0.000	0.097
	Treatment × Packaging	*P*	0.344	1.000	0.404	0.990	0.861	0.896

Gumminess (N)	Organic acid treatment	C	1.070	1.503^{a}	1.499^{a}	1.811^{a}	1.971^{a}	1.919^{a}
		NaL_1	1.082	1.476^{ab}	1.425^{ab}	1.498^{b}	1.449^{b}	1.546^{b}
		NaL_2	1.072	1.418^{abc}	1.368^{bc}	1.428^{b}	1.377b^{c}	1.526^{b}
		Sol.A	0.963	1.320^{bc}	1.288^{c}	1.449^{b}	1.361b^{c}	1.229^{c}
		Sol.B	0.976	1.298^{c}	1.239^{c}	1.407^{b}	1.293^{c}	1.166^{c}
		SE	0.046	0.055	0.042	0.075	0.033	0.067
		P	0.205	0.048	0.001	0.003	0.000	0.000
	Packaging condition	Air	1.037	1.413	1.370	1.539	1.515	1.527
		HiOx	1.031	1.404	1.364	1.519	1.493	1.482
		CO	1.029	1.392	1.358	1.499	1.463	1.422
		SE	0.036	0.043	0.033	0.058	0.026	0.052
		P	0.987	0.941	0.968	0.889	0.377	0.369
	Treatment × Packaging	*P*	1.000	1.000	1.000	1.000	0.996	1.000
Chewiness (Nmm)	Organic acid treatment	C	-3.027^{b}	-3.780^{d}	-4.006^{c}	-4.230^{b}	-4.298^{b}	-3.872^{c}
		NaL_1	-2.344^{a}	-3.206^{bc}	-3.090^{a}	-3.783^{ab}	-3.167^{a}	-3.367^{b}
		NaL_2	-2.506^{a}	-3.079^{ab}	-3.196^{ab}	-3.813^{ab}	-3.096^{a}	-3.094^{ab}
		Sol.A	-2.950^{b}	-2.860^{a}	-3.459^{b}	-3.680^{a}	-2.982^{a}	-2.904^{a}
		Sol.B	-2.991^{b}	-3.463^{c}	-3.791^{c}	-3.767^{ab}	-3.078^{a}	-3.117^{ab}
		SE	0.108	0.109	0.094	0.162	0.062	0.123
		P	0.000	0.000	0.000	0.159	0.000	0.000
	Packaging condition	Air	-2.746	-3.289	-3.499	-3.871	-3.341	-3.352
		HiOx	-2.772	-3.282	-3.517	-3.867	-3.345	-3.308
		CO	-2.773	-3.262	-3.509	-3.826	-3.286	-3.153
		SE	0.084	0.084	0.073	0.125	0.048	0.095
		P	0.968	0.973	0.984	0.962	0.621	0.314
	Treatment × Packaging	*P*	1.000	1.000	1.000	1.000	0.982	0.999

C: control; NaL_1: 1% NaL; NaL_2: 2% NaL; Sol.A: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium acetate; Sol.B: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium lactate; HiOx: 80:20:0/O_2:CO_2:N_2; and CO: 0.4:30:69.60/CO:CO_2:N_2. Means within the same row with different lowercase superscripts are significantly different ($p < 0.05$).

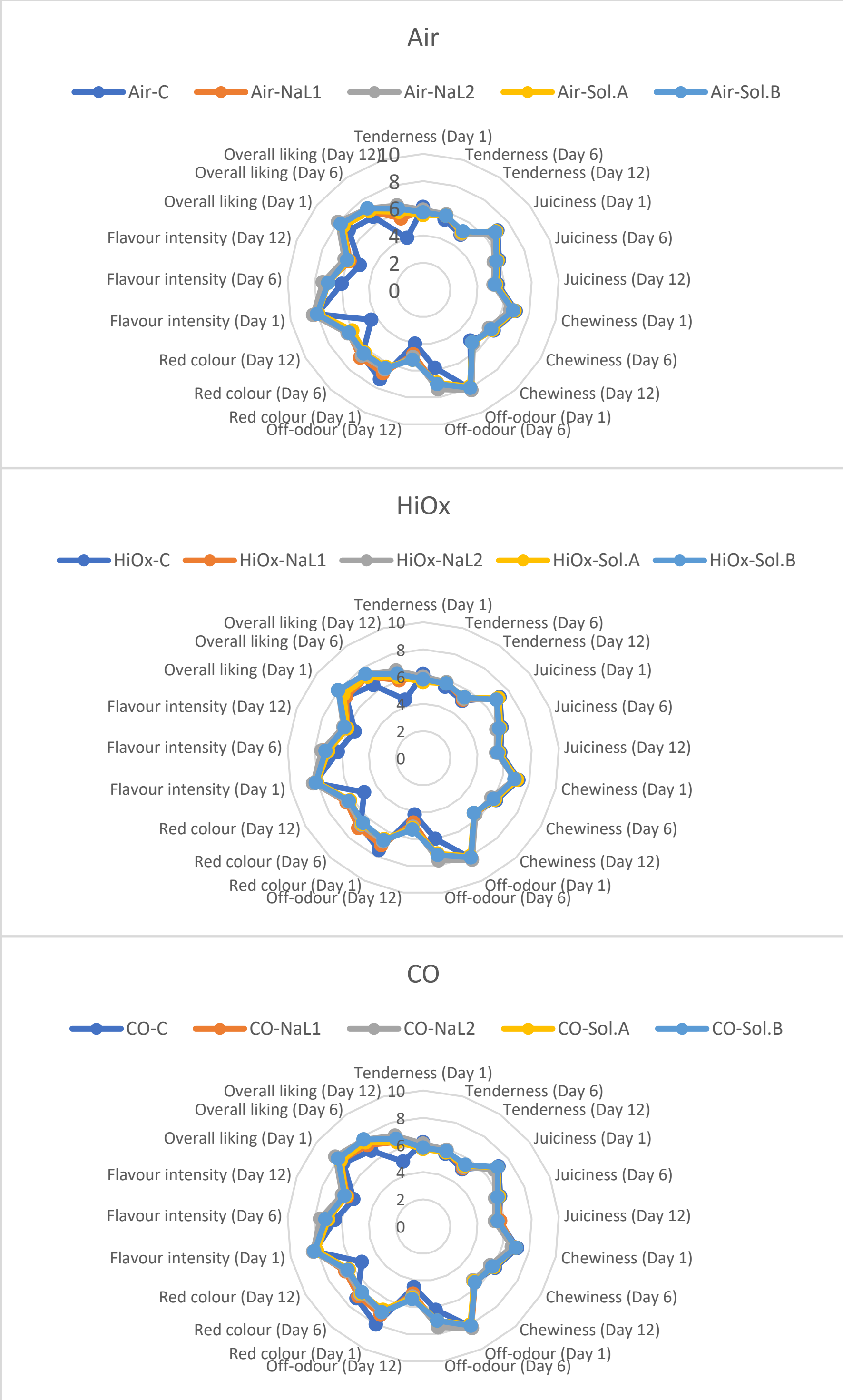

Figure 2. Sensorial properties of meatballs stored at 1°C (tenderness, chewiness, juiciness, off-odour, red colour, flavour intensity, and overall acceptability). C: control; NaL_1: 1% NaL; NaL_2: 2% NaL; Sol.A: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium acetate; Sol.B: 0.5% potassium sorbate + 0.5% sodium citrate + 1% sodium lactate; HiOx: 80:20:0/O_2:CO_2:N_2; and CO: 0.4:30:69.60/CO:CO_2:N_2.

appetising in untreated meatballs in the first six days of storage, the effects of spoilage caused the product to darken by the end of the storage. Meantime, an off-odour occurred during long storage in the same way. Although the flavour intensity diminished over time, the organic acid-added meatballs were regarded more favourably during storage period. No adverse opinions were noted by the panellists regarding the consumption of the product. At the end of the storage period, the overall acceptability of meatballs was determined as NaL_2 > Sol.B > Sol.A > NaL_1 > C, respectively. For the sensory evaluation of the meatballs, packaging conditions were somewhat affected the product quality. CO and HiOx packaging were respectively more beneficial than air packaging and significantly enhanced the quality and shelf-life of the products. In agreement with these findings, Rogers *et al*. (2014) stated that packaging with low level of CO (0.4% CO, 30% CO_2, and 69.6% N_2) exhibited more desirable colour and consumer acceptability during the lighted retail display of ground beef for 20 days under refrigerated condition. Mir and Masoodi (2018) indicated that the quality of meatballs can be improved to a certain extent with the addition of sodium acetate during storage period. The flavour, juiciness, and overall acceptability scores of treated samples were higher than untreated meatballs throughout the storage. Hoffman *et al*. (2008) specified that it is acceptable to apply the phosphates and lactates as injection mixtures to beef meat muscles to increase the sensory properties of the products. Quilo *et al*. (2009) reported higher scores for the overall colour attributes of ground beef samples treated with 3% potassium lactate than untreated ones on days 1 - 3 of display, and lower discoloration was observed when antimicrobial treatments were used. Although, no detectable differences were distinguished on the sensory attributes of ground beef on the first day of display after the addition of antimicrobial agents, lower discoloration were noticed at the end of the storage compared to untreated samples (Quilo *et al*., 2010). On the other hand, Suman *et al*. (2010) indicated that the effect of lactate on the surface discoloration of ground beef was influenced by the packaging systems. While lactate-added patties in PVC and vacuum packages demonstrated lower discoloration than untreated ground beef, no differences existed for lactate-treatments in CO and HiOx packaged samples.

Conclusion

The addition of lactates and other organic acids such as sodium acetate, sodium citrate, and their combinations enhanced the shelf-life of meatballs. Depending on the concentration, organic acids inhibited the microbial growth of both Gram (+) and Gram (-) bacteria. Moreover, organic acids did not cause an undesirable change in the sensory characteristics of the product, and even improved the quality characteristics of meatballs by maintaining the fresh colour, enhancing the taste and flavour, and strengthening the textural properties.

Packaging conditions significantly affected the shelf-life of meatballs. Low level CO-MAP caused a slight increase in total aerobic bacteria, psychrophilic microorganisms, yeast-mould, and *Listeria monocytogenes* counts, and more inhibition in *Pseudomonas* spp. and *Salmonella* Typhimurium counts compared to air and HiOx-MAP meatballs. CO-MAP preserved the red colour of the meatballs, and reduced the lipid oxidation throughout the cold storage.

Therefore, it was concluded that NaL_2 and sodium lactate in combination with sodium citrate and potassium sorbate under 0.4% CO-MAP was most effective in maintaining the storage properties of meatballs without affecting the sensory characteristics for a storage period of up to 12 days.

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