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High hydrostatic pressure treatment of raw sausages: impact on microbiological safety, textural properties and colour

Chiara Angelucci [a], Giulia Tabanelli [b,c,*], Francesca Soglia [a,c], Federica Barbieri [a], Chiara Montanari [a], Gabriele Gardini [d], Rudy Magnani [d], Fausto Gardini [a,c], Massimiliano Petracci [a,c]

[a] Department of Agricultural and Food Sciences, University of Bologna, 47521, Cesena, FC, Italy
[b] Department of Agricultural and Food Sciences, University of Bologna, 40127, Bologna, Italy
[c] Interdepartmental Center for Industrial Agri-Food Research, University of Bologna, 47521, Cesena, FC, Italy
[d] C.I.a.i. Soc. Coop., Via Gambellara 62/A, 40026, Imola, BO, Italy

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ABSTRACT

This study aimed to assess High Hydrostatic Pressure (HHP) process (5 min at 600 MPa) effects on the quality and safety of commercially produced raw pork sausages. In particular, the evolution of key microbial populations was monitored under different storage conditions, including thermal abuses before HHP treatment or during storage. The results showed an increase in shelf-life up to 90 days in HHP-treated samples, compared to 15-20 days for conventional products. Overnight storage at 15 °C before HHP affected the quality only of untreated samples, while storage at 8 °C after HHP strongly increased microbial counts. In addition, challenge tests were performed using *Salmonella* Enteritidis, *Staphylococcus aureus* and *Listeria innocua*. HHP treatment was effective against *S.* Enteritidis, while *L. innocua* remained below the detection limit during the storage, but was qualitatively detected after 90 days. *Staph. aureus* was the most resistant, showing a 3-log reduction after HHP, with stable counts during storage. The study also evaluated the effects of HHP on texture and colour during storage at 4 °C, evidencing increased hardness and chewiness in treated samples, with reduced exudate release. Colour parameters were initially affected by the treatment but showed convergence with control samples over the storage period.

1. Introduction

Raw (not fermented) sausages are the result of a mixture of lean and fatty minced meat, usually of pork origin, with a degree of mincing that may vary according to local recipes (Seman et al., 2018). In Italy, NaCl (2.5-3.5%) and spices (mainly black pepper, but also fennel, garlic, etc.) are generally added to the meat mixture stuffed into natural (sheep or pig) casings. The resulting product is traditionally cooked before consumption, and this guarantees its safety. In fact, due to its high pH (approx. 5.8) and high a_w (≥0.97), it is potentially a favourable substrate for the growth of numerous pathogenic as well as spoilage microorganisms. For these reasons, this type of product must be stored under refrigerated conditions (0-4 °C) and, eventually, under a modified atmosphere, while still having a limited shelf-life (approx. 10-15 days) (Cocolin et al., 2004; Raimondi et al., 2018; Tremonte et al., 2005).

The main microorganisms responsible for the degradation of raw sausages are those arising from the contamination of raw materials, resulting in the production of off-odour and off-flavour, with colour alterations depending on the myoglobin redox state and oxidative reactions due to the high fat content of this product (Hugo & Hugo, 2015). From a quantitative point of view, the initial microbial loads found in raw sausages are in a wide interval, ranging from 4 to 6 log cfu/g (Cocolin et al., 2004; Kamdem et al., 2007; Nuvoloni et al., 2012). Furthermore, initial populations may include pathogenic species that, although the product is cooked before consumption, may result in cross-contamination during preparation, even at the domestic level (De Cesare et al., 2007).

Among the microorganisms responsible for the degradation of raw sausages, microbial groups with psychrotrophic aptitudes belonging to the genera *Brochothrix, Carnobacterium, Serratia,* and *Yersinia* are often

* Corresponding author. Department of Agricultural and Food Sciences, University of Bologna, 40127, Bologna, Italy.
E-mail address: giulia.tabanelli2@unibo.it (G. Tabanelli).

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prevalent. Heterofermenting lactic acid bacteria (LAB) can also cause alterations (*Leuconostoc, Weissella*) along with some lactobacilli (*Latilactobacillus sakei, Latilactobacillus graminis, Latilactibacillus curvatus*) (Angelucci et al., 2024; Benson et al., 2014; Cocolin et al., 2004; Fougy et al., 2016; Raimondi et al., 2018).

High hydrostatic pressure (HHP) is a non-thermal treatment used to reduce the microbial concentration in sausages (Zhu et al., 2022). It consists of the application of pressure of several hundred MPa, usually between 200 and 600 MPa (Aganovich et al., 2021), for different times. The antimicrobial effect of this technique has been proven in several foods, including animal-derived products such as whole or sliced fermented sausages. However, less information is available on fresh meat products, in which promising results can be obtained (Angelucci et al., 2024). Cell death following HHP treatment depends on the accumulation of damages, which may affect molecules that are essential for the survival and growth of microorganisms (e.g. denaturation of proteins/enzymes), structures fundamental for the proper functioning of cells (alteration of membrane fluidity, conformational changes in ribosomes, damage to the cell wall) and nucleotides, such as DNA (Rendueles et al., 2011; Simonin et al., 2012). The main process parameters influencing microbial deactivation are the applied pressure and treatment time. Intrinsic factors include pH, a_w and chemical composition. The physiological state of the cells also plays a role in their deactivation (Aganovich et al., 2021; Hygreeva & Pandey, 2016). Under commercial processing conditions, HHP cannot be considered a sterilising treatment; consequently, treated products require refrigerated storage (0–4 °C) during distribution to ensure both sensory integrity and microbiological safety.

Under these conditions, a significant extension of the shelf-life of foodstuffs subjected to this treatment can be achieved (Angelucci et al., 2024; Rendueles et al., 2011). On the contrary, the occurrence of thermal abuse during manufacturing, storage, transportation or marketing may significantly compromise the shelf-life of the product treated with HHP (de Alba et al., 2012; Duranton et al., 2012). Pathogenic species that may be present in non-fermented sausages include *Salmonella enterica*, *Listeria monocytogenes*, and *Staphylococcus aureus*, as well as *Clostridium* spp., particularly when the product is vacuum-packed (Cocolin et al., 2004; De Cesare et al., 2007).

Although the presence of nitrites can inhibit the viability of *Clostridium* species, the implementation of an appropriate cold chain effectively limits the proliferation of other microbial populations, except *Listeria monocytogenes*, which is capable of growing, albeit slowly, at 4 °C. However, even if unable to duplicate at refrigeration temperature, these cells do not die. The fact that they survive can also cause cross-contamination during the domestic use of these products (de Cesare et al., 2007).

In a previous study, we explored the effectiveness of an HHP treatment for the microbial stabilisation of raw industrial sausages, and a significant extension of the shelf-life (from 15 to 90 days) was observed in products treated at 600 MPa, particularly in samples to which nitrites have been added (Angelucci et al., 2024). Building on these findings, nitrite was included in the product formulation used in the present study for the following reasons. Firstly, nitrite contributes to mitigating the risks associated with the growth of clostridial species (*Clostridium botulinum* and *Clostridium perfringens*), which could grow in vacuum-packaged sausages, but are highly sensitive to the presence of this compound. Secondly, nitrite can prevent or reduce fat oxidation induced by HHP treatment (Cava et al., 2024). Thirdly, HHP treatment leads to an alteration of myoglobin, converting it into metmyoglobin and causing an undesirable dark colour that negatively affects product acceptability (Bak et al., 2019). Nitrite promotes the formation of nitroso-myoglobin, which imparts the characteristic pink colour. In addition, HHP affects meat proteins, including both the sarcoplasmic, strongly related to meat colour, and myofibrillar protein fractions, which may undergo denaturation and subsequent aggregation, thus affecting the textural properties of the meat products (Chevalier et al., 2001; Guyon et al., 2016).

This study aims to investigate the effects of HHP treatment at 600 MPa for 5 min on the quality and safety of commercially produced raw pork sausages. Firstly, the impact of thermal abuse occurring on the products both before HHP treatment and during refrigerated storage was evaluated by monitoring changes in microbiota, which can, consequently, affect product shelf-life. Regarding safety aspects, a challenge test was performed to assess the impact of the HHP treatment on the survival and potential growth of three pathogenic bacteria: *Salmonella* Enteritidis, *Staphylococcus aureus* and *Listeria innocua* (used as a surrogate of *Listeria monocytogenes*). In addition, the influence of HHP treatment on colour and texture was evaluated in sausages stored at 4 °C throughout their shelf life.

2. Materials and methods

2.1. Raw pork sausages preparation

Raw pork meat and adhering subcutaneous fat obtained from hot-deboned shoulder cuts (53 kg) were collected and used for mixture preparation 24 h post-mortem. The ratio of lean meat to fat was approximately 80:20. The raw meat was coarsely ground using a 4–5 mm plate at a temperature of 0 °C and subsequently mixed with 2.1% sodium chloride (NaCl) (w/w), ground black pepper at 0.1% (w/w), ascorbate at 0.04% (w/w), and sodium nitrite ($NaNO_2$) at a concentration of 0.01% (w/w) (Europrodotti Food Ingredients, Concorezzo, MB, Italy). Concerning sodium nitrite, this amount (100 mg/kg) corresponds to a concentration of nitrite ion of 67 mg/kg, complying with the recent EU Regulation 2108/2023 (European Commission, 2023). After mixing, the meat batter was stuffed into natural casing, and sausages of approximately 100 g/each were subsequently vacuum packaged.

2.2. Experimental plan for shelf-life evaluation

The packages were subjected to the experimental plan reported in Fig. 1. Each package contained four sausages, resulting in a total of 132 vacuum-packed units.

High Hydrostatic Pressure (HHP) treatment was applied 24 h after production at a pressure of 600 MPa for 5 min, as described in section 2.3. Before HHP treatment, half of the packages (N = 66) were stored at 15 °C, simulating an initial thermal abuse, while the other samples (N = 66) were stored at 4 °C.

After overnight storage at these two distinct temperatures, a subset of both abused and non-abused samples (N = 72) was subjected to HHP treatment as described below, while the remaining samples (N = 60) were left untreated. HHP treated and not treated samples were then divided into two groups that were subsequently stored throughout the product shelf-life under two different temperature conditions: a portion of the samples (N = 66) was maintained at 4 °C (samples codes: NT-15-4, NT-4-4, 600-15-4, 600-4-4), whereas another subset (N = 66) was stored at 8 °C to simulate a second thermal abuse event during storage and distribution (groups: NT-15-8, NT-4-8, 600-15-8, 600-4-8) (Fig. 1).

2.3. HHP treatment

High-pressure processing of the vacuum-packaged sausages was conducted using an industrial-scale AVURE AV-50X unit (HPP Italia, Traversetolo, PR, Italy). The processing parameters were set to achieve a pressure of 600 MPa and treat the product for 5 min. The samples were rapidly pre-conditioned at a temperature of 4 °C before processing. The application of high pressure induced an average temperature increase of approximately 16 °C in the treated samples.

2.4. Microbiological analysis

For microbiological analyses concerning shelf-life, the pressurized

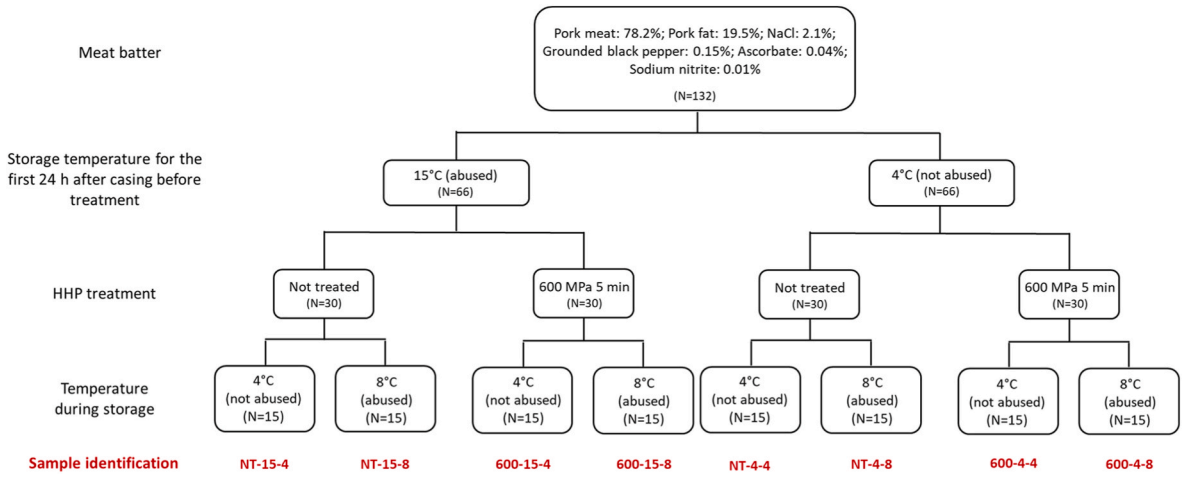

Fig. 1. Experimental plan with sample codes for each condition tested for shelf-life assessment. In red, the sample code used is reported (within brackets, the number of samples is indicated).

sausages were monitored for 90 days (being sampled at t0, t1, t20, t40, t60, and t90 from HHP treatment), while the untreated control was sampled for 20 days (with samples being collected after t0, t9, t15, and t20 days of storage after treatment). For each package, two sausages were combined to obtain a composite sample for microbiological analysis. From this composite, approximately 10 g of sausage were aseptically transferred into sterile stomacher bags, combined with 90 mL of sterile 0.9% (w/v) sodium chloride (NaCl) solution, and homogenised using a Lab Blender Stomacher (Seward Medical, London, UK) for 2 min. Subsequently, appropriate decimal dilutions were prepared and plated onto selective media to quantify specific microbial populations. Total mesophilic counts (TMC) were determined using Plate Count Agar (PCA), while lactic acid bacteria (LAB) were enumerated on de Man-Rogosa-Sharpe (MRS) agar supplemented with 200 mg/L of cycloheximide to inhibit fungal growth. Coagulase-negative cocci (CNC) were counted on Baird-Parker (BP). All media were incubated at 30 °C, and colony enumeration was performed after 48 h. Additionally, Enterobacteriaceae were detected using Violet Red Bile Glucose Agar (VRBGA), following incubation at 37 °C for 24 h. Each microbiological analysis was conducted in three independent repetitions, considering different sausage packaging.

2.5. Texture and colour analysis

Textural and colour measurements were done only on sausages manufactured with batter stored at 4 °C before and after HPP treatment (NT-4-4 and 600-4-4).

Texture Profile Analysis (TPA) was conducted at 22 °C using a TA-Hdi® texture analyser (Stable Micro Systems, UK) equipped with a 25 kg load cell. The analysis was performed on cylindrical samples (1 cm in height and 1.5 cm in diameter), which were compressed up to 50% of their original height using a 5 cm diameter aluminium probe. A 30-s interval was maintained between the two compression cycles. Force-time curves were recorded, and textural parameters, including Hardness (kg), Springiness, Cohesiveness (kg), Chewiness, and Gumminess (kg), were calculated following the method described by Bourne (1978). Ten measurements were performed for each independent repetition (N = 3 sausages).

Colour parameters (CIE L*, a*, b*) were measured using a tristimulus colourimeter (Chroma Meter CR-400, Minolta, Milan, Italy), illuminant C (and a standardised observer angle). To account for the heterogeneous colour distribution of the meat matrix and the presence of fat particles, 10 measurements were performed for each independent repetition (N = 3 sausages).

2.6. Challenge test

For the challenge test, sausages were prepared as previously described in Section 1. The sausages obtained (n = 150) were subsequently divided into three groups, and each group was inoculated with a specific target pathogen: *Salmonella* Enteritidis 155, belonging to the collection of the Department of Agricultural and Food Science of the University of Bologna, *Listeria innocua* DSM 20649 and *Staphylococcus aureus* DSM 20231^{T}.

Each 100 g sausage was inoculated using a sterile syringe with 1 mL of bacterial suspension to achieve an initial contamination level of approximately 5 log cfu/g by subdividing the volume inoculated into ten 0.1 ml aliquots. After inoculation, sausages were packaged under vacuum. The packaged samples were stored approx. 24 h at 0-4 °C before HHP treatment.

After 24 h, the samples were divided into two groups: one group remained untreated and served as the control, while the second group was subjected to high-pressure processing at 600 MPa for 5 min (as described in paragraph 2.3). Throughout the entire storage period, all samples were maintained at 0-4 °C. Untreated samples were analysed during 20 days, whereas sausages subjected to HPP were analysed over a storage period of 90 days. Time 0 corresponds to samples analysed immediately after the inoculum, before HHP treatment, while time 1 refers to samples analysed immediately following the HHP treatment.

The quantitative detection of inoculated pathogens during the challenge test was performed by plate counting, using selective media for each microorganism. Listeria Selective Agar Base (LSO), added with Selective Listeria Supplement, was used to enumerate *L. innocua*, after incubation at 30 °C for 48 h. For *S.* Enteritidis, XLD medium plates were inoculated and incubated at 37 °C for 24 h, while *Staph. aureus* was detected on Baird Parker (BP) plates, incubated at 37 °C for 48 h; the colonies were recognised based on the presence of a halo. Each microbiological analysis was conducted in three independent repetitions, considering different sausage packaging. When *L. innocua* and *S.* Enteritidis were under the detection limit, enrichment of the samples was performed according to Tabanelli et al. (2024). For the qualitative positive/negative assessment, five samples of 25 g were tested for *L. innocua* and three samples of 25 g for *S.* Enteritidis.

All culture media were provided by Oxoid (Basingstoke, UK).

2.7. Statistical analysis

The results concerning microbial counts, colour and textural parameters were analysed by One-way ANOVA to ascertain the main effect of the storage time on the above-mentioned parameters. When

significant, means were subsequently separated by Tukey-HSD test ($p < 0.05$).

3. Results and discussion

3.1. Microbial shelf-life of raw sausages

As described in Fig. 1, the microbial population and shelf-life of sausages were analysed in relation to two different thermal abuse conditions: the first was an initial abuse at 15 °C for 24 h, representing the time between sausage production and HHP treatment; the second was the abuse at 8 °C during storage, resulting from improper commercialisation procedures.

In Fig. 2a and b, the results of microbial counts relative to total mesophilic bacteria, present at an initial concentration of approx. 4 log cfu/g, are reported. The application of the first abuse (15 °C for 24 h) caused a slight increase in the counts (about 0.3 log cfu/g in NT-15-4 and NT-15-8). On the other hand, relevant changes between the samples were induced by the temperature applied during storage (4 or 8 °C). Determining the precise microbial threshold at which changes become perceptible to consumers is generally challenging. However, it is widely recognised that a cell concentration of 7 log cfu/g marks the point at which a product is typically considered spoiled (McMeekin et al., 2006; T. McMeekin & Ross, 1996). Based on this understanding, a threshold of 6.5 log cfu/g was chosen in this study as an indicator of shelf-life. The counts in NT-15-8 (subjected to a double thermal stress) rapidly increased and reached the arbitrary cell concentration considered as a shelf-life indicator after about 10 days. The other sausages stored at 8 °C, not abused before HHP treatment (NT-4-8), had a longer lag phase, but after about 12 days, the limits for shelf-life were reached. Both NT-15-8 and NT-4-8 presented final concentrations after 20 days of storage higher than 8.5 log cfu/g. Concerning the samples not subjected to thermal abuse during storage, sausages kept at 15 °C for 24 h (NT-15-4) exhibited a faster microbial growth rate, reaching the limit for shelf-life after approx. 13 days, while NT-4-4 samples exceeded 6.5 log CFU/g only after 20 days of storage.

The results concerning total mesophilic bacteria in the sausages treated at 600 MPa for 5 min are reported in Fig. 2b. Also in this case, a slight increase in cell counts was observed in the samples stored at 15 °C for 24 h before the treatment (600-15-4 and 600-15-8). The application of HHP treatment determined a reduction of about 2 log cfu/g in all the samples. In this case, a proper temperature storage guaranteed stable counts (never higher than the initial contamination), indicating that, from a microbial point of view, these sausages were still suitable for consumption. In contrast, sausages stored at 8 °C exhibited a low microbial load up to 20 days, after which bacterial concentration increased, reaching the threshold of 6.5 log CFU/g after approximately 43 days (600-4-8) and 53 days (600-15-8). Both samples were characterised by a final concentration of approx. 8 log cfu/g at 90 days.

LAB counts presented similar behaviours when compared with mesophilic bacteria. In the untreated sausages, the initial LAB concentration ranged between 3.6 and 3.8 log cfu/g (Fig. 3a). The thermal abuse at 15 °C for the first 24 h (samples NT-15-4 and NT-15-8) caused an increase of about 0.4 log cfu/g in this microbial population. The sample NT-15-8 was the first to reach the critical concentration of 6.5 log cfu/g after 10 days, followed by NT-4-8 and NT-15-4 (approx. after 13.5 days). The sausages NT-4-4 remained below this threshold even after 20 days.

The HHP treatment (Fig. 3b) determined a relevant decrease in the LAB counts that were below the detection limit or slightly higher immediately after pressurisation. After 20 days, the sausages stored at 8 °C (600-15-8 and 600-4-8) showed a LAB increase, even if the counts were lower than 2 log cfu/g. After 40 days, these sausages were characterised by a relevant increase of LAB counts, which reached the threshold limit (6.5 log cfu/g) after approx. 45 days (600-4-8) and 55 days (600-15-8). The samples stored at 4 °C presented a low increase in LAB counts, which were below 3 log cfu/g even after 90 days.

Regarding the staphylococci counts, no coagulase-positive colony was found. The initial concentration of this microbial group ranged between 3.5 and 3.7 log cfu/g. In the not-treated sausages, this load did not significantly change during the 20 days of storage, regardless of thermal abuse conditions (Fig. 4a). The HHP treatment lowered their concentration by about 3 log cfu/g, after which a further decrease was observed in the sausages stored at 4 °C, while at 8 °C, a small increase was found in staphylococci, even if the final counts after 90 days were always lower than 2.5 log cfu/g (Fig. 4b).

To better illustrate the differences between samples subjected to thermal abuse (either before HHP treatment or during storage), the cell counts for each microbial group are reported in the Supplementary Materials, with uppercase letters indicating significant differences ($p < 0.05$). Tables S1, S2, and S3 refer to total mesophilic counts, lactic acid bacteria, and staphylococci, respectively.

Data concerning Enterobacteria are not shown due to their low initial concentration (1.5 log cfu/g), which did not increase in the non-treated samples, while after HHP treatment, the counts of these bacteria were always under the detection limit.

The initial microbial load of sausages was relatively low (approx. 4 log cfu/g) and comparable with counts found in similar products (Angelucci et al., 2024; Cocolin et al., 2004; Raimondi et al., 2018). Interestingly, LAB counts were similar to total counts, indicating a relevant contribution to this microbial group to the initial contamination of sausages. This could be because sausages were produced in an industrial environment where fermented sausages are usually produced with the use of LAB starter cultures, which can contaminate other productions. However, the initial contamination by LAB of sausages has already been described involving genera such as *Weissella* (Komora et al., 2023), *Leuconostoc* (Cocolin et al., 2004) and *Carnobacterium* (Angelucci et al., 2024). The samples subjected to the first thermal abuse

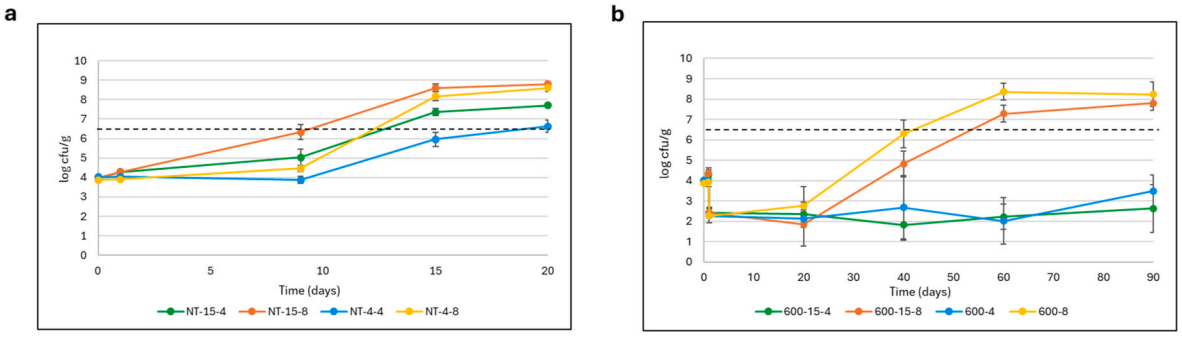

Fig. 2. Total mesophilic counts of not-treated and treated raw sausages during storage (a: not-treated samples; b: samples treated with HHP at 600 MPa for 5 min). The dotted line represents the threshold used as a shelf life indicator (6.5 log CFU/g). Standard deviations are also reported. 15-4: abused temperature before the HHP treatment; 15-8: abused temperature both before HHP treatment and during the shelf-life; 4-4: proper conditions; 4-8: abused temperature during the shelf-life.

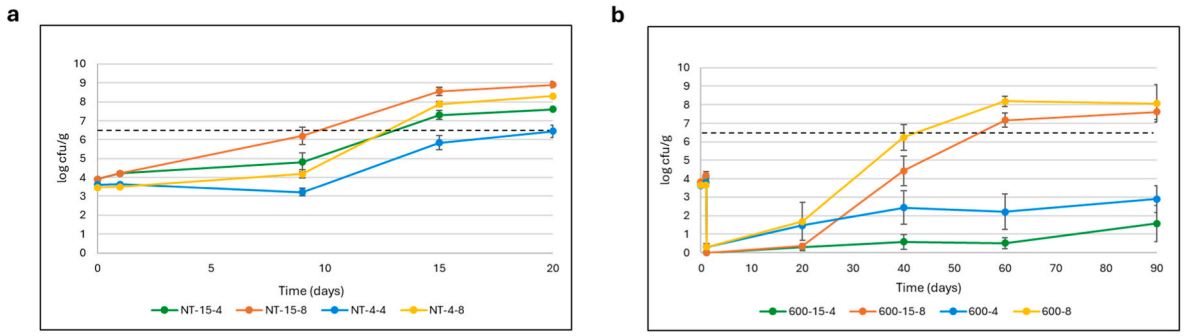

Fig. 3. Lactic acid bacteria counts of not-treated and treated raw sausages during storage (a: not-treated samples; b: samples treated with HHP at 600 MPa for 5 min). The dotted line represents the threshold used as shelf life indicator (6.5 log CFU/g). Standard deviations are also reported. 15-4: abused temperature before the HHP treatment; 15-8: abused temperature both before HHP treatment and during the shelf-life; 4-4: proper conditions; 4-8: abused temperature during the shelf-life.

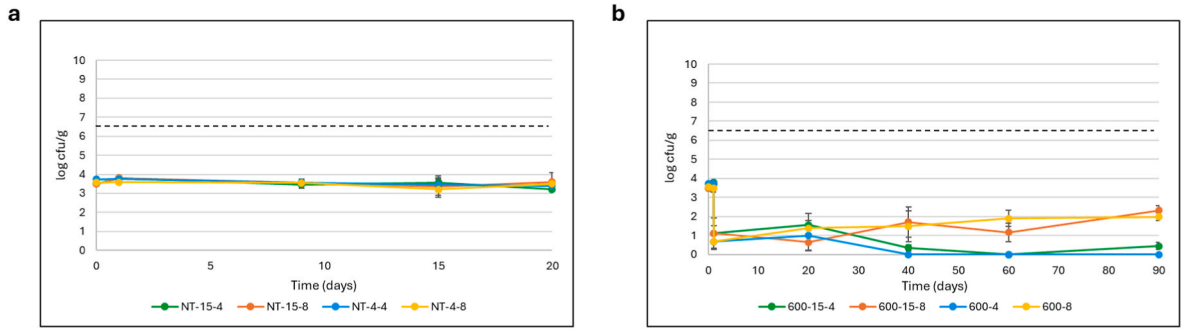

Fig. 4. Staphylococci counts of not-treated and treated raw sausages during storage (a: not-treated samples; b: samples treated with HHP at 600 MPa for 5 min). The dotted line represents the threshold used as shelf life indicator (6.5 log CFU/g). Standard deviations are also reported. 15-4: abused temperature before the HHP treatment; 15-8: abused temperature both before HHP treatment and during the shelf-life; 4-4: proper conditions; 4-8: abused temperature during the shelf-life.

(15 °C for 24 h) showed a reduced increase in bacterial presence. However, this abuse was able to speed up the microbial kinetics in non-pressurized sausages and, in turn, reduce the shelf-life. In contrast, the abuse condition at 8 °C during storage had drastic effects on the shelf-life of the products. This temperature not only speeds up the development kinetics of bacteria, but also allows the recovery and growth of microbial species inhibited by a temperature of 4 °C. This trend emphasises once again that HHP treatment alone cannot be considered sufficient to determine shelf-life, especially in products with the characteristics of raw sausages (Aganovic et al., 2021; Schottroff et al., 2018).

Despite significant reductions in initial microbial populations immediately after treatment, it is difficult to say whether the loss of culturability observed with plate counting results in cell death or, at least in part, leads to sublethal damage to cell structures, inducing the presence of viable but non-culturable (VBNC) cells (Schottroff et al., 2018). The presence of cells not able to grow with plate counting but still possessing metabolic activity following HHP treatment has been demonstrated with flow cytometry (Kimura et al., 2017). These damages can be repaired over time, and, under more favourable conditions, such as higher temperatures, these VBNC cells can re-enter the state of culturability and thus multiply. In addition, it is known that an HHP treatment can retard or inhibit colony formation, inducing the presence of cells with different physiological states (Koseki & Yamamoto, 2006; Kimura et al., 2017).

While the LAB were characterised by the ability to grow at the temperature considered, staphylococci did not. Indeed, the presence of species with psychrotrophic attitudes is reported in many LAB genera, such as *Latilactobacillus, Weissella,* and *Leuconostoc* (Andreevskaya et al., 2018), while temperatures of about 8 °C are already very limiting for staphylococci. In fact, for *Staph. aureus* in particular, 7 °C is generally reported as the minimum growth temperature (Onyango et al., 2012). It is therefore evident that at 8 °C these species are poorly competitive with other bacteria better adapted to grow in these conditions.

3.2. Texture Profile Analysis

The results concerning the textural parameter assessed only on NT-4-4 and 600-4-4 groups, which consisted of sausages kept at 4 °C both after casing and during storage (up to 20 and 90 days for non-treated and treated samples, respectively), are reported in Tables 1 and 2. As for NT samples (Table 1), if compared to the values measured at 0 days, a significant increase in hardness and gumminess was found after 9 days (1.19 *vs.* 1.46 kg; $p < 0.001$ and 0.58 *vs.* 0.64 kg*cm; $p < 0.001$), followed by a decline at the subsequent sampling time (i.e., 15 days). On the other hand, an opposite trend was observed for cohesiveness that exhibited a progressive reduction during storage, with the differences being particularly evident from the initial time (0 days) and after 9 days of storage (0.49 vs. 0.44; $p < 0.001$). The evolution in the textural parameters observed in non-treated sausages may be ascribed to the gel-forming ability of the myofibrillar proteins, which strongly depends on their interaction with the other components (*i.e.*, proteins, fat, water) and develops during meat batter mixing and casing (Sun & Holley, 2011; Toldrà et al., 2014). In fact, it is widely recognised that, in the presence of salt, muscle proteins are extracted from the meat and, after being subjected to a conformational change and dissociation of the myofilaments, are solubilised, thus allowing the onset of protein-water interactions and the formation of a gel-like structure (Toldrà et al., 2014). On the other hand, the evolution in the textural parameters observed during storage is likely the result of the chemical-physical processes (*e.g.*, water movements, protein oxidation, etc.) taking place within the NT sausages (Seibt et al., 2024). The occurrence of oxidative modifications affecting the protein fraction (involving the development of protein cross-links) along with the water entrapment within the meat matrix

Table 1
Evolution in colour and textural parameters (assessed by Texture Profile Analyses) in non-treated samples (NT-4-4) over the storage time. Each value is the mean of 30 measurements performed on three independent samples (10 measurements/sample).

	0 d	9 d	15 d	20 d	sem	P-value
Colour						
Lightness – L*	50.7 ± 4.8 a	49.1 ± 3.9 ab	49.8 ± 4.6 ab	46.2 ± 3.7 b	0.43	***
Redness – a*	21.9 ± 3.9 b	24.6 ± 4.7 a	23.8 ± 3.5 ab	18.5 ± 2.6 c	0.40	***
Yellowness – b*	13.7 ± 1.9 ab	15.1 ± 2.8 ab	17.5 ± 1.9 a	11.0 ± 1.5 b	0.77	*
Texture Profile Analyses						
Hardness (kg)	1.19 ± 0.14 b	1.46 ± 0.14 a	1.24 ± 0.14 b	1.19 ± 0.20 b	0.02	***
Cohesiveness	0.49 ± 0.02 a	0.44 ± 0.03 c	0.45 ± 0.03 bc	0.46 ± 0.02 b	0.01	***
Gumminess (kg*cm)	0.58 ± 0.07 b	0.64 ± 0.07 a	0.56 ± 0.06 b	0.55 ± 0.09 b	0.01	***
Springiness (cm)	0.60 ± 0.06 a	0.58 ± 0.12 ab	0.54 ± 0.07 b	0.55 ± 0.05 ab	0.01	*
Chewiness (kg*mm)	0.35 ± 0.07 ab	0.37 ± 0.11 a	0.30 ± 0.06 b	0.30 ± 0.07 b	0.01	***

sem = standard error of mean.
= $p < 0.05$; *** = $p < 0.001$.
a-c = mean values followed by different letters significantly differ over the storage time ($p < 0.05$).

Table 2
Evolution in colour and textural parameters (assessed by Texture Profile Analyses) in HHP-treated samples (600-4-4) over the storage time. Each value is the mean of 30 measurements performed on three independent samples (10 measurements/sample).

	0 d	1 d	20 d	40 d	60 d	90 d	sem	P-value
Colour								
Lightness – L*	50.7 ± 4.9 de	56.7 ± 2.8 a	53.3 ± 3.3 bc	55.0 ± 2.8 ab	51.8 ± 3.3 cd	48.6 ± 2.2 e	0.32	***
Redness – a*	21.9 ± 3.9 a	18.8 ± 2.0 b	19.1 ± 1.9 b	19.3 ± 1.7 b	16.0 ± 1.4 c	16.5 ± 2.0 c	0.22	***
Yellowness – b*	13.7 ± 1.9 a	8.7 ± 0.9 c	9.0 ± 1.2 c	10.4 ± 1.3 b	9.0 ± 1.2 c	8.3 ± 0.9 c	0.17	***
Texture Profile Analyses								
Hardness (kg)	1.19 ± 0.14 a	2.81 ± 0.45 ab	2.82 ± 0.68 ab	2.44 ± 0.66 bc	2.18 ± 0.53 c	2.17 ± 0.40 c	0.06	***
Cohesiveness	0.49 ± 0.02 d	0.67 ± 0.02 c	0.67 ± 0.03 c	0.72 ± 0.02 b	0.73 ± 0.04 ab	0.75 ± 0.03 a	0.01	***
Gumminess (kg*cm)	0.58 ± 0.07 d	1.89 ± 0.30 a	1.89 ± 0.43 a	1.83 ± 0.49 ab	1.55 ± 0.31 c	1.59 ± 0.27 bc	0.04	***
Springiness (cm)	0.59 ± 0.06 b	0.88 ± 0.04 a	0.93 ± 0.44 a	0.89 ± 0.08 a	0.88 ± 0.09 a	0.85 ± 0.11 a	0.02	***
Chewiness (kg*mm)	0.35 ± 0.07 c	1.66 ± 0.27 ab	1.76 ± 0.86 a	1.65 ± 0.54 ab	1.35 ± 0.28 b	1.35 ± 0.25 b	0.05	***

sem = standard error of mean.
*** = $p < 0.001$.
a-c = mean values followed by different letters significantly differ over the storage time ($p < 0.05$).

likely results in the development of a firmer texture after 9 days (as depicted by higher hardness and gumminess and lower cohesiveness). Then, further oxidative modifications occurring during storage and water redistribution within the meat matrix likely weakened the gel network (Muzolf-Panek et al., 2019; Møller et al., 2011; Toldrà et al., 2014), ultimately resulting in significantly lower hardness, gumminess, and chewiness after 15 days.

As for the textural parameters assessed on HHP-treated sausages (Table 1), the most dramatic differences were observed by comparing the results obtained before (0 days) and after the application of HHP (600 MPa for 5 min, 1 d). In fact, a significant increase in the values measured for all the textural parameters was found by comparing the results obtained at 1 d with those measured at 0 days. These findings can undoubtedly be ascribed to the strong effect exerted by HHP on protein conformation, thus affecting the water-holding and -binding capacity and the gelling properties of the myofibrillar proteins as well (Torres & Velazquez, 2005; Liu et al., 2021). In detail, a disruption of the non-covalent interactions and a concurrent reduction in proteins' volume may occur as a consequence of HHP (Bai et al., 2021). These phenomena, in their turn, result in protein denaturation and exposure of hydrophobic and sulfhydryl groups along with the subsequent development of molecular bonds within or between the protein molecules (Sun and Holley, 2011; Yang et al., 2016) that are responsible for the modifications observed in the textural properties of HHP-treated samples. Moreover, the observed increase in hardness, cohesiveness, and chewiness following HHP treatment suggests the development of a more compact and elastic gel network, which is typically associated with the unfolding and subsequent aggregation of myofibrillar proteins. The enhanced protein-protein interactions likely lead to the establishment of a denser, more continuous matrix capable of entrapping water more effectively, although in some cases this may also restrict the mobility of water within the matrix, thereby increasing rigidity. Similar textural trends have been reported in pressurized meat emulsions and gels, where the strengthening of the protein matrix was attributed to an intensified cross-linking and formation of new disulfide and hydrophobic interactions (Campus, 2010; Liu et al., 2021). It is also important to consider that the effects of HHP on texture are pressure and time-dependent, with increasing pressure levels generally promoting greater structural rearrangements and, consequently, firmer textures. In the present study, the conditions applied (600 MPa for 5 min) appeared sufficient to induce a substantial reorganization of the protein matrix without causing excessive fragmentation or detrimental textural degradation. This balance is crucial, as excessively severe treatments can lead to irreversible protein aggregation, resulting in a brittle texture or loss of juiciness (Wang et al., 2025). Furthermore, the immediate changes observed at 1 d indicate that HHP induces rapid physico-chemical modifications that persist during the early storage period. These changes could influence not only the mechanical properties of the sausage matrix but also its sensory perception, potentially leading to an improved bite and sliceability. However, the long-term stability of these textural improvements should be considered in relation to possible protein–protein rearrangements and water migration phenomena occurring during storage, which may alter firmness and cohesiveness over time (Zhang et al., 2023). Overall, the increase in textural parameters following HHP treatment can therefore be interpreted as the combined effect of protein denaturation, aggregation, and network formation, modulated by the extent of pressure-induced conformational transitions. The results confirm the capacity of HHP to modify the microstructure of meat products in a way that enhances their textural integrity, a feature that can be exploited for improving product quality and consumer acceptability in pressure-treated sausages (Peng et al., 2025).

Then, a significant ($p < 0.001$) reduction in hardness, and consequently in gumminess and chewiness, was observed when comparing the values measured after 1 day with those obtained at subsequent sampling times. This decrease likely reflects microstructural relaxation of the protein network over storage. Evidence has shown that HHP treatments exceeding 300 MPa result in an overall inactivation of endogenous proteolytic enzymes (Homma et al., 1994; Jung et al., 2000; Olsen et al., 2023), limiting protein degradation. In addition, water redistribution within the gel matrix may occur during storage due to oxidative modifications affecting both protein and lipid fractions. Such oxidative processes can alter the water-binding and water-holding capacity of the matrix, leading to a progressive increase in cohesiveness ($p < 0.001$) while subtly altering firmness and chewiness. Recent studies have highlighted that HHP-induced lipid oxidation can influence the textural properties of meat products, including changes in hardness and cohesiveness over time (Chmiel et al., 2025). Additionally, research on seafood muscle subjected to HHP has demonstrated similar effects, with oxidative modifications contributing to alterations in texture and water mobility during storage (Pinheiro et al., 2025).

From a microstructural standpoint, the increase in textural parameters after HHP can be interpreted as a consequence of pressure-driven restructuring of the meat matrix. HHP disrupts non-covalent interactions and promotes partial unfolding of myofibrillar proteins, favoring aggregation and the formation of a more continuous and compact gel network (Aganovic et al., 2021; Liu et al., 2021; Simonin et al., 2012). In comminuted meat systems, this densification is associated with reduced pore size and enhanced matrix continuity, which in turn increases resistance to deformation (Bolumar et al., 2020; Campus, 2010). Concurrently, HHP-induced modifications of myosin functionality can strengthen protein–fat interfacial films and improve emulsion stability, supporting a finer fat globule dispersion within the protein network (Bai et al., 2021; Liu et al., 2021). These structural changes are expected to modify water compartmentalization by increasing immobilized/bound water within the gelled matrix, thereby linking microstructure to the observed variations in water-holding capacity and texture (Hughes et al., 2014; Møller et al., 2011). Similar processing-structure-property relationships have been reported for HHP-treated meat products, where pressure intensity drives the extent of network formation and the resulting hardness/cohesiveness profile (Bolumar et al., 2020; Yang et al., 2016).

3.3. Colour measurements

The evolution of the colour parameters (Lightness - L*, Redness - a*, and Yellowness - b*) during the shelf-life of NT-4-4 and 600-4-4 sausages is reported in Tables 1 and 2 As for NT samples (Table 1), significant variations in L* were observed by comparing the results obtained at the initial sampling time (0 days) and after 20 days. In detail, NT sausages at the end of the shelf life were darker than their fresh counterpart (46.2 vs. 50.7; $p < 0.001$). This significant reduction in L* observed after 20 days may likely be ascribed to moisture losses (and surface dehydration) as well as to both protein and lipid oxidation that, considered on their whole, may affect the light scattering phenomenon which contributes to determine the colour of meat products (Muzolf-Panek et al., 2019; Ruedt & Gibis, 2023; Seibt et al., 2024). In agreement with that, a fluctuating trend was observed for a* that exhibited an increase from 0 to 9 days, followed by a decline at the final sampling time (20 days) ($p < 0.001$). This increase in a* measured after 9 d may result from the surface dehydration of the sausages that may affect their interaction with the incident light, thus enhancing the contribution of myoglobin in defining meat colour (Richards, 2010; Ruedt & Gibis, 2023). Then, a subsequent oxidation of this heme protein is likely responsible for the discolouration (reduction in a*) observed after 20 days (Richards, 2010).

Regarding the colour parameters evaluated in treated sausages (Table 2), the most remarkable differences were found between the samples at the initial sampling time (0 days) and after 1 day of storage (immediately after HHP treatment). In detail, the application of HHP resulted in a sharp increase in L* (50.7 vs. 56.7), which is likely the result of the modifications (*i.e.*, conformational changes, denaturation, subsequent aggregation and development of a gel matrix, etc.) affecting the myofibrillar proteins along with the denaturation of myoglobin (Hughes et al., 2014). In agreement with that, a* and b* exhibited a decline which may be ascribed to the denaturation of myoglobin and to the onset of oxidative modifications affecting the lipid and the protein fraction, respectively (Wackerbarth et al., 2009; Chun et al., 2014; Olsen, & Orlien, 2016). As for the evolution of these parameters during refrigerated storage, overall the trends were similar to those observed in NT sausages with a progressive decline in L*, a*, and b* that may be likely attributed to the evolution of the characteristics and stability of the HHP-induced gel network (which are strongly affected by protein oxidation, moisture losses, etc.) as well as to myoglobin oxidation ultimately resulting in meat discoloration (Møller et al., 2011; Richards, 2010).

3.4. Challenge tests

The survival and eventual growth of *Salmonella* Enteritidis, *L. innocua* and *Staph. aureus* was analysed under controlled thermal conditions (4 °C). The contamination of sausages by these pathogens may occur at many points of the process: the raw meat may be contaminated at the slaughterhouse, during manufacturing, or by contact with surfaces or workers. The results are reported in Table 3. All the pathogens were inoculated in sausages at concentrations ranging from 4.5 to 4.7 log cfu/g. In the samples not treated with HHP, *S.* Enteritidis showed a slight decrease (0.4 log cfu/g) after 20 days of storage, while *Staph. aureus* concentration did not change. *L. innocua* was the only species able to grow under the adopted conditions with an increase of about 0.7 log cfu/g.

Concerning the sausages subjected to HHP treatment, the counts of *L. innocua* and *S.* Enteritidis were below the detection limit (0.3 log cfu/g) during the storage period (90 days). However, while the qualitative

Table 3
Microbiological analysis (log cfu/g) of the control (pressure = 0 MPa) and treated (pressure = 600 MPa) samples inoculated with pathogens and analysed during shelf-life. The results are the average of three independent repetitions (standard deviation is reported) for *S.* Enteritidis and *Staph. aureus*, and the average of five independent repetitions (standard deviation is reported) for *L. innocua*. For samples treated at 600 MPa, 0 days refers to the pre-treatment analysis time, and 1 day refers to the post-treatment analysis time.

Pressure (MPa)	Time (days)	*Salmonella* Enteritidis	*Listeria innocua*	*Staphylococcus aureus*
0	0	4.62 ± 0.23	4.52 ± 0.09	4.69 ± 0.41
0	9	4.41 ± 0.26	4.94 ± 0.51	4.74 ± 0.03
0	15	4.34 ± 0.24	4.91 ± 0.13	4.76 ± 0.08
0	20	4.27 ± 0.19	5.12 ± 0.38	4.64 ± 0.26
600	0	4.62 ± 0.23	4.52 ± 0.09	4.69 ± 0.41
600	1	<1[a]	<1[b]	1.79 ± 0.20
600	9	<1[a]	<1[b]	1.95 ± 0.10
600	20	<1[a]	<1[b]	1.48 ± 0.00
600	40	<1[a]	<1[b]	2.05 ± 0.15
600	60	<1[a]	<1[b]	1.31 ± 1.14
600	90	<1[a]	<1[c]	1.52 ± 0.30

[a] Results of the triple S. Enteritidis research. Negativity was found in all three repetitions.
[b] Results of the five-fold search for *L. innocua.* Negativity was found in all five repetitions.
[c] Results of the five-fold search for *L. innocua.* Negativity was found only in three repetitions.

search for *S*. Enteritidis was always negative, after 90 days, three out of five samples were qualitatively positive for *L. innocua*. On the other hand, after the HHP treatment, *Staph. aureus* showed a reduction of approx. 3 log units if compared to the initial counts. During all the storage, the counts of this species remained below 2 log cfu/g.

Bacterial HHP inactivation is a multi-target process. Cell membrane is among the main targets, though additional damages may be necessary in order to kill bacteria: extensive solute loss, protein denaturation and coagulation, enzyme inactivation and ribosome disruption (Cebrián et al., 2016; Mañas & Pagán, 2005).

In general, HHP microbial inactivation is affected by species, cell morphology and physiological status, surrounding matrix, and detection method (Yamamoto et al., 2021). Among bacteria, spores, gram-positive and gram-negative bacteria present decreasing resistance to HHP. In addition, spheric cells are more resistant than bacilli (Ludwig et al., 2002). For example, *Staph. aureus* shows a relatively high HHP resistance (Cebrián et al., 2016).

Salmonella is usually very sensitive to HHP treatments (Silva, & Evelin, 2023). In addition to the low resistance compared with Gram-positive bacteria (such as *Staph. aureus*), this species inoculated in fish products was also less resistant than *Escherichia coli* (Boziaris et al., 2021). Jofré et al. (2008) demonstrated that *Salmonella enterica* and *L. monocytogenes* concentration, inoculated at approx. 4 log cfu/g in sliced cooked ham, were below the detection limit (10 cfu/g) during the storage period (3 months) under refrigerated (0-6 °C) conditions. In the same work, an inactivation of approx. 1 log cfu/g of *Staph. aureus* was indicated. However, no growth was observed during storage. On the other hand, *Salmonella enterica* and *Staphylococcus aureus* cannot grow at refrigeration temperature, and their recovery could be hindered.

Several studies on the HHP effects on *Staph. aureus* cells confirmed the relatively high resistance of this species (Silva, & Evelin, 2023). Also, for this species, differences in the lethality of HHP were observed in relation to the matrix/medium (O'Reilly et al., 2000). In milk, *Staph. aureus* showed a markedly higher resistance compared to *L. monocytogenes* and *E. coli,* and has had a high recovery potential after 8 days from treatment (Zagorska et al., 2021). A limited reduction of *Staph. aureus* cells (less than 2 log cfu/g) was observed in beef meat treated at 500 MPa (Park et al., 2022).

The effects of HHP treatments on the killing of *L. monocytogenes* have been studied by several authors, often with conflicting results. In turkey meat treated at 600 MPa in the presence of salt and nitrite quantities comparable to those used in this trial, decreases of approximately 4 log cfu/g were observed (Myers et al., 2013) while cooked chicken meat treated at the same pressure was unable to inactivate 3 log cfu of the same species (Patterson et al., 2011). Bover-Cid et al. (2015) demonstrated that the amount of fat could have a protective effect of high fat percentages on the deactivation of *L. monocytogenes* in dry-cured hams when high pressures were applied.

The control of the temperature at 4 °C is essential for avoiding the recovery of damaged cells. According to Bozoglu et al. (2004), among the pathogenic species considered here, only *L. monocytogenes* was able to increase its culturability at this temperature. At higher temperatures, also salmonellae showed the possibility of recovering viability (Ritz et al., 2006). In addition, several studies addressed the evaluation of HHP resistance and highlighted the dependence of the survival of *L. monocytogenes* cells on physiological state and strain (Possas et al., 2017).

4. Conclusions

High-Hydrostatic Pressure (HHP) treatment at 600 MPa significantly extends the shelf life of raw sausages to 90 days, a substantial increase compared to the conventional 15-20 days. When stored properly at 4 °C, the microbial load of HHP-treated sausages remains well below spoilage thresholds throughout the considered period. Concerning safety, challenge tests demonstrated that HHP treatment effectively eliminates high concentrations of Salmonella. For *L. innocua*, while quantitative detection was negative after 90 days of storage at 4 °C, some samples showed qualitative presence when 25g of the product was sampled. However, this qualitative presence is not considered a risk, since raw sausages are cooked prior to consumption. Among the tested pathogenic bacteria, *Staph. aureus* proved most resistant, partially surviving the treatment. Nevertheless, it was unable to multiply at the 4 °C storage temperature.

HHP treatment affected the textural characteristics of raw sausages, leading to an increase in hardness, cohesiveness, gumminess, chewiness, and springiness. These changes also contributed to a reduced release of exudates during storage, improving overall product performance. Regarding colour, HHP treatment causes an immediate decrease in a (redness) and b (yellowness) values**, while L* (lightness) increases. Interestingly, by the end of the storage period, the colour parameters of HHP-treated sausages revert to similar trends observed in raw, untreated sausages.

This research demonstrated that HHP can be a feasible strategy to improve the microbiological quality of raw pork sausages. However, since the process applied conditions cannot be considered sterilising, the maintenance of cold chain (0-4 °C) is needed to guarantee the shelf-life and the safety of HHP-treated raw pork sausages.

CRediT authorship contribution statement

Chiara Angelucci: Writing – original draft, Formal analysis, Data curation. **Giulia Tabanelli:** Writing – review & editing, Conceptualization. **Francesca Soglia:** Methodology, Data curation. **Federica Barbieri:** Methodology, Formal analysis. **Chiara Montanari:** Methodology, Investigation. **Gabriele Gardini:** Formal analysis, Conceptualization. **Rudy Magnani:** Supervision, Investigation. **Fausto Gardini:** Writing – original draft, Data curation. **Massimiliano Petracci:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119293.

Data availability

Data will be made available on request.

References

Aganovic, K., Hertel, C., Vogel, R. F., Johne, R., Schlüter, O., Schwarzenbolz, U., Jäger, H., Holzhauser, T., Bergmair, J., Roth, A., Sevenich, R., Bandick, N., Kulling, S. E., Knorr, D., Engel, K. H., & Heinz, V. (2021). Aspects of high hydrostatic pressure food processing: Perspectives on technology and food safety. *Comprehensive Reviews in Food Science and Food Safety, 20*, 3225–3266. https://doi.org/10.1111/1541-4337.12763

Andreevskaya, M., Jääskeläinen, E., Johansson, P., Ylinen, A., Paulin, L., Björkroth, J., & Auvinen, P. (2018). Food spoilage-associated *Leuconostoc, Lactococcus*, and *Lactobacillus* species display different survival strategies in response to competition. *Applied and Environmental Microbiology, 84*(13). https://doi.org/10.1128/AEM.00554-18. e00554-18.

Angelucci, C., Barbieri, F., Soglia, F., Petracci, M., Montanari, C., Gardini, G., Gardini, F., & Tabanelli, G. (2024). Changes in the microbiota and colour of pork meat batter during refrigerated storage in relation to the application of High hydrostatic pressure. *Food Bioscience, 62*, Article 105419. https://doi.org/10.1016/j.fbio.2024.105419

Bai, Y., Zeng, X., Zhang, C., Zhang, T., Wang, C., Han, M., Zhou, G., & Xu, X. (2021). Effects of high hydrostatic pressure treatment on the emulsifying behavior of myosin and its underlying mechanism. *LWT - Food Science and Technology, 146*, Article 111397. https://doi.org/10.1016/j.lwt.2021.111397

Bak, K. H., Bolumar, T., Karlsson, A. H., Lindahl, G., & Orlien, V. (2019). Effect of high-pressure treatment on the color of fresh and processed meats: A review. *Critical Reviews in Food Science and Nutrition, 59*(2), 228–252. https://doi.org/10.1080/10408398.2017.1363712

Benson, A. K., David, J. R. D., Gilbreth, S. E., Smith, G., Nietfeldt, J., Legge, R., Kim, J., Sinha, R., Duncan, C. E., Ma, J., & Singh, I. (2014). Microbial successions are associated with changes in chemical profiles of a model refrigerated fresh pork sausage during an 80-day shelf-life study. *Applied and Environmental Microbiology, 80*, 5178–5194. https://doi.org/10.1128/AEM.00774-14

Bolumar, T., Orlien, V., Bak, K. H., Aganovic, K., Sikes, A., Guyon, C., Stübler, A. S., de Lambellerie, M., Hertel, C., & Brüggemann, D. A. (2020). High-pressure processing (HPP) of meat products: Impact on quality and applications. In F. J. Barba, E. Puértolas, C. Tonello-Samson, & M. Lavilla (Eds.), *Present and future of high pressure processing* (pp. 221–244). Elsevier. https://doi.org/10.1016/C2018-0-00311-4.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*, 62–66.

Bover-Cid, S., Belletti, N., Aymerich, T., & Garriga, M. (2015). Modeling the protective effect of aw and fat content on the high-pressure resistance of *Listeria monocytogenes* in dry-cured ham. *Food Research International, 75*, 194–199. https://doi.org/10.1016/j.foodres.2015.05.052

Boziaris, I. S., Parlapani, F. F., & DeWitt, C. A. M. (2021). High-pressure processing at ultra-low temperatures: Inactivation of foodborne bacterial pathogens and quality changes in frozen fish fillets. *Innovative Food Science Emerging Technologies, 74*, Article 102811. https://doi.org/10.1016/j.ifset.2021.102811

Bozoglu, F., Alpas, H., & Kaletunç, G. (2004). Injury recovery of foodborne pathogens in high hydrostatic pressure treated milk during storage. *FEMS Immunology and Medical Microbiology, 40*(3), 243–247. https://doi.org/10.1016/S0928-8244(04)00002-1

Campus, M. (2010). High pressure processing of meat, meat products and seafood. *Food Engineering Reviews, 2*(4), 256–273. https://doi.org/10.1007/s12393-010-9028-y

Cava, R., Montero, I., & Ladero, L. (2024). Synergistic impact of pomegranate peel extract and high hydrostatic pressure on controlling discoloration and oxidation in Iberian dry sausage throughout storage. *Food and Bioprocess Technology*. https://doi.org/10.1007/s11947-024-03450-9

Cebrián, G., Mañas, P., & Condón, S. (2016). Comparative resistance of bacterial foodborne pathogens to non-thermal technologies for food preservation. *Frontiers in Microbiology, 7*, 734. https://doi.org/10.3389/fmicb.2016.00734

Chevalier, D., Le Bail, A., & Ghoul, M. (2001). Effects of high-pressure treatment (100–200 MPa) at low temperature on turbot (*Scophthalmus maximus*) muscle. *Food Research International, 34*(5), 425–429. https://doi.org/10.1016/S0963-9969(00)00187-3

Chmiel, M., Świder, O., Roszko, M., Adamczak, L., Cegiełka, A., Pietrzak, D., Florowski, T., Bryła, M., & Florowska, A. (2025). Effect of high-pressure processing and long-time storage on color, lipid oxidation, and free amino acids profile of beef. *Applied Sciences, 15*(5), 2382. https://doi.org/10.3390/app15052382

Chun, J. Y., Min, S. G., & Hong, G. P. (2014). Effects of high-pressure treatments on the redox state of porcine myoglobin and color stability of pork during cold storage. *Food and Bioprocess Technology, 7*, 588–597. https://doi.org/10.1007/s11947-013-1118-4

Cocolin, L., Rantsiou, K., Iacumin, L., Urso, R., Cantoni, C., & Comi, G. (2004). Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. *Applied and Environmental Microbiology, 70*, 1883–1894. https://doi.org/10.1128/AEM.70.4.1883–1894.2004

de Alba, M., Bravo, D., & Medina, M. (2012). High pressure treatments on the inactivation of *salmonella* Enteritidis and the characteristics of beef carpaccio. *Meat Science, 92*(4), 823–828. https://doi.org/10.1016/j.meatsci.2012.07.008

De Cesare, A., Mioni, R., & Manfreda, G. (2007). Prevalence of *Listeria monocytogenes* in fresh and fermented Italian sausages and ribotyping of contaminating strains. *International Journal of Food Microbiology, 120*, 124–130. https://doi.org/10.1016/j.ijfoodmicro.2007.06.009

Duranton, F., Guillou, S., Simonin, H., Chéret, R., & de Lamballerie, M. (2012). Combined use of high pressure and salt or sodium nitrite to control the growth of endogenous microflora in raw pork meat. *Innovative Food Science and Emerging Technologies, 16*, 373–380. https://doi.org/10.1016/j.ifset.2012.08.004

European Commission. (2023). Commission Regulation (EU) 2023/2108 of 6 October 2023 amending annex II to regulation (EC) no 1333/2008 of the European Parliament and of the Council and the annex to Commission Regulation (EU) no 231/2012 as regards food additives nitrites (E 249-250) and nitrates (E 251-252). *Official Journal of the European Union, Series L*. Regulation - EU - 2023/2108 - EN - EUR-Lex.

Fougy, L., Desmonts, M. H., Coeuret, G., Fassel, C., Hamon, E., Hézard, B., Champomier-Vergès, M. C., & Chaillou, S. (2016). Reducing salt in raw pork sausages increases spoilage and correlates with reduced bacterial diversity. *Applied and Environmental Microbiology, 82*, 3928–3939. https://doi.org/10.1128/AEM.00323-16

Guyon, C., Meynier, A., & de Lamballerie, M. (2016). Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science & Technology, 50*, 131–143. https://doi.org/10.1016/j.tifs.2016.01.026

Homma, N., Ikeuchi, Y., & Suzuki, A. (1994). Effects of high pressure treatment on the proteolytic enzymes in meat. *Meat Science, 38*(2), 219–228. https://doi.org/10.1016/0309-1740(94)90111-2

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science, 98*(3), 520–532. https://doi.org/10.1016/j.meatsci.2014.05.022

Hugo, C. J., & Hugo, A. (2015). Current trends in natural preservatives for fresh sausage products. *Trends in Food Science & Technology, 45*, 12–23. https://doi.org/10.1016/j.tifs.2015.05.003

Hygreeva, D., & Pandey, M. C. (2016). Novel approaches in improving the quality and safety aspects of processed meat products through high pressure processing technology-A review. *Trends in Food Science & Technology, 54*, 175–185. https://doi.org/10.1016/j.tifs.2016.06.002

Jofré, A., Garriga, M., & Aymerich, T. (2008). Inhibition of *Salmonella* sp. *Listeria monocytogenes* and *Staphylococcus aureus* in cooked ham by combining antimicrobials, high hydrostatic pressure and refrigeration. *Meat Science, 78*(1-2), 53–59. https://doi.org/10.1016/j.meatsci.2007.06.015

Jung, S., Ghoul, M., & de Lamballerie-Anton, M. (2000). Changes in lysosomal enzyme activities and shear values of high pressure treated meat during ageing. *Meat Science, 56*(3), 239–246. https://doi.org/10.1016/S0309-1740(00)00048-6

Kamdem, S. S., Patrignani, F., & Guerzoni, M. E. (2007). Shelf-life and safety characteristics of Italian Toscana traditional fresh sausage (Salsiccia) combining two commercial ready-to-use additives and spices. *Food Control, 18*, 421–429. https://doi.org/10.1016/j.foodcont.2005.11.013

Kimura, K., Morimatsu, K., Inaoka, T., & Yamamoto, K. (2017). Injury and recovery of *Escherichia coli* ATCC25922 cells treated by high hydrostatic pressure at 400-600 MPa. *Journal of Bioscience and Bioengineering, 123*, 698–706. https://doi.org/10.1016/j.jbiosc.2017.01.007

Komora, N., Maciel, C., Isidro, J., Pinto, C. A., Fortunato, G., Saraiva, J. M., & Teixeira, P. (2023). The impact of HPP-assisted biocontrol approach on the bacterial communities' dynamics and quality parameters of a fermented meat sausage model. *Biology, 12*(9), 1212. https://doi.org/10.3390/biology.12091212

Koseki, S., & Yamamoto, K. (2006). Recovery of *Escherichia coli* ATCC 25922 in phosphate buffered saline after treatment with high hydrostatic pressure. *International Journal of Food Microbiology, 110*(1), 108–111. https://doi.org/10.1016/j.ijfoodmicro.2006.01.039

Liu, H., Xu, Y., Zu, S., Wu, X., Shi, A., Zhang, J., Wang, Q., & He, N. (2021). Effects of high hydrostatic pressure on the conformational structure and gel properties of myofibrillar protein and meat quality: A review. *Foods, 10*(8), 1872. https://doi.org/10.3390/foods10081872

Ludwig, H., van Almsick, G., & Schreck, C. (2002). The effect of hydrostatic pressure on the survival of microorganisms. In biological systems under extreme conditions. In Y. Taniguchi, H. E. Stanley, & H. Ludwig (Eds.), *Biological and medical physics series* (pp. 239–256). Berlin, Heidelberg: Springer.

Mañas, P., & Pagán, R. (2005). Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology, 98*, 1387–1399. https://doi.org/10.1111/j.13652672.2005.02561.x

McMeekin, T. A., Baranyi, J., Bowman, J., Dalgaard, P., Kirk, M., Ross, T., Schmid, S., & Zwietering, M. H. (2006). Information systems in food safety management. *International Journal of Food Microbiology, 112*, 181–194. https://doi.org/10.1016/j.ijfoodmicro.2006.04.048

McMeekin, T., & Ross, T. (1996). Shelf-life prediction: Status and future possibilities. *International Journal of Food Microbiology, 33*, 65–83. https://doi.org/10.1016/0168-1605(96)01138-5

Møller, S. M., Grossi, A., Christensen, M., Orlien, V., Søltoft-Jensen, J., Straadt, I. K., … Bertram, H. C. (2011). Water properties and structure of pork sausages as affected by high-pressure processing and addition of carrot fibre. *Meat Science, 87*(4), 387–393. https://doi.org/10.1016/j.meatsci.2010.11.016

Muzolf-Panek, M., Kaczmarek, A., Tomaszewska-Gras, J., Cegielska-Radziejewska, R., & Majcher, M. (2019). Oxidative and microbiological stability of raw ground pork during chilled storage as affected by plant extracts. *International Journal of Food Properties, 22*(1), 111–129. https://doi.org/10.1080/10942912.2019.1579834

Myers, K., Montoya, D., Cannon, J., Dickson, J., & Sebranek, J. (2013). The effect of high hydrostatic pressure, sodium nitrite and salt concentration on the growth of *Listeria monocytogenes* on RTE ham and Turkey. *Meat Science, 93*(2), 263–268. https://doi.org/10.1016/j.meatsci.2012.09.007

Nuvoloni, R., Pedonese, F., Fratini, F., Torracca, B., Turchi, B., Serra, A., & Preziuso, G. (2012). Microbiological and physicochemical profile of traditional salsiccia toscana during storage. *Italian Journal of Animal Science, 11*, 317–322. https://doi.org/10.4081/ijas.2012.e59

O'Reilly, C. E., O'Connor, P. M., Kelly, A. L., Beresford, T. P., & Murphy, P. M. (2000). Use of hydrostatic pressure for inactivation of microbial contaminants in cheese. *Applied and Environmental Microbiology, 66*(11), 4890–4896. https://doi.org/10.1128/AEM.66.11.4890-4896.2000

Olsen, K., Bolumar, T., Rode, T. M., & Orlien, V. (2023). Effects of high-pressure processing on enzyme activity in meat, fish, and egg. *Effect of High-Pressure Technologies on Enzymes*, 241–267. https://doi.org/10.1016/B978-0-323-98386-0.00003-8

Olsen, K., & Orlien, V. (2016). High pressure processing for modification of food biopolymers. In K. Knoerzer, P. Juliano, & G. Smithers (Eds.), *Innovative food processing techniques* (pp. 291–313). Cambridge: Woodhead Publishing Limited. https://doi.org/10.1016/B978-0-08-100294-0.00011-0.

Onyango, L. A., Dunstan, R. H., Gottfries, J., von Eiff, C., & Roberts, T. K. (2012). Effect of low temperature on growth and ultra-structure of *Staphylococcus* spp. *PLoS One, 7*(1), Article e29031. https://doi.org/10.1371/journal.pone.0029031

Park, S., Park, E., & Yoon, Y. (2022). Comparison of nonthermal decontamination methods to improve the safety for raw beef consumption. *Journal of Food Protection, 85*(4), 664–670. https://doi.org/10.4315/JFP-21-243

Patterson, M. F., Mackle, A., & Linton, M. (2011). Effect of high pressure, in combination with anti-listerial agents, on the growth of *Listeria monocytogenes* during extended storage of cooked chicken. *Food Microbiology, 28*(8), 1505–1508. https://doi.org/10.1016/j.fm.2011.08.006

Peng, H., Yang, Q., Zhou, C., Wang, L., Pan, D., Cao, J., Sun, Y., Zhang, H., & Xia, Q. (2025). Processing–microstructure–properties relationship of meat myofibrillar

proteins as defined by high hydrostatic pressure. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/acs.jafc.5c09120

Pinheiro, A. C. D. A. S., Tappi, S., Soglia, F., Baldi, G., Hernandez, J. B. M., Laghi, L., Dalla Rosa, M., Petracci, M., & Rocculi, P. (2025). Effect of high hydrostatic pressure (HHP) on proteins, lipids, and water state of three different thawed seafood products. *Italian Journal of Food Science, 37*(3). https://doi.org/10.15586/ijfs.v37i3.3106

Possas, A., Pérez-Rodríguez, F., Valero, A., & García-Gimeno, R. M. (2017). Modelling the inactivation of *Listeria monocytogenes* by high hydrostatic pressure processing in foods: A review. *Trends in Food Science & Technology, 70*, 45–55. https://doi.org/10.1016/j.tifs.2017.10.006

Raimondi, S., Nappi, M. R., Sirangelo, T. M., Leonardi, A., Amaretti, A., Ulrici, A., Magnani, R., Montanari, C., Tabanelli, G., Gardini, F., & Rossi, M. (2018). Bacterial community of industrial raw sausage packaged in modified atmosphere throughout the shelf-life. *International Journal of Food Microbiology, 280*, 78–86. https://doi.org/10.1016/j.ijfoodmicro.2018.04.041

Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT - Food Science and Technology, 44*, 1251–1260. https://doi.org/10.1016/j.lwt.2010.11.001

Richards, M. P. (2010). Heme proteins and oxidation in fresh and processed meats. In E. A. Decker (Ed.), *Oxidation in foods and beverages and antioxidant applications* (pp. 76–104). Woodhead Publishing.

Ritz, M., Pilet, M. F., Jugiau, F., Rama, F., & Federighi, M. (2006). Inactivation of *Salmonella Typhimurium* and *Listeria monocytogenes* using high-pressure treatments: Destruction or sublethal stress? *Letters in Applied Microbiology, 42*(4), 357–362. https://doi.org/10.1111/j.1472-765X.2006.01868.x

Ruedt, C., Gibis, M., & Weiss, J. (2023). Meat color and iridescence: Origin, analysis, and approaches to modulation. *Comprehensive Reviews in Food Science and Food Safety, 22*(4), 3366–3394. https://doi.org/10.1111/1541-4337.13191

Schottroff, F., Fröhling, A., Zunabovic-Pichler, M., Krottenthaler, A., Schlüter, O., & Jäger, H. (2018). Sublethal injury and viable but non-culturable (VBNC) state in microorganisms during preservation of food and biological materials by non-thermal processes. *Frontiers in Microbiology, 9*, 2773. https://doi.org/10.3389/fmicb.2018.02773

Seibt, A. C. M. D., Nerhing, P., Pinton, M. B., Santos, S. P., Leães, Y. S. V., De Oliveira, F. D. C., Robalo, S. S., Casarin, B. C., dos Santos, B. A., Barin, J. S., Wagner, R., de Menezes, C. R., Campagnol, P. C. B., & Cichoski, A. J. (2024). Green technologies applied to low-NaCl fresh sausages production: Impact on oxidative stability, color formation, microbiological properties, volatile compounds, and sensory profile. *Meat Science, 209*, Article 109418. https://doi.org/10.1016/j.meatsci.2023.109418

Seman, D. L., Boler, D. D., Carr, C. C., Dikeman, M. E., Owens, C. M., Keeton, J. T., Pringle, D. T., Sindelar, J. J., Woerner, D. R., de Mello, A. S., & Powell, T. H. (2018). Meat science lexicon. *Meat and Muscle Biology, 2*, 127. https://doi.org/10.22175/mmb2017.12.0059

Silva, F. V. M., & Evelin. (2023). Pasteurization of food and beverages by high pressure processing (HPP) at room temperature: Inactivation of *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Salmonella*, and other microbial pathogens. *Applied Sciences, 13*(2), 1193. https://doi.org/10.3390/app13021193

Simonin, H., Duranton, F., & de Lamballerie, M. (2012). New insights into the high-pressure processing of meat and meat products. *Comprehensive Reviews in Food Science and Food Safety, 11*, 285–306. https://doi.org/10.1111/j.1541-4337.2012.00184

Sun, X. D., & Holley, R. A. (2011). Factors influencing gel formation by myofibrillar proteins in muscle foods. *Comprehensive Reviews in Food Science and Food Safety, 10*(1), 33–51. https://doi.org/10.1111/j.1541-4337.2010.00137.x

Tabanelli, G., Montanari, C., Gómez-Caravaca, A. M., Díaz-de-Cerio, E., Verardo, V., Zadeh, F. S., Vannini, L., Gardini, F., & Barbieri, F. (2024). Microbiological safety and functional properties of a fermented nut-based product. *Foods, 13*, 3095. https://doi.org/10.3390/foods13193095

Toldrá, F., Knipe, C. L., Flores, M., Ockerman, H. W., & Basu, L. (2014). Sausages type of. In M. Dickeman, & D. Carrick (Eds.), *Encyclopedia of meat sciences* (2nd ed., Vol. 3, pp. 241–266). Academic Press.

Torres, J. A., & Velazquez, G. (2005). Commercial opportunities and research challenges in the high-pressure processing of foods. *Journal of Food Engineering, 67*(1-2), 95–112. https://doi.org/10.1016/j.jfoodeng.2004.05.066

Tremonte, P., Sorrentino, E., Succi, M., Reale, A., Maiorano, G., & Coppola, R. (2005). Shelf-life of fresh sausages stored under modified atmospheres. *Journal of Food Protection, 68*, 2686–2692. https://doi.org/10.4315/0362-028x-68.12.2686

Wackerbarth, H., Kuhlmann, U., Tintchev, F., Heinz, V., & Hildebrandt, P. (2009). Structural changes of myoglobin in pressure-treated pork meat probed by resonance Raman spectroscopy. *Food Chemistry, 115*, 1194–1198. https://doi.org/10.1016/j.foodchem.2009.01.027

Wang, X., Han, R., Ramaswamy, H., Wang, C., & Duan, H. (2025). Effects of high-pressure processing on physicochemical, structural and emulsifying properties of chicken myofibrillar proteins. *LWT - Food Science and Technology*. , Article 118168. https://doi.org/10.1016/j.lwt.2025.118168

Yamamoto, K., Zhang, X., Inaoka, T., Morimatsu, K., Kimura, K., & Nakaura, Y. (2021). Bacterial injury induced by high hydrostatic pressure. *Food Engineering Reviews*, 1–12. https://doi.org/10.1007/s12393-020-09271-8

Yang, H., Khan, M. A., Yu, X., Zheng, H., Han, M., Xu, X., & Zhou, G. (2016). Changes in protein structures to improve the rheology and texture of reduced-fat sausages using high pressure processing. *Meat Science, 121*, 79–87. https://doi.org/10.1016/j.meatsci.2016.06.004

Zagorska, J., Galoburda, R., Raita, S., & Liepa, M. (2021). Inactivation and recovery of bacterial strains, individually and mixed, in milk after high pressure processing. *International Dairy Journal, 123*, Article 105147. https://doi.org/10.1016/j.idairyj.2021.105147

Zhang, H., Tang, D., Yang, H., Liu, X., Cheng, J., Wang, X., Zou, J., & Lin, Y. (2023). Effects of high hydrostatic pressure assisted enzymatic tenderization on goose meat texture and myofibril protein. *LWT - Food Science and Technology, 184*, Article 114845. https://doi.org/10.1016/j.lwt.2023.114845

Zhu, Y., Yan, Y., Yu, Z., Wu, T., & Bennett, L. E. (2022). Effects of high-pressure processing on microbial, textural and sensory properties of low-salt emulsified beef sausage. *Food Control, 133*, Article 108596. https://doi.org/10.1016/j.foodcont.2021.108596