



## Separate and combined effects of lactic acid, chitosan and modified atmosphere packaging on the shelf life of quail carcass under chilled conditions



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### ABSTRACT

The shelf life of quail carcass was examined after imposing separate and combined treatments of spraying with lactic acid (LA, 1% v/v), dipping in chitosan (CH, 1% w/v) and placing the carcass in modified atmosphere package (MAP, 65% CO<sub>2</sub> + 30% N<sub>2</sub> + 5% O<sub>2</sub>) at 4 ± 1 °C for 20 days. The control group was packed under similar atmospheric condition but without any antimicrobial agents. Microbiological populations, physicochemical properties and sensory attributes (color, odor and overall appearance) were monitored at 4-day intervals. All antibacterial treatments effectively lowered microbial numbers, compared to the control during chilled storage. The total viable count, as an indicator of meat spoilage, on day 8 of the storage in treated samples was 0.5–3.4 log CFU/g which was lower than the control. The weakest and strongest effects were caused by LA and LA + CH + MAP treatments, respectively. Similar impact intensity occurred in other microbial tests during refrigerated storage. Binary combination treatments of MAP and chitosan showed synergistic effects on controlling microbial growth, and had no significant difference with LA + CH + MAP. Evaluations on days 4 and 8 showed that all treatments significantly controlled microbial growth, pH value, peroxide value and reactive substances of thiobarbituric acid in comparison with the control ( $P < 0.05$ ). Interestingly, total volatile basic nitrogen (TVB-N) values were sharply increased in all treatments, whereas the CH + MAP and LA + CH + MAP retained the TVB-N content below 30 mg/100 g, which is an indication of meat freshness. The LA + CH + MAP caused the lowest TVB-N value on days 4, 12, 16 and 20 ( $P < 0.05$ ). On day 8 of storage, the scores of sensory attributes for the control group were less than the minimum score of acceptance (i.e. 5 points). The results of this study indicate that the LA + CH + MAP treatment rendered the samples favorable in terms of overall appearance (> 7) during the entire chilled storage. It is noteworthy that organoleptic evaluations correlated well with physicochemical and microbiological data. Based on microbiological data and the overall appearance results of the carcasses, the shelf lives of the control, LA, CH, MAP, LA + MAP, LA + CH, CH + MAP and LA + CH + MAP samples were 6, 7, 11, 12, 12, 15, 20 and 20 days, respectively, under chilled conditions.

### 1. Introduction

There is a gradual increase in popularity of quail meat among many populations in the world. The aim of breeding quails is to enhance the nutritional diet of humans, since quail meat is currently less consumed by the average human population compared to the conventional chicken meat which is more frequently consumed. Quails belong to the species *Coturnix coturnix japonica*. Their first country of domestication was Japan, but they are currently distributed worldwide, however they are more popular among Asian countries (Boni et al., 2010). Quail meat is characterized by a suitable fatty acid profile, high protein content,

relatively low fat and cholesterol levels, bioavailable sodium, potassium, iron and notable vitamins such as vitamin B6, niacin, thiamin, pantothenic acid and riboflavin (Aminzade et al., 2012). These features make it qualitatively different compared to other fowls. However, the perishability of fresh poultry meat becomes evident after 4–5 days of being stored under refrigeration conditions (Morshedy and Sallam, 2009). The most important factor that causes meat to spoil and shelf life to shorten is microbiological growth. In poultry meat industry, total viable count (TVC), lactic acid bacteria (LAB), *Enterobacteriaceae*, *Pseudomonas* spp., yeast and mold on the meat are controlled as indicators of processing hygiene and microbiological quality throughout

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the storage and the distribution (Latou et al., 2014; Petrou et al., 2012; Vasilatou and Savvaidis, 2013). Considering these issues, the development of new preservation techniques for quail meat is necessary. Chemical preservatives are among the most effective methods of food preservation. However, in recent years, due to increasing public awareness of the adverse effects of chemical preservatives, consumers prefer to use foods that do not contain chemical preservatives (Gitrakou et al., 2010).

MAP can best be described as a means of packing food products in atmospheres other than the standard atmosphere of air. The gases commonly used for fresh meat are O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> which are added to the package headspace in different proportions and combinations, depending on the product type, temperature and employed packaging system (Campbell, 2015). The United States Food and Drug Administration has allowed the use of lactic acid (LA) as a substance to be used against spoilage in poultry meat (USDA-FSIS, 2017) because LA can safely extend the shelf life of poultry meat by inhibiting microbial growth (Bolton et al., 2014; Burfoot and Mulvey, 2011). Recently, the use of edible coatings has gained popularity in food packaging. Chitosan (CH) is an edible coating and is known to have antioxidant, anti-inflammatory, non-toxic and biodegradable properties, while it also has broad antimicrobial activity against bacteria and fungi (Sharafati Chaleshtori et al., 2016).

An extensive review of the scientific literature shows that there are little or no reports on preservation techniques for improving the quality of quail carcass (QC) and the maintenance of its microbial, physico-chemical and sensorial properties during chilled storage. The aim of the present study was to evaluate the effect of single and combined applications of CH (1% w/v), LA (1% v/v) and MAP CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub> (65:30:5%) on the shelf life of quail carcass. Various parameters were controlled, i.e. microbial quality, pH, Peroxide Value (PV), ThioBarbituric Acid Reactive Substances (TBARS), Total Volatile Basic Nitrogen (TVB-N) and sensory attributes during 20 days of storage under chilled condition (4 ± 1 °C).

## 2. Materials and methods

### 2.1. Quail carcasses (Qces)

A total number of 144 quails (*Coturnix coturnix japonica*) of 35-day-old were used in this study. After slaughter and dressing, Qces were weighed, and the average weighing of each carcass was 195 ± 15 g. Each QC was packaged in a separate sterile plastic bag after being dressed and transferred to the laboratory using an ice box. The application of treatments began about 3 h after the collection of samples.

### 2.2. Preparation of chitosan

One gram of chitosan had 75–85% deacetylation and a low molecular weight (MWt; 340,000) (Sigma-Aldrich Co., China), was used to prepare 1% CH solution according to the scientific literature outlined by Latou et al. (2014).

### 2.3. Treatment application

The Qces (n = 144) were divided into 4 groups and each group was prepared accordingly. The first group did not receive any antimicrobial agents. In the second group, the Qces were dipped in 200 ml of chitosan solution (1% w/v) for 1.5 min in an open sterile packaging pouch. Then, they were decanted and allowed to dry for 20 min under a biological containment hood. For the third group, a solution (1% v/v) of lactic acid (Merck, Germany) was sprayed onto the interior and exterior sides of the Qces according to the method used by Burfoot and Mulvey (2011). The amount of acid applied in the present study was 100 g of lactic acid solution per kg of Qces. After spraying the Qces, it was decanted and allowed to dry for 20 min under a biological containment

hood. For the fourth group, the lactic acid solution was sprayed on the Qces and then the chitosan solution was applied. Each group was then divided into two subgroups. The first subgroup was packed under normal atmospheric conditions and the second subgroup was packed under MAP conditions (65% CO<sub>2</sub> – 30% N<sub>2</sub> – 5% O<sub>2</sub>). Qces were packed under atmosphere or MAP condition using a packaging machine (model 200-A Henkelman, Netherlands), with polyethylene terephthalate (PET) semi-rigid trays (Tb shimi, Iran). They were packed in low density polyethylene/polyamide or low density polyethylene (LDPE/PA/LDPE) barrier pouches (80 µm in thickness, with an O<sub>2</sub> transmission rate of 45.3 cm<sup>3</sup>/m<sup>2</sup>/24 h/atm, a CO<sub>2</sub> transmission rate of 174 cm<sup>3</sup>/m<sup>2</sup>/24 h/atm and a water vapor transmission rate of 2.6 g/m<sup>2</sup>/24 h, at 85% RH and 23 °C). The ratio of gas to meat was 2:1 (v/v). Based on a previous study by Latou et al. (2014), the needle in the upper part was pierced in order to ensure the aerobic condition of the samples which were packed under normal atmosphere conditions. All packed samples were stored at 4 ± 1 °C for 20 days. Analyses were performed at intervals on days 0, 4, 8, 12, 16 and 20 during the chilled storage.

### 2.4. Microbiological analysis

Each QC was washed thoroughly for microbiological sampling. Similar to the method described by Georgsson et al. (2006), each individual QC was weighed and aseptically transferred to a sterile bag containing 225 ml of 0.1% sterile peptone water. It was then vigorously massaged by hand for 2 min. The QC was relocated aseptically after rinsing and the microbiological analysis was performed by using the rinse peptone water. Serial decimal dilutions of carcass rinse were made in 0.1% peptone water. To enumerate the total viable count (TVC, by surface spread method), 0.1 ml samples of serial decimal dilutions were spread on Plate Count Agar (PCA, Lioflichem, Italy) medium and the plates were incubated at 30 °C for 48 h. The *Pseudomonas* spp. were identified on Pseudomonas Agar Base (Quelab, Canada) with a selective supplement (CFC, Biolife, Italy) incubated at 25 °C for 2 days. The pour-over method was used in order to count the *Enterobacteriaceae* by using Violet Red Bile Glucose (VRBG) agar. Plates containing *Enterobacteriaceae* were incubated at 37 °C for 24 h. LAB were counted in the pour plate and the overlay with the same medium on de Man Rogosa Sharpe (MRS) agar (Lioflichem, Italy). LAB were counted after being incubated at 30 °C for 3 days. Yeasts and molds were identified on Rose Bengal Chloramphenicol (RBC) selective agar (Lioflichem, Italy) that were incubated at 25 °C for 3–5 days. Microbiological data were converted to log CFU/g.

### 2.5. Physicochemical analysis

The pH was measured by homogenizing a 5 g sample with 45 ml distilled water. Ground skin and deboned cuts including the breast, thigh and drumstick (GSDCs) of carcasses were sampled from each group of treatments. The pH of the resultant suspension was measured by a digital pH meter (Labnic, USA). The International Dairy Federation (IDF) method 74A:1991 was applied to measure the peroxide value (PV) of extracted lipids. Finally, PV was reported as milliequivalent of peroxides per kilogram of lipid (Shantha and Decker, 1993). For measuring TBARS (µmol malondialdehyde/kg GSDCs), Aliquots of 5 g of the GSDCs sample were homogenized in 15 ml 7.5% trichloroacetic acid (TCA) with 0.10% propylgallate and 0.10% EDTA for 45 s using a homogenizer (Ultra-Turrax T-25 basic, IKA Company, Germany). The procedure followed methods introduced by Vyncke (1970), with modifications according to Sørensen and Jørgensen (1996). The method described by Goulas and Kontominas (2005) was used in order to measure total volatile basic nitrogen (TVB-N) content of the GSDCs sample. Results were reported as mg nitrogen/100 g GSDCs.

## 2.6. Sensory analysis

For sensory assessment, a panel of 60 untrained members (60% women and 40% men) was selected among graduate students, aged between 18 and 30, non-smokers, and regular consumers of quail carcass. The panelists were requested to score color, odor and overall appearance of Qces according to a 9-point hedonic scale, where 9- expressed 'liked extremely' and 1- showed 'disliked extremely'. The lower limit of acceptability was an overall appearance score of 5 (Trindade et al., 2012).

## 2.7. Data analysis

All obtained data, were analyzed by SAS version 9.1 software (SAS Institute INC., U.S.A. Enterprise Guide 4.1.) using a fully randomized factorial design ( $6 \times 8$ ) with two fixed factors and nine determinations (three independent packages per treatment and sampling day) to make a random order of determinations (Latou et al., 2014). The first factor included time (6 different levels), while the second factor was comprised of 8 different treatments. Microbial counts were transformed to log CFU/g and the comparison of mean values of the physicochemical data was performed with Duncan test at 5% level. Correlation between variables was calculated using the software package mentioned above.

## 3. Results and discussion

### 3.1. Microbiological changes

The shelf life of poultry meat is primarily estimated by TVC. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the acceptable limit for TVC in fresh poultry meat is 7.0 log CFU/g. Fig. 1a illustrates changes in TVC of different QC treatments in relation to storage time, as the initial TVC of fresh Qces was between 4.5 and 4.6 log CFU/g.

As Fig. 1a shows, MAP controlled the growth of TVC in Qces more effectively than LA did, and the difference between MAP and CH was slight in this regard. CO<sub>2</sub> is the most important gas of MAP which effectively inhibits molds and some aerobic bacteria growth by increasing the lag phase and slowing down the exponential growth of microorganisms (Campbell, 2015).

One of the most important reasons for the resistance of microorganisms to antimicrobial agents can be the mechanism of action of the antimicrobial agents. Furthermore, the defense mechanism, membrane permeability characteristics, genetic responses and intracellular biochemical properties of microorganism can also be other reasons for the difference in the microbial population of various treatments (Zhou et al., 2015).

Binary combination treatments of MAP and antimicrobial agents (especially chitosan) show synergistic effects on controlling the TVC (Fig. 1a). Other studies have also reported the antimicrobial effect of chitosan and MAP in reducing the microbial load of poultry meat. Petrou et al. (2012) reported that the TVC decreased in fresh chicken breast meat when treated with chitosan (1.5% w/v) and MAP, and the chicken's shelf life was extended by 10 days compared with the control samples. In a similar study, the microbiological shelf-life of chicken breast meat was extended by > 9 days when samples were coated with chitosan solution (1% w/v) and when later stored under MAP (Latou et al., 2014).

The LA + CH + MAP and CH + MAP were the most effective treatments for controlling the growth of TVC. So, during the 20 days of storage, TVC was < 6 logarithmic cycles. Our result shown, adding LA treatment to CH + MAP did not have a significant synergistic effect on growth of TVC.

LAB produce organic acids which change the pH of food such as meat, and are therefore not desirable because the change in pH influences the product's physical and chemical properties (Yesudhasan et al.,

2014). The initial count of LAB increased quickly in control samples (Fig. 1b). Qces treated with LA, MAP, CH, LA + CH and LA + MAP did not show a clear difference in their LAB count during the first 4 days of storage, but the differences became clear on the last day the counting was performed. A comparison between the CH and LA + CH treatments showed that the two were not significantly different in their effect on the LAB population until the 12th day. Among the binary combination treatments, the best result was observed by applying CH + MAP which caused the LAB population to be 3.4 log CFU/g less, compared to the control treatment, by the eighth day of storage. Similar reports have addressed chicken breast meat (Latou et al., 2014; Petrou et al., 2012). Based on our results, the most effective treatment to inhibit the growth of LAB was LA + CH + MAP which reduced or maintained the initial LAB counts during the storage period. Accordingly, after 8 days of storage, the treatment by LA + CH + MAP caused the population of LAB in samples to be 3.7 log CFU/g less, compared to the control, and thereafter the counts of LAB increased with a moderate slope.

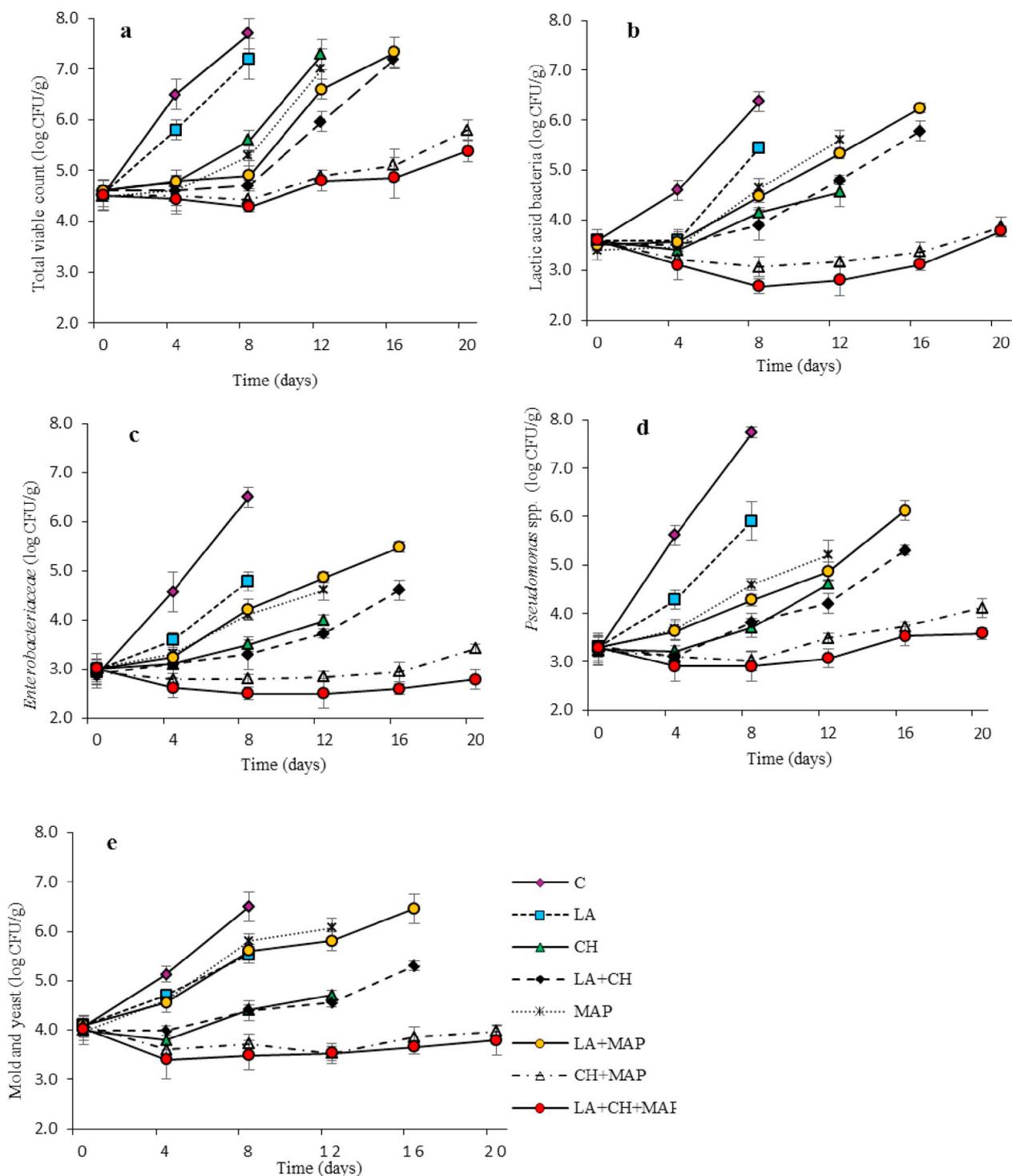
*Enterobacteriaceae* are bacteria which belong to the coliform category, and are served as organisms that indicate pathogenic growth in food. EC (2005) has labeled out a safe limit of 2.5–3.0 log CFU/cm<sup>2</sup> for *Enterobacteriaceae* count for carcasses of cattle, sheep, goats, horses and pigs. The initial count of *Enterobacteriaceae* in Qces was 2.9–3.0 log CFU/g (Fig. 1c) which indicates good hygiene condition throughout processing work surfaces and handling of Qces. The initial counts of *Enterobacteriaceae* in the control sample sharply increased. The populations of *Enterobacteriaceae* in treated samples were less, in comparison with the control sample on the eighth day of storage. The decrease in the number of *Enterobacteriaceae* has previously been reported by other studies on chicken breast meat coated by chitosan (Sharafati Chaleshtori et al., 2016), chicken carcass dipped in 5% citric acid (Bolton et al., 2014) and spray-washed with 1.5% lactic acid (Liu et al., 2016). In this research, the LA + CH + MAP treatment caused the *Enterobacteriaceae* population to be kept less than three logarithmic cycles until the 20th day.

*Pseudomonas* strains can produce many enzymes that functionally break down the chemical components in poultry products. Some of these enzymes separate amino acids from meat, thereby increasing the TVB-N in compounds. Therefore, the integrity of poultry meat depends on the control of *Pseudomonas* spp. growth (Gracey et al., 1999). As previously noted for LAB and *Enterobacteriaceae*, all treatments retarded *Pseudomonas* spp. growth (Fig. 1d), with the CH + MAP and LA + CH + MAP treatments being more effective in inhibiting *Pseudomonas* spp. than other treatments during the chilled storage period. The CH + MAP treatment managed to bring the population of *Pseudomonas* spp. to about 4.1 log CFU/g after 20 days. A similar report was published by Latou et al. (2014). The best result was obtained by the LA + CH + MAP treatment which, on the eighth day, inhibited the growth of *Pseudomonas* spp. most effectively since its population became 4.8 log CFU/g less than the population in the control sample. This result is a unique one of its kind and is hereby reported for the first time. *Pseudomonas* spp. is an aerobic bacterium which is also able to grow under reduced oxygen concentrations (Economou et al., 2009), and therefore MAP could not prevent its growth completely.

Yeasts and molds have been reported to be a minor part of the microbial complex in fresh poultry meat (Bazargani-Gilani et al., 2015). The initial counts of yeasts and molds (measured on the first day of storage) increased rapidly in all samples except by the CH + MAP and LA + CH + MAP treatments (Fig. 1e). As previously noted, for other groups of bacteria, CH + MAP and LA + CH + MAP treatments effectively suppressed the growth of yeasts and molds by maintaining their populations below four logarithmic cycles until the end of the storage time (20 days).

### 3.2. Physicochemical changes

The pH value plays an important role in the quality of meat and



**Fig. 1.** Changes in total viable count (a), lactic acid bacteria (b), *Enterobacteriaceae* (c), *Pseudomonas* spp. (d) and yeasts and molds (e) of quail carcass under chilled conditions ( $4 \pm 1$  °C) for up to 20 day. C: packed under atmospheric condition, without any antimicrobial agents; LA: sprayed with lactic acid solution 1% (v/v); CH: coated with chitosan solution 1% (w/v); MAP: packed under MAP, without any antimicrobial agents; LA + CH: sprayed with lactic acid solution 1% (v/v) + coated with chitosan solution 1% (w/v); LA + MAP: sprayed with lactic acid solution 1% (v/v) + packed under MAP; CH + MAP: coated with chitosan solution 1% (w/v) + packed under MAP; LA + CH + MAP: sprayed with lactic acid solution 1% (v/v) + coated with chitosan solution 1% (w/v) + packed under MAP. Results are mean of three independent experiments.

consumer satisfaction. The highest value of pH was seen in the control sample on the fourth ( $6.0 \pm 0.1$ ) and eighth ( $6.6 \pm 0.1$ ) days of storage (Table 1), which is probably due to the production of volatile bases e.g.,  $\text{NH}_3$  and TMA via protein breakdown by bacteria (Chaijan et al., 2005). This is probably because of the acidic nature of chitosan and lactic acid. In this context, Mohan et al. (2012) reported that the pH

values of fish samples decreased significantly when the samples were coated with a chitosan solution (1 and 2% w/v with pH = 4), compared to the control samples ( $P < 0.05$ ).

Furthermore, the proliferation of LAB under MAP and the dissolution of  $\text{CO}_2$  in both muscle and fat tissues can also be responsible for decrease of pH value (Lerasle et al., 2014). The changes of pH showed a

**Table 1**  
The physicochemical changes of quail carcass under chilled condition (4 ± 1 °C) for up to 20 day.

Attributes	Treatment	Days of storage					
		0	4	8	12	16	20
pH	C	5.6 ± 0.1 <sup>a,C</sup>	6.0 ± 0.1 <sup>a,B</sup>	6.6 ± 0.1 <sup>a,A</sup>	–	–	–
	LA	5.6 ± 0.2 <sup>a,C</sup>	5.8 ± 0.1 <sup>b,B</sup>	6.4 ± 0.1 <sup>b,A</sup>	–	–	–
	CH	5.6 ± 0.1 <sup>a,C</sup>	5.5 ± 0.1 <sup>c,C</sup>	5.8 ± 0.1 <sup>c,B</sup>	6.1 ± 0.1 <sup>a,A</sup>	–	–
	MAP	5.6 ± 0.2 <sup>a,C</sup>	5.6 ± 0.1 <sup>c,C</sup>	5.9 ± 0.2 <sup>c,B</sup>	6.2 ± 0.2 <sup>a,A</sup>	–	–
	LA + CH	5.6 ± 0.1 <sup>a,C</sup>	5.4 ± 0.0 <sup>d,D</sup>	5.6 ± 0.1 <sup>de,C</sup>	5.8 ± 0.1 <sup>b,B</sup>	6.2 ± 0.1 <sup>b,A</sup>	–
	LA + MAP	5.6 ± 0.1 <sup>a,C</sup>	5.6 ± 0.1 <sup>c,C</sup>	5.7 ± 0.1 <sup>cd,C</sup>	6.1 ± 0.0 <sup>a,B</sup>	6.4 ± 0.1 <sup>a,A</sup>	–
	CH + MAP	5.6 ± 0.2 <sup>a,B</sup>	5.5 ± 0.0 <sup>cd,BC</sup>	5.4 ± 0.0 <sup>ef,C</sup>	5.4 ± 0.1 <sup>c,C</sup>	5.5 ± 0.1 <sup>c,BC</sup>	5.9 ± 0.1 <sup>a,A</sup>
	LA + CH + MAP	5.6 ± 0.2 <sup>a,AB</sup>	5.4 ± 0.1 <sup>d,CD</sup>	5.3 ± 0.1 <sup>f,D</sup>	5.3 ± 0.0 <sup>c,D</sup>	5.5 ± 0.1 <sup>c,BC</sup>	5.7 ± 0.1 <sup>b,A</sup>
	PV (meq peroxide/kg)	C	0.115 ± 0.005 <sup>a,C</sup>	0.907 ± 0.021 <sup>a,B</sup>	1.200 ± 0.013 <sup>a,A</sup>	–	–
LA	0.111 ± 0.006 <sup>ab,B</sup>	0.653 ± 0.015 <sup>b,B</sup>	0.977 ± 0.015 <sup>b,A</sup>	–	–	–	
CH	0.118 ± 0.003 <sup>a,D</sup>	0.307 ± 0.017 <sup>c,C</sup>	0.710 ± 0.017 <sup>c,A</sup>	0.633 ± 0.009 <sup>b,B</sup>	–	–	
MAP	0.100 ± 0.010 <sup>b,D</sup>	0.410 ± 0.020 <sup>c,C</sup>	0.780 ± 0.026 <sup>d,A</sup>	0.707 ± 0.019 <sup>a,B</sup>	–	–	
LA + CH	0.119 ± 0.007 <sup>a,E</sup>	0.337 ± 0.012 <sup>d,D</sup>	0.590 ± 0.011 <sup>f,A</sup>	0.563 ± 0.012 <sup>c,B</sup>	0.363 ± 0.012 <sup>b,C</sup>	–	
LA + MAP	0.120 ± 0.008 <sup>a,E</sup>	0.410 ± 0.018 <sup>c,D</sup>	0.837 ± 0.008 <sup>c,A</sup>	0.703 ± 0.006 <sup>a,B</sup>	0.437 ± 0.017 <sup>a,C</sup>	–	
CH + MAP	0.107 ± 0.004 <sup>ab,E</sup>	0.253 ± 0.007 <sup>f,C</sup>	0.373 ± 0.006 <sup>g,A</sup>	0.350 ± 0.014 <sup>d,B</sup>	0.243 ± 0.007 <sup>c,C</sup>	0.223 ± 0.013 <sup>a,D</sup>	
LA + CH + MAP	0.118 ± 0.009 <sup>a,D</sup>	0.230 ± 0.010 <sup>f,B</sup>	0.300 ± 0.010 <sup>h,A</sup>	0.303 ± 0.008 <sup>e,A</sup>	0.207 ± 0.019 <sup>d,C</sup>	0.197 ± 0.015 <sup>a,C</sup>	
TBARS (µmol MDA/kg)	C	4.07 ± 0.06 <sup>a,C</sup>	5.13 ± 0.20 <sup>a,B</sup>	7.18 ± 0.16 <sup>a,A</sup>	–	–	–
	LA	4.02 ± 0.09 <sup>a,B</sup>	3.73 ± 0.05 <sup>c,C</sup>	5.81 ± 0.18 <sup>b,A</sup>	–	–	–
	CH	4.11 ± 0.13 <sup>a,C</sup>	3.42 ± 0.11 <sup>d,D</sup>	4.53 ± 0.05 <sup>d,A</sup>	4.32 ± 0.10 <sup>a,B</sup>	–	–
	MAP	4.01 ± 0.10 <sup>a,C</sup>	3.98 ± 0.17 <sup>b,C</sup>	5.02 ± 0.09 <sup>c,A</sup>	4.34 ± 0.07 <sup>a,B</sup>	–	–
	LA + CH	4.00 ± 0.04 <sup>a,B</sup>	3.47 ± 0.04 <sup>d,C</sup>	4.31 ± 0.07 <sup>d,A</sup>	4.09 ± 0.11 <sup>b,B</sup>	4.48 ± 0.19 <sup>b,A</sup>	–
	LA + MAP	4.10 ± 0.12 <sup>a,C</sup>	3.62 ± 0.09 <sup>cd,D</sup>	4.60 ± 0.23 <sup>d,B</sup>	4.38 ± 0.14 <sup>a,BC</sup>	5.22 ± 0.30 <sup>a,A</sup>	–
	CH + MAP	4.07 ± 0.11 <sup>a,A</sup>	3.07 ± 0.07 <sup>e,D</sup>	3.70 ± 0.10 <sup>cd,BC</sup>	3.49 ± 0.05 <sup>c,C</sup>	3.78 ± 0.10 <sup>c,AB</sup>	3.55 ± 0.24 <sup>a,C</sup>
	LA + CH + MAP	4.03 ± 0.07 <sup>a,A</sup>	2.85 ± 0.23 <sup>c,C</sup>	3.23 ± 0.27 <sup>f,B</sup>	3.27 ± 0.21 <sup>c,B</sup>	3.51 ± 0.22 <sup>c,B</sup>	3.25 ± 0.20 <sup>a,B</sup>
	TVB-N (mg N/100 g)	C	8.4 ± 0.2 <sup>a,C</sup>	25.4 ± 0.7 <sup>a,B</sup>	38.8 ± 0.9 <sup>a,A</sup>	–	–
LA	8.4 ± 0.2 <sup>a,C</sup>	19.3 ± 0.4 <sup>b,B</sup>	35.7 ± 0.4 <sup>b,A</sup>	–	–	–	
CH	8.6 ± 0.3 <sup>a,C</sup>	14.4 ± 0.6 <sup>c,C</sup>	26.2 ± 0.3 <sup>c,B</sup>	36.4 ± 0.4 <sup>a,A</sup>	–	–	
MAP	8.6 ± 0.2 <sup>a,D</sup>	14.9 ± 0.3 <sup>c,C</sup>	25.9 ± 0.5 <sup>c,B</sup>	33.2 ± 0.7 <sup>b,A</sup>	–	–	
LA + CH	8.4 ± 0.1 <sup>a,E</sup>	12.9 ± 0.2 <sup>d,D</sup>	22.0 ± 0.6 <sup>d,C</sup>	27.9 ± 0.1 <sup>d,B</sup>	31.4 ± 0.6 <sup>b,A</sup>	–	
LA + MAP	8.5 ± 0.1 <sup>a,E</sup>	14.8 ± 0.1 <sup>c,D</sup>	22.8 ± 0.3 <sup>d,C</sup>	31.6 ± 0.6 <sup>c,B</sup>	39.0 ± 1.0 <sup>a,A</sup>	–	
CH + MAP	8.4 ± 0.2 <sup>a,F</sup>	11.6 ± 0.2 <sup>e,E</sup>	15.8 ± 0.7 <sup>e,D</sup>	21.5 ± 0.9 <sup>c,C</sup>	24.6 ± 1.1 <sup>c,B</sup>	27.5 ± 0.9 <sup>a,A</sup>	
LA + CH + MAP	8.7 ± 0.1 <sup>a,F</sup>	10.2 ± 0.8 <sup>f,E</sup>	15.3 ± 0.2 <sup>c,D</sup>	18.6 ± 1.1 <sup>f,C</sup>	21.9 ± 0.8 <sup>d,B</sup>	24.2 ± 1.0 <sup>b,A</sup>	

Each value is the mean of 3 replicates with standard deviation. Means with different lowercase letters in the same column are significantly different (P < 0.05). Means with different capital letters in the same line are significantly different (P < 0.05).

**Table 2**  
Correlation of microbiological, physicochemical and sensory parameters of quail carcass under chilled condition (4 ± 1 °C) for up to 20 day.

Pearson correlation												
	TVC	LAB	Enterobacteriaceae	Pseudomonads	Yeast & mold	pH	PV	TBARS	TVB-N	Color	Odor	Overall appearance
TVC	1											
LAB	0.926*	1										
Enterobacteriaceae	0.909*	0.979*	1									
Pseudomonads	0.941*	0.967*	0.986*	1								
Yeast & mold	0.757*	0.932*	0.923*	0.881*	1							
pH	0.984*	0.972*	0.946*	0.966*	0.852*	1						
PV	0.896*	0.992*	0.965*	0.938*	0.945*	0.948*	1					
TBARS	0.946*	0.987*	0.985*	0.987*	0.896*	0.978*	0.962*	1				
TVB-N	0.979*	0.969*	0.928*	0.946*	0.836*	0.994*	0.950*	0.966*	1			
Color	-0.939*	-0.875*	-0.883*	-0.926*	-0.698 <sup>N.S.</sup>	-0.919*	-0.843*	-0.896*	-0.906*	1		
Odor	-0.919*	-0.943*	-0.972*	-0.971*	-0.821*	-0.929*	-0.910*	-0.975*	-0.916*	0.890*	1	
Overall appearance	-0.930*	-0.991*	-0.981*	-0.976*	-0.916*	-0.967*	-0.985*	-0.978*	-0.962*	0.915*	0.940*	1

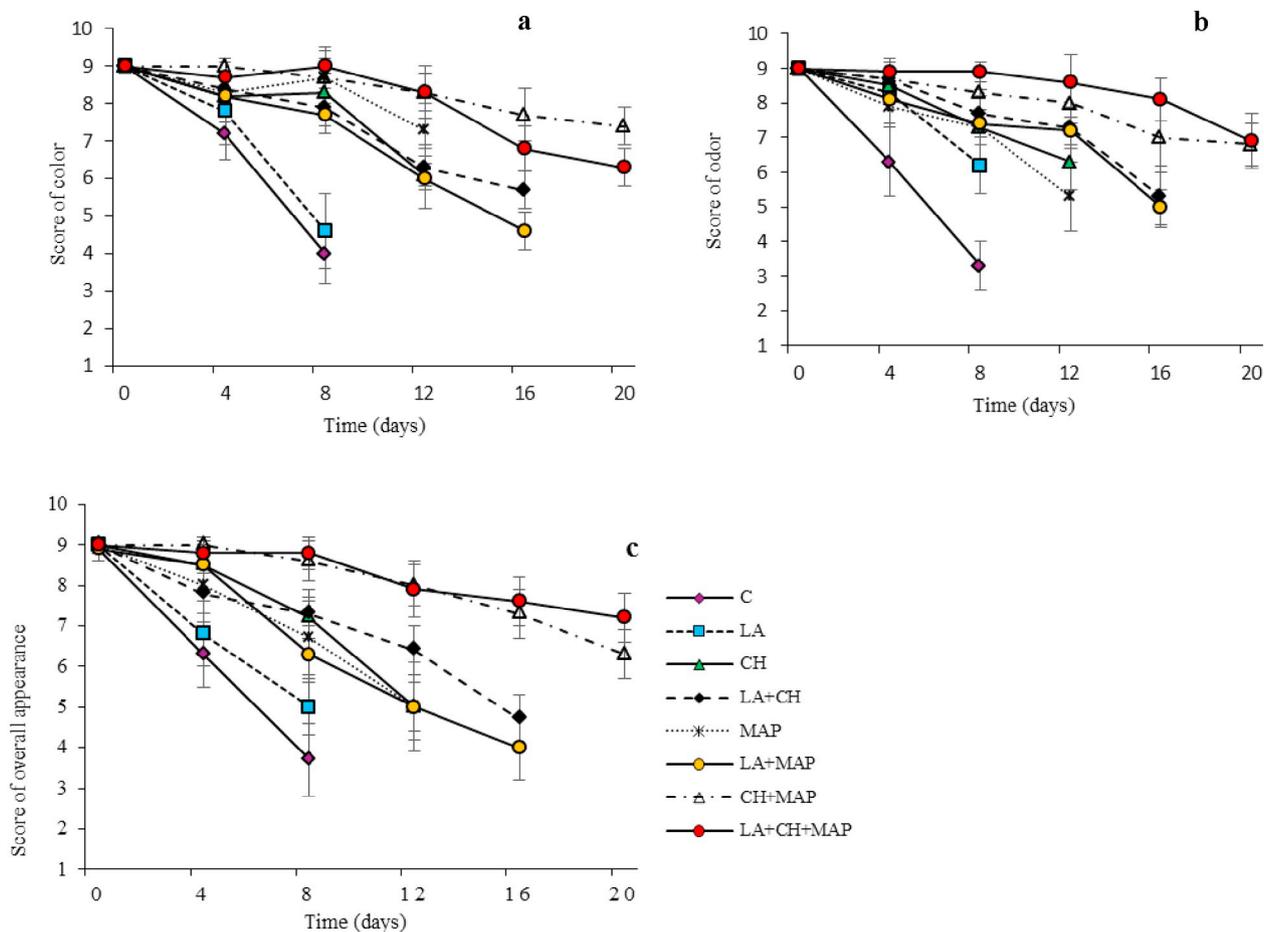
Significance: N.S.: Correlation is not significant at the 0.05 level.

\* Correlation is significant at the 0.05 level.

positive correlation with TVC, *Pseudomonas* spp., LAB, *Enterobacteriaceae* and to a higher extent with TVB-N (Table 2).

Hydroperoxides are the main primary products of oxidation, which would be accumulated during the initiation and propagation steps of the oxidation process. Control samples showed the highest amount of peroxide value on days 4 and 8 of the storage period (Table 1). The results showed that all treatments of quail carcass, as compared to control samples, had significantly (P < 0.05) lower PVs, and samples treated with CH + MAP and LA + CH + MAP exhibited the most substantial inhibition of lipid oxidation. This is probably due to the low concentration of oxygen used in the MAP and the antioxidant properties

of chitosan. Hydroperoxides accumulated to reach a maximum level during 8 days of refrigerated storage (4 ± 1 °C), as a result of treatments except the control and the LA treatment (Table 1). After the eighth day, hydroperoxides declined. Likewise, Shahidi and Wanasundara (2008) reported that hydroperoxides drop was observed when the rate of its decomposition into secondary products exceeded its formation rate. In this study, hydroperoxides were considered as primary oxidation products which predominantly occurred in the early phase (0–8 days) of the storage. Then, the secondary oxidation products were dominant in the latter phase (8–20 days) of the refrigerated storage. So far, no study has investigated the dynamics of hydroperoxides



**Fig. 2.** Changes in the color (a), odor (b) and overall appearance (c) of quail carcass under chilled conditions ( $4 \pm 1^\circ\text{C}$ ) for up to 20 day. C: packed under atmospheric condition, without any antimicrobial agents; LA: sprayed with lactic acid solution 1% (v/v); CH: coated with chitosan solution 1% (w/v); MAP: packed under MAP, without any antimicrobial agents; LA + CH: sprayed with lactic acid solution 1% (v/v) + coated with chitosan solution 1% (w/v); LA + MAP: sprayed with lactic acid solution 1% (v/v) + packed under MAP; CH + MAP: coated with chitosan solution 1% (w/v) + packed under MAP; LA + CH + MAP: sprayed with lactic acid solution 1% (v/v) + coated with chitosan solution 1% (w/v) + packed under MAP. Results are mean of three independent experiments.

in the Qces, but similar increases and thereafter decreases in the PV were reported by other study regarding other meat products during storage (Bazargani-Gilani et al., 2015). Table 2 shows a good correlation between PV and total microbial population ( $r = 0.896$ ,  $P < 0.05$ ). Zanardi et al. (2002) results as microorganisms, especially LAB, oxidize unsaturated fatty acids of meat, are in consistent with results of this study.

TBARS test is one of the oldest methods used for the detection of secondary oxidation products in meat. Table 1 shows no significant differences among the TBARS in samples of Qces as they were treated differently on day 0 of the storage time ( $P > 0.05$ ). During the periods of evaluation, TBARS values for all QC samples varied between  $2.85 \pm 0.23$  and  $7.18 \pm 0.16$   $\mu\text{mol MDA/kg}$ . These values were safely below the  $9.08$   $\mu\text{mol MDA/kg}$  (or  $2$   $\text{mg MDA/kg}$ ) threshold where rancid off-flavors come into notice (Buyn et al., 2003). Control samples showed that the TBARS value progressively increased, as compared to the other treatments until the 8th day of storage. CH + MAP and LA + CH + MAP treatments showed the lowest amount of TBARS value on the 4th, 12th, 16th and 20th day of storage, while the LA + CH + MAP treatment showed the lowest amount on the 8th day ( $P < 0.05$ ).

The comparison of mean values of TBARS between samples treated with LA, CH and MAP showed that, until the eighth day of storage, CH treatment exhibited the highest effects on inhibiting the growth of TBARS. Adding the CH treatment to LA and MAP treatments caused a

synergistic effect on controlling the growth of TBARS ( $P < 0.05$ ). Another report has indicated that chitosan is able to prevent lipid oxidation in chicken breast meat (Petrou et al., 2012). Chitosan has a strong capacity of scavenging free radicals and metal ions chelating activity. Free radicals can initiate lipid peroxidation and cause chain reactions that affect food quality (Yen et al., 2008). Meanwhile, Weist and Karel (1992) reported that stable fluorospheres with volatile aldehydes such as malondialdehyde can be formed by primary amino groups of chitosan, which arise from the breakdown of fats during oxidation. High  $\text{CO}_2$  concentrations in MAP cause inhibitory effects on lipid oxidation in fresh meat (Economou et al., 2009). In contrast, Latou et al. (2014) reported that MAP and, more interestingly, chitosan did not significantly ( $P < 0.05$ ) affect oxidative rancidity of fatty acids in chicken breast fillets. In our study, the CH + MAP and LA + CH + MAP were the most effective treatments in inhibiting oxidative rancidity in QC samples.

TVB-N is commonly used for the evaluation of quality and freshness of meat because it occurs concurrently alongside bacterial spoilage or the degradation of enzymes. Comparison of the mean TVB-N values of QC samples are given in Table 1. The initial values of TVB-N significantly ( $P < 0.05$ ) increased in all samples during the 20 days of the storage period. TVB-N values of the control sample and those treated with LA increased more sharply as compared to the other treatments. Accordingly, the highest values of the control sample and LA treatment were obtained on the eighth day ( $38.7 \pm 0.9$  and  $35.7 \pm 0.4$   $\text{mg N/}$

100 g, respectively) ( $P < 0.05$ ). In this study, spraying lactic acid (1% v/v) on Qces, as compared to the control sample, had significantly ( $P < 0.05$ ) lower TVB-N values. In contrast, Liu et al. (2016) reported that 1.5% lactic acid, did not significantly ( $P > 0.05$ ) affect TVB-N content of chicken meat during 60 days of storage. Comparison of mean values of TVB-N in samples treated with LA, MAP and CH showed that CH was more effective in decreasing the TVB-N value. Combination of those treatments, as compared to their single applications (except MAP and LA + MAP on the 4th day), showed a greater effect on the inhibition of growth in TVB-N value. The lowest amount of TVB-N was observed on the 4th, 12th, 16th, 20th days by LA + CH + MAP and on the 8th day of storage by CH + MAP and LA + CH + MAP treatments. Interestingly, the TVB-N value in samples treated with CH + MAP and LA + CH + MAP remained below the upper limit of acceptability (30 mg N/100 g) during storage time. The present results are similar to those of Patsias et al. (2008) who reported that the limit of 30 mg N/100 g was exceeded when considering the time scale between day 4 and 5 of storage for chilled raw chicken fillets in atmospheric air. In contrast, Balamatsia et al. (2007) and also Economou et al. (2009) suggested 40 mg N/100 g as the tolerance limit of TVB-N value in the chicken meat when stored aerobically and under MAP, respectively. Differences in the type of meat and antimicrobials may impact the results of TVB-N content of poultry meat. It must be mentioned that a positive correlation was observed between total microbial growth and TVB-N content in the Qces.

### 3.3. Sensory changes

The impact of different treatments on sensory attributes (color, odor and overall appearance) during chilled storage is shown in Fig. 2a–c. The results show that the sensory attributes of Qces decreased during the 20 days of the storage period. The highest rates of reduction in sensory attributes were observed in control samples and then in LA treatment. Accordingly, until the 8th day of storage, the scores were  $< 5$ . Meredith et al. (2013) also reported that organic acids did not significantly ( $P > 0.05$ ) affect sensory characteristics of chicken meat. On the other hand, Burfoot and Mulvey (2011) showed that 4% lactic acid solution negatively affected the appearance of chicken and turkey carcasses and in addition to a greying of the leaf fat, caused an obvious discoloration of treated samples.

Various acid solutions exist in different types, temperatures, pH values, concentrations and application methods, which can affect the outcomes of sensory assessment of poultry meat. Furthermore, Zanardi et al. (2002) stated that the oxidative processes in red meat and meat products lead to the degradation of lipids and proteins (including pigments), which in turn, contribute to the deterioration in color, texture (cross linking binding) and flavor. Therefore, differences in the sensory attribute results of some studies noted above may be due to the different amounts of pigments in various meats. The inspection of sensory properties of treatments on the twentieth day showed that CH + MAP treatment had the highest color score ( $7.4 \pm 0.5$ ), LA + CH + MAP treatment ( $7.2 \pm 0.6$ ) had the highest overall appearance score, and finally, treatments of CH + MAP and LA + CH + MAP earned very close odor scores, which was above the minimum score of acceptance (5 points). Therefore, based on our results, MAP and chitosan had a synergistic effect on each other in preserving sensory attributes, but lactic acid did not considerably affect the color, odor and overall appearance of the Qces. Likewise, Latou et al. (2014) reported MAP and chitosan can improve sensory attributes of chicken breast meat and ready-to-cook poultry products, respectively. Table 2 shows that sensory data correlated well with physicochemical and microbiological data (except correlation of yeast and mold with color). The results show that there is a weak correlation between the color of quail meat and PV. However, this value is higher for odor and the overall acceptance (Table 2).

## 4. Conclusion

The results of this study indicated that treating quail carcasses with lactic acid (1% v/v), chitosan (1% w/v) and modified atmosphere package (65% CO<sub>2</sub> + 30% N<sub>2</sub> + 5% O<sub>2</sub>) can extend carcasses shelf-life. The longest shelf life of samples, based on microbiological data and the features of overall appearance, was considered as the last day of chilled storage (day 20) and this was obtained under treatments of CH + MAP and CH + MAP + LA. Combining the LA, CH and MAP together caused substantial decrease of microbial growth, lipid oxidation and total volatile basic nitrogen, compared to the CH + MAP treatment. Finally, these findings demonstrate specific extensions of hurdle technology to poultry meat packaging for preservation industries.

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