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Journal Pre-proofs

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Title

Spoilage of fresh turkey and pork sausages: Influence of potassium lactate and modified atmosphere packaging

Journal

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Abstract

Fresh poultry and pork meat products represent highly perishable products which are susceptible to spoil within a few days after production. Lactate addition and modified atmosphere packaging are common preservation strategies used to overcome spoilage. This study aimed to identify the effects of these strategies and their possible interactions on spoilage indicators simultaneously on fresh pork and turkey sausages. Ten batches of raw meat (turkey or pork) sausages were industrially produced with different lactate concentrations (0, 1 or 2% w/w in turkey and 0, 0.57 and 1.13% w/w in pork), packed under different gas mixtures (air, MAP1: 70% O₂ – 30% CO₂ and MAP2: 50% CO₂ - 50% N₂) and chill stored during 22 days. Spoilage responses including enumeration of total aerobic mesophilic and lactic acid bacteria, measurement of pH and colour, evaluation of visual defects and off-odour, were monitored. Effects of lactate and modified atmosphere packaging (MAP) as well as random effect of the batch variability were studied using a mixed effect model. Despite initial batch variability, significant effects of lactate and gas packaging were observed but in a different way in turkey and pork. Our results suggest that for fresh turkey sausages, the gas mixture enriched in oxygen enhanced off-odour perception and sausage discolouration from red to dark grey / brown colour. Unlike turkey sausages, in pork sausages, lactate did not significantly influence the monitored spoilage responses, whereas MAP (70% O₂-30% CO₂) reduced the off-odour perception. The developed model could be useful to estimate the effect of preservation strategies on spoilage occurrence while considering industrial batch variability.

Keywords

Meat spoilage, Poultry sausages, Lactic Acid Bacteria, Off-odour, Off-colour, Acidification, Mixed-effect model, Biological Variability, Batch Variability

Highlights

- High oxygen atmosphere increases off-odour and discolouration of turkey sausages.
- Unlike turkey, lactate barely affects spoilage-associated responses in pork sausages.

- Spoilage dynamics of pork and turkey sausages vary greatly between production batches

Abbreviations

- CFU Colony Forming Unit
- LAB Lactic Acid Bacteria
- MAP Modified Atmosphere Packaging
- REML Restricted Maximum Likelihood
- TAMB Total aerobic mesophilic bacteria

Colour should be used for all figures in print

Journal Pre-proofs

1. Introduction

Fresh meat products represent highly perishable products since spoilage may occur within a few days following production. Spoilage is associated with food rejection and food losses because products become unacceptable for the consumer (Koutsoumanis, Stamatiou, Drosinos, & Nychas, 2008). Spoilage of meat products is mainly due to microbial growth and chemical defects such as lipid and protein oxidation. It results in sensory alterations with change of colour or texture and development of off-odours, slime, *etc.* (Erkmen & Bozoglu, 2016 ; Pellissery, Vinayamohan, Amalaradjou, & Venkitanarayanan, 2020). Meat products may be contaminated by microorganisms, especially bacteria, transmitted from intestinal track or skin of the animal as well as from the factory environment (water, air, soil), surfaces (storage bins, cutting knives, equipment) and human manipulations (clothing and hands) following slaughtering processes (Chaillou et al., 2015; Hultman, Rahkila, Ali, Rousu, & Bjorkroth, 2015) and also final operations such as slicing, mixing and grinding. Food manufacturers have to ensure food safety and quality by controlling microbial contamination through good hygiene practices and by limiting microbial growth. Deterioration of the meat quality associated with microbial growth (Borch, Kant-Muermans, & Blixt, 1996; Cervený, Meyer, & Hall, 2009; Gram et al., 2002) can vary greatly depending on the characteristics of the products, such as the meat matrices or the type of product (red/white or raw/processed meat) (Dave & Ghaly, 2011). Factors influencing microbial growth include intrinsic factors (natural composition and added ingredients, pH, redox potential and water activity), as well as extrinsic factors (storage temperature and atmosphere of the packaging) (Iulietto, Sechi, Borgogni, Beniamino, & Cenci-Goga, 2015 ; Pellissery et al., 2020). The influence of intrinsic factors (e.g. meat composition, ingredients, pH and a_w) as well as extrinsic factors (e.g. temperature, atmosphere) on meat spoilage has been recently reviewed (Pellissery et al., 2020). Spoilage generally results from the contamination and growth of several bacterial groups, their possible interactions, and their metabolic activities. Several strategies to preserve fresh products in order to overcome meat deterioration have been proposed (Abdel-Aziz, Asker, Keera, & Mahmoud,

2016; Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; Hasapidou & Savvaidis, 2011). They generally include the addition of ingredients like food preservatives, essential oils (Abdel-Aziz et al., 2016; Gammariello, Conte, & DelNobile 2015; Hultman et al., 2015; Pellissery et al., 2020), storage under chilled conditions and under modified atmosphere packaging (MAP) (Farber, 1991b; Genigeorgis, 1985). Nevertheless, the influence of organic salts as lactate for example, on spoilage occurrence has been poorly documented, with studies mainly focused on their effect on pathogenic species or yeasts (Houtsma, de Wit, & Rombouts, 1993; Linton, Connolly, Houston, & Patterson, 2014; Mataragas, Zwietering, Skandamis, & Drosinos, 2010). Moreover, to our knowledge, the influence of these hurdle strategies on spoilage occurrence, has not been studied simultaneously on fresh processed sausage products from different types of meat.

The aim of this work was to study the influence of both potassium lactate concentration and atmosphere packaging on several common spoilage-associated responses in two types of fresh sausages while considering process variability. Fresh turkey or pork sausages were produced on an industrial scale in two distinct French food companies following specific instructions regarding product formulation. The process variability was considered by monitoring ten batches for each sausage type, produced over a 6-month period. Including this variability in analyses of the effects of the preservations strategies on spoilage was possible with the use of an appropriate statistical treatment assuming a random effect of production batches.

2. Material and Methods

2.1 Data acquisition

The data and all the experimental protocols included in this study come from a large collaborative project between academic partners, technical centres, and two industrial producers, which have been deposited and published in a data paper (Poirier et al. 2020)

2.1.1 Sausage production and packaging

In France, raw sausages are made either with pork or poultry meat which are also the two meat types the most consumed. Since the industrial process of these two meat products were similar, we found it interesting to compare the influence of common preservation strategies on these two products. Turkey and pork sausages were produced by two French food companies. Each kind of sausages was provided from ten independent productions (batches) from July to December 2017. We considered that the variability of the meat quality which may be especially attributed to the animal diet, was encompassed in the global variability of the products which we decided to tackle by analysing ten different sausage batches. Each production was made with three formulations of potassium lactate. These formulations were respectively 2% (w/w), 1% (w/w) and 0% (w/w) in turkey sausages [turkey meat 75% (w/w), pork fat 15% (w/w), water, mix of salt and spices 10% (w/w)], and 1.13% (w/w), 0.57% (w/w) and 0% (w/w) in pork sausages (pork shoulder 80% (w/w), pork cutting fat 20% (w/w), sodium acetate 0.27%, sodium ascorbate 0.006%, spices]. The formulations of 2% (w/w) in turkey and 1.13% (w/w) in pork sausages were equivalent to the ones generally used by these food companies. For simplification purposes here, for each meat matrix, the three formulations were denoted 'Current dose of lactate', 'Half-dose of lactate' and 'Without lactate', respectively.

Sausages were afterwards packed by five (or six) in PET (polyethylene terephthalate) trays one day and two days respectively after production of turkey (or pork). Sausages were conditioned under three modified atmosphere packaging (MAP) and sealed with thin high barrier polyester-based film (PET/EVOH (Co-polymer of Ethylene and Vinyl alcohol)/PE (PolyEthylene)). The physical properties of the packaging film were as follows: oxygen transmission rate $< 5 \text{ cm}^3/\text{m}^2 \cdot 24 \text{ h}^{-1} \cdot \text{bar}^{-1}$, CO_2 transmission rate $< 25 \text{ cm}^3/\text{m}^2 \cdot 24 \text{ h}^{-1} \cdot \text{bar}^{-1}$. The initial gas compositions in the used MAPs were 'Air packaging': 21% O_2 -78% N_2 -1% of other gases including CO_2 ; 'MAP1': 70% O_2 -30% CO_2 and 'MAP2': 50% CO_2 -50% N_2 . The two latter ones are gas compositions commonly used by the two supply French companies. Nine different combined conditions of lactate contents and MAP were then considered. For each condition, at least three trays were prepared to ensure a sufficient quantity of sausages for the measurement of

the various responses. For each matrix and each sausage production batch, 90 trays of sausages were packed in total and used for sampling (Fig. 1).

After packaging, sausage trays were stored at 4°C until the 5th day after the production, then at 8°C until the 22nd day. This storage protocol was inspired by the French Norm NF V01-003 used to establish the shelf-life of chilled perishable and highly perishable food (AFNOR, 2018). It consists in storing samples at 4°C for one third of the shelf-life and then, at 8°C during the remaining two third of the storage duration. The use-by-date (UBD) established by the producers for the sausages containing the normal dose of lactate and conditioned under modified atmosphere, was 15 days for both types of meat. Therefore, sausages were first stored at 4°C during 5 days and then placed at 8°C during the remaining storage duration. To be able to observe spoilage occurrence, the storage duration was extended to one UBD and half, *i.e.* 22 days. Several microbiological and sensory responses were measured at different sampling time points, *i.e.* 2, 8, 15 and 22 days after the production. Despite the differences in the packing date between the two types of sausages, it was chosen to monitor the different responses at the same times to enable comparisons. The first analyses conducted at day 2 after production for both matrices were considered as the initial conditions of the experiments.

2.1.2 Microbiological analyses

Analyses were independently performed on pork and turkey with harmonized protocols in two separate laboratories, which explained the difference in equipment and media used (Poirier et al., 2020). Around 50 g of sausages (corresponding to one fresh sausage) were placed into a stomacher bag with a filter (Bagpage, Interscience, France). It was then homogenised with 200 ml of sterile peptone water (Biorad, France), containing 1% Tween 80 (Sigma, Ilkirch) emulsifier in a masticator blender (Bagmixer, Interscience, France) for 120 seconds at room temperature. For each sample, appropriate serial decimal dilutions were prepared in buffered peptone water. Total aerobic mesophilic bacteria (TAMB) were enumerated in Plate Count Agar medium (PCA, Biomérieux or Biorad, France) incubated at 30°C for 48h. Lactic Acid Bacteria (LAB) were determined on the Man Rogosa

Sharpe Agar medium (Biokar or Biomérieux, France) incubated at 30°C for 48h. After incubation, plates with 30–300 colonies were counted. Microbiological data were transformed into decimal logarithms of the number of colonies forming units per gram of sausage sample (log CFU/g).

2.1.3 pH measurement

pH was measured using a pH meter (InLab Solids Pro or FiveGo, Mettler Toledo) by direct insertion of the electrode into sausage samples. Three measurements were carried out on three different sausages from the same tray (corresponding to one condition of packaging and lactate) as previously described in Lerasle et al. (2014).

2.1.4 Spoilage characterisation

Visual defects

Two visual defects were evaluated at each sampling time point, *i.e.* packaging swelling and presence of exudate, as already performed by Fougy et al. (2016). These evaluations were always performed on sealed trays (before opening). Packaging swelling was determined visually or by pressing softly on the sealing film to check its resistance. Exudate was evaluated by tilting the tray in order to check if there was a liquid accumulated at the bottom of the tray. Qualitative assessment of these visual defects was characterized by 'presence' or 'absence'.

Perception of off-odour

Sensory analyses were performed by 15 trained panellists in order to evaluate off-odours, according to standard procedures (NF ISO, 2010, 2017). The panel was trained during an 8-month period before performing analyses. The objective of the training sessions was the acquisition of the ability to distinguish spoiled from unspoiled products by olfactive perception through triangular tests. Normative documents were used as support of these training sessions (ISO, 1994; NF EN ISO, 2007, 2016; NF ISO, 2006) .

Ten sessions were then performed to evaluate all pork and turkey products. Two batches (pork or turkey) were analysed during the same session. Between 10 to 15 panellists participated to each session. The samples were presented to the panellists according to a predefined, balanced tasting plan, different for each panellist and for each session. One tasting plan per meat matrix was created for all the sessions, which represented in total 20 equilibrated latine square designs, automatically generated (Fizz software, Version 2.51 , Biosystèmes, Couternon, France).

Evaluations were done on trays frozen at -20°C , as commonly performed in sensorial analysis (Mace et al., 2014). Freezing packed samples at different sampling time points was necessary to keep all off-odour characteristics during the 6-month sampling period and transport, before sensorial analysis. Freezing different samples also enabled panellists to perform sequential monadic evaluation, *i.e.* randomly and sequentially evaluate different types of products during the same session (different monitored time points, different lactate or MAP conditions). Two hours before each session, samples were thawed. Then, samples of 20 g of sausage were prepared and presented to the panellist in a 250-mL Erlenmeyer flask in opaque glass and with ground glass stopper, codified with three random numbers. For each evaluation, a control sample (fresh sausage containing lactate frozen immediately after production), was presented to the panellists as the reference, to avoid random difference between different sessions. This control sample was present in two copies to be able to assess the panel effect. All panellists evaluated the same samples against a control sample. By comparing each sample to the control one, each panellist denoted the odour as 'spoiled' or 'not spoiled' after sniffing. The off-odour score for each sample (in continuous scale between 0 and 1) was represented by the proportion of the panel denoting the sample as 'spoiled'. The off-odour of sausages formulated with the "Half-dose of lactate" was not assessed.

Colour measurement

Colour profile of sausages was measured using the Minolta CR400 ChromaMeter (Grosseron, Nantes, France) in CIE-Lab scale (CIE, 1978). The measurements determined chromatic coordinates of L^*

(brightness), a^* (green-red balance) and b^* (blue-yellow balance). Measurements were made in three replicates for each condition, each sampling time and each production batch. The colour of sausages formulated with the “Half-dose of lactate” was not monitored.

2.2 Statistical analysis

Seven quantitative responses were considered in this study, *i.e.* enumeration of TAMB (in log CFU/g), enumeration of LAB (in log CFU/g), pH value, measurement of the ‘brightness’ (CIE L^* value), ‘green-red’ (CIE a^* value), ‘blue-yellow’ (CIE b^* value) balances and the off-odour score (in continuous scale between 0 and 1). Analyses were carried out independently for turkey and pork sausages. Correlations between responses were evaluated using non-parametric Spearman’s rank correlation test. Linear mixed effects analyses were performed to study the influence of the storage time, packaging and lactate formulation on each considered spoilage response. For each meat matrix, production batches were associated with random effects whereas storage time, packaging and lactate formulation were considered as fixed effects. Including three fixed factors and the random effect of batches required an adapted procedure for building a mixed effect model enabling to describe all the above effects. For each meat matrix, considering firstly a reference model denoted M_0 which was written as described below:

$$(Eq.1)(M_0) : Response \sim Time + Lactate + Packaging + (1 | Batch) + \epsilon,$$

where $(1|Batch)$ represents the random effect of production batches on the intercept coefficient. Including this random effect in the model enabled to consider the non-independence of the data since sausage samples with different lactate contents and packed from the same product batch were not independent. The model adjustment assumptions were checked by diagnosing residual plots to check and remove data presenting any deviations from homoscedasticity or normality. Data were considered as outliers following residual diagnosing over all experimental conditions and all sampling time points. Based on these criteria, one or two batches considered as outlier data in turkey and pork sausages respectively, were removed from mixed-effects analyses. Fitting mixed effect models to data was

performed by maximising the likelihood of the data (Restricted Maximum Likelihood approach, REML) using the *R* package *lme4* (Bates, Maechler, Bolker, & Walker, 2014). The p-value corresponding to each fixed effect was obtained by likelihood ratio tests of M_0 against the model without the effect in question. The p-value corresponding to each possible interaction between main factors (Storage time x Packaging, Storage time x Lactate formulation, Packaging x Lactate formulation) was obtained by likelihood ratios tests of M_0 against the model including the interaction in question. In order to reduce the number of estimated parameters in the model with interaction, the three-factor interaction (Storage time x Gas Composition x Lactate) was not included. The significant main factors and interactions presenting a p-value < 0.05 were retained in the final model for describing significant effects. The estimates of each retained effect were represented by the coefficient corresponding of each modality of the effect as shown in Table 2 in the next section. The R script for the analysis is provided in Supplementary data.

Two qualitative responses were also considered in this study and corresponded to two visual defects, *i.e.* package swelling and presence of exudate. Each qualitative response took value 0 or 1 corresponding to the 'absence' or 'presence' of each defect and was described by a mixed-effects logistic (generalised linear) model. The *logit* function was used for transforming these qualitative responses into the occurrence probability of the considered responses. Mixed effects generalised linear analyses were performed in the same way as described above for quantitative responses to describe the relationships between the transformed response and the three fixed factors in question.

3. Results and discussion

3.1 Evolution and correlations between spoilage responses during chilled storage

Evolution of different spoilage responses

Despite an important inter-batch variability in both matrices and in all conditions, off-odour scores increased globally over time as shown in Table 1, generally reaching more than 0.8 no matter the meat matrix. It is noteworthy that the off-odour increased earlier in turkey than in pork, with a difference between turkey and pork sausages of 0.4 approximately on the 8th day after the production, especially in absence of lactate and under 'Air packaging' or 'MAP1 (70% O₂-30% CO₂)'. Then off-odour scores of turkey and pork sausages seemed to converge, reaching on the 15th day, mean values between 0.6- and 0.77, and more than 0.8 at the end of the storage. The evolution of all the monitored spoilage responses was plotted and provided in Fig.2, Fig.4 and Supplementary data (Figs S.1-6).

Meat colour is closely related to the proportion of the three forms of myoglobin (oxymyoglobin: bright-red, deoxymyoglobin: dark purplish-red, and metmyoglobin: brownish) (AMSA, 2012). Regarding colour characteristics, L^* values were shown to slightly increase in both meat matrices during storage for all conditions, from 44.32 to 48.36 and from 42.01 to 44.4, respectively in turkey and pork (Table 1). These values suggested then a "whitening" trend in pork and turkey sausages over time, whatever conditions. L^* variability has been shown by Lindahl, Lundstrom, and Tornberg (2001) to be mostly explained by pigment content and fractions of metmyoglobin and oxymyoglobin. Since storage is assumed to favour myoglobin oxidation into metmyoglobin, L^* was expected to decrease. However, other phenomena such as protein denaturation or water exudation, both enhanced at low pH, may have resulted in this slight L^* increase (Alvarado, Wenger, & O'Keefe, 2005). However, the amplitude of this increase seemed to be higher in turkey (9%) than in pork (4%). In addition to the "whitening"

effect, a decrease of the CIE a^* values from 13.55 to 9.64 and 16.75 to 14.77 respectively in turkey and pork matrices suggested a loss of the 'red shade' in sausages. Such discolouration has already been observed in other studies (Bouju-Albert, Pilet, & Guillou, 2018; Martinez, Djenane, Cilla, Beltran, & Roncales, 2005). The decrease of a^* was ascribed to oxidation and formation of metmyoglobin by Saucier, Gendron, and Garipey (2000) and Veberg et al. (2006). At last, the CIE b^* value increased from 8.87 to 10.71 over time in turkey sausages, contrarily to pork sausages with a slight decrease for from 11.05 to 10.30. The increase of b^* associated with enhanced yellowness under high oxygen atmosphere may be due to lipid oxidation in turkey sausages. Indeed, increased b^* values correlated with lipid oxidation have also been observed in turkey patties conditioned under similar gas atmosphere (Veborg et al., 2006). Globally, major alterations of the CIE L^* , a^* , b^* values were observed during the first 15 days of storage, corresponding to a discolouration from 'red' in the fresh sausages into 'less red/yellow/brown' in the spoiled ones. The average trend of these values suggested that the colour seemed to be stabilised between the 15th and the 22nd day after production.

Considering microbiological enumerations, at the beginning of storage (day 2), the enumeration of TAMB varied between batches and was 5.7 ± 0.6 log CFU/g and 4.5 ± 0.7 log CFU/g in average, in turkey and pork sausages, respectively (Table 1). In both matrices, the TAMB increased over time but earlier in turkey than in pork samples, reaching 8.5 ± 0.5 log CFU/g in average, on the 8th day of storage as opposed to 6.2 ± 0.8 log CFU/g in pork. However, on the 15th day of storage, the average count of TAMB reached the same level in both matrices at approximately 8.2-9 log CFU/g, at which stabilisation seemed to occur.

Similar trends were observed for the enumeration of LAB, with a faster increase in turkey than in pork, reaching 7.2 ± 0.8 log CFU/g at day 8, as opposed to 5.9 ± 0.8 log CFU/g in pork. Globally, the one-log higher population in turkey at the beginning of the storage, disappeared from day 15 at which stabilisation occurred at around 8 log CFU/g in both matrices. LAB seemed to be dominant in both

meat matrices which is commonly observed, as also found by Benson et al. (2014) in fresh pork sausages.

Regarding pH, at the beginning of the storage, the average pH value of sausages was 6.40 ± 0.14 in turkey and 5.98 ± 0.10 in pork. Globally, acidification was observed over time in both matrices as shown in Fig. 2. However, the pH value decreased earlier in turkey than in pork, since an important drop of 0.5 pH units was observed between the 2nd and 8th day, whatever the process conditions. In contrast, in pork samples, the pH did not change before the 8th day of storage. From this time point, the pH decreased and reached 5.5 approximately in both matrices on the 15th day, but continued dropping in pork to 5.2 at day-22, unlike turkey in which stabilisation occurred. Acidification is a common effect observed during storage of meat products and is due to acid production from microbial metabolism. The pH in pork sausages was shown to follow a curve with three phases, *i.e.* lag, acidification and stabilisation similarly as observed by Deumier and Collignan (2003) in dry fermented chicken sausages or by Benson et al. (2014) in fresh pork sausages. A similar two-phase decrease of pH (without lag phase) was also observed in chicken frankfurters (Tovunac, Galić, Prpić, & Jurić, 2011).

Not surprisingly, the storage time significantly affected all spoilage responses measured in this study, stressing the dynamical nature of spoilage (mixed model analysis, Table 2). Moreover, the results suggested that pork and turkey sausages were altered differently, especially regarding the onset of important changes which occurred earlier in turkey than in pork sausages. In turkey sausages, the fairly initial higher pH and closer to neutrality and higher content in sulfur-containing amino-acids of turkey sausages may explain this faster spoilage. Simultaneous changes have been observed between acidification, discolouration, off-odour perception and evolution of microbiological indicators. This suggested then potential correlations between these indicators.

Correlation analysis between physico-chemical and microbiological indicators

For both matrices of turkey and pork sausages, significant correlations were observed between most of the studied indicators, except for the CIE b^* value which was only correlated with the CIE a^* value

in pork sausages (Fig. 3). Strong correlations were observed between the off-odour score, pH and the enumeration of TAMB and LAB in both matrices, with variations in the same directions (positive or negative correlation) in both matrices (absolute correlation between 0.62 and 0.95; p -value < 0.05). Hence, these results suggested that acidification was strongly positively associated with the off-odour perception and the bacterial count, whatever the meat matrix. In particular, in pork sausages, the colour indicators were poorly correlated with the other spoilage responses. Few studies have performed such correlation analysis. For instance, Fouguy et al. (2016) used this approach to link the nature of spoilage odours to the type of packaging for raw pork sausages.

3.2 Effect of lactate formulation and modified atmosphere packaging on spoilage indicators: turkey *versus* pork sausages

The effects of storage time, gas composition and lactate formulation on the off-odour score, colour attributes, pH and enumeration of LAB and TAMB and corresponding p -values estimated from mixed effect analyses are summarised in Table 2. Since these factors influenced differently on different responses, the model including the retained significant effect was different from one response to another. For each response presented in Table 2, the intercept value of the model, denoted α , corresponded to a reference condition ("Current dose of lactate – Air packaging – Day 2"). The effects of lactate, packaging and time point, if significant, were estimated through the estimation of their corresponding coefficients, *i.e.* δ , γ and β , respectively, gathered in Table 2. Each response (TAMB, LAB, off-odour score, colour and pH) at a condition was estimated from the sum of the intercept value of the model α and the coefficients corresponding to the considered modality of significant main effects or interactions. The random effects of production batches on each spoilage response were expressed in standard deviation. For simplification purpose, significant interactions were represented in plots provided in Supplementary data (Fig. S7)

3.2.1 Random effect of batches

The mixed effect model enabled firstly to estimate random effects of production batches on each response and to take into account non-independence of data. These random effects corresponding to the biological variabilities between production batches could represent an important effect. For example, the random effect between batches on the pH value, expressed in standard deviation, was estimated at 0.12 for turkey sausages (Table 2). This effect was nearly close to the one of lactate on the same response, since using 'Half dose' or 'Zero lactate' formulations instead of the 'Current dose' in turkey only reduced pH only from 0.10 to 0.11. Hence, these results suggested that the acidification was firstly different from one production batch to another and the evolution was rather important over time. Similar patterns were observed for the enumeration of total aerobic mesophilic bacteria in turkey: the random effect of production batches, estimated at 0.20 log CFU/g in standard deviation, was close to the effect of lactate where 'Half dose' and 'Zero lactate' formulations increased 0.11 and 0.42 log CFU/g, respectively. These results suggested that the evolution of the bacterial growth over time could possibly be influenced by the random variability on initial bacterial load in the meat matrices as well as by preservation strategies. Therefore, random biological variabilities from different production batches could have notable influence on spoilage comparing with the storage time, packaging or formulation and should not be overlooked.

3.2.2 Effects of lactate formulation

The content of potassium lactate differently affected the measured responses, depending on the meat matrices (Table 2), with more significant effects in turkey than in pork.

Similar significant effects of lactate on both matrices were observed on the CIE L^* value and on the enumeration of TAMB. The obtained results suggested that increasing the dose of lactate could limit the 'whitening' trend in both matrices. This effect of lactate on L^* was observed especially in turkey, since the L^* value at the end of storage was more than one point higher in the 'Zero lactate'

formulation than in the 'Current dose' one. The slowed changes of the colour shading were also observed by Bouju-Albert et al. (2018) in pork sausages containing lactate.

In turkey sausages exclusively, the model estimation suggested other potential effects of lactate addition. Hence, increasing the initial lactate contents could limit significantly the off-odour perception, pH acidification and 'yellow discolouration'. Indeed, the formulation 'Current dose' in turkey (2% w/w) reduced the off-odour score of nearly 6% at every time point (Table 2) compared to the 'Without lactate' one. The pH value was significantly higher in sausages with the 'Current dose' than with lower doses (drop of 0.10 and 0.11 in pH if half or the entire dose was removed from the formulation). These results were consistent with previous findings. An increase of pH consequently to lactate addition was also found by Bouju-Albert et al. (2018) in pork sausages and by Tovunac et al. (2011) in chicken frankfurters, conferring an acidification inhibitor power to lactate. Earlier studies in the literature have also indicated a positive effect of lactate additive on colour stability in turkey meat products (Cegielska-Radziejewska & Pikul, 2004). At last, despite the significant effect of lactate on TAMB in turkey, removing the entire dose of lactate from sausages was estimated to increase only their enumeration of 0.42 log CFU/g.

In pork sausages, lactate addition was shown to have limited effects. The only other effect identified was associated with the slowing growth of LAB. However, as for TAMB, this effect should be considered cautiously since increasing the dose of lactate would only reduce about 0.10-0.30 log CFU/g despite the significant statistical effects. These results on pork sausages were consistent with previous studies of Bradford, Huffman, Egbert, and Jones (1993) and Drosinos, Mataragas, Kampani, Kritikos, and Metaxopoulos (2006). The former showed that potassium lactate addition reduced slightly the aerobic bacterial population of about 0.3 log CFU/g but did not affect sensory properties, while the latter suggested that 3% of a mixture of potassium lactate and potassium acetate slightly inhibited LAB growth in untreated frankfurters.

3.2.3 Evolution of gas composition and effects of modified atmosphere packaging

The use of different initial gas compositions in packaging was shown to differently affect discolouration, off-odour perception and acidification in turkey and in pork. First, for both matrices, the 'MAP1 (70%O₂-30% CO₂)' seemed to accelerate the loss of the 'red shade' of the sausages comparing with the 'Air packaging', since the CIE a^* value decreased significantly in both matrices (from 13.55 to 9.15 in average in turkey and from 16.8 to 13.05 in pork at day 22, Table 1). In the presence of oxygen, metmyoglobin is generated because of the formation of oxygen radicals stimulated by metal ions (iron, copper) (AMSA, 2012). In contrast, the CIE b^* value was observed to increase in turkey (from 8.87 to 11.73 at day 22) and decrease in pork (from 11.1 to 9.67 at day 22), whereas the CIE L^* values (lightness) increased by two points at day 22 especially in pork, but not in turkey. The loss of the red shade under MAP1 was unexpected since high oxygen concentration favours transformation of myoglobin in meat into bright red coloured oxymyoglobin and generally used to maintain the red pigment in red meat products such as beef steaks (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2003) or ostrich steaks (Leygonie, Britz, & Hoffman, 2011). Nevertheless, it has to be noted that the O₂ concentration initially present at 70% at the beginning of the storage had decreased to only 3.1 – 5% at day 15 (data published in data paper by Poirier et al. 2020), presumably due to bacterial metabolism and oxidation reactions, rather than oxygen leakage from the package, considering the high barrier film with very low transmission rate. Also, as reported by Benson et al. (2014), the red pigment retention by high-O₂ packaging only lasts for a short time and the CIE a^* value could decrease quickly after 8 days of storage, which could explain the drop of a^* in our study. Moreover, oxidation and formation of metmyoglobin might be responsible for the a^* decrease as suggested by Veberg et al. (2006). Second, using 'MAP2:50% CO₂-50% N₂' instead of 'Air packaging' had very little effects on the CIE b^* value. Globally, the use of 'MAP1:70% O₂-30% CO₂' was associated with a discolouration into a 'yellow-brown' in turkey sausages presumably due to lipid oxidation and a 'loss of red' in pork sausages, and the 'MAP2:50% CO₂- 50% N₂' was associated with a 'loss of red-yellow' off-colour in turkey and 'light red/purplish' in pork. Regarding the off-odour perception, using

'MAP2: 50% CO₂-50% N₂' seemed more relevant to reduce the off-odour perception. Turkey sausages stored under this MAP represented off-odour scores at the 8th day after production (0.45-0.52) lesser than in 'Air' or 'MAP1: 70%O₂-30%N₂' (0.62 – 0.74) (Table 1, Fig. S7-C1). However, in pork sausages, using MAP2 instead of 'Air packaging' increased slightly the spoilage off-odour of pork sausages (up to 6%, Table 2). The gas atmosphere MAP1 enriched in oxygen was shown to be especially detrimental for turkey sausages. High concentration of oxygen is known to promote lipid oxidation and especially in meats which have been minced or homogenized because disruption of muscle cell structure exposes lipids to oxygen, metals and heme (Sato & Hegarty, 1971; Veberg et al., 2006). Veberg et al. (2006) observed that turkey patties were more susceptible to lipid oxidation when stored under high oxygen concentration (70% O₂ – 30% CO₂) than pork patties. The authors explained this difference by the composition of turkey meat which is richer in lipids (turkey:9.6%, pork 3.1%) and especially in unsaturated fatty acids such as oleic (C18:1n-9) and linoleic (C18:2n-6) acids.

Slowing the onset of clearly recognizable off-odour or off-flavour by using MAP containing 30% to 65% CO₂ instead of air, was also observed by Balamatsia, Patsias, Kontominas, and Savvaidis (2007) in breast chicken. The increased off-odour could be explained by the promoting oxidation effect of CO₂ on lipids (Martinez et al., 2005).

Considering the acidification, MAP1 seemed to slow down acidification over time in turkey, more efficiently than MAP2 (with a higher percentage of CO₂). In pork sausages, the initial gas composition, and especially the presence of CO₂ in the packaging did not decrease the pH. Similar results have been found by Rubio et al. (2007) in dry fermented pork sausages where acidification was not influenced by MAP and especially CO₂, even if decrease in pH values was generally attributed to the activity of lactobacilli and the dissolution of CO₂ into meat product. The absorption of CO₂ by meat leading to a lowering of pH has been reported for instance by Martinez et al. (2005) in fresh pork sausages.

At last, our analyses also identified no or very low effects (less than 0.10 log CFU/g) of MAP on the enumeration of TAMB and LAB in both matrices. These results are in agreement with the findings of Balamatsia, Paleologos, Kontominas, and Savvaidis (2006) and Rossaint, Klausmann, and Kreyenschmidt (2015) in breast chicken and Nieminen, Dalgaard, and Björkroth (2016) in raw pork loins. Hence, the well-known antimicrobial effect of CO₂ (Farber, 1991a; Jakobsen & Bertelsen, 2002) could not be shown by enumerating bacterial counts but is likely to occur by inhibiting some aerobic bacteria (Säde, Murros, & Björkroth, 2013).

3.2.4 Effects on visual defects

The evolution of the visual defects (including packaging swelling and exudate) are shown in Fig. 4. Globally, no defect was observed on the first day of storage, and defects occurred earlier in turkey than in pork.

The number of samples presenting package swelling increased over time, but earlier and more often in turkey than in pork sausages. Swelling is generally associated with the so-called 'blown pack' phenomenon. It is most often due to production of CO₂ by bacteria but it can also be associated with other volatile molecules (Dousset, Jaffrès, & Zagorec, 2016). This spoilage-associated defect is commonly due to metabolism of *Enterobacteriaceae* and cold-tolerant *Clostridium* spp., in particular *Clostridium estertheticum* and *Clostridium gasigenes* (Remenant, Jaffrès, Dousset, Pilet, & Zagorec, 2015; Zagorec & Champomier Vergès, 2017). In turkey, swelling occurred earlier (from the 8th day of storage) in the 'Air packaging' than in 'MAP2: 50% CO₂-50% N₂' (from the 15th day of storage), and in 'MAP1: 70% O₂-30% CO₂', no package swelling was observed along the whole storage duration.

Exudate formation is another spoilage-associated defect. Very few samples presented exudate before the 15th day of storage. From the 15th day, exudate occurred in some samples of pork sausages, and in almost all turkey samples from this time point. Chicken muscle is recognised as producing exudate easily (Varnam & Sutherland, 1995). Moreover, mincing operations are also known to induce more physical disruption to turkey than to other meats and then favour myofibrillar protein extraction and

exudate formation (Arnaud, Santchurn, & Collignan, 2014). Fig. 4 suggested that (i) the two studied visual defects seemed to be independent on the lactate contents, and (ii) MAP seemed to influence exclusively the package swelling in turkey sausages, and exudate formation in pork sausages. Indeed, package swelling and exudate formation seemed to be higher under MAP2 containing high CO₂ concentration. High CO₂ concentration favours protein solubilisation which is necessary to exudate formation whereas package swelling may occur following saturation of the meat with CO₂ (Gill, Harrison, & Penney, 1990). Unfortunately, the statistical analyses for identifying effects of storage time, lactate and packaging on the qualitative responses for visual defects could not be done as for quantitative responses in the previous section, essentially due to their important variability between conditions. Indeed, the important changes in the number of samples presenting a defect from one storage time point to another (e.g. for exudate: Day 8 (no batch presenting an exudate) versus Day 15 (all batches presenting an exudate)) or one packaging to another (e.g. 'Air packaging' versus 'MAP1') could be a reason of several difficulties in the adjustment of the logistic model.

3.3 Correlated responses of spoilage and relationships with preservation strategies

The results above allowed us to perceive the complexity of the spoilage phenomenon. The effects of lactate and MAP on the three highly correlated measures responses, *i.e.* pH, LAB count and off-odour score were illustrated in Fig. 5.

In both matrices, the three responses were strongly correlated. In turkey, the increase in initial potassium lactate content decreased the off-odour score and acidification but did not influence LAB growth. In contrast, in pork, lactate had no effect on defects such as off-odour or acidification. The mechanism underlying the effect of lactate would not be linked to LAB growth (Fig. 5a). Potassium lactate was first used as humectant and flavour enhancer in beef and turkey meat products with increased cooking yields and water holding capacity (Shelef, 1994). It has also shown antimicrobial properties at the recommended levels of 2% (w/w) with growth inhibition effects on *Listeria monocytogenes*, coliforms, aerobes, and *Clostridium*, by lowering the internal pH of bacteria (Shelef,

1994). Lactate is supposed to act into two steps. At first, it decreases water activity due to its hygroscopicity. Then it crosses the cytoplasmic membrane of microbial cells and dissociates, thereby releasing protons and acidifying the internal pH, which inhibits bacterial growth (Tovunac et al., 2011). However, the influence of lactate on spoilage occurrence has been poorly documented.

As shown in Fig. 5b, in turkey, the three measured responses were positively correlated. MAP2:50% CO₂-50% N₂ slightly increased LAB counts and pH, but decreased the off-odour despite the positive correlation. The atmosphere deprived of O₂ but enriched in CO₂ would have favoured LAB growth, leading to intensified acidification through lactic acid production. The selection pressure of high-CO₂ atmospheres toward psychrotrophic and strictly or facultative anaerobic microbes like LAB is well-known (Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015). However, the bacteria responsible for the off-odour perception have not been identified and might not be only LAB. Volatile organic compounds (VOCs) of microbial origin including organic acids, volatile fatty acids, ethyl esters, sulphur compounds, ketones, aldehydes, alcohols, ammonia and other molecules have been associated with off-odours. LAB may produce acids but also alcohols, aldehydes and sulphur compounds (Pothakos et al., 2015). Hence, the involvement of LAB in spoilage was not straightforward. In contrast, in pork, MAP2:50% CO₂-50% N₂ without O₂ had no effect either on pH or LAB count, but increased off-odour perception through an unknown mechanism. Finally, as shown in Fig. 5c, the use of MAP1 enriched in O₂ decreased the off-odour although it had no effect on LAB count and pH value. LAB constitute a heterogeneous group with specific metabolic abilities. Despite the same bacterial count, there may be differences in LAB species. Therefore, the cause-effect mechanism between these three correlated measured responses was then difficult to understand and would require in-depth characterisation of the bacterial communities.

4. Conclusion

In this study the influence of batch variability, lactate formulation and MAP were studied on industrially produced pork and turkey sausages. The random effect of production batches was found to be not negligible and justified the necessity of considering biological variability, as well as non-independent experiment design in studies relative to spoilage. Despite initial batch variability, the studied preservation strategies combining MAP and lactate addition were shown to significantly influence the studied spoilage responses but in a different way for turkey and pork. Our results suggested that for fresh turkey sausages, the lactate formulation of 2% (w/w) combining with MAP (50% CO₂-50% N₂) reduced significantly acidification, off-odour perception and prevented discolouration of sausages from red to dark grey / brown colour. In pork sausages, the lactate formulation of 1.13% (w/w) currently used in the French industry did not significantly influence the monitored spoilage responses compared to products without lactate, but the MAP (70% O₂-30% CO₂) slightly reduced the off-odour perception. Our results allow us to perceive the complexity of the spoilage phenomenon which was characterised by different correlated spoilage responses. The cause-effect mechanism between these correlated responses and the choice of an appropriate preservation strategy would require in-depth characterisation of the bacterial ecosystem structure.

The mixed effect model method used enabled to consider non-independencies between conditions from the same batch and random variabilities between batches. The results showed that the modified atmosphere enriched in oxygen should be avoided for turkey sausages because of the off-odours presumably due to lipid oxidation. Regarding pork sausages, addition of lactate should be questioned since the shelf-life of pork sausage did not seem to be longer in the presence of lactate. However, the influence of lactate on pathogenic microorganism loads should be studied.

This study revealed that the relevance of certain industrial practices, sometimes empirical, regarding the use of food additives and modified atmosphere needs to be regularly reassessed while taking advantage of the advancement of knowledge and methods.

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Table 1. Evolution of average inter-batch physico-chemical and microbiological responses recorded during the chilled storage of pork and turkey sausages. Each value is the mean of 10 (resp. 11) batches of poultry (resp. pork) with standard deviation. ND: Not Determined.

Time (days)	Poultry									pork									
	Current dose			1/2 dose			Without lactate			Current dose			1/2 dose			Without lactate			
	Air	MAP1	MAP2	Air	MAP1	MAP2	Air	MAP1	MAP2	Air	MAP1	MAP2	Air	MAP1	MAP2	Air	MAP1	MAP2	
L*	2	44.32 ± 2.3			ND			44.32 ± 2.3			42.07 ± 1.7			ND			42.07 ± 1.7		
	8	46.19 ± 1.3	46.71 ± 2.2	46.97 ± 2.0	ND			47.94 ± 2.1	48.72 ± 1.5	47.02 ± 1.0	42.3 ± 2.0	43.0 ± 1.5	43.3 ± 1.9	ND			44.4 ± 2.6	44.7 ± 2.2	43.8 ± 2.6
	15	47.76 ± 2.0	48.17 ± 1.5	47.70 ± 1.8	ND			48.44 ± 1.4	49.35 ± 1.9	49.49 ± 1.8	42.6 ± 1.9	44.7 ± 2.6	44.7 ± 2.7	ND			44.6 ± 2.3	45.1 ± 2.8	43.2 ± 2.6
	22	47.91 ± 1.5	47.64 ± 0.98	47.77 ± 1.9	ND			49.69 ± 1.5	48.33 ± 1.6	48.81 ± 1.3	43.0 ± 1.3	45.3 ± 2.3	44.4 ± 1.6	ND			44.3 ± 2.4	46.1 ± 1.8	43.4 ± 1.8
a*	2	13.55 ± 1.2			ND			13.55 ± 1.2			16.8 ± 1.3			ND			16.8 ± 1.3		
	8	12.81 ± 0.73	10.75 ± 1.2	12.56 ± 1.8	ND			12.77 ± 1.4	10.59 ± 1.1	12.99 ± 0.89	16.8 ± 1.6	15.6 ± 1.1	18.9 ± 2.2	ND			15.6 ± 1.4	16.1 ± 1.7	19.2 ± 1.4
	15	10.98 ± 0.8	8.88 ± 1.5	10.61 ± 0.6	ND			11.51 ± 1.4	8.91 ± 2.0	11.49 ± 0.8	16.7 ± 1.4	13.3 ± 1.4	17.2 ± 2.5	ND			15.6 ± 1.9	13.4 ± 1.4	17.3 ± 1.9
	22	10.21 ± 1.2	8.79 ± 1.8	9.56 ± 1.1	ND			10.00 ± 1.1	9.51 ± 1.6	9.80 ± 1.4	15.7 ± 1.4	13.6 ± 1.4	15.6 ± 1.6	ND			15.2 ± 1.2	12.5 ± 1.1	16.0 ± 1.0
b*	2	8.87 ± 1.3			ND			8.87 ± 1.3			11.1 ± 1.3			ND			11.1 ± 1.3		
	8	9.53 ± 0.94	9.06 ± 1.2	9.09 ± 1.0	ND			10.35 ± 0.85	10.65 ± 1.1	9.78 ± 1.0	10.8 ± 1.6	10.9 ± 1.2	12.0 ± 1.7	ND			11.4 ± 2.1	11.2 ± 1.7	12.7 ± 1.2
	15	9.91 ± 1.1	10.47 ± 1.2	9.24 ± 1.1	ND			10.08 ± 0.8	12.03 ± 2.1	10.47 ± 1.1	10.9 ± 1.9	9.50 ± 1.6	11.2 ± 1.4	ND			11.8 ± 1.7	10.0 ± 1.4	11.1 ± 1.7
	22	10.17 ± 0.9	11.45 ± 0.97	9.26 ± 0.71	ND			10.67 ± 1.4	12.01 ± 1.4	10.72 ± 1.4	10.4 ± 1.8	9.91 ± 1.6	10.6 ± 2.1	ND			10.8 ± 1.7	9.42 ± 0.73	10.7 ± 1.5
TAMB	2	5.5 ± 0.7			5.6 ± 0.6			5.9 ± 0.5			4.5 ± 0.7			4.6 ± 0.7			4.4 ± 0.7		
	8	8.6 ± 0.2	8.5 ± 0.4	8.2 ± 0.7	8.6 ± 0.4	8.7 ± 0.3	8.1 ± 0.6	8.8 ± 0.3	8.9 ± 0.3	8.4 ± 0.4	6.3 ± 0.8	6.0 ± 0.7	5.9 ± 1.0	6.6 ± 0.8	6.5 ± 0.8	6.2 ± 1.0	6.4 ± 0.8	6.3 ± 0.7	5.9 ± 0.7
	15	9.1 ± 0.5	9.2 ± 0.4	8.9 ± 0.5	9.1 ± 0.5	9.1 ± 0.3	9.0 ± 0.4	9.1 ± 0.4	9.1 ± 0.4	9.0 ± 0.5	8.2 ± 0.3	8.1 ± 0.4	8.1 ± 0.4	8.4 ± 0.3	8.3 ± 0.3	8.2 ± 0.5	8.3 ± 0.3	8.3 ± 0.4	8.3 ± 0.3
	22	8.8 ± 0.3	8.9 ± 0.3	8.9 ± 0.4	8.9 ± 0.3	8.9 ± 0.3	9.0 ± 0.5	8.7 ± 0.3	8.8 ± 0.2	9.0 ± 0.7	8.4 ± 0.4	8.2 ± 0.4	8.4 ± 0.4	8.5 ± 0.3	8.4 ± 0.2	8.5 ± 0.3	8.5 ± 0.3	8.4 ± 0.3	8.6 ± 0.3
LAB	2	3.5 ± 0.7			3.4 ± 0.5			3.5 ± 0.6			2.3 ± 0.4			2.7 ± 0.5			2.4 ± 0.3		
	8	7.2 ± 0.8	6.9 ± 0.9	7.3 ± 0.8	7.2 ± 0.6	6.9 ± 0.8	7.6 ± 0.6	7.2 ± 0.7	7.0 ± 0.7	7.5 ± 0.8	5.8 ± 1.0	5.7 ± 0.8	5.4 ± 1.0	6.2 ± 0.8	6.2 ± 0.8	5.9 ± 1.0	5.9 ± 0.6	6.0 ± 0.6	5.6 ± 0.8
	15	8.3 ± 0.6	8.1 ± 0.5	8.5 ± 0.4	8.2 ± 0.4	7.8 ± 0.6	8.5 ± 0.3	8.1 ± 0.5	7.8 ± 0.5	8.4 ± 0.3	8.1 ± 0.4	8.0 ± 0.4	7.9 ± 0.4	8.3 ± 0.3	8.1 ± 0.4	8.1 ± 0.5	8.2 ± 0.4	8.1 ± 0.4	8.2 ± 0.3
	22	8.2 ± 0.5	8.0 ± 0.6	8.4 ± 0.4	8.1 ± 0.5	8.0 ± 0.7	8.4 ± 0.4	7.9 ± 0.4	7.8 ± 0.5	8.5 ± 0.7	8.2 ± 0.4	8.0 ± 0.4	8.3 ± 0.4	8.4 ± 0.3	8.3 ± 0.2	8.5 ± 0.3	8.4 ± 0.3	8.4 ± 0.3	8.1 ± 0.9
Off-odour odour score	2	0.18 ± 0.1			ND			0.24 ± 0.1			0.30 ± 0.14			ND			0.35 ± 0.13		
	8	0.65 ± 0.1	0.62 ± 0.1	0.45 ± 0.1	ND			0.74 ± 0.1	0.66 ± 0.1	0.52 ± 0.2	0.33 ± 0.14	0.43 ± 0.22	0.41 ± 0.14	ND			0.35 ± 0.20	0.36 ± 0.14	0.49 ± 0.14
	15	0.79 ± 0.1	0.78 ± 0.1	0.69 ± 0.2	ND			0.75 ± 0.2	0.83 ± 0.1	0.80 ± 0.1	0.64 ± 0.11	0.68 ± 0.14	0.73 ± 0.25	ND			0.63 ± 0.11	0.64 ± 0.13	0.69 ± 0.16
	22	0.85 ± 0.1	0.79 ± 0.1	0.80 ± 0.1	ND			0.92 ± 0.1	0.84 ± 0.1	0.93 ± 0.1	0.84 ± 0.15	0.75 ± 0.11	0.87 ± 0.11	ND			0.84 ± 0.07	0.79 ± 0.08	0.90 ± 0.08

Table 2. Linear mixed effects analyses for relationship between spoilage responses and the storage time, packaging and lactate formulation: significance and estimations.

	Effect	Model adjustment	Poultry				Pork			
			Estimates ± sd	p-Value	Random effects of batches (sd)	Residual of random effects	Estimates ± sd	p-Value	Random effects of batches (sd)	Residual of random effects
Off-odour score	Reference modality (Intercept) α		0.22 ± 0.02		0.01	0.11	0.30 ± 0.03		0.02	0.14
	Storage time	Day 8	+0.39 ± 0.02				+0.06 ± 0.03			
		β	Day 15	+0.55 ± 0.02	< 0.0001	***	+0.34 ± 0.03	< 0.0001	***	
		Day 22	+0.63 ± 0.02				+0.51 ± 0.03			
	Packaging	MAP1	-0.00 ± 0.03	0.0028	**		-0.01 ± 0.02	0.0092	**	
		γ	MAP2	-0.00 ± 0.03			+0.06 ± 0.02			
	Lactate	Zero lactate		+0.06 ± 0.02	0.0002	***			0.4439	
		Interaction	Storage time x Packaging		<i>Fig. S7-C1</i>	< 0.0001	***			0.3550
			Storage time x Lactate			0.5474				0.7969
			Packaging x Lactate			0.4380				0.7103
pH value	Reference modality (Intercept) α		6.46 ± 0.05		0.12	0.12	5.98 ± 0.03		0.08	0.11
	Storage time	Day 8	-0.44 ± 0.04				-0.04 ± 0.02			
		β	Day 15	-0.87 ± 0.04	< 0.0001	***	-0.50 ± 0.02	< 0.0001	***	
		Day 22	-0.86 ± 0.04				-0.76 ± 0.02			
	Packaging	MAP1	+0.00 ± 0.03	< 0.0001	***			0.7484		
		γ	MAP2	+0.00 ± 0.03						
	Lactate	Half dose	-0.10 ± 0.03	< 0.0001	***			0.0544		
		δ	Zero lactate	-0.11 ± 0.03						
	Interaction	Storage time x Packaging		<i>Fig. S7-C4</i>	< 0.0001	***			0.6099	
		Storage time x Lactate		<i>Fig. S7-C3</i>	0.0020	**			0.6068	
Packaging x Lactate			0.7496				0.9963			
Enumeration of Lactic Acid Bacteria	Reference modality (Intercept) α		3.36 ± 0.10	NR	0.25	0.37	2.26 ± 0.08		0.11	0.47
	Storage time	Day 8	+3.67 ± 0.10				+3.41 ± 0.08			
		β	Day 15	+4.80 ± 0.10	< 0.0001	***	+5.76 ± 0.08	< 0.0001	***	
		Day 22	+4.64 ± 0.10				+5.92 ± 0.08			
	Packaging	MAP1	+0.00 ± 0.10	< 0.0001	***			0.0935		
		γ	MAP2	+0.00 ± 0.11						
	Lactate	Half dose		0.7067			+0.30 ± 0.07	< 0.0001	***	
		δ	Zero lactate				+0.10 ± 0.07			
	Interaction	Storage time x Packaging		<i>Fig. S7-C7</i>	< 0.0001	***			0.2195	
		Storage time x Lactate			0.9999				0.3469	
Packaging x Lactate			0.6703				0.7820			
Enumeration of Mesophilic aerobic bacteria	Reference modality (Intercept) α		5.43 ± 0.12		0.20	0.40	4.18 ± 0.12		0.18	0.44
	Storage time	Day 8	+3.11 ± 0.15				+2.06 ± 0.13			
		β	Day 15	+3.69 ± 0.15	< 0.0001	***	+4.08 ± 0.13	< 0.0001	***	
		Day 22	+3.33 ± 0.15				+4.27 ± 0.13			
	Packaging	MAP1	+0.00 ± 0.12	0.0128	*			0.0940		
		γ	MAP2	-0.00 ± 0.12						
Lactate	Half dose	+0.11 ± 0.11	0.0361	*		+0.17 ± 0.07	0.0157	*		

	δ	Zero lactate	+0.42 ± 0.11			+0.03 ± 0.07		
		Storage time x Packaging	<i>Fig. S7-C2</i>	0.0002	**	<i>Fig. S7-P1</i>	0.0105	*
	Interaction	Storage time x Lactate	<i>Fig. S7-C10</i>	0.0368	*		0.1976	
		Packaging x Lactate		0.9947			0.9679	
		Reference modality (Intercept) α	44.13 ± 0.43			41.17 ± 0.42		0.45 1.97
		Day 8	+2.24 ± 0.40			+2.17 ± 0.41		
	Storage time β	Day 15	+3.69 ± 0.40	< 0.0001	***	+2.60 ± 0.41	< 0.0001	***
		Day 22	+3.65 ± 0.40			+2.73 ± 0.41		
	Packaging γ	MAP1		0.8847		+0.89 ± 0.35	0.1216	*
		MAP2				+0.08 ± 0.35		
L* value	Lactate δ	Zero lactate	-0.00 ± 0.04	< 0.0001	***	+0.80 ± 0.29	0.0060	**
		Storage time x Packaging		1.0000			1.0000	
	Interaction	Storage time x Lactate	<i>Fig. S7-C6</i>	0.0027	*		0.5541	
		Packaging x Lactate		0.6750			0.0280	
		Reference modality (Intercept) α	13.55 ± 0.32			16.81 ± 0.43		0.82 1.20
		Day 8	-0.64 ± 0.37			-0.74 ± 0.43		
	Storage time β	Day 15	-2.34 ± 0.37	< 0.0001	***	-1.12 ± 0.43	< 0.0001	***
		Day 22	-3.41 ± 0.37			-1.45 ± 0.43		
	Packaging γ	MAP1	-0.00 ± 0.37	< 0.0001	***	+0.24 ± 0.45	< 0.0001	***
		MAP2	-0.00 ± 0.37	< 0.0001	***	+0.24 ± 0.45	0.0001	***
a* value	Lactate δ	Zero lactate		0.1976			0.2302	
		Storage time x Packaging	<i>Fig. S7-C5</i>	< 0.0001	***	<i>Fig. S7-P2</i>	<	***
	Interaction	Storage time x Lactate		0.5700			1.0000	
		Packaging x Lactate		0.6164			0.3791	
		Reference modality (Intercept) α	8.74 ± 0.34			10.85 ± 0.47		1.02 1.13
		Day 8	+0.57 ± 0.39			+0.47 ± 0.41		
	Storage time β	Day 15	+0.69 ± 0.39	< 0.0001	***	+0.36 ± 0.41	< 0.0001	***
		Day 22	+1.31 ± 0.39			-0.04 ± 0.41		
	Packaging γ	MAP1	-0.00 ± 0.33	< 0.0001	***	+0.20 ± 0.42	0.0005	***
		MAP2	-0.00 ± 0.33	< 0.0001	***	+0.20 ± 0.42		
b* value	Lactate δ	Zero lactate	+0.00 ± 0.27	< 0.0001	***	+0.29 ± 0.17	0.0884	
		Storage time x Packaging	<i>Fig. S7-C8</i>	0.0050	**	<i>Fig. S7-P3</i>	0.0019	**
	Interaction	Storage time x Lactate	<i>Fig. S7-C9</i>	0.0171	*		1.0000	
		Packaging x Lactate		0.2682			0.4564	

For each response and each matrix, the intercept coefficient corresponds to the reference modality ('Current dose of lactate – Air packaging – Day 2'). The reference model (M0) included three main fixed effects (storage time, packaging and lactate formulation) and random effects of production batches on intercept. For each fixed effect, the coefficient corresponding to each modality estimated by maximisation of the likelihood of (M0). The p-value corresponding to each fixed effect was obtained by testing the log-likelihood of (M0) against the model without the effect in question (χ^2 likelihood ratio test). p-value significant codes: '*' < 0.05, '**' < 0.01, '***' < 0.001. The significant effects with p-value < 0.05 were retained in model for estimations. The coefficients corresponding to different modalities of each significant main effect are given in the column 'Estimates – sd'. The coefficients corresponding to different cross modalities of each significant interaction effect are plotted in Fig. S7. The random effects of production batches and residual from random effects, expressed in standard deviation, were estimated from model with only significant main and interaction effects.

Figure Captions

Figure 1. Experimental design. For each sausage type, from day 8, 3 trays were necessary to monitor all responses per lactate dose, gas atmosphere and sampling time (per sampling time: 3 lactate doses x 3 gas compositions x 3 sampling days x 3 trays = 81 trays). For day 2, gas composition was not considered since trays had just been conditioned (3 lactate doses x 1 gas composition x 3 trays = 9 trays).

Figure 2. pH evolution as a function of experimental conditions (concentration of lactate and atmosphere packaging). For each condition, the blue (resp. red) curves correspond to the turkey (resp. pork) sausages. The different thin curves correspond to different batches. The thick curves correspond to the inter-batch average curve.

Figure 3. Pairwise plots between sensory, physico-chemical and microbiological responses monitored during chilled storage of pork and turkey sausages. The lower graphs correspond to the scatter plot between responses. The blue (resp. red) points correspond to the turkey (resp. pork) sausages. The Spearman's correlation rank sum coefficients between responses are shown in the upper graph. * $p < 0.05$ for Spearman's correlation rank sum test. Example for reading values: the correlation coefficient between b* value and pH was 0.12 with $p > 0.05$ - not significant (resp. -0.26 with $p < 0.05$ - significant) in pork (resp. turkey) sausages.

Figure 4. Visual alterations of sausages during chilled storage (Packaging swelling and Exudate). The point size is correlated to the to the number of spoiled batches. The smallest points correspond to 0 spoiled batch (day 2), the biggest points correspond to 10 spoiled batches (e.g. turkey, under Air packaging at day 22). The green (resp. orange and blue) points and lines correspond to the "Air packaging" (resp. "MAP1" and "MAP2"). MAP1:70%O₂-30%CO₂, MAP2:50%CO₂-50%N₂. For each packaging, the lightness of the points corresponds to the lactate formulation (very light colour: "Without lactate", light colour: "Half dose of lactate" and dark colour "Current dose of lactate"). Turkey: "Without lactate" = 0g/kg, "Half dose of lactate" = 10g/kg, "Current dose of lactate" = 20g/kg. Pork: "Without lactate" = 0g/kg, "Half dose of lactate" = 5.65g/kg (visual defects not evaluated), "Current dose of lactate" = 11.3g/kg.

Figure 5. Schematic representation of the effects of process parameters on pH acidification, growth of LAB count and off-odour perception score. a. Influence of increasing potassium lactate. b. Effect of the replacement of air by the MAP1 70% O₂ – 30% CO₂. c. Effect of the replacement of air by MAP2 50% CO₂ – 50% N₂. The positive (resp. negative) signs correspond to the significant positive (resp. negative) correlation between spoilage responses (Spearman's correlation). The ascending arrows (resp. descending arrows and crosses) representing the significant increases (resp. decreases or absence of the effect) of the response by the used preservation strategy according to mixed effect analyses.

Figure S.1. Off-odour score evolution as a function of experimental conditions (concentration of lactate and atmosphere packaging). For each condition, the blue (resp. red) curves correspond to the turkey (resp. pork) sausages. The different thin curves correspond to different batches. The thick curves correspond to the inter-batch average curve.

Figure S.2. Lactic acid bacteria enumeration as a function of experimental conditions (concentration of lactate and atmosphere packaging). For each condition, the blue (resp. red) curves correspond to the turkey (resp. pork) sausages. The different thin curves correspond to different batches. The thick curves correspond to the inter-batch average curve.

Figure S.3. Total mesophilic aerobic bacteria enumeration as a function of experimental conditions (concentration of lactate and atmosphere packaging). For each condition, the blue (resp. red) curves correspond to the turkey (resp. pork) sausages. The different thin curves correspond to different batches. The thick curves correspond to the inter-batch average curve.

Figure S.4. Evolution of the CIE L* value as a function of experimental conditions (concentration of lactate and atmosphere packaging). For each condition, the blue (resp. red) curves correspond to the turkey (resp. pork) sausages. The different thin curves correspond to different batches. The thick curves correspond to the inter-batch average curve.

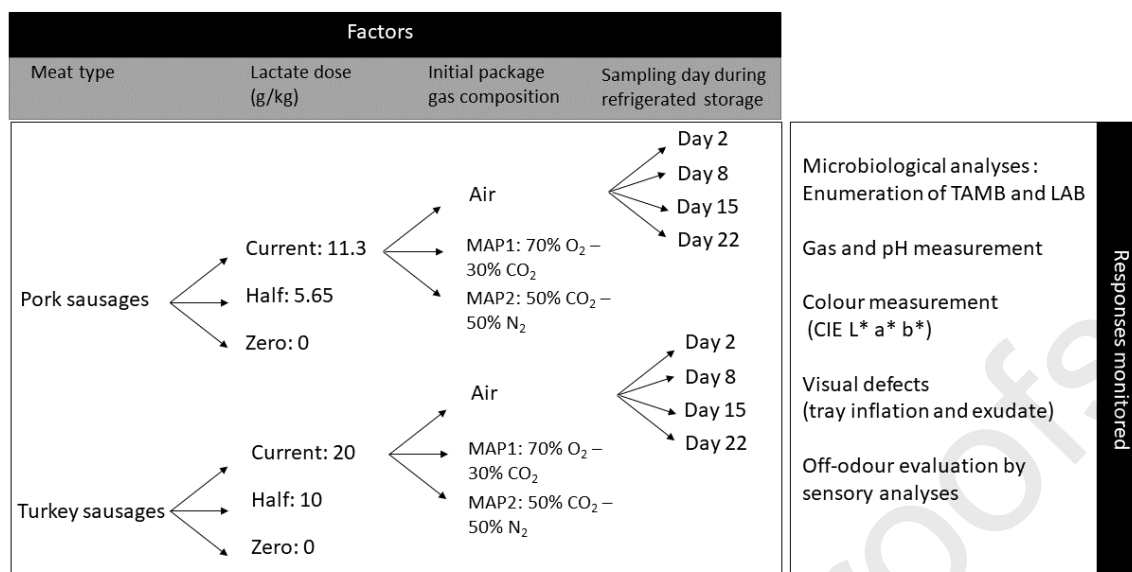
Figure S.5. Evolution of the CIE a* value as a function of experimental conditions (concentration of lactate and atmosphere packaging). For each condition, the blue (resp. red) curves correspond to the turkey (resp. pork) sausages. The different thin curves correspond to different batches. The thick curves correspond to the inter-batch average curve.

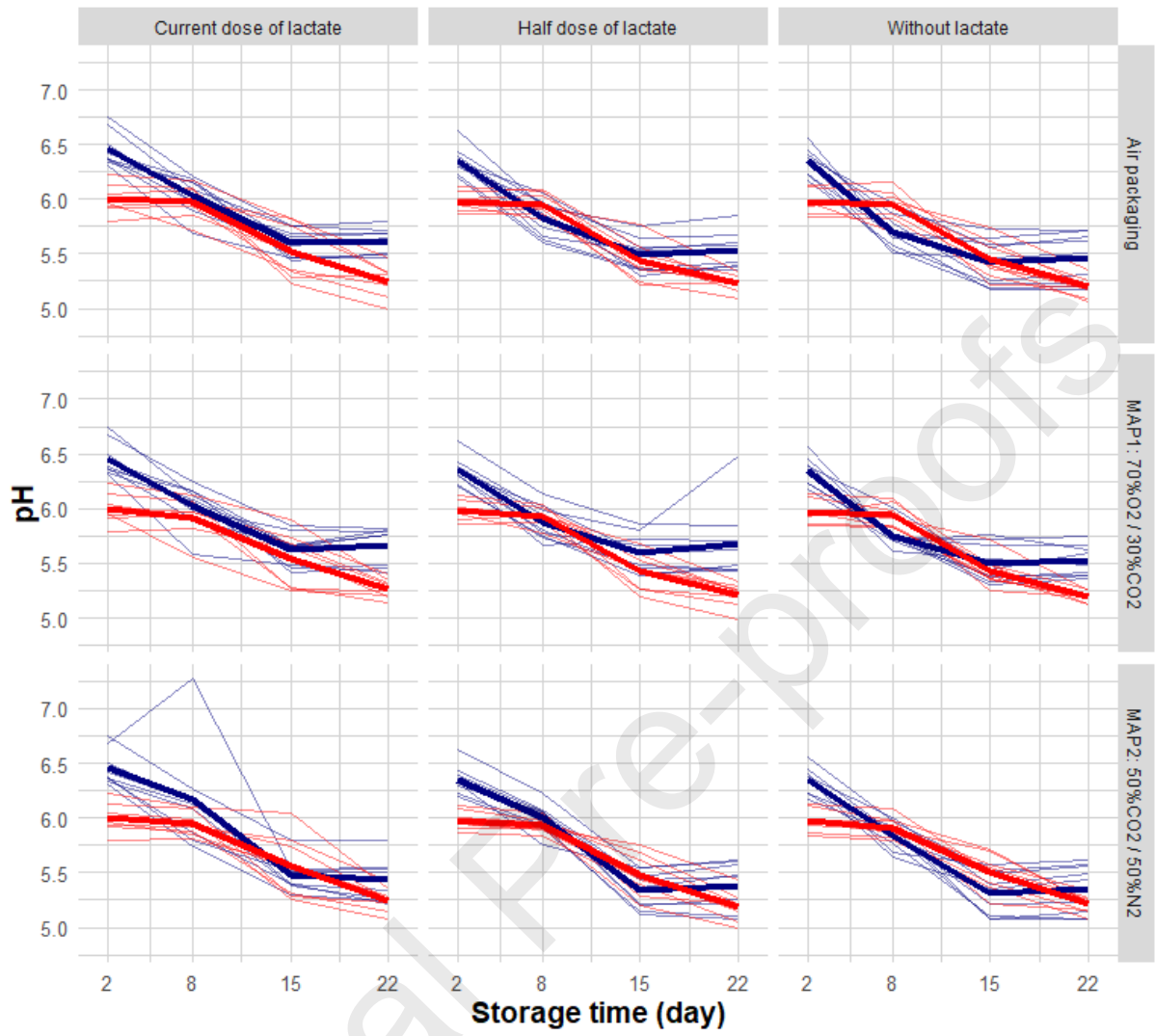
Figure S.6. Evolution of the CIE b* value as a function of experimental conditions (concentration of lactate and atmosphere packaging). For each condition, the blue (resp. red) curves correspond to the turkey (resp. pork) sausages. The different thin curves correspond to different batches. The thick curves correspond to the inter-batch average curve.

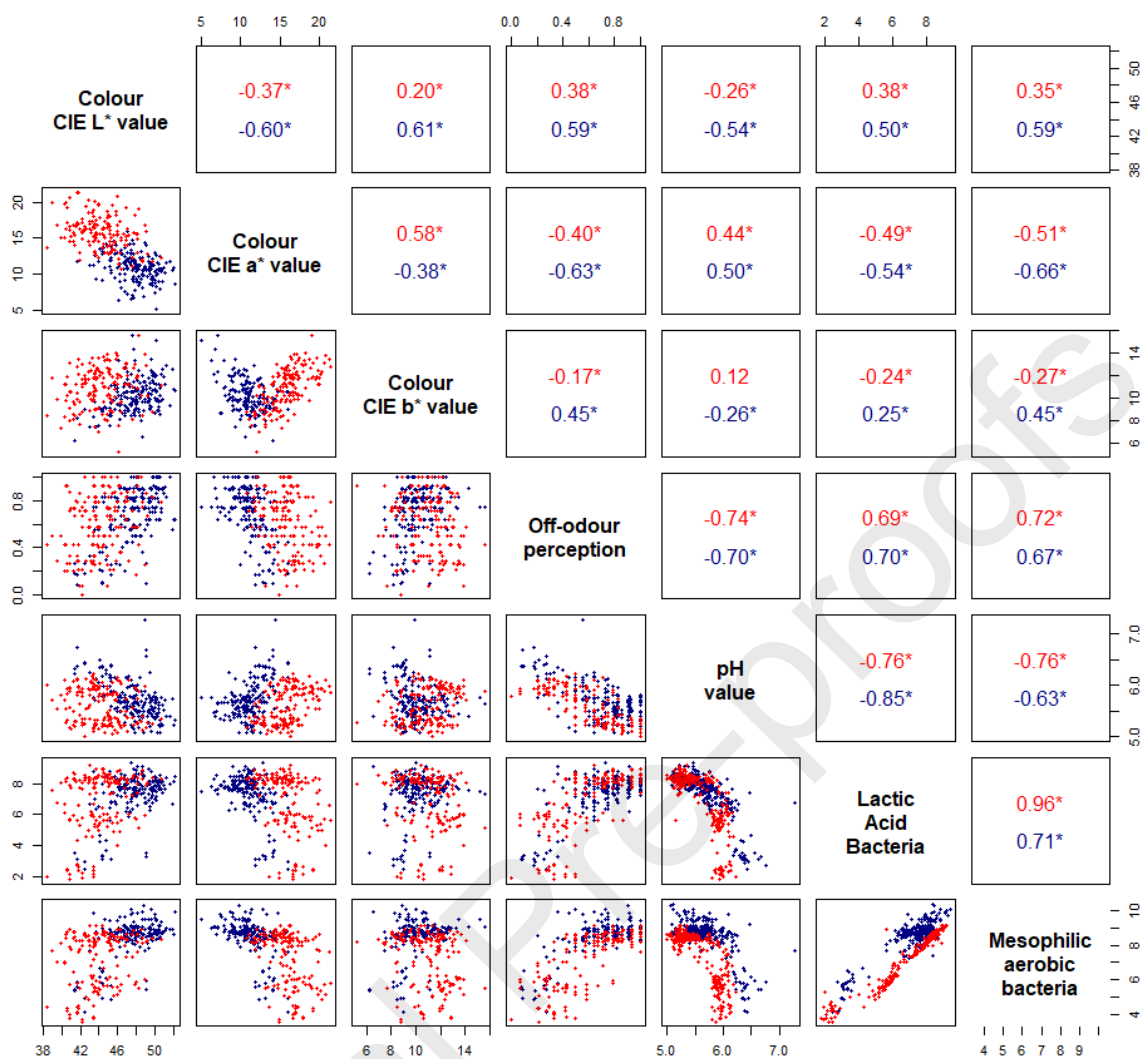
Figure S.7. Estimated coefficients for interactions between fixed factors of storage time, packaging or lactate on spoilage responses in pork and turkey sausages. C: Turkey, P: Pork. Only significant interactions (p -value < 0.05) are shown (Table 2). The coefficients are plotted by point estimates and 95% confidence interval using the R package 'sjPlot'. The different colours correspond to the different packaging and initial concentrations of lactate.

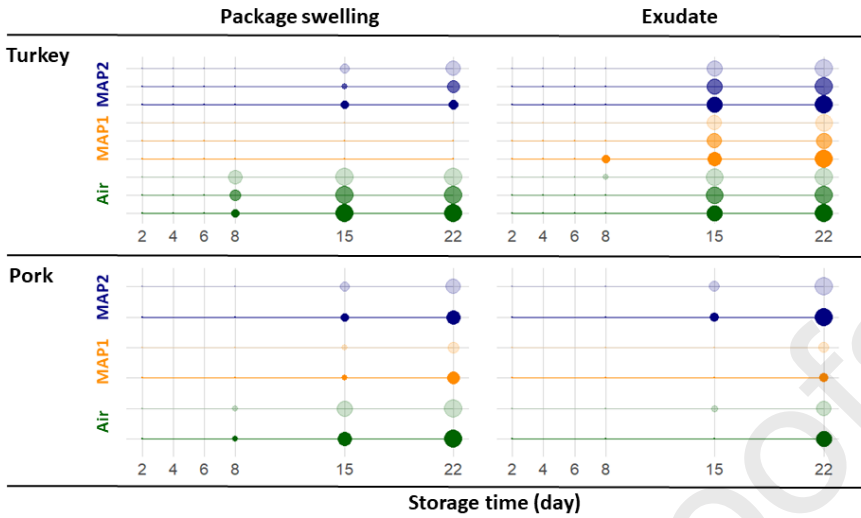
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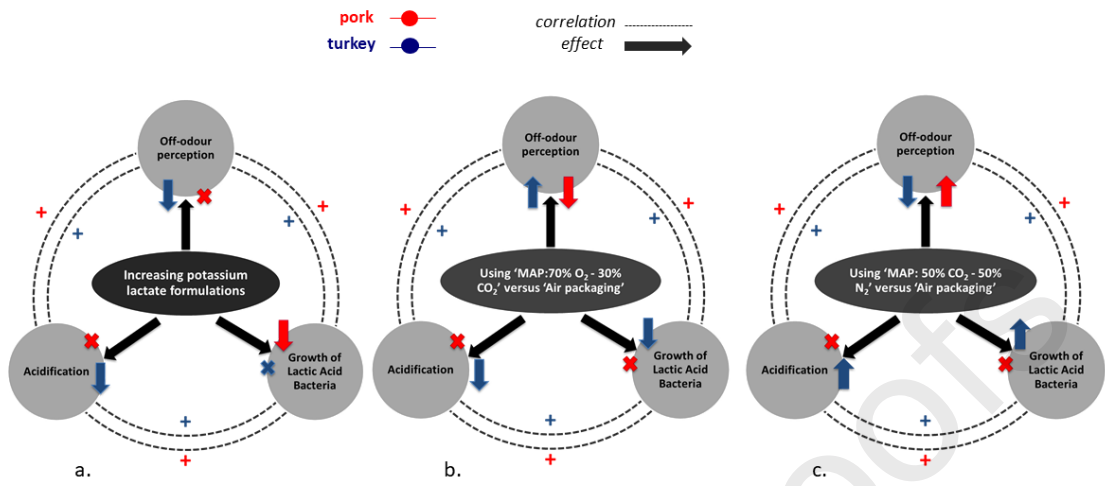
Ngoc-Du Martin LUONG: Original draft writing, Data acquisition, Methodology, Data analysis, **Sabine JEUGE**: Data acquisition, Data analysis, writing ; **Louis COROLLER** : Methodology, Supervision, Reviewing ; **Carole FEURER** : Data acquisition, supervision, reviewing ; **Marie-Hélène DESMONTS** : Data acquisition and analysis ; reviewing ; , **Nicolas MORICEAU** : Data acquisition ; **Valérie ANTHOINE** : Data acquisition ; **Sophie GAVIGNET** : Data acquisition ; **Adeline RAPIN** : Data acquisition ; **Bastien FREMAUX** : Data acquisition ; **Emeline ROBIEU** : Data acquisition ; **Monique ZAGOREC** : Data acquisition, Conceptualisation, Data acquisition, Supervision, Reviewing ; **Jeanne-Marie MEMBRE** : Methodology, Supervision, reviewing ; **Sandrine GUILLOU** : Data acquisition, Supervision, Writing, reviewing.

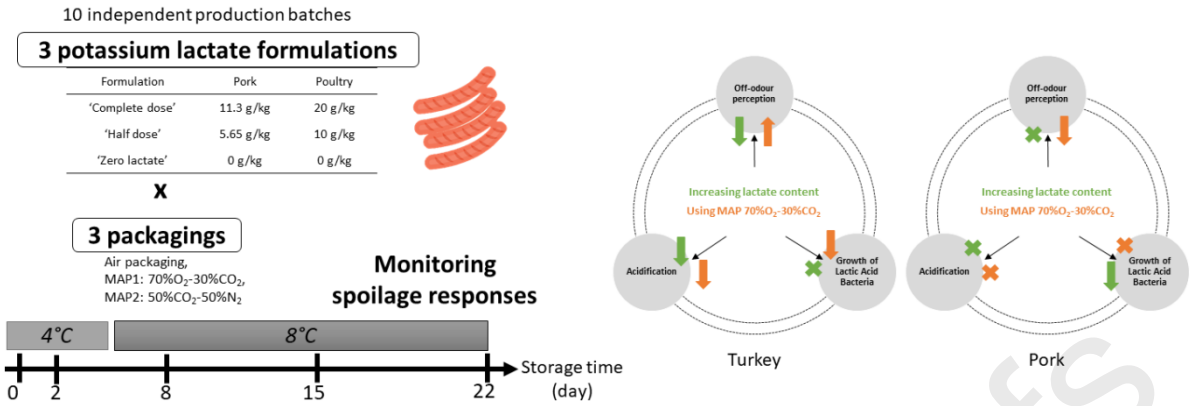












Highlights

- High oxygen atmosphere increases off-odour and discolouration of turkey sausages.
- Unlike turkey, lactate barely affects spoilage-associated responses in pork sausages.
- Spoilage dynamics of pork and turkey sausages vary greatly between production batches

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