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Original scientific paper

Changes in bacterial status and aw values during the maturation of fermented sausages

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A R T I C L E I N F O

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A B S T R A C T

This study presents the results related to changes in a_w value and bacteriological status of fermented sausages during maturation without and with added starter culture, stuffed into a narrower and wider casing. Values of a_w of narrower and wider diameter sausages with and without added starter cultures decreased during ripening, and were close to values of 0.9. *Enterobacteriaceae* in narrower diameter sausages were not detected on day 18, i.e., the end of the ripening process, and these bacteria were not detected in wider diameter fermented sausages on day 25 or at the end of ripening (day 35). The increase in the lactic acid bacteria in narrow and wider diameter sausages without added starter culture was slower than the increase in the number of these bacteria in sausages with added starter culture.

1. Introduction

Fermented sausages are among the oldest and most valuable meat products. Today, this type of sausage is produced in all parts of the world, especially since the advent of controlled microclimatic conditions in chambers (temperature, humidity, air circulation) for drying, smoking, and maturation. It is understood that the characteristics of fermented sausages vary greatly worldwide. Even within the same geographic region, or rather locality, there are different varieties of fermented sausages with typical regional recipes and established production technologies (*Leroy et al*., 2015). They are most commonly made from ground meat and fat tissue (mostly firm pork fat) with the addition of salt and spices, and are stuffed into natural (pork, sheep, beef) or artificial (collagen and cellulose) casings (*Savić & Savić*, 2002).

The basic and most important strategy for meat preservation, including fermented sausages, has been based on salting for centuries and its importance in controlling the growth of spoilage bacteria (especially pathogens), achieving the desired texture, and enabling slicing (*Montanari et al*., 2022). The use of starter cultures in the production of fermented sausages began after 1950, and just ten years later, a mixture of different bacteria (lactic acid bacteria, micrococci) was used as a starter (*Laranjo et al*., 2019). In the mid-1990s, the meat industry started using selected bacterial cultures that act antagonistically towards pathogenic bacteria and bacteria that produce toxins. Water content is closely related to the a_w (water activity) value of meat products, and fermented sausages are considered shelf-stable if their a_w value is around 0.92 (https://www.meatsandsausages.com/sausage-types/fermented-sausage).

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The aim of this study was to investigate changes in a_w values and selected bacterial groups during the maturation and drying of two different diameter fermented sausages, with and without the addition of a starter culture.

2. Materials and methods

For the purposes of the experiment, pork meat of the first and second category (back meat, shoulder, leg, meat with fat and connective tissue), as well as a certain proportion of pork back fat, were used. A meat grinder was used to grind these ingredients to the desired degree of fineness. The ground meat was left to cool at 0 to 5°C. After 24 hours, a spice mixture for tea sausage (containing glucose, table salt, spices, and spice extracts) and nitrite salt were added in a quantity of 2.3%, and after that the ground meat was divided into four groups. The first group (KI) consisted of narrower diameter sausages of the control group without a starter culture, the KII group consisted of wider diameter sausages of the control group without a starter culture, the KIII group consisted of narrower diameter sausages of the control group with a starter culture, and the KIV group consisted of wider diameter sausages of the control group with a starter culture. The starter culture contained lactic acid bacteria (*Lactobacillus sakei, Staphylococcus carnosus*, and *Staphylococcus xylosus*). The starter culture was added at a rate of 20 g per 200 kg of ground meat. The filling was stuffed into casings of narrower (34 mm) and wider diameter (55 mm). The sausages were labeled by groups, and placed on racks in the smokehouse (smoking at a temperature of 20–23°C, drying at 17°C and a relative air humidity of 75%). The production process lasted 18 days for sausages of narrower diameter and 35 days for sausages of wider diameter.

Microbiological analyses were conducted using the following methods: *Enterobacteriaceae* count according to ISO (2009); lactic acid bacteria count according to ISO (1998). Water activity (a_w) values were determined using the *Gimenéz and Dalgaard* (2004) method.

The obtained results were compared through statistical analysis using Microsoft Excel 2010 and GraphPad Prism software, version 8.00 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). Mean values and measures of variation for the bacterial count and a_w value were calculated. The mean bacterial counts and a_w values in the sausages are presented graphically.

3. Results

The changes in the a_w value of sausages with a narrower diameter, with and without the addition of a starter culture, are shown in comparison with the changes in enterobacteria and lactic acid bacteria (LAB) in Figures 1–4.

The a_w value of the sausages with a narrower diameter without a starter culture (group KI) was 0.9695 ± 0.0006 on day 0 and decreased during the maturation process to 0.9110 ± 0.0010 on day 18 (Figures 1a, 2a). On day 0, the a_w value of the sausages with a narrower diameter and the addition of a starter culture (group KIII) was 0.9697 ± 0.0007, and on day 18, at the end of the maturation process, it was 0.9211 ± 0.0006 (Figure 1b, 2b).

The average number of enterobacteria in the group KI sausages was 4.64 ± 0.32 log CFU/g on day 0, which decreased to 3.01 ± 0.09 log CFU/g on day 7. In the group KIII sausages, the average number of enterobacteria was 4.64 ± 0.08 log CFU/g on day 0, and on day 7, it was 2.47 ± 0.08 log CFU/g. Enterobacteria were not detected in the sausages with a nar-

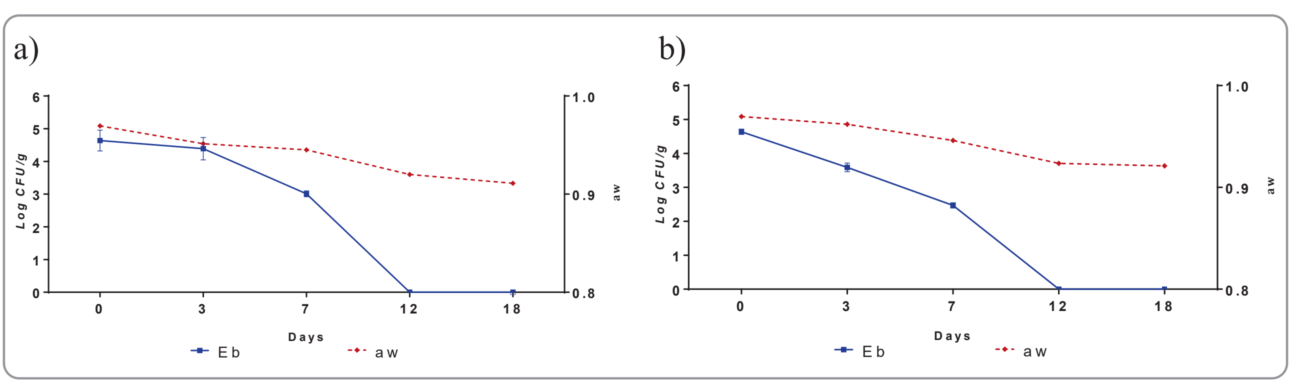

Figure 1. Numbers of *Enterobacteriaceae* (Eb) and a_w values during maturation of narrower diameter sausages without (a) group KI, and with added starter culture (b) – group KIII.

rower diameter, both with and without the addition of a starter culture, on days 12 and 18 of maturation (Figures 1a and 1b). The LAB counts in the sausages in groups KI and KIII during maturation are presented in Figures 3a and 3b. The average LAB count in group KI sausages was 5.09 ± 0.25 log CFU/g on day 0, while in group KIII, it was 5.21 ± 0.08 log CFU/g. The highest LAB count in group KI sausages was observed on day 12 of maturation (9.25 ± 0.08 log CFU/g) (Figure 2a), while in group KIII, it was on day 7 of maturation (9.56 ± 0.01 log CFU/g) (Figure 2b).

The a_w values of sausages with a wider diameter, with and without the addition of a starter culture, are shown in comparison with the bacterial status of sausages in Figures 3 to 4. The average a_w value of sausages with a wider diameter without the addition of starter cultures (group KII) was 0.9695 ± 0.0006 on day 0 and decreased during maturation to 0.9346 ± 0.0003 on day 35 (Figure 3a, 4a). Similar results were obtained for the sausages with added starter culture (group KIV). On the day 0, the a_w value of the filling was 0.96950 ± 0.0008, and it

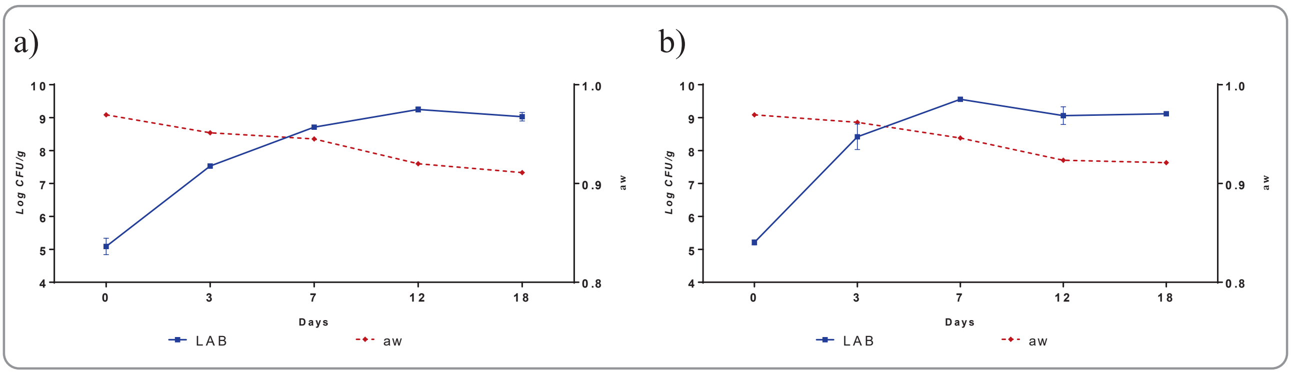

Figure 2. Numbers of lactic acid bacteria (LAB) and a_w values during maturation of narrower diameter sausages without (a) – group KI, and with added starter culture (b) – group KIII.

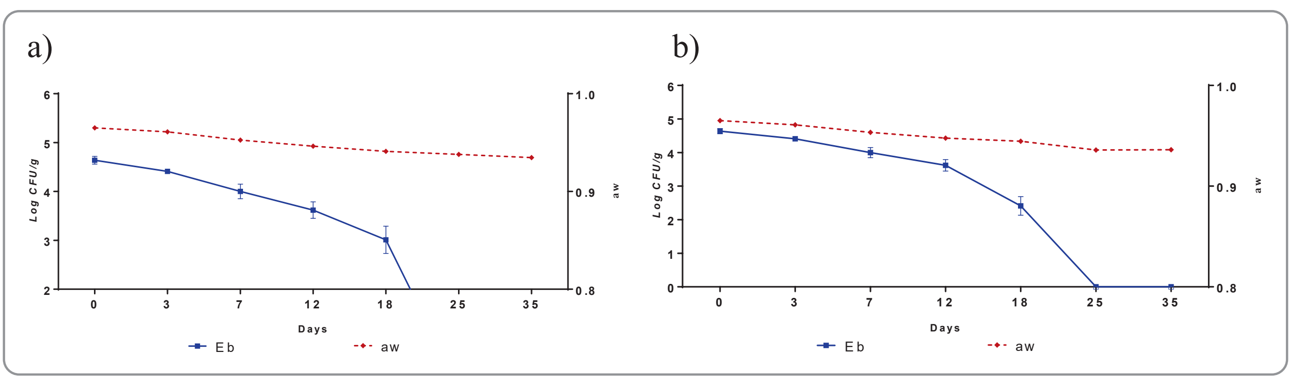

Figure 3. Numbers of *Enterobacteriaceae* (Eb) and a_w values during maturation of wider diameter sausages without (a) – group KII, and with added starter culture (b) – group KIV.

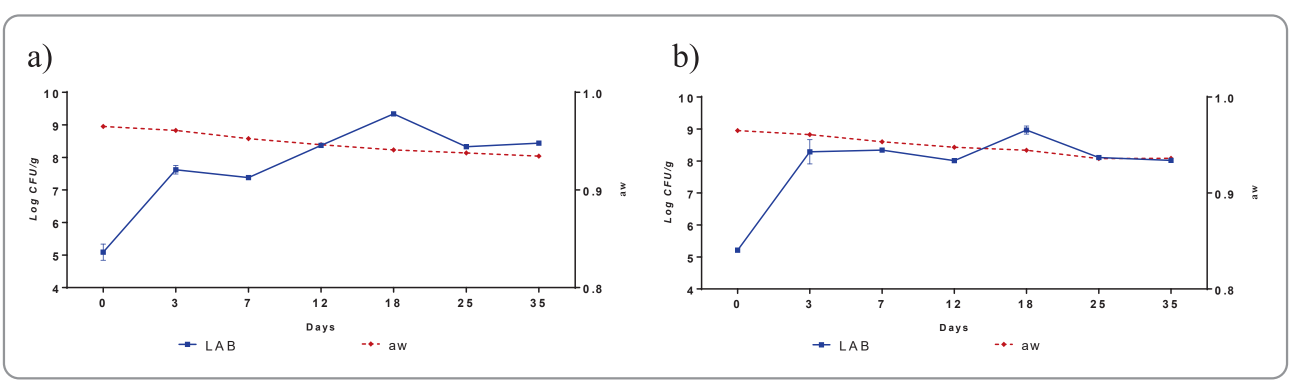

Figure 4. Numbers of lactic acid bacteria (LAB) and a_w values during maturation of wider diameter sausages without (a) – group KII, and with added starter culture (b) – group KIV

decreased during maturation to 0.9361 ± 0.0006 on day 35 (Figure 3b, 4b).

On the day of maturation, the average number of enterobacteria in the filling of group KII sausages was 4.46 ± 0.32 log CFU/g, and it did not significantly change until day 7. On day 12, it was 3.85 ± 0.12 log CFU/g, and on day 18, it was 3.01 ± 0.05 log CFU/g (Figure 3a). The average number of enterobacteria initially in the group KIV sausages was 4.64 ± 0.08 log CFU/g, and it consistently decreased during maturation, reaching 2.41 ± 0.28 log CFU/g on day 18 (Figure 3b). Enterobacteria were not detected in the filling of sausages in groups KII and KIV on days 25 and 35 of maturation (Figures 3a and 3b). The average LAB count initially in the filling of sausages in group KII was 5.09 ± 0.25 log CFU/g, and it increased until day 18 of maturation, when it reached 9.34 ± 0.22 log CFU/g. It then decreased by one logarithm, measuring 8.33 ± 0.16 log CFU/g on day 25 and 8.44 ± 0.22 log CFU/g at the end of maturation on day 35 (Figure 4a). In the filling of group KIV sausages, the average LAB count at the beginning of testing was 5.21 ± 0.11 log CFU/g, and it rapidly increased by day 3, reaching 8.29 ± 0.38 log CFU/g. It then remained stable until the day 35, measuring 8.02 ± 0.06 log CFU/g (Figure 4b).

4. Discussion

In industrial and traditional meat processing, fermented sausages have significant economic importance. The traditional production of fermented sausages with spontaneous fermentation contributes to the characteristic attributes associated with specific locations or rural environments. These attributes include physical properties (sausage diameter), physicochemical properties (pH and a_w), and the microbiota of sausages (total bacterial count, enterobacteria, enterococci, LAB, yeasts) *(Leroy et al*., 2015; *Palavecino et al.*, 2021). Traditional fermented sausages, especially those with geographical indication protection, are mainly produced without the addition of starter cultures. The preservation strategy relies on the addition of salt to reduce the water activity, control the growth of pathogens, and promote the formation of a desirable product texture.

In traditional fermented sausages, the a_w values are generally higher in sausages with a larger diameter. The a_w value depends on the water and salt content, as increasing water content in sausages increases the a_w, while increasing salt content reduces it. However, in industrial food production, the salt content is being reduced in all meat products, including fermented sausages, for health and nutritional reasons. Therefore, special importance is given to the use of starter cultures that contribute to the safety and quality of the final product. Lactic acid bacteria have the ability to lower the pH of the filling, thereby inhibiting the growth of spoilage bacteria, while also bringing the meat proteins to their isoelectric point, promoting water loss and the maturation phenomenon of sausages (*Montanari et al*., 2022).

The use of starter cultures in the production of fermented sausages dates back to the mid-20th century, initially using a mixture of LAB and micrococci as a starter culture. The primary goal was to achieve the desired color of the filling, control bacterial spoilage, and shorten the production process. Later, attention was given to starter cultures that prevent the growth of pathogens and bacteria that produce toxins. Today, the most commonly used starter cultures include LAB, *Staphylococcus*, and yeasts (*Laranjo et al*., 2019; *Cocconcelli & Fontana*, 2014; *Berni*, 2014; *Holck et al.*, 2017). According to *Montanari et al.* (2022), changes in a_w values during the maturation of fermented sausages are not dependent on the starter culture and casing selection. In the tested groups of sausages with different diameters and with the addition of different starter cultures, the a_w values were 0.97 in fresh filling, 0.96 after four days, and at the end of maturation, i.e., 30 days, ranged from 0.92 to 0.93. Numerous bacteria species are tolerant to an a_w value of 0.92. For example, *C. botulinum* does not grow below an a_w value of 0.93, *B. cereus*, *C. jejuni*, and *Salmonella* spp. do not grow below 0.95, *E. coli* does not grow below 0.93, and *L. monocytogenes* does not grow below 0.92 (source: https://www.meatsandsausages.com/sausage-types/fermented-sausage). *Baka et al.* (2011) determined that the initial LAB count (different strains of *L. sakei*) at the beginning of their study ranged from 5.7 to 6.9 log CFU/g. By the eighth day, for most strains, it increased to between 8 and 9 log CFU/g and remained relatively stable until the 28th day, the end of the maturation process. In sausages without the addition of starter cultures, the bacterial count was 4 log CFU/g at the beginning, 7.5 log CFU/g on the 16th day, and 8 log CFU/g at the end of the 28th day. In all sausage groups (experimental and control), the count of enterobacteria was 3.5 log CFU/g and decreased to below the detection limit by the 16th day. When different starter cultures and casings were used in fermented sausages (*Montanari et al.*, 2022), on day 0 of the study, the LAB count ranged from 6.94 to 7.08 log CFU/g, on the fourth day it ranged from 8.02 to 8.84 log CFU/g, and on the 30th day, it ranged from 7.50 to 8.44 log CFU/g. In the same sausage groups, the count of enterobacteria on day zero ranged from 1.81 to 2.00 log CFU/g, on the fourth day it ranged from 1.00 to 1.77 log CFU/g, and on the 30th day, it ranged from 1.30 to 2.16 log CFU/g (*Montanari et al.*, 2022). In addition to the production of organic acids (mainly lactic acid), the pH decrease, and the a_w reduction, the preserving effect of LAB is also based on the production of antimicrobial components known as bacteriocins. Our results regarding a_w values and the bacteriological status of fermented sausages largely align with the findings of other authors.

5. Conclusion

During the ripening of fermented sausages, there is a decrease in the a_w value and number of enterobacteria, and an increase in lactic acid bacteria. The production of fermented sausages with a narrower diameter ends at 18 days, and with a wider diameter at 35 days.

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