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Article

Use of Lactoperoxidase Inhibitory Effects to Extend the Shelf Life of Meat and Meat Products

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Abstract: Lactoperoxidase (LP) is an important enzyme of the salivary and mammary glands. It has been proven to increase the shelf life of raw milk by inhibiting the growth of bacteria, especially *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* spp. The aim of this work was to verify the use of LP to extend the shelf life of meat products. *In vitro* experiments showed inhibitory effects against selected bacteria (*Listeria innocua* (ATCC 33090), *Staphylococcus saprophyticus* (CP054440.1), and *Pseudomonas fluorescens* (ATCC 13525) due to prolongation of the lag phase of growth curves. A lower increase in viable counts ($P < 0.05$) was also found by testing the pork cubes surface treated with LP solution (5%) + *L. innocua* and stored for 7 days at 15 °C. LP has also been studied at concentrations of 0.25 and 0.50% in meat products (pork ham and pâté) during refrigerated storage (4 °C for 28 days). Lower viable counts were observed throughout the storage experiment, especially for 0.50% LP ($P < 0.05$). Meat products containing LP also showed lower levels of oxidation (MAD) ($P < 0.05$). According to these results, LP could extend the shelf life of a wider range of products.

Keywords: lactoperoxidase; meat products; shelf life; antibacterial effect; *Listeria innocua*; TBARS

1. Introduction

Currently, there are many licensed chemical preservatives (especially nitrites) that are used to extend the shelf life of meat products. However, we can increasingly find claims that question these preservatives due to their possible negative effects on human health [1–3].

Lactoperoxidase (LP) is an enzyme that belongs to the peroxidase group. It is a group of widespread natural enzymes that are found in the secretions of the mammary (colostrum and milk), salivary and lacrimal glands [4]. The main synthesis of LP occurs in the mammary glands, where it then performs a protective function against pathogenic microorganisms [5]. The principle of the inhibitory effect is the catalytic function of LP during the formation of hypothiocyanite ions $[OSCN]^-$ as inhibitors [6] affecting the metabolic enzymes of microorganisms [7] and cell function, e.g. membranes integrity and transport systems of membranes (release of polypeptides, potassium ions, and amino acids and cellular uptake of purines, pyrimidines, glucose, and amino acids is inhibited) [8,9]. The antibacterial effect of LP against different groups of microorganisms shows varying degrees of sensitivity, potentially having a bactericidal or bacteriostatic effect depending on factors such as the type of microorganism, the type of electron donor in membrane proteins, pH, temperature, incubation time, and concentration of microorganisms [10].

The inhibitory activity of LP against bacteria also depends on the different resistances of the bacteria themselves. For example, if gram-negative bacteria (*Salmonella* spp., *Pseudomonas* spp., *Escherichia coli*, *Shigella* spp., *Klebsiella* spp.) are exposed to LP, then the bacteria are killed or inhibited [11,12]. Regarding the effect on gram-positive bacteria such as lactic acid bacteria or gram-positive foodborne microorganisms (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus* spp., *Clostridium perfingens*, etc.), LP has an inhibitory effect against these bacteria [12–15]. The advantage of using LP

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as a preservative is the GRAS (generally recognized as safe), leading to numerous expert publications on its use as a natural food preservative instead of chemical preservatives [15,16].

LP shows activity in the pH range from 4 – 11 and maximum activity is reached at pH 6. However, it is also resistant to acidic pH values up to 3. It is also characterized by high thermal stability and is used as an indicator of the efficiency of milk pasteurization. LP is one of the most heat-stable enzymes in milk, and its thermal inactivation has been accepted as a criterion for high milk pasteurization. It is inactivated after 30 minutes when milk is heated to 75 °C and after 30 seconds when milk is heated to 80 °C. It is also necessary to mention the increased sensitivity of LP to high temperatures at a low pH value [7,17]. Marks et al. (2001) reported that a still active LP system was found after pasteurization of cow's milk at 72 °C for 15 s, which is still able to maintain the quality of milk inoculated with the bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus thermophilus* [18].

Due to the thermal stability of LP, there is potential for this enzyme to be used for meat production and to extend the shelf life of meat products. Meat products are often heat-treated and in the case of the Czech Republic, meat products of the "Heat-treated" group according to Decree No. 69/2016 Coll. must be heat-treated in the core of the product for 10 minutes at a temperature of 70 °C [19]. At present, as far as the authors know, there are not many publications that have examined the potential of LP to extend the shelf life of meat products. For illustration, the study by Elliot et al. (2004) on the effect of LP on microbial contamination of the surface of beef cubes inoculated with approximately 10^4 CFU/cm^2 of *L. monocytogenes* and incubated at different temperatures (37 and 12 °C). LP was found to have a greater effect at lower non-permissive temperatures for rapid bacterial growth with strong growth inhibition [9]. The temperature dependence of the inhibitory effect of LP was also confirmed in a study by Kennedy et al. (2000) by monitoring the growth of *E. coli* O157:H7, *L. monocytogenes* L45, and *S. aureus* R37 in broth (37 °C) and ground beef (0, 6, and 12 °C). The inhibitory effect was confirmed and, in addition, *L. monocytogenes* was found to be more sensitive to LP compared to the other bacteria [1].

Considering the possible use of LP for the preservation of meat products, the inhibitory effect of LP on *L. innocua* in liquid broth (different incubation temperatures) and on the surface of pork cubes as well as directly in meat products (pork ham and pâté) was investigated in this experiment.

2. Materials and Methods

2.1. Lactoperoxidase

In this work a mixture of active enzymes and substrates based on the lactoperoxidase system HYDRO LP (Hubka-Petrášek a vnuci, Ltd.; Czech Republic) was used. The dose of this mixture in products ranges from 4 to 6 g per kg of product.

2.2. Testing of LP in Liquid Broth

The inhibitory effect of LP was tested *in vitro* on the growth of bacteria *Listeria innocua* (ATCC 33090) in liquid broth of TSB (tryptic soy broth) (Merck KGaA; Darmstadt, Germany), *where* (CP054440.1) (isolated from pork meat) in liquid broth of BHI (brain heart infusion) (Merck KGaA; Darmstadt, Germany), and *Pseudomonas fluorescens* (ATCC 13525) in TSB broth. Non-pathogenic *L. innocua* was selected as a replacement for pathogenic *L. monocytogenes*. In this part of the experiment, the development and changes in bacterial growth curves (time dependence of the optical density of microorganisms) were monitored using a laboratory bioreactor Biosan RTS-8 (Biosan; Latvia) according to Beňo et al. (2022) [20] with modifications. The measurement is performed at a wavelength of 850 ± 15 nm to eliminate the effect of the colour of the culture medium and the plastic tube used. Measurements were carried out in parallel in sterile conical tubes (LaboServ, Czech Republic) with a volume of 50 mL into which 14 mL of TSB broth + 1 mL of *L. innocua* inoculum (3.1×10^7 CFU/mL) + 1 mL of 1% LP (in sterile distilled water) were transferred. The tubes thus prepared were placed in a bioreactor and cultured at 15, 20, 25, and 40 °C with a speed of 2000 rpm. The same procedure was carried out with bacteria *P. fluorescens* (2.4×10^8 CFU/mL). To prevent oxygen access,

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the measurement process of *S. saprophyticus* (1.3×10^7 CFU/mL) was modified - rpm 2000 only before the measurement itself, not continuously.

2.3. Testing of LP on Pork Cubes

The inhibitory effect *ex vivo* of LP on the surface of pork (shoulder) cubes (2 × 2 × 2 cm), which were divided into 3 groups - control, cubes soaked in the SafeFAST Classic *L. innocua* (2.5×10^6 CFU/mL), and cubes soaked in *L. innocua* (2.5×10^6 CFU/mL) + LP (5 g/100 mL) in sterile distilled water, was also investigated. Soaking the cubes in the solution took 1 minute and took place under sterile conditions in flow box (Schoeller Instruments, Ltd., Czech Republic), as did cutting of the meat into cubes. After soaking, the cubes were kept in drain excess water and placed to sterile conical tubes and transferred to a POL-EKO ST2B40 (POL-EKO, Poland) at 15 °C. This was followed by microbial analyses at different storage time intervals (days 1, 4, and 7). Inoculations were performed on PCA (plate count agar) (Penta; Prague, Czech Republic) and culture was carried out at 30 °C for 3 days.

2.4. Testing of LP in Meat Products

To test the effect of LP in a meat product on its microbiological stability, a heat-treated meat products (pork ham and pâté) were chosen and produced under laboratory conditions. Pork leg meat (purchased from the market) was used to produce restructured ham as well as the remaining raw materials for the production of pâté.

A total of 3 hams were produced and the composition is as follows: pork leg meat (330.0 ± 1.0 g) + curing salt (5.0 g) + water (66.0 g) + 0.25 %, respectively 0.50 % LP. The composition of pâtés is as follows: pork liver (380.5 ± 1.0 g) + pork lard (400.0 ± 1.0 g) + pork flank (760.5 ± 1.0 g) + pork broth (165.5 ± 0.5 g) + curing salt (31.0 g) + dried onion (70.5 ± 0.1 g) + garlic. (15.1 ± 0.1 g) + spice (12.1 ± 0.1 g) + 0.25 %, respectively 0.50 % LP.

The pork cubes (1 × 1 × 1 cm) together with the curing salt were mixed at first for 15 minutes, and then ½ volume of water was added. After another 15 minutes, the rest of the water was added and mixing continued for a further 55 minutes. The mixture was transferred to polyamide bags and plastic cylinders mold before heat treatment. The pâtés were made using the Thermomix TM5 (VORWERK, Germany). First, the liver was crushed with curing salt to mousse, then lard, boiled flank and broth was added and mixed to soft consistency. Finally, onions, garlic, spices, and LP were added. The finished mixture was filled into jars (content about 75 g) and heat treated. Subsequently, the hams and pâtés were cooked in a Memmert WB22 water bath (Memmert GmbH, Germany) to the Czech legislative requirements of 70 °C in the core of the product for 10 minutes. The finished meat products were then cut into 1 cm slices, wrapped in a polyamide bag, and placed in a POL-EKO ST2B40 (POL-EKO, Poland) and stored at 4 °C with constant illumination by led lamps. The meat products were then subjected to a storage experiment and microbiological analyses were carried out on days 1, 7, 14, 21, and 28.

2.5. Microbial Analysis of Meat Products

The sample (10 g) was weighed in a polymer bag along with 90 mL of saline and homogenized in a homogenizer MIXW 1002 (MIXWEL®; Bruz, France) at the highest intensity for 3 minutes. The saline solution was prepared by dissolving 8.5 g of NaCl (Penta; Prague, Czech Republic) and 1 g of peptone (Merck KGaA; Darmstadt, Germany) in 1000 mL of distilled water and followed by autoclaving. The homogenisation of the sample in saline was followed by dilution of the homogenate in saline solution and then the inoculum was mixed well. After solidification, the Petri dishes were placed in a thermostat for cultivation. The product has been microbiologically tested in PCA (plate count agar) (Penta; Prague, Czech Republic) for 3 days at 30 °C for determination total viable count (TVC) and MRS (Man Rogosa Sharpe agar) (Penta; Prague, Czech Republic) for 5 days at 30 °C for determination of the lactic acid bacteria (LAB) count. The results were recalculated as $\log_{10}$ CFU/g.

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2.6. TBARS

The level of oxidation of the meat products during storage was determined by the formation of reactive thiobarbituric acid (TBARS) expressed as malondialdehyde (MDA) content (mg/kg) measured at 538 nm. The homogenized sample (10 g) was transferred to a distillation tube containing 2.5 mL HCl (Penta; Prague, Czech Republic) diluted with distilled water (*V:V*; 1:2; HCl:H_2O) and 97.5 mL of distilled water. The distillation tube was then placed in a BÜCHI K-355 distillation unit (BÜCHI; Switzerland) and at 30% steam level the sample was distilled for 10 min until approximately 50 mL of distillate has been collected. An aliquot of the distillate (5 mL) was then added to the boiling tube with 5 mL of 0.02M 2-thiobarbituric acid (Merck KGaA; Darmstadt, Germany) (in a solution 90% acetic acid (Penta; Prague, Czech Republic) solution with distilled water) and the tube was boiled in a boiling water bath for 35 min. The absorbance of the complex was then measured at a given wavelength on an Onda V-10-plus (Giorgio Bormax S.r.l.; Italy) spectrophotometer. The concentration of MDA was calculated according to the equation:

$$c_{MDA}(\text{mg/kg}) = 7{,}8 \cdot A \cdot \frac{m_V \cdot m_D}{10 \cdot 50} \quad (1)$$

where A is the measured absorbance of the sample, m_V is the weight of the sample (g) and m_D is the weight of the distillate (g), 10 is the theoretical weight of the sample (g) and 50 is theoretical weight of the distillate (g).

2.7. Statistical Analysis

The statistical significance of the use of LP to reduce the concentration of malondialdehyde (n = 5) in the products was calculated using STATISTICA 12.0 CZ software (StatSoft; Prague; Czech Republic). Before statistical evaluation, the Dien-Dixon test was performed to exclude outliers. Mixed-design ANOVA was applied to the collected data. The product storage days were treated as a fixed effect and the MDA concentrations were included in the model as a random effect (MIXED procedure). The HSD Tukey test was then used to compare the mean values between samples. Microorganism counts were transformed into $\log_{10}$ CFU/g before determining means and performing statistical analyses by ANOVA. All microbiological analyses were done in triplicate. Statistical significance was defined as $P < 0.05$ in all analyses.

3. Results

3.1. Effect of LP in Liquid Broth

The first part of this work was to observe and determine the effect of LP on the growth of selected bacteria in liquid broths. The results are expressed as growth curves of the microorganisms, which were generated from the dependence of the optical density of the microorganisms on the cultivation time. Growth curves can be seen in Figures 1 and 2. The testing was carried out at different temperatures 15, 20, 25, and 40 °C. Figure 1 presents the results of the growth curves of *L. innocua* in TSB broth. It can be seen that LP has inhibitory effects, especially at lower temperatures. A significant inhibitory effect ($P < 0.05$) was observed at culture temperatures of 15 and 20 °C, when the lag phase of the bacteria was prolonged for approximately 25 h. On the contrary, at higher temperatures (30 and 40 °C) bacterial growth occurred more rapidly and even at 40 °C a higher optical density was detected. Inhibitory effects at lower temperature can also be seen in the growth curve of *S. saprophyticus* (Figure 2A) and *P. fluorescens* (Figure 2B). The lag phase of *S. saprophyticus* was prolonged by approximately 10 hours at 15 and 20 °C. The lag phase of *P. fluorescens* was not prolonged, however, a slower increase in the exponential phase can be seen.

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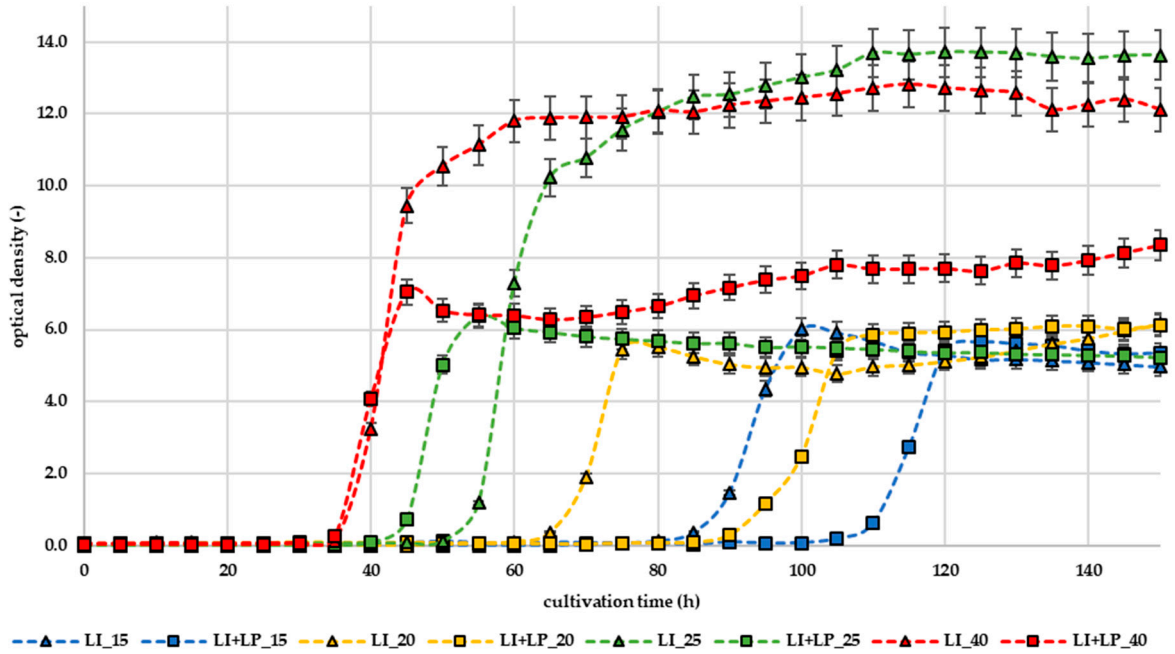

Figure 1. Effect of LP on *Listeria innocua* (LI) growth curves; C (▲) – control bacteria; LP (■) – bacteria + lactoperoxidase; 15, 20, 25, and 40 – temperatures (°C).

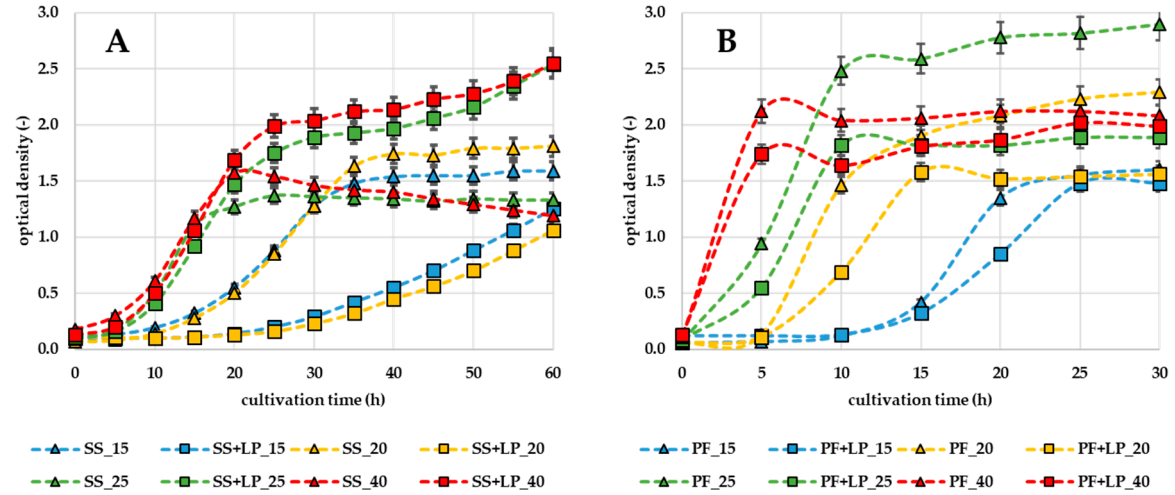

Figure 2. Effect of LP on *Staphylococcus saprophyticus* (SS) (**A**) and *Pseudomonas fluorescens* (PF) (**B**) growth curves; C (▲) – control bacteria; LP (■) – bacteria + lactoperoxidase; 15, 20, 25, and 40 – temperatures (°C).

3.2. *Effect of LP on the Surface of Pork Cubes*

At a storage temperature of 15 °C, the inhibitory effect was examined in untreated pork cubes, cubes inoculated with a solution of *L. innocua*, and cubes inoculated with bacteria together with LP (5 g/100 mL). Microbiological analyses on PCA agar were performed on days 1, 4, and 7 (see Table 1.) Rapid growth of viable aerobic bacteria on PCA agar was observed in all pork cubes at 15 °C. Strong inhibition ($P < 0.05$) on the treated pork cubes using LP was obvious immediately during the first day of cultivation compared to control and control + *L. innocua* samples. The most significant difference ($P < 0.05$) in viable counts was at day 4. The difference between the LP samples and the others was between 0.8 and 1.3 logs CFU/g. On day 7, the total counts in all samples were already between 8.8 – 9.8 log and the LP sample proved the inhibition of the number of bacteria present.

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These results, including the *in vitro* experiment from liquid broth, prove the potential for using LP in meat products during storage.

Table 1. Testing the effect of LP on the surface of the pork cubes (2 × 2 × 2 cm) at 15 °C.

Treatment	Total viable count (log_{10} CFU/g ± SD[1])		
	day 1	day 4	day 7
control	$5.2^{c} \pm 0.1$	$7.7^{bc} \pm 0.2$	$9.1^{bc} \pm 0.2$
control + *L. innocua*	$5.3^{c} \pm 0.1$	$8.2^{ac} \pm 0.1$	$9.8^{ac} \pm 0.0$
L. innocua + LP	$4.7^{ab} \pm 0.1$	$6.9^{ab} \pm 0.1$	$8.8^{ab} \pm 0.1$

[1] values presented are the mean of three replicates ± standard deviation; means followed by the different lowercase letter in the column did differ significantly ($P < 0.05$).

3.3. Effect of LP in Pork Ham and Pâté

Due to the positive *in vitro* and *ex vivo* results, the number of microflora present in the selected meat products was also monitored. In addition to the number of microorganisms, the oxidative stability of meat products was also tested, which is closely related to the oxidative processes during the rendering process [21]. The results in Table 2 show what effect the addition of LP had on the development of microorganisms in pork ham and pâté. It should be noted that no lactic acid bacteria (LAB) were found in the pork pâté product. The authors explain this phenomenon by a combination of a higher heat treatment temperature than that of ham and the addition of a seasoning mixture containing, for example, dried onions and garlic.

Table 2. Microbiological analysis of meat products with/without the addition of LP.

Meat product	Storage (days)	Count (log_{10} CFU/g ± SD[a])					
		TVC			LAB		
		control	LP1	LP2	control	LP1	LP2
Pork ham	1	$4.0^{bc} \pm 0.1$	$3.7^{ac} \pm 0.1$	$3.4^{ab} \pm 0.1$	$2.7^{bc} \pm 0.1$	$2.4^{a} \pm 0.0$	$2.5^{a} \pm 0.1$
	7	$4.5^{c} \pm 0.1$	$4.4^{c} \pm 0.0$	$3.9^{ab} \pm 0.2$	$4.0^{c} \pm 0.2$	3.8 ± 0.1	$3.7^{a} \pm 0.1$
	14	$5.0^{c} \pm 0.2$	4.5 ± 0.0	$4.3^{a} \pm 0.1$	$4.5^{bc} \pm 0.2$	$3.8^{a} \pm 0.0$	$3.8^{a} \pm 0.1$
	21	$5.2^{bc} \pm 0.1$	$5.1^{a} \pm 0.1$	$4.9^{a} \pm 0.1$	$5.1^{c} \pm 0.1$	4.9 ± 0.1	$4.7^{a} \pm 0.1$
	28	$6.3^{bc} \pm 0.1$	$6.2^{ac} \pm 0.1$	$5.9^{ab} \pm 0.1$	$6.1^{c} \pm 0.1$	5.9 ± 0.1	$5.8^{a} \pm 0.0$
Pork pâté	1	$4.2^{c} \pm 0.2$	4.0 ± 0.1	$3.8^{a} \pm 0.1$	< 1.0	< 1.0	< 1.0
	7	$5.0^{c} \pm 0.0$	$4.8^{c} \pm 0.2$	$4.5^{a} \pm 0.1$	< 1.0	< 1.0	< 1.0
	14	$5.9^{bc} \pm 0.1$	$5.4^{ac} \pm 0.0$	$4.7^{ab} \pm 0.2$	< 1.0	< 1.0	< 1.0
	21	$6.3^{c} \pm 0.2$	6.2 ± 0.1	$5.1^{a} \pm 0.0$	< 1.0	< 1.0	< 1.0
	28	$6.6^{bc} \pm 0.1$	$6.1^{ac} \pm 0.1$	$5.6^{ab} \pm 0.0$	< 1.0	< 1.0	< 1.0

[a] values presented are the mean of three replicates ± standard deviation; means followed by the different lowercase letter in the row did differ significantly ($P < 0.05$); TVC – total viable count; LAB – lactic acid bacteria.

Table 2 shows the positive effect of LP on microbial contamination in both ham and pâté. Especially for the higher addition (0.5 %) of LP2. A similar effect was also observed for the oxidation grade of the products, which showed lower levels of rancidity with the addition of LP1 and LP2, respectively. Slower growth of viable counts was found for both meat products. The higher concentration of LP2 inhibited bacterial growth more intensively compared to LP1, as expected. Throughout the pork ham LP2 reduced all viable counts by approximately 0.5 log CFU/g ($P < 0.05$). Even more pronounced inhibitory effects were observed during the storage of pork pâté. We can observe up to 1 log CFU/g reduced viable counts for LP2 pâté. Table 1 shows that lactic acid bacteria accounted for the majority of the total viable counts. LP, even in this experiment, as we can see, did not completely stop the growth of bacteria, but inhibited and prolonged the growth. The results thus suggest that the shelf life of the products could be extended by up to 7 days.

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The level of oxidation of meat products is shown in Figure 3. Similar results to the microbiological stability were confirmed for the oxidation level. Meat products with a higher concentration of LP2 showed less rancidity as they contained a lower malondialdehyde content (mg/kg). The MDA content of the sample was always lower with increasing addition of LP. The reduction of MDA in LP2 was significant during all tests. On day 7, the MDA content of LP2 ham was almost half that of the control ($P < 0.05$) same as on day 28. The MDA content of LP1 was also lower, although not necessarily significant. The MDA content of the ham at the beginning of the experiment was close to zero mg/kg. On the contrary, on the first day of the experiment for pork pâté, oxidation was observed due to the higher fat content. A more significant slowing down of oxidation processes is evident in the pork pâté. The MDA content of LP2 pâté was in most cases approximately 3 times lower during each day of the experiment compared to the control sample. There was also a significant reduction in LP1, about 2 times lower. In general, an MAD concentration of more than 2 mg/kg already causes a negative sensory flavor in products, a typical rancid aroma and a pungent and bitter taste. The level of 2 mg/kg for ham was not reached, while the control sample of pâté rigidly exceeded the level already on day 7. Therefore, the shelf-life of the products can be extended again because of these findings on oxidation processes.

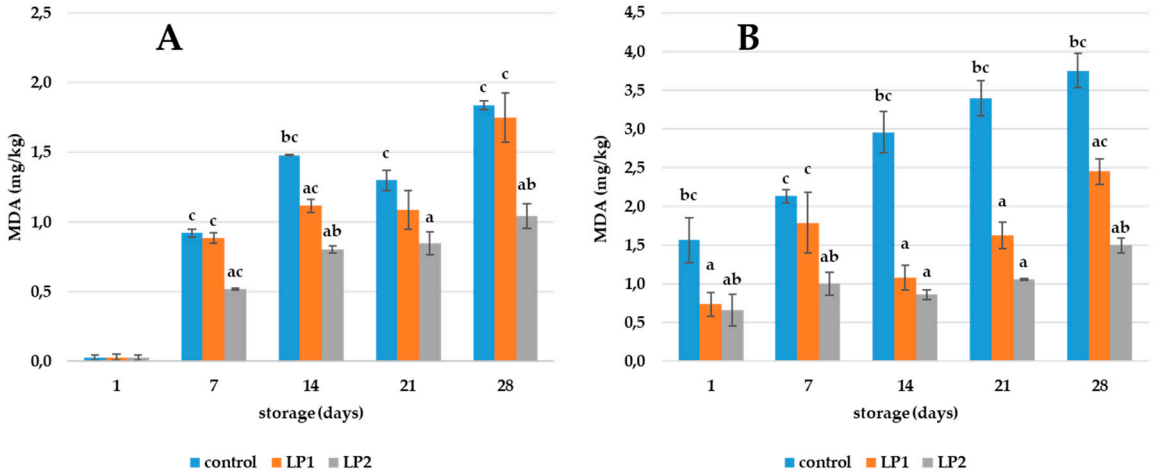

Figure 3. Oxidation level (MDA concentration (mg/kg) of meat products (**A** – ham, **B** – pâté) with/without the addition of LP; LP1 – meat products with 0.25 % of LP; LP2 – meat products with 0.50 % LP; **a-c** means statistical difference ($P < 0.05$) between control and L1, respectively LP2.

4. Discussion

The microbial stability of meat products depends on many factors related to the whole process of meat production and the manufacture of meat products. In particular, temperature that has the greatest effect on the potential of microbes to reduce the quality and shelf life of products. In addition, technologists are also faced with an increasing customer demand for safe additives that do not affect the quality of the final product and human health. LP as a substitute for chemical preservatives could have good potential to ensure the microbial safety of meat products [9,22]. Most often, we can find professional publications dealing with the use of LP in the dairy industry, especially as a preservative in developing countries where rapid cooling of milk is difficult [15,23–25].

LP shows a strong dependence on the temperature at which it is applied. The dependence of LP activity on temperature is described in the study by Silva et al. (2020), where it was found that the highest LP activity increases with decreasing temperature to 4 °C of milk [15]. The results of our study are consistent with this claim. Since according to Figures 1 and 2 we can see that inhibitory activity against *L. innocua*, *S. saprophyticus*, and *P. fluorescens* bacteria was highest at the tested temperature of 15 and 20 °C. Therefore, it was found that the lag phase of *L. innocua* was prolonged up to two times. Vice versa, at higher temperatures, the mentioned bacteria had a shortened lag phase. Elliot et

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al. (2004) reported similar trends in their work on inhibition of foodborne bacteria in beef cube system [9] and by Kennedy et al. (2000) in their work dealing with LP and its inhibitory effect on *E. coli* 0157:H7, *L. monocytogenes*, and *S. aureus* [1].

Meat and meat products can be infected by a wide range of microorganisms and, given the confirmed inhibitory effects of LP on gram-negative bacteria and some gram-positive bacteria in milk and dairy products [11–15], a similar effect can be expected in meat and meat products. There are not many studies dealing with LP in meat as far as the authors are aware. Kennedy et al. (2000) first investigated the LP inhibitory effects on the growth of *E. coli* O157:H7, *L. monocytogenes* L45, and *S. aureus* R37 in 37 °C broth system and in ground red meat (0, 6, and 12 °C). *L. monocytogenes* was shown to be the bacterium most susceptible to LP, and after a 4 h culture period, LP significantly reduced the growth of microbial populations [1]. A similar study by Elliot et al. (2004) focused on the surface microflora of beef meat cubes. The cubes were inoculated with approximately 10^4 CFU cm^{-2} of bacteria and treated with LP. Incubation was carried out under different conditions (24 h at 37 °C, 7 days at 12 °C, 7 days at 12 to -1 °C, 4 weeks at -1 °C) [9]. Inhibition by LP was effective at temperatures suboptimal for the growth of the chosen bacteria. Strong inhibition in cubes was found at 12 °C and a reduction of viable pathogens at refrigeration temperatures (especially *Pseudomonas* spp.). However, there was no arrest in the development of native lactic acid bacteria. The results of our work investigating LP influences on the surface of pork cubes agree with Elliot et al. (2004) [9] and Kennedy et al. (2000) [1]. Pork cubes inoculated with *L. innocua* had 1 log CFU/g of higher viable findings at the end of the storage trial (day 7 at 15 °C). Different concentrations (0, 3, 5, and 7% w/w) of LP were also used in whey protein isolate to treat roast turkey [26]. The results showed a reduction of 3 and 2 log CFU/g in inoculated samples of *S. enterica* and *E. coli* O157: H7, respectively. The inhibitory effect was confirmed against both bacteria at storage temperatures of 4 and 10 °C. Thus, the results of our study and the previous studies mentioned suggest the possibility of prolonging the shelf life of meat and meat products using LP, either in the form of a film on the surface or mixed into the mixture during manufacture.

One of the more recent studies by Ehsami et al. (2020) investigated the effect of different types of active biodegradable films containing LP on the shelf life of fish burgers [27]. Storage of fish products at 4 °C for 20 days. The study confirmed lower total viable counts for products wrapped in LP + albumin and LP + chitosan films at day 1. At the end of the experiment, there was a reduction in viable counts of approximately 3 log. On contrast, on day 1, the total counts were higher for products wrapped only in films containing albumin or chitosan compared to biodegradable films containing LP only. Similar results were found for the growth of psychrophilic microorganisms and *Pseudomonas* spp. and *Shewanella* spp. Our results for pork meat products are therefore consistent with the results of this study. However, the differences in TVC in our study were not as marked (see Table 2) but it is worth noting that LP2 showed its inhibitory efficacy especially in pork pâté ($P < 0.05$). Differences in LP efficacy between studies may have been due to differences in methodology as Ehsami et al. (2020) wrapped fish products in LP film. In our study, LP was added to the product during manufacturing. Another factor that may have caused the difference is the increase in antimicrobial activity of the LP solution incubated at 23 ± 2 °C for 24 hours.

The rate of lipid oxidation in the products was determined using the thiobarbituric acid test, in which the MDA content was measured. MDA is formed as a secondary oxidation product from hydroperoxides of the lipids present in the products [28]. Lipids are susceptible to degradation and are one of the main non-microbial reasons for the reduced quality of meat and meat products [29]. The effect of LP on oxidation rates has been described in several publications. A study by Farshidi et al. (2018) found that the combination of the lactoperoxidase system and the whey protein used as shrimp coating increased TBARS during storage at 4 °C [30]. A similar result was also published in a study by Shokri et al. (2015), Rostami et al. (2017), and Jasour et al. (2015), who report no significant changes in MDA formation in LP-treated rainbow trout and pikeperch fillet samples [31–33]. Reduced levels of MDA during cold storage of shrimp were confirmed by Ehsani et al. (2020). However, the reason for the reduced oxidation rate was not directly due to LP but to the biodegradable film containing LP that caused a barrier of meat to oxygen and light [27]. Therefore,

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our work does not confirm or agree with these studies. In fact, from the results of our work, we can see (Figure 3) a significantly lower level of MDA ($P < 0.05$) in products containing LP, especially at a concentration of 0.50%. Our hypothesis is reduced MDA production due to lower concentrations of microorganisms (Table 2) as a result of the inhibitory effect of LP against bacteria. In fact, bacteria are capable of lipid peroxidation products in cell membranes, including malondialdehyde [34,35]. Therefore, from our results we can say that LP extends the shelf life of meat products.

5. Conclusions

Currently, there is a lack of scientific publications that focus on the preservation of meat and meat products by lactoperoxidase. From the results of this work, it is possible to note the positive effect of lactoperoxidase on inhibition of the growth of *L. innocua*, *S. saprophyticus*, and *P. fluorescens*. The effectiveness is limited by temperature, being most effective at temperatures that are not optimal for bacterial growth. This fact was exploited for the storage of pork hams and patties at 4 °C. There was a reduction in viable counts without affecting lactic acid bacteria. Another positive finding was a reduction in malondialdehyde formation as a product of lipid secondary oxidation of lipids. Thus, in addition to its use in the dairy industry, there is also the potential to use lactoperoxidase to preserve meat or meat products.

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