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Improved microbial and sensory quality of chicken meat by treatment with lactic acid, organic acid salts and modified atmosphere packaging



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ABSTRACT

Microbial contamination and growth play important roles in spoilage and quality loss of raw poultry products. We evaluated the suitability of three commercially available organic acid based antimicrobial compounds, Purac FCC80 (L-lactic acid), Verdad N6 (buffered vinegar fermentate) and Provian K (blend of potassium acetate and diacetate) to prevent growth of the innate microbiota, reduce spoilage and enhance the sensory quality of raw chicken under vacuum, high CO₂ (60/40% CO₂/N₂), and high O₂ (75/25% O₂/CO₂) modified atmosphere (MA) storage conditions. Solutions were applied warm (50 °C) or cold (4 °C) to reflect treatments prior to (Prechill) or after (Postchill) cooling of chicken carcasses, respectively. Single postchill treatments of raw chicken wings with 5% Verdad N6 or Provian K solutions and MA storage enabled complete growth inhibition during the first seven days of storage before growth resumed. Enhanced bacterial control was obtained by combining Prechill lactic acid and Postchill Verdad N6 or Provian K treatments which indicated initial reductions up to 1.1 log and where total bacterial increase after 20 days storage was limited to 1.8-2.1 log. Antibacterial effects were dependent on the concentration of the inhibiting salts used, pH and the storage conditions. Bacterial community analyses showed increased relative levels of Gram-positive bacteria and with reductions of potential spoilage organisms in samples treated with the organic acid salts Verdad N6 and Provian K. Sensory analyses of raw, treated wings showed prominent lower scores in several spoilage associated odour attributes when compared with untreated chicken wings after 13 days storage. For heat-treated chicken, only minor differences for 22 tested attributes were detected between seven antimicrobial treatments and untreated control chicken. Immersion in commercially available organic acid/salt solutions combined with MA storage can reduce bacterial levels, improve microbial and sensory quality, and potentially improve shelf life and reduce food waste of chicken products.

1. Introduction

Poultry meat production steadily increases and reached 130 million tons in 2019, a reported 31% increase in production last 10 years (FAO, 2019). Worldwide consumption reached 15.6 kg per capita in 2018 with Israel ranked first (67.5 kg/capita) and United States of America (>50 kg/capita) as the largest consumers in the developed western world. Increased consumption also occurs in European countries and has made chicken meat the most consumed animal protein source in many countries (OECD, 2021). Factors behind the increased demand of chicken meat include changes in consumer preferences for nutritious, healthy, more environmental-friendly and versatile alternatives to red meat at an affordable price. The increasing demands for fresh, easy-to-use, consumer-friendly poultry products challenge the meat industry in providing fresh, safe, high quality meat with a reasonable shelf life to the consumers.

The successive steps from slaughtering to meat processing and packaging include stages that can have microbial reduction effects but also processes that promote microbial transfer and contamination (Rouger et al., 2017, 2018; Samapundo et al., 2019). Poultry meat is therefore prone to extensive microbial contamination. Along with its high pH level, nutrient and water content, chicken meat is a perishable product with short shelf life and where growth of spoilage microorganisms during storage may lead to large economical losses and extensive food waste. Different studies reported total viable counts at the beginning of the storage period in the range 4.3 to 5.7 log/g increasing to 6.5–9 log after storage and to be dependent on cuts, presence of skin or not least processing and storage conditions (e.g. time, temperature

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Received 25 June 2021; Received in revised form 22 October 2021; Accepted 2 December 2021 Available online 7 December 2021 0168-1605/© 2021 Elsevier B.V. All rights reserved. and atmosphere; Al-Nehlawi et al., 2013; Balamatsia et al., 2007; Bjorkroth, 2005; Capita et al., 2002, 2013; Chouliara et al., 2007; Zhang et al., 2012). The data suggest large quantitative and qualitative variations in microorganisms present on chicken meat and a need to evaluate strategies to reduce this variation and provide fresh, high quality chicken products throughout their shelf life.

To enhance chicken product quality, research efforts have increasingly been directed towards control of the indigenous microbiota including spoilage microorganisms. Dominant contaminants include psychrotrophic or mesophilic bacteria of *Pseudomonas* spp., *Enterobacteriaceae* (e.g. *Serratia*, *Hafnia*, *Rhanella*, *Yersinia*), *Shewanella*, *Brochothrix thermosphacta* and lactic acid bacteria (LAB) e.g. *Carnobacterium* spp. and *Lactobacillus* spp. (see references in Rouger et al., 2017; Silva et al., 2018). Spoilage potential varies within and between genera and species, and the above-mentioned bacteria could be regarded as potential spoilers with the ability to grow and exert metabolic spoiling activities under relevant storage conditions.

Storage temperature is the most important factor to control microbial growth, but recommended temperatures close to 0 °C cannot prevent microbial activity and product deterioration of raw, fresh chicken. Modified atmosphere packaging (MAP) is therefore widely used as an additional hurdle to prolong shelf life and reduce product and financial losses in the food chain. MAP is therefore considered to provide an overall positive environmental impact compared to traditional packaging (Schumann and Schmid, 2018; Thoden van Velzen and Linnemann, 2008).

MAP combining CO₂ with N₂ as an inert filler gas (with residual low O₂) is commonly used for fresh poultry. High O₂ is used in red meat packing for the formation of oxymyoglobin and enhancement of the red meat colour. This is not relevant for white meat poultry, still a growing number of producers use high concentrations of oxygen in poultry product packaging (Holl et al., 2016; Rossaint et al., 2015). The growth inhibitory effect of CO₂ is proportional to the CO₂ partial pressure. If the partial pressure of CO₂ is high enough both an extended lag phase and reduced metabolic activity will contribute to increased shelf life. Microbial sensitivity to CO2 varies considerably between different microorganisms. Aerobic bacteria such as Pseudomonas and Acinetobacter are CO2 sensitive while mesophilic LAB were favoured by high CO2-levels (Holck et al., 2014; Rossaint et al., 2015). Detailed descriptions on how different MAP gas mixtures affect bacterial growth on raw chicken are scarce, but various proportions of CO2/N2/O2 in the MAP mixtures shape bacterial growth and dynamics and the composition of the chicken microbiota during storage. This may also lead to MAPdependent changes in spoilage characteristics (McKee, 2007).

Shelf-life extensions from five-six days for aerobically stored chicken to 12 and 15 days when stored in 30% $CO_2/70\%$ N₂ or 70% $CO_2/30\%$ N₂, respectively, have been reported (Chouliara et al., 2007). Holck et al. reported a shelf-life extension of seven days when 100% CO_2 was used compared with 60% CO_2 . Observed increase in drip loss at 100% CO_2 storage could be overcome by application of CO_2 -emitters, easily implemented in industrial packaging lines (Holck et al., 2014).

An effective cool chain and MAP packaging are the two most effective microbial growth preventing strategies applied in the European Union (EU). These still have their limitations as sole strategies for shelflife extension and food waste reductions for fresh poultry products. Additional strategies should therefore be evaluated to obtain further improvements in food safety, microbial quality and shelf- life. Various physical and chemical methods to reduce microorganisms on poultry carcasses and products have been studied such as use of chlorine, ozone, phosphates, organic acids, steam, high pressure processing, irradiation and ultraviolet light (Alonso-Hernando et al., 2013; Bolton et al., 2014; Kure et al., 2020; Loretz et al., 2010; Lu et al., 2019; McLeod et al., 2018; Olaimat et al., 2018; Silva et al., 2018; Zhang et al., 2018). Spraying 4% lactic acid, pH 3.7, on chicken carcasses, reduced aerobic plate counts with up to 2.1 log (Burfoot and Mulvey, 2011), while spraying with 2% lactic acid gave 0.47–0.83 log reduction of total viable counts (Duan et al., 2017). Less is known about the use of salts of organic acids or fermentates. Buffered vinegar was used to increase the shelf-life of chicken retail cuts at pH 6.1 from 12 to 20 days when packaged in carbon dioxide (Desai et al., 2014). Lactic acid/sodium lactate buffer has also been employed to reduce contamination of Campylobacter jejuni on raw chicken legs during storage (Rajkovic et al., 2010). Some of the above mentioned methods are applied in certain countries and markets (e.g. USA, Australia) despite negative effects and limitations for use due to chemical residues with potential adverse human health effects, negative sensory effects (e.g. colour, smell, texture), generally low consumer acceptance, corrosiveness on equipment, high costs or low effectivity (Chousalkar et al., 2019; Moore et al., 2017; Soro et al., 2020). Antimicrobial treatments of poultry meat are highly restricted in the EU due to regulations and the concerns mentioned above. It is therefore a need for further evaluation of strategies that not only focus on maximizing the antimicrobial effect, but also on applying strategies and conditions that could be relevant and acceptable according to both industry needs, consumer acceptance and microbial and sensory effects.

The aim of the current study was to determine if the microbial and sensory quality of raw chicken, using chicken wings as a suitable chicken product, could be improved by treatments with solutions of commercially available salts of organic acids, fermentates and lactic acid as single or combined treatments implemented in the processing chain of raw poultry. Effects of the treatments on bacterial counts and communities were evaluated during storage under different atmospheric conditions commonly applied for fresh, raw chicken. The sensory quality of treated raw chicken and untreated controls as well as heat-treated chicken was determined.

2. Materials and methods

2.1. Chicken samples

Raw, fresh chicken wings were obtained from a local poultry slaughterhouse. The chicken wings were transported to the laboratory under refrigerated conditions (4 °C) within 3 h after slaughter and kept at 0 °C \pm 0.5 °C overnight.

2.2. Antimicrobial treatments

Three types of antimicrobials applied as single treatments or as consecutive, combined treatments of raw, fresh chicken were used. The three antimicrobials were Purac FCC80 (L-lactic acid; Corbion, Amsterdam, The Netherlands), Verdad N6 (a white distilled vinegar produced by fermentation; Corbion) and Provian K (potassium acetate/diacetate, Niacet, Tiels, The Netherlands). The Purac FCC80 (hereafter termed lactic acid) was diluted in sterile water and pH-adjusted with 10 M NaOH to provide in-use solutions of 2.5% and 5%, both with pH of 3.0 and 3.9. In-use solutions of Verdad N6 (2.5%; 5%) and Provian K (5%) were prepared by solubilization of the salts (w/w) in sterile water. The solutions were freshly made and kept at 4 °C or 50 °C according to use.

Prior to antimicrobial treatments, the chicken wings were manually mixed to obtain an approximately equal distribution of microorganisms per square centimeter on the meat surface within the complete batch of chicken wings. Antimicrobial treatments were done by immersion of the chicken wings in solutions of lactic acid, Verdad N6 and Provian K. Different treatment scenarios were included, reflecting treatments at different sites in the poultry slaughter line. These included treatment of freshly slaughtered chicken prior to refrigeration (termed Prechill treatment), treatment of slaughtered chicken after refrigeration (termed Postchill treatment) and combinations of Prechill and Postchill treatments (termed Combined treatment). Prechill treatments were performed with solutions at 50 °C and performed in 50 °C water bath with chicken wings preheated to 37 °C to reflect the temperature of a freshly slaughtered chicken at the slaughter line. Postchill treatments were performed with 4 °C solutions on refrigerated, fresh chicken wings.

not otherwise stated, 30 s exposure time per solution applied was used before the wings were gently shaken to remove excess of liquid solution. Up to 36 chicken wings (approximately 1.1-1.2 kg) were treated per liter of solution before these were discarded and replaced by fresh solutions for further treatments. When combined treatments were used, the wings were kept at 4 °C for 2 h between Prechill and Postchill treatments to reflect the cooling step in poultry processing. Control samples were not treated. Prior tests with immersions in sterile water showed the immersion procedure to have negligible effects on bacterial reductions (not shown). Details of the total of nine different single antimicrobial treatments (Table 1) and eight combined treatments (Table 2) are shown.

2.3. Packaging and storage conditions

Samples of treated wings and controls were packed under three atmospheric conditions; vacuum and two MAP conditions; 60% CO₂ and 40% N₂ (termed High CO₂) and 75% O₂ and 25% CO₂ (termed High O₂). Vacuum packaging was done by placing chicken wings in vacuum bags consisting of polyamide/polyethylene (PA/PE) (Allfo, Waltenhofen, Germany; oxygen transmission rate 50 cm³/m²/day, at 23 °C, 75% RH) on an Intevac IN30machine (Intevac Verpackungsmaschinen, Wallenhorst, Germany). MAP packaging was performed on a Multivac T200 tray sealing machine (Multivac, Wolfertschwenden, Germany) by using food grade gas mixtures (Linde, Oslo, Norway). Trays of polypropylene (P2187-1Q Natur PP; Færch plast, Denmark) were sealed with a top film (Bialon 65 PP, Wipak Biaxer, Wipak Oy, Finland). Oxygen transmission rates (OTR) for the top film was 7 $\text{cm}^3/\text{m}^2/24$ h at 4 °C and 50% relative humidity (RH). OTR for the complete package of tray and top web was measured by the Ambient Oxygen Ingress Rate (AOIR) method (Larsen et al., 2000; Larsen and Liland, 2013) to 1.31 \pm 0.05 cm³/m²/day at 4 °C, 70% RH. The gas to meat ratio was approximately 3.6. All samples were stored at 4 °C.

2.4. Gas analyses

The atmosphere (levels of CO_2 and O_2) of MAP-packed samples were checked immediately after packaging (three to five random samples for each MAP condition) and at day of sampling (one package per treatment). The CO_2 and O_2 concentrations were determined using a CheckMate 9900 instrument (PBI Dansensor, Ringsted, Denmark).

2.5. Culture dependent and independent microbial analyses

Total counts were recorded at day 0 (untreated control samples only), 1, 7, 14, and 20 after treatment if not otherwise stated. Each sample of chicken wing was transferred to a stomacher bag and added 90 ml of peptone water. The samples were stomached for 1 min and appropriate 10-fold dilutions in peptone water was plated on Plate count agar (PCA) and incubated aerobically at 15 $^{\circ}$ C for 5–7 days.

Tabl	le 1
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Single	treatments	of	chicken	wings.
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Treatment	Compound ^a	Concentration (%)	pН	Treatment temperature (°C)
S1	Verdad N6	2.5	6.0	Postchill (4)
S2	Verdad N6	5.0	6.0	Postchill (4)
S3	Provian K	5.0	5.7	Postchill (4)
S4	Lactic acid	2.5	3.9	Prechill (50)
S 5	Lactic acid	2.5	3.0	Prechill (50)
S6	Lactic acid	5.0	3.9	Postchill (4)
S7	Lactic acid	5.0	3.9	Prechill (50)
S8	Lactic acid	5.0	3.9	Postchill (4)
S9	Lactic acid	5.0	3.0	Prechill (50)

^a Lactic acid treatments were performed using Purac FCC80 (L-lactic acid; Corbion, Amsterdam, Netherlands).

Microbiota profiling using high-throughput sequencing of bacterial 16S rRNA gene amplicons (MiSeq, Illumina) was performed on selected samples of stored chicken wings. Sampling for profiling was performed on day 20 or on the first sampling day (day 24 or day 30) after bacterial levels exceeded log 9 CFU/sample to determine the dominating bacteria. For sample preparations, 1 ml of stomacher solution was centrifuged at 13,000 $\times g$ for 5 min and the pellets stored at -20 °C. DNA was extracted from thawed pellets using the DNeasy PowerSoil HTP-96 kit according to the manufacturers protocol (Qiagen, Hilden, Germany) and cell lysis in FastPrep-96 homogenizer (MP Biomedicals, Solon, OH) at 1600 rpm for 2×1 min. PCR was performed in triplicates with amplification of the V4-V5 region of the 16SrRNA gene using region specific primers according to Caporaso et al. (2011, 2012) with redesign performed by Parada et al. (2016) and Apprill et al. (2015). The forward primer was redesigned with a 12-base barcode sequence that supported pooling of different samples (Walters et al., 2016). The triplicate samples were pooled and purified with AMPure XP (Agencourt Bioscience Corporation, Beverly, MA, USA) and quantified by the Quant-iT Picogreen dsDNA Assay (Invitrogen, Life Technologies, Dynal AS, Oslo, Norway) before pooling. The sample pool was purified and quantified as described above, diluted to 4 nM and sequenced using the MiSeq Reagent Kit v3 on a MiSeq (Illumina) following the protocol provided by Illumina using 6.3 pM sample. In addition to the experimental samples, the MiSeq run also contained a control library made from PhiX Control v3, which, in this run, accounted for 24% of reads. The MiSeq Control Software (MCS) version used was RTA 1.18.54. Paired end sequencing $(2 \times 151 \text{ bp})$ was performed using sequencing primers Read1 seq. primer, Read2_seq.primer and Index_seq.primer according to the protocol of Walters et al. (2016).

The sequences were processed in QIIME2 Studio (qiime2-2019.1; Bolyen et al., 2019). Briefly, the data were: demultiplexed using demux, paired ends were joined using vsearch (Rognes et al., 2016), quality filtered based on a q-score above 30, denoised using deblur, and taxonomy was achieved using classify-sklearn with the Greengenes 16S 13_8 database (Amir et al., 2017; Bokulich et al., 2018; McDonald et al., 2012; Pedregosa et al., 2011). The level 6 (genus) table derived from Qiime2 Studio was used for bar chart illustrations. The relative frequency table was exported to a text file and further processed in Excel.

2.6. Chemical analyses

The pH of raw chicken wing samples was measured in the stomached solution using a sensION + pH 31 pH meter (Hach Company, Loveland, CO, USA).

2.7. Sensory analyses

Sensory analyses were performed to determine effects of selected antimicrobial treatments of chicken wings on relevant sensory quality parameters. A highly trained panel of ten assessors (women; aged 37–64 years) at Nofima (Ås, Norway) performed sensory descriptive analysis (DA) according to the "Generic Descriptive Analysis" as described by (Lawless and Heymann, 2010) and the ISO standard 13299. The assessors are regularly tested and trained according to ISO standard 8586, and the sensory laboratory follows the practice of ISO standards 8589.

Sensory analyses were performed on both raw (odour, visual appearance) and heat-treated (odour, flavour/taste) wings. Sensory analyses of raw chicken were performed on wings stored at 4 °C in High CO_2 for 6 and 13 days after treatment. For raw chicken, twelve treatments were evaluated using thirteen sensory attributes (total odour, sourness odour, acetic odour, ammonia odour, sweet odour, cloying odour, sour/fermented odour, sulphur odour, burnt odour, colour hue, colour strength, whiteness, glossiness; Supplemental Table S1). Each assessor was served a single chicken wing at refrigerated temperature. This test was a screening with one replicate, with the intention of selecting samples for the heat-treated test.

Table 2		
Combined	treatments of chicken	wings.

Treatment	Treatment #1 (Prechill, 50 °C)			Treatment #2 (Postchill, 4 °C)		
	Compound ^a	Concentration (% ^b)	pH	Compound	Concentration (%)	pH
C1	Lactic acid	2.5	3.9	Verdad N6	5.0	6.0
C2	Lactic acid	2.5	3.9	Provian K	5.0	5.7
C3	Lactic acid	2.5	3.0	Verdad N6	5.0	6.0
C4	Lactic acid	2.5	3.0	Provian K	5.0	5.7
C5	Lactic acid	5.0	3.9	Verdad N6	5.0	6.0
C6	Lactic acid	5.0	3.9	Provian K	5.0	5.7
C7	Lactic acid	5.0	3.0	Verdad N6	5.0	6.0
C8	Lactic acid	5.0	3.0	Provian K	5.0	5.7

^a Lactic acid treatments were performed using Purac FCC80 (L-lactic acid; Corbion, Amsterdam, Netherlands).

^b Concentration of lactic acid in user solution.

Sensory analyses of heat-treated wings were evaluated on wings stored at 4 °C in High CO2 for 6 days after treatment. For heat-treated chicken, seven treatments were evaluated for 22 sensory attributes: (total odour, sourness odour, sweet odour, acetic odour, ammonia odour, cloying odour, sour/fermented odour, sulphur odour, burnt odour, total flavour, sourness taste, sweet taste, salt taste, bitter taste, acetic taste, ammonia taste, cloying taste, fermented taste, sulphur taste, burnt taste, metal taste, rancid taste; Supplemental Table S1). In a pretest session, the assessors were calibrated on two samples that were considered the most different. The wings were served in white plastic beakers covered with a metal lid. For heat-treated chicken evaluation, each assessor was served twice with one wing per treatment. Heat treatment of chicken wings were performed in a combi-oven (Electrolux Air-o-steam, Model AOS061EANQ) at 200 °C for 20 min. The chicken wings had a core temperature of 75 °C. Samples were served in preheated porcelain bowls covered with a warm metal lid. The samples were placed on a hot plate for keeping them warm until assessing. All attributes were evaluated and graded on a continuous non-structured scale ranging from the lowest intensity (value 1.0) to the highest intensity" (value 9.0). Each assessor evaluated all samples at individual speed on a computer system for direct recording of data (EyeQuestion, Software Logic8 BV, Utrecht, the Netherlands). All samples were served to the panel coded with a three-digit number in duplicates following a balanced block design. Tap water and unsalted crackers were available for palate cleansing.

2.8. Statistics

Effects of antimicrobial treatments on bacterial growth: Two to four biological replicates were performed for all factor combinations (treatments and storage conditions). Each biological replicate was performed on different days with separate batches of chicken wings and included three parallels (six for untreated controls) for each factor combination. According to variations in the initial microbial levels present on the chicken between biological replicates, the day 0 levels were normalized between the replicates to allow comparative analyses of bacterial growth during storage: if y'(t, d, r) is the logarithm of total counts per sample at day *d* for replicate *r* and treatment *t*, then the normalized values *y* are given by y(t, d, r) = y'(t, d, r) - y'(t, d = 0, r).

For all statistical tests, a significance level of $\alpha = 0.05$ was used, meaning that tests were considered statistically significant for *P*-values < 0.05. Two approaches were used to determine statistically significant effects on bacterial growth of treated versus untreated control chicken: 1) paired *t*-test to compare the effect of a treatment at a given day and 2) a linear model describing growth during days 7 and 14. Before day 7 there was a variable lag time before onset of growth and after day 14 the bacterial levels approached the stationary phase. The linear model is a two-way effects model with "days" and "treatment" as the two ways, expressed as:

 $y(t, d, r) = y_c + \tau_t + \beta_d + (\tau\beta)_{t,d} + \epsilon_{t,d,r}$

where y_c is the value of the control at day 7, τ_t is the effect of treatment t, β_d is the effect of growth from day 7 to day 14, $(\tau\beta)_{t, d}$ is the interaction between treatment and growth and finally the error term $\epsilon_{t, d, r}$ for each treatment, day and replicate is assumed to be an independent, identically distributed, zero-mean, Gaussian noise term with variance σ^2 . The linear model relies on the assumption of equal variance in the error term (homoscedasticity). Levene's test of equal variance was used to verify this assumption. This assumption was discarded in some cases, and the above linear model was not considered. Measured values for bacterial growth and standard deviations at each sampling day are provided in Supplemental Table S2. To address the effect of atmosphere in addition to the two-ways model above, we calculated the average effect between atmospheres using estimated marginal means (R package emmeans) where differences are less sensitive to imbalances. If $\overline{y}_{a}(t,d)$ is the average of the logarithm of total counts per sample for the atmosphere a, treatment t and day d, then using estimated marginal means amounts to calculating the average difference between these means for the remaining factor combinations. If $z_a(d) = 1/N \sum_t \overline{y}_a(t, d)$ where *a* is the atmosphere and N is the number of factor combinations, then the differences $z_{vacuum}(d) - z_{O2}(d)$ or $z_{vacuum}(d) - z_{CO2}(d)$ were considered. Such an approach provided a single, average effect of atmosphere over treatments for each day 7 and 14.

Effects of antimicrobial treatments on sensory properties: For the heat-treated chicken wings, eight groups were assessed in two repetitions. The data were modelled in a two-way ANOVA test using F-tests if there were significant differences between the groups for each of the sensory properties (5% level of significance). For those properties where the F-test is significant, Tukey's multiple comparison test was also performed to determine which samples are different. If the difference between two mean values is greater than the critical value calculated by the test, it means that these two groups are significantly different. The results are summarized using mean values, standard deviations and *p*-values. The mean values are an average of assessors and two replicates. The raw chicken wings were assessed in one repetition as a screening with mean values and standard deviations of assessors' score of one replicate reported.

3. Results

3.1. Total counts in untreated raw chicken

Fresh, raw chicken wings were obtained directly from a local slaughterhouse and stored at 0 $^{\circ}$ C overnight prior to antibacterial treatments (day 0). Total viable counts (TVC) on fresh, raw chicken wings of four production batches from different time periods showed variations in the range 4.6–5.4 log/sample with an average level of 5.21 log CFU/sample (wing) corresponding to 3.7 log CFU/g prior to the antibacterial treatments (day 0). According to these TVC differences at day 0, total counts were normalized to allow comparison of different treatments and storage effects among all batches. Total counts were

therefore recorded as an increase or decrease in bacterial counts per sample relative to the zero value which was the bacterial counts determined prior to treatment and storage at day 0. Setting the spoilage limit to 7 log CFU/g means that spoilage is reached after approx. 3.3 log growth. This spoilage limit is indicated on Figs. 1 through 5.

For untreated chicken, bacterial growth was dependent on the storage condition (Fig. 1). MAP with High CO₂ and High O₂ was able to significantly delay bacterial growth (p < 0.001) giving an increase of 0.9 and 1.7 log after seven days of storage, respectively, compared with vacuum storage (3.2 log increase). Significantly lower bacterial levels (p= 0.004) were also observed after 14 days storage in High CO₂ compared with vacuum. After 20 days of storage, total counts were close to the stationary phase (>9 log CFU/sample) for all storage conditions.

3.2. Antimicrobial effects of single treatments of chicken wings

To determine if the bacterial levels on chicken meat could be controlled by treatments with a natural fermentate (Verdad N6; a white distilled vinegar), samples were treated with 2.5% or 5% Verdad N6 followed by packaging and storage under three conditions (Fig. 1). Results showed reduced initial growth and overall reduced bacterial levels in the treated chicken wings compared with untreated controls. The growth inhibitory effects were dependent on levels of Verdad N6, the immersion/exposure time (not shown), storage time and storage atmosphere. Complete growth inhibition was observed for 5% Verdad N6 treated chicken the first seven days of storage at 4 °C under MA conditions (High-CO2 and High-O2). Bacterial growth resumed in treated samples beyond seven days storage, but with levels 1.0-1.5 log lower compared with untreated controls after 14 days and with lower average levels for chicken treated with 5% Verdad (p < 0.05). Storage in MA compared to vacuum provided significant effects on bacterial levels and with High CO₂ having more growth inhibiting effects than High O₂ (Supplemental Table S3) After 20 days MA storage, the differences in bacterial counts between Verdad N6 treated and untreated chicken wings were less pronounced (0.4-0.9 log difference).

Treatments with Provian K solutions provided bacterial inhibitory effects comparable to those of Verdad N6 at the same solute concentration (5%; Fig. 2). Combinations of 5% Provian K treatments and High-CO₂ or High-O₂ MAP also provided complete growth inhibition during the first seven days of storage. The observed differences in bacterial counts between treated and untreated chicken meat stored under the same condition at day 7, day 14 and day 20 were in the range 0.9–2.6 log, 0.8–1.8 log and 0.7–1.2 log, respectively. The average bacterial count values were generally lower in High CO₂ stored samples than in vacuum and High O₂ samples although with no statistically significant differences obtained between the two MAP conditions (p > 0.05).

Treatments with Verdad N6 and Provian K clearly showed bacterial growth inhibitory effects. This gave estimated shelf-life extension of approx. six to seven days providing a shelf-life of about 20 days for the most effective treatments and storage regimes according to the 3.3 log increase in TVC needed to reach the spoilage limit of 7 log/g (Figs. 1 and 2). However, these treatments provided no bactericidal effects and also showed somewhat limited effects on TVC during extended storage (up to 20 days). We therefore evaluated the effects of potential bactericidal treatments using lactic acid applied either at elevated temperature (50 °C) on prewarmed (37 °C) chicken wings (termed Prechill treatment) or using cold (4 °C) solutions on cooled chicken (termed Postchill treatments). The Prechill and Postchill treatments were applied to reflect treatment of freshly slaughtered chicken prior to cooling and after the cooling step in chicken processing, respectively.

Bactericidal effects of Prechill lactic acid treatments indicated up to 0.9 log reductions in bacterial levels the day after treatment (day 1) with higher reductions obtained using high concentration (5.0%) and low pH (3.0) LA (Fig. 3). Although bacteria surviving the treatment were able to grow during storage, nearly all treatments showed statistically significant effects (p < 0.05) on bacterial levels. This indicated that a shelf-life beyond 20 days could be possible for the most effective treatment and storage atmosphere used in this study (Fig. 3B). Similarly, nearly all Postchill treatments showed significant effects on bacterial levels during storage although initial bactericidal effects at day 1 (until 0.3 log) appeared marginal (p > 0.05) (Supplemental Fig. S1).

3.3. Antimicrobial effects of combined treatments of chicken wings

Double treatments combining bactericidal lactic acid applied Prechill using warm lactic acid (50 °C) followed by Postchill treatment with cold (4 °C) Verdad N6 prior to packaging and storage under three conditions (4 °C) indicated initial bacterial reductions up to log 1.1 the day after treatment (day 1) followed by growth inhibition during storage (Fig. 4). Growth inhibition was stimulated by the low pH (pH 3.0 and pH 3.9) LA treatments and was also dependent on the level of the fermentate. The most prominent inhibitory effects were obtained using LA 5.0%, pH 3.0 followed by 5.0% Verdad N6 Postchill treatments and storage at High-CO₂. For this treatment, no growth was recorded the first seven days of storage and bacterial levels remained below those present on the fresh untreated chicken meat at day 0 for more than 10 days (Fig. 4B). During the storage period, bacterial levels in untreated control samples increased in the range 3.5–4.3 log under the three storage conditions used.

Results obtained for combined Prechill lactic acid and Postchill Provian K treatments were comparable with those of the combined Prechill lactic acid and Verdad N6 treatments (Fig. 5). Initial bacterial reductions (up to log 0.9 at day 1) were followed by effective bacterial growth inhibition the first seven days under all storage conditions. Further storage gave only limited growth in chicken stored for 14 days under MAP conditions while approximately 2.0-log total increases in bacterial levels were apparent after 20 days of storage. For all combined lactic acid and Provian K treatments, bacterial levels in chicken stored

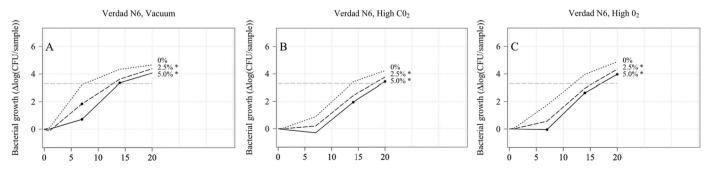


Fig. 1. Total bacterial growth on chicken wings treated with Verdad N6 (2.5% or 5%) and untreated controls (0%) and stored under Vacuum (A), High CO_2 (B) and High O_2 (C). Average values are shown. Values statistically different from untreated control at given sampling days are marked with a dot. Treatments providing statistically different effects on bacterial growth relative to the untreated control for days 7 and 14 are also shown (*). The dashed horizontal line indicates the spoilage limit.

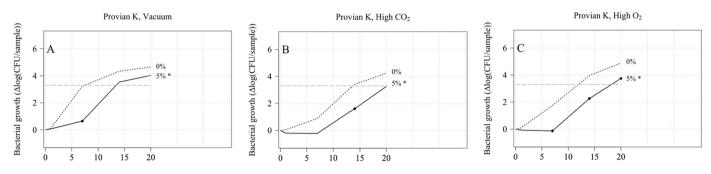


Fig. 2. Total bacterial growth on chicken wings treated with Provian K (5%) and untreated control (0%) and stored under Vacuum (A), High CO_2 (B) and High O_2 (C). Values statistically different from untreated control at given sampling days are marked with a dot. Treatments providing statistically different effects on bacterial growth relative to the untreated control for days 7 and 14 are also shown (*). The dashed horizontal line indicates the spoilage limit.

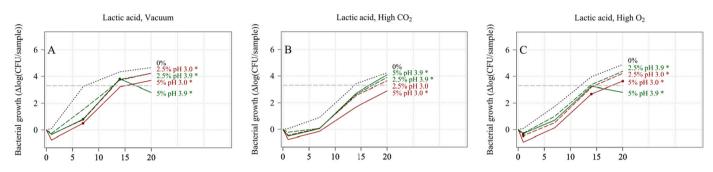


Fig. 3. Total bacterial growth on chicken wings treated with lactic acid (2.5% or 5% at pH 3.0 or 3.9) at 50 °C (Prechill treatments) and untreated control (0%). The chicken wings were stored under Vacuum (A), High CO₂ (B) and High O₂. Values statistically different from untreated control at given sampling days are marked with a dot. Treatments providing statistically different effects on bacterial growth relative to the untreated control for days 7 and 14 are also shown (*). The dashed horizontal line indicates the spoilage limit.

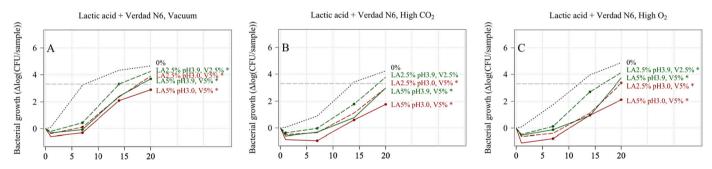


Fig. 4. Total bacterial growth on chicken wings treated with lactic acid at 50 °C (Prechill treatment) and Verdad N6 at 4 °C (Postchill treatment) and stored under vacuum (A), High-CO₂, (B) and High-O₂ (C). Chicken wings were immersed in solutions of lactic acid (2.5% or 5% at pH 3.0 or 3.9) and cooled for 2 h prior to treatments in Verdad N6 (2.5% or 5%) solutions. Values statistically different from untreated control at given sampling days are marked with a dot. Treatments providing statistically different effects on bacterial growth relative to the untreated control for days 7 and 14 are also shown (*). The dashed horizontal line indicates the spoilage limit.

for 20 days were below the levels of non-treated control samples stored for 14 days under the same conditions (p < 0.05). The most effective combined LA and organic acid salt treatments indicated a shelf-life well beyond 20 days under all applied storage conditions compared to the approx. seven to 13 days shelf-life obtained for non-treated chicken (Fig. 5).

3.4. Changes in gas atmospheres and pH during storage

High-CO₂ packages showed a decrease in measured CO₂ during storage from day 0 (56.5%) to day 20 (41.0%) with the most pronounced drop during the first day of storage. This is mainly due to CO₂ being dissolved in the meat during storage. Oxygen levels were low (0.02% to 0.7%) during the storage period. For packages with High-O₂, levels of O₂ (range 71.3–74.6%) and CO₂ (19.2–22.8%) remained relatively stable

throughout the 20 days storage period. The antimicrobial treatments had no significant effect on the gas composition during storage.

The pH of untreated control chicken wings remained stable (range pH 6.5–6.6) irrespective of storage condition during the 20 days storage period. Only slight changes in pH were also observed in the Verdad N6 (pH 6.3–6.6) or Provian K (pH 6.3–6.4) treated chicken for 20 days storage. The lactic acid treatments provided a pH drop to pH 5.8–6.2 at day 1 but values increased to pH 6.0–6.6 after 20 days storage. In the combined Prechill and Postchill treatments we observed reduced pH levels (pH 5.6–6.1) the day after treatment (day 1) according to the prechill lactic acid treatments. After 20 days storage, pH levels were in the range 6.1–6.4.

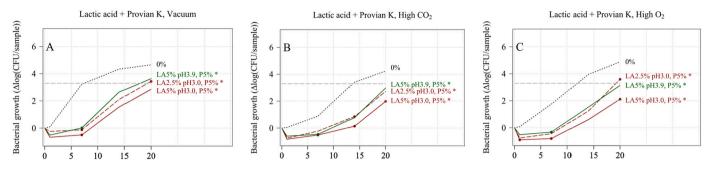


Fig. 5. Total bacterial growth on chicken wings treated with lactic acid at 50 °C (Prechill treatment) and Provian K at 4 °C (Postchill treatment) and stored under vacuum (A), High-CO₂, (B) and High-O₂ (C). Chicken wings were immersed in solutions of lactic acid (2.5% or 5% at pH 3.0 or 3.9) and cooled for 2 h prior to treatments in Provian K (5%) solutions. Values statistically different from untreated control at given sampling day are marked with a dot. Treatments providing statistically different effects on bacterial growth relative to the untreated control for days 7 and 14 are also shown (*). The dashed horizontal line indicates the spoilage limit.

3.5. Microbiota of chicken wings after antimicrobial treatments and storage

Bacterial community analyses at the end of storage showed that only a few genera dominated for each treatment and storage condition of chicken wings (Fig. 6). The bacterial composition was clearly dependent on both antibacterial treatment and storage condition. In vacuum-stored chicken, Pseudomonas was abundant irrespective of treatments with Verdad N6, Provian K, lactic acid or combinations. Morganella was abundant in chicken stored in High CO2. For High O2 stored chicken, Pseudomonas and Acinetobacter were dominant. In untreated controls, significant proportions of Shewanella and bacteria within the families Enterobacteriaceae and Aeromonadaceae were also present under all three storage conditions. However, treatments with the organic acid salt compounds Verdad N6 and Provian K provided a shift with increased relative levels of the Gram positives Vagococcus, Carnobacterium, Lactobacillales and Brochothrix and diminished levels of Gram negative Shewanella, Enterobacteriaceae and Aeromonadaceae. Single treatments with lactic acid provided more limited shift in the microbiota composition compared with untreated samples. Combined treatments with lactic acid and Verdad N6 or Provian K showed Morganella and Pseudomonas to remain dominant bacteria under vacuum storage. At MA conditions (High CO₂ or High O₂) combined treatments provided further stimulation of lactic acid bacteria and with reduced relative levels of Acinetobacter at High O₂ MAP conditions.

3.6. Sensory analyses of antimicrobial-treated chicken wings

For raw chicken wings, the sensory effects of 12 different antimicrobial treatments (including non-treated control) were evaluated. The test was performed as a screening with one replicate to determine overall sensory effects on raw samples and to select treatments to be included in sensory analyses of heat-treated chicken. The analyses were performed on raw chicken wings stored for both 6 and 13 days after treatment (Fig. 7, Supplemental Table S4). Overall, changes in odour attributes were most apparent while changes in appearance were minor (not shown). After six days of storage, small differences (<1-2 intensity score units) were obtained for nearly all odour attributes. One exception was for the attribute Vinegar odour where samples treated using Verdad N6 and Provian K showing intensity scores in the range 2.3-3.8 compared with 1.2 for the controls. Sensory analysis of chicken wings stored for 13 days showed prominent differences in several odour attributes between treated samples and untreated controls. Treated samples showed intensity scores ranging 1.8-5.1 units lower than those of untreated controls for the attributes Total odour, Ammonia odour, Sweet odour, Cloying odour, Sour/fermented odour and Sulphur odour. The highest intensity scores of controls were for Cloying odour (7.1) and Total odour (6.9). There were generally small changes in sensory scores

within antimicrobial treated samples stored for six versus 13 days although with lactic acid (pH 3.9) treated samples closest to the untreated control for several of the odour attributes after 13 days storage.

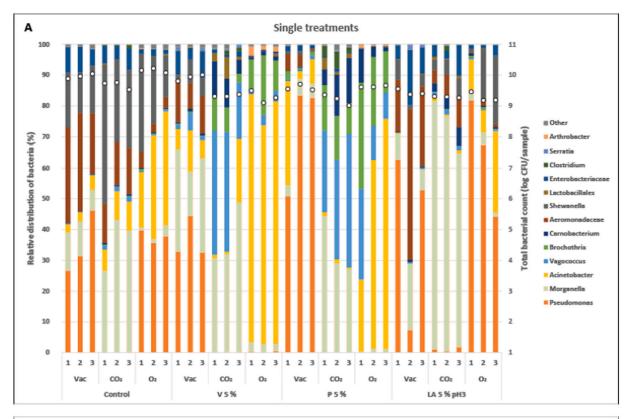
Due to the acceptable sensory quality provided and small differences in sensory scores for raw chicken wings subjected to the different treatments, the antimicrobial treatments for sensory analyses of heattreated samples were selected based on the antibacterial effects. For the seven treatments included, statistically significant differences (p < 0.05) between samples were obtained for five sensory attributes out of 22, while differences between treated samples and untreated control samples were only obtained for two attributes (Fig. 8, Supplemental Table S5). Vinegar odour gave a higher intensity score for combined treatments with lactic acid (pH = 3) and Verdad N6 or Provian K (3.3–3.5) compared with 1.8 for untreated control (p < 0.001).

4. Discussion

The range of initial bacterial counts from the four chicken wing batches investigated were 3.1–3.9 log/g (average 3.7 log/g). These levels were lower than those of previous studies reporting ranges of 4.1–5.7 log/g in freshly processed raw chicken and approximately 7 log CFU/g in processed products (Al-Nehlawi et al., 2013; Alvarez-Astorga et al., 2002; Capita et al., 2013; Chouliara et al., 2007; Samapundo et al., 2019). Reduced bacterial contamination during processing in addition to strategies preventing bacterial growth and metabolism during storage should have the potential to extend the quality of chicken products beyond the reported 4–10 days shelf-life under chilled storage (Al-Nehlawi et al., 2013; Bolton et al., 2014; Chen et al., 2020; Meredith et al., 2014).

Microbial contamination and growth during storage is one of the most important factors contributing to quality loss of chicken meat (Rouger et al., 2017). The critical spoilage level as an indication of end of shelf-life of raw chicken is often set at total counts of 7 log CFU/g (Nychas et al., 2008; Okolocha and Ellerbroek, 2005). Katiyo et al. showed high correlations between odour and microbial growth on chicken legs (Katiyo et al., 2020). Negative sensory attributes along with total counts exceeding 8 log CFU/g were obtained for aerobic, 4 °C storage longer than seven days. At such high bacterial levels, other deterioration effects including slime formation, compromised meat texture and off-flavours provided by spoilage organisms with proteolytic and lipolytic properties may occur (Borch et al., 1996; Nychas et al., 2008; Wang et al., 2017a).

In the current study, vacuum storage provided an increase in bacterial levels of 3.2 log during the first seven days of storage, thus reaching levels close to the microbial spoilage level of 7 log. Under High CO_2 MAP conditions, a prolonged lag phase was observed with CO_2 providing increased generation times (Holck et al., 2014; Patsias et al., 2008), particularly for aerobic bacteria. At High O_2 MAP, reduced



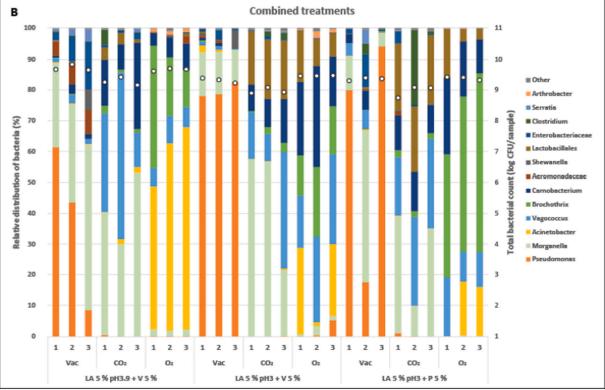


Fig. 6. Total bacterial counts and relative abundance of bacterial genera in untreated and treated chicken wings after storage under vacuum (Vac), High CO_2 (CO_2) and High O_2 (O_2). (A): Chicken wings subjected to no treatment (Control) and single treatments of 5% Verdad N6 (V), 5% Provian K (P), and 5% lactic acid (LA). (B): Chicken wings subjected to combined treatments with 5% lactic acid (LA) pH 3.9 or pH 3.0 and Verdad N6 (V) or Provian K (P). Only taxa with average over all samples above 0.2% or max value above 2% are represented. The remaining taxa are represented as "Other". The taxa are coloured according to family or genus affiliation. The dashed horizontal line indicates the spoilage limit.

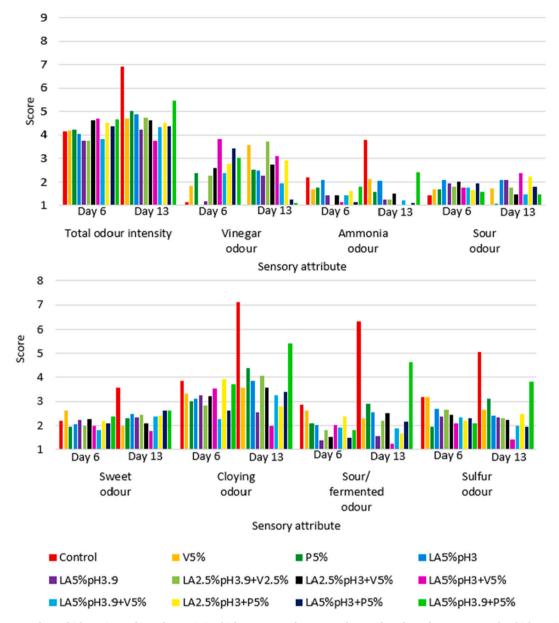


Fig. 7. Sensory scores of raw chicken wings subjected to antimicrobial treatments after storage for 6 and 13 days after treatment. The chicken wings were treated with Verdad N6, Provian K, lactic acid or combinations of lactic acid and Verdad N6 or Provian K and stored in High CO₂ at 4 °C until sensory analyses. Mean scores of odour sensory attributes are shown.

growth was also evident. Thus, bacterial levels did not exceed 7 log/g until 12–14 days storage at 4 °C at High CO_2 or High O_2 conditions. These data corroborate studies reporting shelf-life of 14 days under refrigerated MA storage (Jimenez et al., 1997; Patsias et al., 2008; Rokka et al., 2004). The effect of MAP on shelf-life extension depends on several factors including the microbial quality of the meat, microbiota composition and characteristics, storage temperature and packaging properties.

Several reported strategies intended to control microorganisms in poultry meat (e.g. steaming, irradiation, cold plasma, high hydrostatic pressure, chlorine wash, ozone, phosphate-based compounds, peracetic acid) have their limitations due to e.g. health issues, negative sensory effects, limited antibacterial effects and consumer acceptance (see reviews of Loretz et al., 2010; Lu et al., 2019; Silva et al., 2018; Soro et al., 2020). Organic acid salts are natural antimicrobial products that include "clean-label" products that make them particularly interesting for use by the food industry. Organic acid salts and fermentates show *Listeria*inhibiting effects on salmon (e.g. Heir et al., 2019, 2021; Neetoo et al., 2008; Tang et al., 2013), but no studies are available on the effect of pHneutral salts of acetic acids and diacetates on the indigenous flora of fresh poultry.

In the current study, the commercially available "clean-label" fermentate Verdad N6, and the potassium acetate/diacetate based Provian K provided growth inhibition by extending the lag time before bacterial growth resumed. Thus, the bacterial spoilage level of 7 log was not reached until days 14–20 after these treatments with best antimicrobial effects obtained in combination with storage at High CO_2 MA conditions. The similarities in inhibitory effects of the acetate rich Verdad N6 and Provian K were as expected.

While no significant bactericidal effects were observed using Verdad N6 and Provian K, treatments with lactic acid showed tendencies of bactericidal effects depending on acid concentration, pH and exposure time and temperature. This agrees fairly well with previous concentration dependent reductions of 0.17–2.48 log (Bolton et al., 2014, and references therein).

The most effective bacterial control was obtained by combined

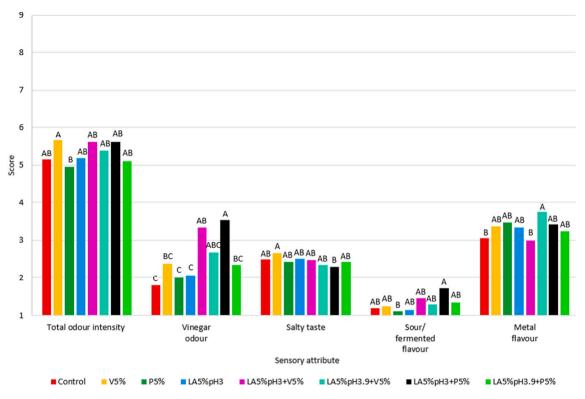


Fig. 8. Sensory scores of heat-treated chicken wings subjected to antimicrobial treatments, analysed in a descriptive analysis (DA). The chicken wings were treated with Verdad N6 (V), Provian K (P), lactic acid (LA) or combinations of lactic acid and Verdad N6 or Provian K and stored in High CO_2 at 4 °C for 6 days after treatment. Only attributes which gave statistically significant differences between treatments are shown. Mean scores of sensory attributes of samples of chicken wings are shown. Samples with different letters indicate statistically different scores.

bactericidal and growth inhibiting treatments performed using Prechill lactic acid followed by Postchill Verdad N6 or Provian K. For these treatments, increase in bacterial levels after 20 days storage could be limited to 1.8-2.1 log meaning that bacterial levels after 20 days storage were below the 7 log/g spoilage limit for all storage conditions (vacuum, High CO₂, High O₂).

The main antimicrobial effect of the organic acids is caused by undissociated acids being able to penetrate the bacterial membrane and acidify the interior of the cell (Alakomi et al., 2000; Stanojevic-Nikolic et al., 2016). Thus, the lactic acid treatments were effective during an initial surface drop in pH, but lactic acid could not maintain the pH at low levels during storage for 20 days. Chicken meat and skin have pH buffering capacity reducing the antimicrobial effect of lactic acid treatments (Riedel et al., 2009). Also, the organic acids will diffuse throughout the meat during storage and thereby lowering the local surface concentration and increasing the pH with time. Acetic acid, being a weaker acid, therefore showed better inhibition during subsequent storage than lactic acid. Effects of lactic acid may vary with differences in treatments, concentrations, pH, exposure time, temperature, application method (spray, immersion) and the hygienic status of the poultry meat (Bolton et al., 2014; Gonzalez-Fandos et al., 2020; Okolocha and Ellerbroek, 2005). Since the death and growth curves for treatments with 5% pH 3.9, 2.5% pH 3.9 and 2.5% pH 3.0 are very similar (Fig. 3), it is difficult to determine the relative importance of pH and undissociated lactic acid from these experiments.

Optimal preservation and MA conditions for fresh poultry cannot be determined only by total CFU but depend on factors including sources and composition of the contaminating microbiota, its growth and spoilage characteristics and the effect of the applied strategies on sensory attributes. Studies applying high throughput sequencing to describe production processes, antimicrobial interventions and storage strategies on the microbiome of raw chicken meat are increasing (Chen et al., 2020; Dourou et al., 2021; Handley et al., 2018; Kim et al., 2017; Wang

et al., 2020). In the current study, dominant bacteria after storage included several psychrotrophic or psychrotolerant genera such as Pseudomonas, Acinetobacter, Shewanella, Carnobacterium, Brochothrix. Acinetobacter and Pseudomonas associated with aerobic growth, were dominating at High O2 MA conditions. Other genera like Shewanella were less affected and showed similar relative levels independent of storage under vacuum, High CO₂ or High O₂. Different MA conditions often affect spoilage characteristics and are also likely to be dependent on the innate microbiota present. The various antimicrobial treatments indicated a competition between different genera. This was particularly evident for treatments where Verdad N6 or Provian K were included. Of specific notice was the reduced relative levels of Shewanella in this chicken at all storage conditions. A recent study determined Shewanella to be among the dominant bacteria (along with Lactococcus and Carnobacterium) at the end of shelf life and in spoiled MA-stored chicken meat (Wang et al., 2017b). The same treatments provided increased relative levels of Gram-positive bacteria (Brochothrix, Carnobacterium, Lactobacillus, Vagococcus) under High CO2 atmosphere. Of note, Vagococcus fluvialis was the only bacterium to cause reduced levels of off-odours and extensions in sensory quality of salmon gravlax (Wiernasz et al., 2017). A recent review analysing quantitative effects of selected processing factors on meat spoilage pointed out possible advantages of removing O2 in packaging to delay spoilage and a protective role of lactic acid bacteria (Luong et al., 2020). In our study, Pseudomonas, a dominant spoilage organism in raw poultry (Nychas et al., 2008), showed reduced relative levels in the chicken treated by combinations of lactic acid and the organic acid salt compounds under MA storage. The results support potential advantageous effects of combining lactic acid/organic acid salt treatments and High CO2 storage on chicken meat spoilage development.

The variability in the microbial contaminants present in spoiled poultry meat was also illustrated by detection of genera not commonly found in other studies as well as the opposite — no detection of bacteria reported in other studies e.g. *Vibrio* (Nieminen et al., 2012). This suggests a high diversity of contaminating bacteria originating from the animal microbiota and the slaughterhouse and processing environments. Once contamination occurs, applied treatments and storage conditions shape the microbial dynamics (Chen et al., 2020; Rouger et al., 2017, 2018; Samapundo et al., 2019). Further studies are needed to direct the microbiota development towards a composition having less spoilage potential.

Acceptable control strategies should reduce spoilage-associated sensory attributes during storage and provide raw and heat-treated chicken with similar or enhanced overall quality. After six days of storage, only minor changes were observed, in line with the limited time for bacterial growth and off-odours to develop. After 13 days of storage, substantial intensity in several spoilage-associated odour attributes (e.g. total odour, ammonia odour and cloying odour) had developed for untreated samples, while no or minor odour changes were attained for the treated chicken. All treatments showed small effects on the appearance of the chicken wings after both six and 13 days of storage. For lactic acid, negative changes in flavour and colour (burnt appearance) have been reported at higher levels (8%) and low pH treatments (Burfoot et al., 2015). In the present study, all treatments were selected to minimize negative sensory effects.

The results further support that odour is a critical characteristic of perceived raw chicken meat quality with significant correlations between microbial quality and odour attributes (Katiyo et al., 2020; Sarfraz et al., 2021). They also indicate that the applied antimicrobial treatments have potential to substantially reduce spoilage odour attributes and prolong shelf-life. Treatments with the acetate-containing salts Verdad N6 and Provian K, provided somewhat increased Vinegar odour but still with low intensity scores; 1.8–3.8 on a 1–9 point scale. Odour intensity below 3 is considered low and is generally believed to be not recognizable by majority of consumers (Sarfraz et al., 2021).

For heat-treated chicken, only Vinegar odour (score up to 3.5) could be at levels recognized by the majority of consumers. The heat-treated chicken wings were served plain without any use of spices. Common consumer preparations would likely further mask any Vinegar odour obtained using acetate-containing salts. Odour affects consumer perception of raw chicken (Katiyo et al., 2020). The sensory results of the present study indicate that common negative odour attributes can be reduced during refrigerated storage of fresh chicken and that lactic acid and organic acid salt treatments applied provide small or negligible quality changes for both raw and cooked products.

The EC regulation No 853/2004 allows decontamination treatments to be considered if shown to be safe and effective and not employed to conceal poor hygiene practices. Lactic acid up to 5% is approved for decontamination of bovine carcasses and organic acid surface treatment of pork carcasses and cuts recently been evaluated in EU (EFSA, 2011, 2018). In the US, lactic acid and a large number of compounds and technologies have also been approved for poultry decontamination by the Food safety and Inspection Service of the U.S. Department of Agriculture (https://www.fsis.usda.gov/inspection/compliance-guidance/n ew-technology/new-technology-information-table). These have been assayed for antimicrobial efficacy, often towards Salmonella and Campylobacter, and verified for continuous effectiveness during implementation (Buncic and Sofos, 2012; Moore et al., 2017). Some of these strategies could be attractive for implementation in the worldwide poultry slaughtering process in order to reduce current contamination of carcasses and restrict microbial regrowth during storage. Approval in the EU of substances other than water will depend on thorough scientific evaluations by the European Food Safety Authority of public health risks involved in their use (EFSA, 2010).

The present study allowed only rough estimates on shelf-life with basis in the time required for total viable counts to reach the spoilage limit of 10^7 CFU/g (Bolton et al., 2014; Charles et al., 2006; Nychas et al., 2008). The shelf-life extension estimates using fermentates were in line with Desai et al. reporting eight days extension to a shelf-life of 20

days using buffered vinegar (Desai et al., 2014). Treatments with lactic acid showed greater TVC reductions at higher concentrations as reported by others (Bolton et al., 2014 and references therein). The enhanced effects using combinations of organic acids and/or organic acids salts to control microbial growth have also been demonstrated (Gonzales-Fandos et al., 2021; Zhu et al., 2016). Overall, treatments with lactic acid and fermentates provide improvement of the microbiological quality of raw chicken that further extend the shelf-life of poultry meat. However, comparisons and conclusions on the effects of these treatments on shelf-life extension is difficult as a number of variable parameters affecting microbial growth and spoilage are not evident in these studies.

In conclusion, treatments of raw chicken with lactic acid and organic acid salts/fermentates, provided enhanced bacterial growth control, microbial compositional changes and reductions in several spoilageassociated sensory attributes indicating potential for increased product shelf-life for treated raw chicken products. The study provides poultry processors with easy to implement bacterial control strategies thus promoting sustainable food production and consumption with potential for acceptance by consumers. The study also supports the benefit of combined intervention strategies along the poultry processing line to reduce microbial contamination thus contribute to reduction of food waste and consumer acceptance linked to poultry production and consumption. Although the evaluated interventions reduced the bacterial loads on poultry carcasses to some extent, decontamination treatments always must be considered part of an integral food quality and safety system.

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Declaration of competing interest

The authors declare that there is no conflict of interest.

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