

Effect of high-pressure processing and natural antimicrobials on the shelf-life of cooked ham

MARKÉTA ADAMCOVÁ^{1*}, VINCENT VAN ANDEL¹, JAN STROHALM², MILAN HOUŠKA²,
RUDOLF ŠEVČÍK¹

¹Department of Food Preservation, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Prague, Czech Republic

²Food Research Institute Prague, Prague, Czech Republic

*Corresponding author: marketa.adamcova@vscht.cz

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Abstract: The need to reduce the content of questionable health preservatives leads to the search for new methods to extend the shelf-life of meat products. The spectrum of possible approaches includes physical methods and the use of additives from natural sources. In this study, we examined the influence of the combination of high-pressure processing (HPP) and the addition of natural antimicrobials on the shelf-life of cooked ham. The samples of cooked ham were produced in a professional meat processing plant. One half of the samples were produced according to a traditional recipe, and the other was enriched with potassium lactate in the form of a commercial product PURASAL[®] Hirer P Plus. This product is produced via sugar fermentation and contains high levels of potassium lactate, a compound with high antimicrobial activity. Cooked hams were inoculated by bacteria *Serratia liquefaction*, vacuum packaged and treated by HPP. Packaged ham samples were stored at 3°C for 40 days and the total microbial count was examined during this storage period in defined intervals. The combination of HPP and potassium lactate from natural sources significantly reduced the total microbial counts in cooked hams and, thus, could be a suitable solution for the meat industry.

Keywords: hurdle technology; meat products; microbial stability; potassium lactate; preservation; *Serratia liquefaciens*

One of the main limiting factors of shelf-life of meat products is microbial spoilage, which can be slowed down by some technological procedures, such as the addition of preservatives, heat treatment, fermentation, packaging, drying, high-pressure processing, etc. These individual procedures are usually not applied as separate processes, but in an optimised combination with each other, creating a so-called hurdle effect, to increase the effectiveness of applied preservation processes (MARCOS *et al.* 2008; HYGREEVA & PANDEY 2016; HORITA *et al.* 2018).

In the last decade, there has been a search for optimised combinations of hurdles, which will extend

the shelf-life of the product and, at the same time, respond to an increased demand for meat products produced by alternative methods. This demand is related to the increasing knowledge of consumers and increasing popularity of alternative dietary patterns. Such products should contain as few preservatives as possible, and the shelf-life should be ensured by methods which ideally do not negatively affect the nutritional values (HUGAS *et al.* 2002; HYGREEVA & PANDEY 2016). A possible approach to accomplish such requirements is to combine a physical method, such as high-pressure processing, with addition of antimicrobial substances from natural sources.

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This combination could effectively reduce microbial counts and extend the shelf-life (HUGAS *et al.* 2002; MARCOS *et al.* 2008; HYGREEVA & PANDEY 2016).

High-pressure processing (HPP) is a non-thermal processing technique, for a wide range of food materials (HUGAS *et al.* 2002; BELLETI *et al.* 2013). HPP effectively reduce microbial numbers and, at the same time, practically do not influence the nutritional and sensory properties of fresh and processed meat products and is therefore more gentle than commonly used heat treatment. Hence, one of its primary purposes is a final hygienisation treatment after production and packaging (BELLETI *et al.* 2013). However, high-pressure processing in meat production should always be used about the possible negative impact on texture and water holding capacity (MARCOS *et al.* 2007; BAJOVIC *et al.* 2012).

The effectiveness of high-pressure processing depends on the process conditions (levels of pressure, processing time, temperature, etc.) and physicochemical parameters of treated meat product (pH, a_w , salt content, initial contamination, etc.). High-pressure processing effectively deactivates eukaryotic cells and Gram-negative bacteria; Gram-positive bacteria are generally more resistant to HPP (HUGAS *et al.* 2002; HORITA *et al.* 2018). During the pressurisation, microbial cells are inactivated by a combination of factors, such as the damage of the cytoplasmic membrane, the denaturation of microbial proteins, and the inactivation of metabolic enzymes. According to GARRIGA *et al.* (2004) and BAJOVIC *et al.* (2012) the level of pressure between 400–600 MPa for 3–7 min is sufficient for the pasteurisation of meat products.

Without a doubt, preservatives are a significant part of the composition of meat products. Sodium chloride and sodium nitrite (E250) are amongst the most commonly-used preservatives in the meat industry (PIPEK *et al.* 2016). Both compounds have essential functions such as improving technological, organoleptic and safe shelf-life properties of meat products. Both compounds are safe in recommended doses and ways of treatment (ALAHAKOON *et al.* 2015). However, extensive intake of sodium chloride is associated with an increased risk of high blood pressure and the subsequent development of cardiovascular diseases (KAMENÍK *et al.* 2017). Sodium nitrite can pose a risk due to highly carcinogenic nitrosamines that are formed during heat treatment (>130°C) (ALAHAKOON *et al.* 2015). Thus, many studies have examined various methods to decrease the content of sodium chloride and sodium nitrite without negatively affecting the shelf-life of meat products. One

of the possible approaches is to partially replace these compounds with natural antimicrobial properties such as lactic acid (E270) and its sodium (E325) and potassium (E326) salts, which are commonly used additives (MARCOS *et al.* 2008; ALAHAKOON *et al.* 2015).

Lactic acid is produced via sugar fermentation, and sodium and potassium lactates are its derivatives (MARCOS *et al.* 2008; PIPEK *et al.* 2016). The use of lactates, especially potassium lactate, has several advantages in the meat industry, as it has no significant impact on the pH of meat products. Moreover, it has a mild salty taste, and can therefore partially replace sodium chloride (HOUSTMA *et al.* 1993; ADAMCOVÁ *et al.* 2016). Last but not least, lactates can significantly extend the shelf-life of meat products (HOUSTMA *et al.* 1993; MARCOS *et al.* 2008), by extending the lag-phase of microorganisms, i.e. slowing down their metabolism, and inhibiting some metabolic pathways (CARPENTER & BROADBENT 2009; BRADLEY *et al.* 2011). According to previous studies, addition of 2–3 wt% potassium lactate effectively reduces microbial counts, and at the same time does not significantly influence the quality of tested meat products (BRADLEY *et al.* 2011; ADAMCOVÁ *et al.* 2016).

Even though high-pressure processing and lactates treatments are already commercially used in the meat industry across the world, there are only a few studies on the combined effect of these methods on the shelf-life of meat products. MARCOS *et al.* (2008) examined the effectiveness of HPP and potassium lactate on the growth of *Listeria monocytogenes* in cooked meat products. They found that the combination of HPP and lactate significantly reduced the counts of *Listeria monocytogenes* during 84 days storage period at 5°C. Due to the limited available knowledge on the combined effects of HPP and lactates, there is an increasing need to examine the application of these methods on a broader range of spoilage and pathogenic microorganisms. Therefore, we examined the effectiveness of this combination on the reduction of bacteria *Serratia liquefaciens*, which is one of the most important spoilage microorganisms in cooked ham (BELLETI *et al.* 2013).

MATERIAL AND METHODS

Cooked ham manufacturing. Cooked hams were prepared according to a traditional recipe (Table 1) and procedure in the meat production plant (MP Krásno a.s., Czech Republic). Samples were divided into

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Table 1. The composition of cooked hams

Raw materials	Cooked ham	Potassium lactate (wt%)
Pork shoulder	71.4	71.4
Ice	20.7	19.2
Aroma, Naturcoloid, Naturham	6.2	6.2
Sodium chloride	1.6	0.8
Potassium lactate	–	2.3
Sodium nitrite	0.008	0.008

two batches. The first batch was prepared with the addition of sodium chloride only and the second batch contained both – sodium chloride and potassium lactate. Whereas 50% of the sodium chloride was equimolarly replaced by potassium lactate (PURASAL[®] HiPure P Plus; Corbion, Netherland). Samples were packaged in polyamide bags and cooked according to Czech regulation 69/2016 (for 10 min with a temperature at 70°C in the centre of the product). The whole pieces of cooked hams were transported to University of Chemistry and Technology Prague (UCT) under cooling conditions and stored at 3°C before further treatment (slicing, inoculation of microorganisms, packaging and HPP).

Bacterial strain and culture preparation. The bacterial strain of *Serratia liquefaciens* (CTC1691) was obtained from the Czech State Veterinary Administration Jihlava. The bacteria were transported to UCT Prague on Blood Agar plates (Merck KGaA, Germany). Subsequently the inoculum culture was prepared. One bacterial colony was transferred in 5 ml of Tryptic Soy Broth (Merck KGaA, Germany) and incubated overnight at 30°C. The prepared bacterial culture was afterward used for the inoculation of the cooked ham.

Sample preparation and high-pressure processing. Cooked ham ($a_w = 0.98$ and $\text{pH} = 6.12$) and cooked

ham with added potassium lactate ($a_w = 0.97$ and $\text{pH} = 6.20$) were aseptically unpacked and sliced into 2 cm thick slices, which were subsequently inoculated by the appropriate dilution of the culture of *Serratia liquefaciens* (CTC1691) to obtain an inoculation level of 10^3 CFU/g. The inoculum was equally spread over the surface of the slice. After the absorption of the inoculum (2 min), samples were vacuum-packaged (95% vacuum) in polyethylene-polyamide bags (Wipak[®], Finland) and stored at 3°C for 48 h before HPP.

After the storage period, inoculated samples were treated under different HPP conditions (Table 2). The pressurisation was carried out in an experimental HPP unit (ŽĎAS a.s. joint stock Co., Czech Republic) at the Food Research Institute Prague. The volume of the pressure chamber was 2 l, and the pressurisation fluid was drinking water. After the pressurisation process, samples were transported to UCT Prague under cooling conditions 3°C and stored at 3°C for 40 days.

Microbiological determinations. Samples of cooked hams were analysed according to Czech Standards ČSN EN ISO 6887-1 and ČSN EN ISO 6887-2.

Ten grams of the inoculated ham from each sample was transferred into a sterile blender bag (VWR[®], USA) and mixed with 90 ml of sterile physiological solution. The samples were then, homogenised in a Stomacher laboratory blender (Seward, United Kingdom) for 2 minutes. One milliliter of appropriate dilution was pipetted onto a sterile Petri dish and mixed with Plate Count Agar (Merck KGaA, Germany). The inoculated plated were incubated at 30°C for 72 hours.

Three repetitions for each sample were analysed on day 1 (after packaging), day 4 (first day after pressurisation), day 7, 11, 18, 25, and 40 of the storage period.

Statistical analysis. The inactivation of *S. liquefaciens* was expressed by log-reduction of microbial counts before and after pressurisation, i.e., $\log(N_0/N)$.

Table 2. *Serratia liquefaciens* after different HPP conditions

	Samples	Pressure (MPa)	Time (min)	Temperature (°C)	Inactivation $\log(N_0/N)^*$
Cooked ham	A	0	–	–	–
	B	400	10	14.8	2.73 ± 0.03
	C	400	20	15.7	2.81 ± 0.05
Potassium lactate	D	0	–	–	–
	E	400	10	16.2	2.33 ± 0.25
	F	400	20	16.6	2.32 ± 0.04

*values are means \pm standard deviation

Data obtained from microbiological determinations were analysed for statistical significance with analysis of variance (ANOVA). Significant differences among results were determined using Tukey's t-test ($P < 0.05$) in Statistica 13.1 (TIBCO Software Inc., USA).

RESULTS AND DISCUSSION

High-pressure processing (400 MPa/10 min; 400 MPa/20 min) significantly reduced ($P < 0.05$) the initial contamination of the samples in the range of 2.3–2.8 log CFU/g (Table 2). After pressurisation, the population counts of *Serratia liquefaciens* were lower than the initial inoculation count and were near the detection limit of the enumeration method. This consequential inactivation effect might have occurred due to the fact that *Serratia liquefaciens* is sensitive to HPP (BELLETI *et al.* 2013). The level of reduction of *S. liquefaciens* presents similar microbial inactivation in comparison with similar studies (GARRIGA *et al.* 2004; JOFRÉ *et al.* 2008; BELLETI *et al.* 2013; ARGYRI *et al.* 2018). Pressures between 400–600 MPa caused various microbial count reductions for various microorganisms in different meat products. The microbial count of *S. liquefaciens* in dried ham, *Pseudomonas* spp. in chicken fillets, *Salmonella* spp. and *Enterobacteriaceae* spp. both in cooked ham, was reduced to at least 2.6 log CFU/g at 450 MPa/5 min (BELLETI *et al.* 2013), 2.8 log CFU/g at 500 MPa/10 min (ARGYRI *et al.*

2018), 3.5 log CFU/g at 600 MPa/5 min (JOFRÉ *et al.* 2008), and 4 log CFU/g at 600 MPa/6 min (GARRIGA *et al.* 2004) respectively.

According to our results (Figure 1), the population of *S. liquefaciens* in the pressurised samples without the addition of potassium lactate remained low at the beginning of the storage. However, the population increased throughout the storage period depending on the holding time of pressure and storage day. Nevertheless, the lag-phase of microorganisms was significantly extended in HPP treated samples compared to unpressurised samples ($P < 0.05$). There might be several explanations for this phenomenon, but the most probable is that HPP causes changes in the permeability of the microorganisms' cell membrane, followed by the inhibition of enzymes, and the destabilisation of the DNA (HUGAS *et al.* 2002; AYMERICH *et al.* 2008). Similar results were obtained in the studies of ARGYRI *et al.* (2018) and GARRIGA *et al.* (2004), who analysed the growth curve of various representatives of family *Enterobacteriaceae* after HPP. Moreover, HUGAS *et al.* (2002) and RENDUELES *et al.* (2011) found, that by extending the pressure holding time, the more significant extension of the lag-phase could be obtained.

The partial replacement of sodium chloride by potassium lactate in the composition of cooked ham had a positive effect on the shelf-life of both the unpressurised and the pressurised samples (Figure 1). In regards to the addition of potassium lactate in unpressurised samples, the numbers of *S. liquefaciens* were significantly lower (0.31–1.16 log CFU/g) in samples with added potassium lactate compared to without potassium lactate during the entire storage period ($P < 0.05$). The statistically significant difference between pressurised samples with and without potassium lactate was noticeable from the 11th (400 MPa/10 min) and 25th (400 MPa/20 min) day of storage. It is obvious that the different mechanisms of microbial inactivation by HPP and potassium lactate works in synergism, as HPP causes damage to the cell wall and the changes in microbial metabolism and thus make bacteria more vulnerable to the antimicrobial effects of organic acids and their salts (HUGAS *et al.* 2002; MARCOS *et al.* 2008).

According to the above, both methods alone can significantly ($P < 0.05$) reduce the numbers of *S. liquefaciens* in cooked ham. However, their combination along with the longer holding time of pressure seems to be the most effective method to extend the lag-phase of *S. liquefaciens*.

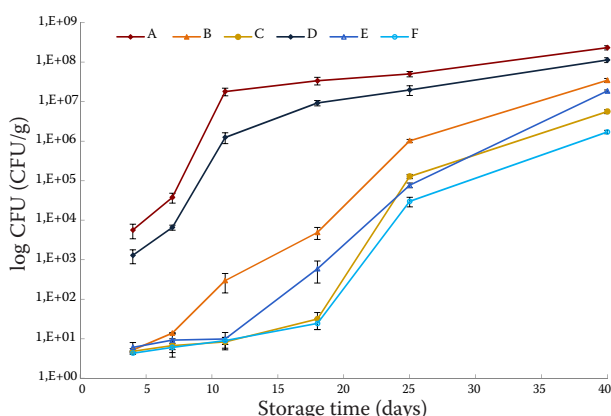


Figure 1. Changes in the population of *S. liquefaciens* during storage of cooked. A – unpressurised cooked ham; B – 400 MPa for 10 min; C – 400 MPa for 20 min; D – unpressurised with added lactate; E – with added lactate and treated by 400 MPa for 10 min, F – with added lactate and treated by 400 MPa for 20 min

CONCLUSIONS

High-pressure processing along with the addition of lactates are the well-known methods to assure the microbial stability of a wide range of meat products. However, to best of our knowledge, our study was one of the first to describe the combined effect of these methods on the growth rate of *Serratia liquefaciens*, an important representative of spoilage microflora in the meat industry. The reduction of *S. liquefaciens* by the combination of high-pressure processing and the addition of potassium lactate was significantly higher than in the pressurised samples without the addition of lactate, which was probably caused by the synergistic effect of these methods. An additional but essential variable in these experiments appeared to be the holding time of pressure. Extended pressurisation time significantly extended the lag-phase of the studied microorganisms. Moreover, the combination of HPP and potassium lactate allowed us to significantly reduce the content of sodium chloride without negatively affecting the shelf-life of cooked ham. Our results suggest that the combination of both methods could be useful for the industrial treatment of meat products.

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