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**Influence of composition and processing of Frankfurter sausages on the growth
dynamics of *Listeria monocytogenes* under vacuum**

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ABSTRACT

Ready-to-eat (RTE) meat products, such as Frankfurter sausages, are often linked to cases of listeriosis, which is a disease caused by the foodborne pathogen *Listeria monocytogenes*. Frankfurter sausages vary, from manufacturer to manufacturer, in many aspects: (i) composition, (ii) physicochemical characteristics, (iii) background flora, (iv) processing technology and (v) organoleptic characteristics. Some of these factors have been widely investigated for their effect on *L. monocytogenes* on food products. However, studies on the specific effect of composition and processing technology on *L. monocytogenes* dynamics between two sausages are lacking. In this study, the growth dynamics of *L. monocytogenes* on two types of Frankfurter sausages, fresh and in brine, were studied at constant storage temperatures (4, 8 and 12°C). Additionally, the physicochemical and compositional characteristics of both types of sausages were analysed. In order to study the isolated effect of preservatives, *L. monocytogenes* growth dynamics, at 4°C and 30°C, were studied in two types of liquid systems. These were prepared with the same level of preservatives as in the two types of sausages. Results indicated no major significant differences in physicochemical characteristics for the two types of sausages; but, statistically significant variability was detected in the concentration of preservatives. In liquid systems, the maximum specific growth rate ($\mu_{\max}$) remained unaffected by the effect of preservatives, but the lag phase was longer in the system mimicking fresh sausages. In sausages, the 'in brine' type had two-fold higher $\mu_{\max}$ at all temperatures and shorter lag phase at 4°C. The presence/absence of sausage skin, which was found to be impermeable from *L. monocytogenes* cells and was present in the fresh sausage, could explain those differences. In conclusion, this study highlighted the influence of processing factors, and specifically of the sausage casing on *L. monocytogenes*

growth dynamics. Therefore, an edible membrane, which is heat resistant and impermeable to the cells, could be a hurdle strategy to control the microbiological food safety.

1. Introduction

Consumers' demand for ready-to-eat (RTE) meat products, such as Frankfurter sausages, is continuously increasing, worldwide. This type of products are often linked to cases of listeriosis, which is a disease caused by the foodborne pathogen *Listeria monocytogenes*. Listeriosis has a high fatality rate, 17.8% in 2012 (EFSA/ECDC, 2014). This pathogen can survive even at harsh environmental conditions such as, refrigeration temperature, high NaCl level, modified atmospheres or vacuum, a fact that makes it a major threat in food industry.

The food industry is continuously assessing processing techniques, compositional characteristics and the packaging of their products. Their assessments focus on ensuring the microbiological safety and producing healthier products, i.e., with reduced NaCl and fat content. Thus, the food industry aims to retain the organoleptic properties of their products by altering processing techniques and composition, in order to make them healthier.

Variety of products, sold as Frankfurters, are manufactured by different companies and are considered as similar products by consumers. Nevertheless, they vary significantly in terms of composition, production technology packaging conditions and background flora. All these variations among different brands, apart from differences in organoleptic properties, they might cause variations in microbial growth dynamics as well. As the effect of packaging atmosphere and background flora has extensively been studied and found to be influential to the growth of *L. monocytogenes* on meat products (Wimpfheimer et al., 1990; Tsigarida et al., 2000; Samelis et al., 2001; Pal et al., 2008; Baka et al., 2014), this study focuses only on the investigation of compositional and processing factors, the presence of the sausage casing of this type of products and their influence on the growth dynamics of *L. monocytogenes*. The sausage casing might act as a bidirectional impermeable barrier, which prevents (i) cells from penetrating into the

sausage and also (ii) nutrients to migrate from the interior of the sausage to the surface. Nevertheless, there is not available information on this subject.

To the knowledge of the authors, there are no studies where the composition, processing or the influence of the sausage casing have been studied on the growth dynamics of *L. monocytogenes*. However, there are studies in liquid microbiological media and on real food products, where the preservatives' level, as part of the total composition has been investigated on *L. monocytogenes* growth (Shelef and Yang, 1991; Razavilar and Genigeorgis 1998; Mbandi and Shelef, 2001, 2002; Golden et al., 2007).

In this study, the growth dynamics of *L. monocytogenes* were studied on two types of Frankfurter sausages, fresh and in brine, with different casing, packed under vacuum and stored at 4, 8 and 12°C. Preliminary studies among three *L. monocytogenes* strains, isolated from meat products, were conducted. The fastest growing among them will be further used in this study, representing the worst case scenario of growth on sausages. Both types of sausages were analysed for their physicochemical (pH and a_w) and compositional characteristics. Two types of liquid systems were also prepared, mimicking in composition the two types of sausages, where the isolated effect of the composition was assessed on the microbial dynamics of *L. monocytogenes* was studied. Qualitative and quantitative cell penetration experiments were performed to this sausage casing, in order to investigate if microbial cells are able to penetrate it and quantify them.

2. Materials and methods

2.1. Growth dynamics of L. monocytogenes in Frankfurter sausages

2.1.1. Preculture conditions

L. monocytogenes LMG 23775 strain was acquired from the BCCM/LMG bacteria collection of Ghent University in Belgium and was stored at -80°C in Brain Heart Infusion broth (BHI, Oxoid), supplemented with 25% glycerol (v/v) (Acros Organics). For the inoculum

preparation, a loopful of the frozen culture was inoculated into a 100 mL Erlenmeyer flask containing 20 mL BHI, which was incubated in a temperature controlled incubator at 30°C (model KBP6151, Termaks). After 22 h, 20 µL were transferred into 20 mL fresh BHI and again incubated at 30°C for 22 h.

2.1.2. Culture conditions in Frankfurter sausage

Two types of Frankfurter sausages were purchased from a Belgian supermarket: ‘type 1’, in a jar with brine (30 g/sausage) and ‘type 2’, fresh in packs (80 g/sausage). Immediately after their transfer to the laboratory, the ‘type 2’ sausages were maintained at 4°C before use and the ‘type 1’ sausages at room temperature. Prior to inoculation, sausages were dipped in boiling water for 30 s, in order to eliminate the background flora (Radin et al., 2006). Then, they were allowed to dry on sterile bandages and cool down under aseptic conditions for approximately 5 min (Baka et al., 2014). Early stationary phase cultures of *L. monocytogenes* were inoculated, with a target initial inoculum of 10^2 CFU/g. Preliminary tests were performed in order to select the most appropriate inoculation volume for the size / available surface of the sausages. The final volume was 30 and 80 µL for ‘type 1’ and ‘type 2’ sausages, respectively, in order to cover the maximum sausage surface and to minimise the drying period, before vacuum packaging. Although the occurrence of *L. monocytogenes* is legally prohibited in ready-to-eat meat products, a limit concentration of 10^2 CFU/g is allowed for products at the consumption stage. This value has been used here to mimic the worst permitted scenario, as it also allows cell enumeration above the detection limit. After inoculation, the cell solution was spread with a pipette tip over the entire sausage surface. Inoculated sausages were aseptically transferred into plastic bags of Polyamide / Polyethylene (PA/PE 90), and left at 4°C for 15 min, to allow cell attachment (Byelashov et al., 2008). Finally, they were packaged (one sausage per bag) under 99% vacuum in a vacuum machine (Boxer 42, Henkelman) and incubated at 4, 8 and 12°C for maximum 30 days. These temperatures were selected based on the normal

storage temperature of the fresh type of meat products (4°C) and temperature-abuse conditions (8 and 12°C). All samples were vacuum packed prior to incubation (one sausage per bag).

2.1.3. Microbiological analysis

Evolution of *L. monocytogenes* concentration over time was monitored by viable plate counting. Standard volume, 210 and 240 mL of peptone water (10 g/L bacteriological peptone (Oxoid) and 5 g/L NaCl (AnalaR, Normapur®, VWR International)) was aseptically poured in the vacuum bag, and mixed for 2 min by stomaching (basic masticator, Led techno) with the whole sausages of 'type 1' and 'type 2', respectively. The dilution factor was different for each type of sausage and different from the decimal one (1:10), which is commonly used. The reason was two-fold: (i) reduce as much as possible the dilution volumes and improve the efficiency of the procedure and (ii) facilitate the sampling of the stomached sausages by pipetting, considering their different weight and resistance to stomaching. Then, the corresponding serial decimal dilutions were plated with a Spiral Plate System (Eddy Jet IUL Instruments) on Palcam agar base with Palcam selective supplement (Oxoid), a selective and differential medium for the enumeration of *L. monocytogenes*. Plates were incubated at 30°C for 48 h before enumeration. The sampling frequency, until growth curves reached the stationary phase, was 1, 2 and 3 samples/day at 4, 8 and 12°C, respectively. Afterwards, sampling time frequency was reduced up to 1 to 2 samples/day per temperature. It is noteworthy that a number of sausages (between 20 and 30) were inoculated simultaneously for each incubation temperature and one sausage per temperature was sampled at each time interval. Every experiment was independently performed in duplicate.

2.1.4. Modelling growth dynamics and parameter estimation

To estimate the growth parameters of *L. monocytogenes*, the Baranyi and Roberts (1994) model, described by equations (1), was fitted to experimental data.

$$\frac{dN(t)}{dt} = \frac{Q(t)}{1+Q(t)} \cdot \mu_{\max} \cdot \left(1 - \frac{N(t)}{N_{\max}}\right) \cdot N(t) \quad (1)$$

$$\frac{dQ(t)}{dt} = \mu_{\max} \cdot Q(t)$$

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with $N(t)$ [CFU/g] for sausages and [CFU/mL] for BHI, the cell density at time t ; $N_{\max}$ [CFU/g] for sausages and [CFU/mL] for BHI, the maximum value for $N(t)$; $Q(t)$ [-], the physiological state of the cells; and $\mu_{\max}$ [1/h], the maximum specific growth rate. When lag estimate approached the biologically lower bound of zero, with unrealistically large confidence intervals, then lag was set equal to zero hours (Baka et al, 2014). Model parameters were estimated from the set of experimental data via the minimisation of the sum of squared errors, using the lsqnonlin routine of the Optimization Toolbox of Matlab

$$SSE = \sum_{i=1}^M (n(t_i) - n(t_i, \mathbf{p}))^2 \quad (2)$$

$$MSE = \frac{SSE}{M - \nu}$$

version R2010b (The Mathworks Inc.). For parameter estimation based on one experiment (with M measurements), the Mean Squared Error (MSE) is described by equation (2).

With SSE the Sum of Squared Errors, $n(t_i)$ the natural logarithm of the cell density by measurements and $n(t_i, \mathbf{p})$ the natural logarithm of the cell density by the model prediction at time t_i . The vector $\mathbf{p}$ contains the considered parameters of the model. ν stands for the number of degrees of freedom.

2.2. Growth dynamics of *L. monocytogenes* in liquid systems

Preculture conditions, modelling the growth dynamics and parameter estimation for the growth of *L. monocytogenes* in liquid systems is identical to those described for Frankfurters, sections 2.1.1 and 2.1.4, respectively.

2.2.1. Culture conditions in liquid systems

Two types of BHI liquid solutions (3.7% w/v) were produced, mimicking in composition (NaCl, NaNO₂, citric acid, ascorbic acid, pentasodium triphosphate) the two types

of Frankfurters. Both liquid systems were inoculated with *L. monocytogenes* cells, at a target inoculum level of 10^2 CFU/mL. Afterwards, they were divided in vacuum bags (4 mL/bag), vacuum packed at 99% vacuum and incubated at the appropriate temperature (4 and 30°C). Temperatures were selected in order to work (i) at stressing temperature (4°C), which was the most stressing of the three studied on Frankfurters and (ii) at optimal temperature (30°C), in order to study the isolated effect of the compositional variability on the growth of *L. monocytogenes* in liquid systems, without interfering with any other stressing factor.

2.2.2. Microbiological analysis

Evolution of *L. monocytogenes* concentration over time was monitored by viable plate counting, as for Frankfurters. 1 mL of sample was obtained from each vacuum bag. Then, the corresponding serial decimal dilutions were plated with a Spiral Plate System (Eddy Jet IUL Instruments) on BHI-agar (BHI supplemented with 1.4% (w/v) agar technical No.3, Oxoid). Plates were incubated at 30°C for 48 h before enumeration. The sampling frequency until growth curves reached the stationary phase was 1 samples/day and 1 sample/hour the first day, at 4 and 30°C, respectively. Every experiment was independently performed in duplicate.

2.3. Physicochemical and compositional analysis

For both types of sausages and for each analysis, three replicates from different commercial batches were used. pH and water activity (a_w) measurements were performed with a pH electrode (PY-P11, Sartorius), and an a_w -Kryometer (AWK-40, NAGY MessystemeGmbH), respectively.

Water content, fat, NaCl, and NaNO₂ were measured based on the analytical methods published by the Association of Official Analytical Chemists (AOAC, 1998). The carbohydrate content was extracted and measured by the phenol-sulfuric method, as described by BeMiller (2010). The protein concentration was measured by using the Lawry method (Lawry et al., 1951). The sample was prepared as described by Peterson (1977) and the extraction of the

proteins as instructed by Sözgen et al. (2006). Both preservatives ascorbic acid and citric acid were analysed by a validated RP-HPLC method (Iammarino and Di Taranto, 2012). Sample preparation was as follows: 4 g sample of each sausage, homogenised in a blade homogeniser, was mixed in 40 mL of phosphate buffer 10^{-2} M pH 3.5. The mixture was vortexed for one minute and then centrifuged for 5 min at 250 x g at room temperature. The supernatant was filtered through filter paper and then through 0.2 μ m syringe filters directly to vials. The standards used were citric acid monohydrate (VWR Prolabo, Assay: 100%) and L-Ascorbic acid (Duchefa Biochemie, Assay: >99%). The chromatographic separation was carried out by a RP-HPLC method at an Agilent 1200 series module (Agilent Technologies, Palo Alto, CA, USA) equipped with a Diode Array detector. The column was an Aminex HPX-87H 300 m x 7.8 mm, 0.9 μ m particle size (BioRad, CA, USA), operating at 23°C. The mobile phase used, was 100% H_2SO_4 0.005 M, at a flow rate of 0.4 mL/min within a run time of 30 min. The injection volume was 20 μ L and the detection was by absorbance at 212 nm and 244 nm for citric acid and ascorbic acid, respectively. Acid concentrations were determined by numerical integration of the area under the corresponding peaks.

2.4. Statistical analysis

Analysis of variance (ANOVA) test was performed to determine whether there are any significant differences amongst means of compositional values, at a 95.0% confidence level ($\alpha=0.05$). Fisher's Least Significant Difference (LSD) test was used to distinguish which means were significantly different from which others. Standardised skewness and standardised kurtosis were used to assess if data sets came from normal distributions. These analyses were performed using Statgraphics Centurion XVI.I® Package (Statistical Graphics, Washington, USA). Test statistics were regarded as significant when P was ≤ 0.05 .

2.5. Cell penetration studies in sausage casings

The 'type 1' sausages were skinless, in contrast to the 'type 2' sausages which had a casing. Therefore, no penetration experiments were performed with the 'type 1' sausages. The casing of the 'type 2' sausages consisted of: (i) collagen: 47-52%, (ii) water: 22-22.5%, (iii) humectant: 16-17%, (iv) cellulose: 5%, (v) vegetable oil: 3.5-5%, (vi) colour components: 0-3% (information provided by manufacturer). The ability of *L. monocytogenes* cells to penetrate the casing of 'type 2' sausages was examined qualitatively and quantitatively. For both cases, the pre-treatment of the sausages was the same. The sausages were dipped in boiling water for 30 s, in order to eliminate the background flora and left cool down, under aseptic conditions. Then, the sausage casing was peeled in small pieces. For the qualitative analysis, the piece of the sausage casing was placed on a BHI-agar plate in triplicate. Then, 3 μ L of 10^2 and 10^9 CFU/mL of *L. monocytogenes* cells were placed on the sausage skin as i) a compact drop or ii) spread on the surface of the sausage skin with the pipette tip. The samples were allowed to dry under aseptic conditions for 1 h. Then, the sausage skin was removed from the agar-plate and thrown away. Furthermore, the agar-plate was incubated at 30°C for 24 h, when it was checked for the presence or absence of colonies. For the quantitative analysis, the procedure was based on the one described by Perni et al. (2008). A small piece of the sausage casing was placed on the top of a filter (25mm diameter, 0.2 μ m porosity, Whatman) aseptically attached on a BHI-agar plate. Then, 3 μ L of 10^2 and 10^9 CFU/mL of *L. monocytogenes* cells were placed on the sausage skin as previously described. The liquid was allowed to dry under aseptic conditions for 1 h. Then, the piece of the sausage skin was removed and the filter was immersed in peptone water and stomached to release the migrating cells. Then, the appropriate dilutions in peptone water were plated on BHI-agar plates, which were incubated for 24 h at 30°C. At a next step, the plates were enumerated for potential colonies presence.

3. Results and Discussion

3.1. Growth dynamics of *L. monocytogenes* on the two types of sausages

L. monocytogenes grew faster on the skinless ‘type 1’ sausages, than on the ‘type 2’ with the synthetic casing, at all storage temperatures 4, 8 and 12°C (Figure 1a, 1b, 1c and Table 1). The maximum specific growth rate ($\mu_{\max}$) was approximately two-fold higher at the ‘type 1’ sausages than at the ‘type 2’, at all temperatures. Lag phase (λ) was absent at 8 and 12°C, but at 4°C the growth dynamics of *L. monocytogenes* on ‘type 2’ sausages exhibited higher lag estimate than at the ‘type 1’ sausages. In order to unravel the reason of the different growth dynamics on the two types of sausages, further investigation was necessary. Firstly, both types of sausages were analysed for their physicochemical and compositional characteristics. Firstly, the penetration of the ‘type 2’ sausage casing by *Listeria* cells will be assessed and the influence of the sausage casing will be discussed.

3.2. Influence of the physicochemical and compositional characteristics of the two types of sausages on the growth dynamics of *L. monocytogenes*

No statistically significant differences were found between the pH, a_w , moisture, proteins, carbohydrates and NaNO₂ content of both types of sausages, although they differed significantly in the concentration of fat, NaCl, citric acid and ascorbic acid levels (Table 2). However, NaCl variance was too small to justify such a big difference between the growth dynamics of *L. monocytogenes* at both types of sausages. On the other hand, the variance in fat concentration could suppress the growth of *L. monocytogenes* in the ‘type 1’ sausage, where fat concentration is higher. It is known that microorganisms grow in aqueous phase (Wilson et al., 2002) and not in the fat/oil phase. Therefore, the ‘type 1’ sausage having a higher concentration of not favourable for growth, fat environment, could exhibit growth inhibition for *L. monocytogenes*. Nevertheless, growth of *L. monocytogenes* was lower in ‘type 2’ sausage than in ‘type 1’. Regarding the citric and ascorbic acid levels, a six-fold higher concentration of citric acid and a two-fold less level of ascorbic acid were found, in the ‘type 1’ sausages, in comparison to the ‘type 2’ sausages. Finally, pentasodium triphosphate was contained in the

‘type 2’ sausage, but not in the ‘type 1’. Nevertheless, the concentration of pentasodium triphosphate contained in ‘type 2’ sausages was lower than 2% (w/w), which is the reported limit at which the growth of *L. monocytogenes* remains unaffected (Zhang and Farber, 1996).

In order to assess the influence of the different levels of preservatives in both types of sausages, growth dynamics were also determined in BHI-based, liquid systems. Those systems were enriched with the preservatives, NaCl, NaNO₂, citric acid and ascorbic acid at the same concentrations as they were quantified in both types of sausages. These experiments were conducted at 4 and 30°C. The growth parameters of *L. monocytogenes* in both types of BHI are presented in Table 3 and the corresponding growth curves are illustrated in Figure 2a and 2b. It is observed that $\mu_{\max}$ estimates for both types of BHI were almost equal at both temperatures. Yet, there was a significant difference between the lag time estimates at 4°C. At 30°C, it was almost negligible. This observation is logical, as 4°C is stressing temperature, in contrast to optimal 30°C, and therefore enforces the influence of other factors causing mild stress, such as preservatives, on the growth dynamics. It is noteworthy, that lag estimates of *L. monocytogenes* on Frankfurters were not affected by differences in preservatives concentrations at low, and consequently stressing, temperatures (4, 8 and 12°C). Furthermore, *L. monocytogenes* growth dynamics in liquid (BHI) and solid systems (Frankfurters) exhibited significant differences, although they had equal preservatives’ level. This is due to the additional effect of the solid food structure (Wilson, 2000), which suppresses microbial growth. It has been reported that a solid environment promotes colony growth, instead of planktonic growth found in liquids and induces limitations on the mechanical distribution of water (Hills et al., 1996, 1997; Wilson et al., 2002), the chemical redistribution of food preservatives (Brocklehurst et al., 1993; Brocklehurst and Wilson, 2000), and also physical constraints on the mobility of microorganisms (Mattila and Frost, 1988 a, b; Dodd, 1990; Dodd and Waites, 1991; Robins et al., 1994; Robins and Wilson, 1994; Wimpenny et al., 1995). Additionally, concentration

gradients of nutrients and metabolites, and also O₂ and pH, within and around the colonies affect microbial growth, as a consequence of diffusional limitations in solid systems. Therefore, the experiments in BHI could only characterise the isolated effect of the preservatives' levels found at the two types of sausages, but not the stress from the 'solid environment'. The compositional (concentration of preservatives) variability in liquid systems did not influence $\mu_{\max}$, but affected lag times. Differences on the lag estimates of *L. monocytogenes* on Frankfurters were only observed at 4°C and were not comparable to those from BHI. An explanation could be the fact that the undissociated organic acids (preservatives) are lipophilic compounds (Gordon and Reid, 1922; Bodansky, 1928; von Schelhorn, 1964; Leo et al., 1971) and their migration to the lipid phase increases by the presence of solutes, such as NaCl, in the water phase (Gooding et al., 1955; Sofos and Busta, 1981). Thus, in the 'type 1' sausages, with higher fat concentration, the action of undissociated organic acids might have been diminished. This could explain the more extended lag phase at 4°C and the lower $\mu_{\max}$ values on the 'type 2' sausages.

3.3. Influence of the sausage casing on the growth dynamics of *L. monocytogenes*

In this section, the influence of the sausage casing will be discussed. The ability of the cells to penetrate the sausage casing of the 'type 2' sausages was examined, whereas the skinless 'type 1' sausages were not examined for cells penetration. Results indicated that there were no colonies formed on the BHI-agar plate, where the sausage casing with a drop of *L. monocytogenes* cells was placed (qualitative method). Additionally, for the quantitative method, no cells were found on the BHI-agar plates after plating variable dilutions of peptone water, where the filter paper was immersed. Thus, results were negative for penetration from both the qualitative and quantitative assays. Consequently, in 'type 2' sausages, *L. monocytogenes* cells had a barrier, the sausage casing, which hampered their direct contact with the surface of the sausage and its nutrients, in contrast to the 'type 1' sausages, which were

in direct contact. Specifically, the sausage casing could bidirectionally act as a barrier: (i) for the nutrients to reach the cells attached on the sausage casing and (ii) for the cells to get in touch with the filled paste of the sausage, where the nutrients are. The surface of the skinless Frankfurter is composed of coagulated protein with small cavities (McKellar et al., 1994), which permit cells' penetration. Therefore, the cavities formation and the direct contact of the cells with the sausage surface favour the proliferation of *L. monocytogenes*, by increasing the space availability and the nutrients intake on 'type 1' sausages. This could explain the higher $\mu_{\max}$ values of *L. monocytogenes* on 'type 1' sausages, than on 'type 2' sausages. At this step, it is noteworthy to discuss how and where *L. monocytogenes* proliferates on 'type 2' sausages. As it could not penetrate the casing, it could only grow due to the nutrients' (soluble in the water phase) diffusion from the interior of the sausage to the surface. In addition to this water and nutrients' diffusion, it has been reported that dipping the sausages in boiling water prior to inoculation, to eliminate the background flora, a water film is created, where microorganisms can proliferate (Buchanan and Klawitter, 1991, Noriega et. al., 2010, Notermans and Kampelmacher, 1975). So, the growth of *L. monocytogenes* at 'type 2' sausages corresponds to surface growth, or to a liquid micro-environment on the surface of the sausage. Nevertheless, a few of the components of the casing in 'type 2' sausages could cause water and, consequently, water-soluble nutrients diffusional limitations. It has been reported by Pavlath and Orts (2009) that fat in edible films, such as the sausage casing, are used to reduce the water transmission. Additionally, cellulose has reduced water transmission than average polysaccharides (Pavlath and Orts, 2009). Therefore, the specific sausage casing contained compounds that reduce the water transmission capacity. This could also justify the slower growth of *L. monocytogenes* on 'type 2' sausages.

4. Conclusion

This study illustrated that two different types of Frankfurter sausages, varying in composition and in processing techniques, can have significant differences at microbial growth. The two products were similar in physicochemical (pH and a_w) but varied in compositional characteristics and at the sausage casing. The 'type 1' was skinless and the 'type 2' had a synthetic casing. *L. monocytogenes* cells could not penetrate the sausage casing of 'type 2' sausages. The differences between preservatives' level in liquid systems exhibited no variations at the $\mu_{\max}$ estimates, but the lag estimates at 'type 1' were longer than 'type 2'. At the sausages, the $\mu_{\max}$ estimates of 'type 1' were higher than of 'type 2' and lag estimates at 4°C were longer at 'type 2' than at 'type 1'. These results demonstrated that *L. monocytogenes* growth dynamics at two different types of sausages vary significantly, not only due to compositional variability, but also due to other factors, related to the processing technologies, presence of sausage casing and structure of sausages. Therefore, further investigation is required with respect to the compositional and processing effect of meat products, and subsequently of other food products on their microbiological safety. This study demonstrated that the sausage casing suppresses the growth of *L. monocytogenes*. Therefore, an edible membrane, surrounding processed foods, which is heat resistant and impermeable from the cells, could be a hurdle strategy to control the microbiological food safety.

Table captions

Table 1. *L. monocytogenes* growth parameters estimates, their standard error and the Mean Squared Error (MSE) of the fit on i) the 'type 1' and 'type 2' Frankfurter sausages at 4, 8 and 12°C.

Table 2. Analysis of physicochemical, compositional and preservatives' level of the two types of Frankfurter sausages.

Table 3. *L. monocytogenes* growth parameters estimates, their standard error and the Mean Squared Error (MSE) of the fit on i) the ‘type 1’ and ‘type 2’ BHI at 4 and 30°C.

Figure captions

Figure 1. Growth dynamics of *Listeria monocytogenes* on ‘type 1’ and ‘type 2’ sausages, at 4 (a), 8 (b) and 12°C (c). The experimental data of the ‘type 1’ sausage are represented with (○) and the ‘type 2’ with (○). The model fit is depicted by (-) and (-) for the ‘type 1’ and ‘type 2’ sausages, respectively.

Figure 2. Growth dynamics of *Listeria monocytogenes* on ‘type 1’ and ‘type 2’ BHI at 4 (a) and 30°C (b). The experimental data of the ‘type 1’ BHI are represented with (○) and the ‘type 2’ with (○). The model fit is depicted by (-) and (-) for the ‘type 1’ and ‘type 2’ BHI, respectively.

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Figure 1

Figure 2

Table 1

Growth parameters	Type 1			Type 2		
	4°C	8°C	12°C	4°C	8°C	12°C
N₀ (ln(CFU/g))	5.39 ± 0.34	4.62 ± 1.88	5.78 ± 0.42	4.95 ± 0.51	5.08 ± 0.55	5.39 ± 0.70
N_{max} (ln(CFU/g))	18.85 ± 0.27	20.17 ± 0.20	20.18 ± 0.08	17.79 ± 1.79	19.14 ± 0.23	19.11 ± 0.26
μ_{max} (1/h)	0.03 ± 0.00	0.06 ± 0.00	0.12 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
Lag (h)	36.26 ± 14.98	0.00 ± 0.00*	0.00 ± 4.45	50.21 ± 40.91	0.00 ± 0.00*	0.00 ± 0.00*
MSE	0.17	0.31	0.18	0.54	0.34	0.63

*Values bearing an asterisk as superscript are not estimated but fixed to zero.

Table 2

Parameter	Type 1	Type 2
Physicochemical characteristics		
pH	6.13 ± 0.13 ^a	6.11 ± 0.09 ^a
a_w	0.9809 ± 0.0028 ^a	0.9811 ± 0.0027 ^a
Compositional characteristics		
Moisture (%)	65.47 ± 1.49 ^a	66.89 ± 0.52 ^a
Fat (%)	19.02 ± 1.13 ^a	14.46 ± 1.96 ^b
Proteins (%)	12.59 ± 1.84 ^a	10.86 ± 2.13 ^a
Carbohydrates (%)	1.04 ± 0.31 ^a	0.85 ± 0.20 ^a
NaCl (%)	2.18 ± 0.14 ^a	1.91 ± 0.06 ^b
NaNO₂ (ppm)	8.00 ± 1.10 ^a	14.40 ± 4.60 ^a
Preservatives concentration		
Ascorbic acid (ppm)	49.38 ± 0.06 ^a	83.72 ± 0.07 ^b
Citric acid (ppm)	375.37 ± 0.64 ^a	<63.50 ± 12.74 ^b
Pentasodium triphosphate (ppm)	--	956.44 ± 17.03

¹For each parameter, values bearing different superscripts (no characters in common) are significantly different (P ≤ 0.05)

Table 3

Growth parameters	Type 1		Type 2	
	4°C	30°C	4°C	30°C
N_0 (ln(CFU/mL))	4.62 ± 0.22	4.26 ± 0.17	4.46 ± 0.24	4.15 ± 0.26
$N_{\max}$ (ln(CFU/mL))	20.36 ± 0.16	21.10 ± 0.06	20.52 ± 0.12	21.25 ± 0.09
$\mu_{\max}$ (1/h)	0.04 ± 0.00	1.02 ± 0.02	0.04 ± 0.00	1.01 ± 0.02
Lag (h)	27.88 ± 7.76	2.06 ± 0.24	2.62 ± 7.63	1.76 ± 0.35
MSE	0.07	0.04	0.06	0.07