EFFECTS OF HIGH PRESSURE COMBINED WITH CRUDE BACTERIOCINS AND THAI HERB EXTRACTS ON MICROBIAL AND PHYSICAL QUALITY OF SLICED PORK LOINS

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Abstract – The effects of high hydrostatic pressure (HHP) treatment and in combination with selected local Thai herbs and crude bacteriocins on microbial growth and physical changes of pork loins during refrigerated storage were investigated. HHP at 200 and 300 MPa with combination of 0.2% crude bacteriocins produced from *Lactococcus lactis* TISTR1401, and 1% selected Thai herbs (*Spondias pinnata* (L.f.) Kurz and *Schinus terebinthifolius*) were applied to pork loins. The treated pork samples were aerobically packaged and stored at 4 ± 1 ºC for 9 days, taken every 3 days for microbial enumeration, and weight loss, texture and color measurement. Throughout storage time, total plate counts of 200 MPa treated meats were not different and slightly increased towards the end of storage. Combination of 300 MPa HHP with crude bacteriocins and the herb extracts could reduce total plate counts by 1-2 log cycles. Sample weight losses from all treatments were not different (p>0.05). However, pork tenderness increased (p<0.05) when crude bacteriocins and both herb extracts were combined, and with increasing storage time except for those from 300 MPa combined with crude bacteriocins. Lighter color and less redness (p<0.05) of meats were observed when 300 MPa and in combination with crude bacteriocins and both herb extracts were applied.

Key Words – Antimicrobial efficacy, Color, Texture, Weight loss

I. INTRODUCTION

In recent years, many attempts have been emphasized on the searches of natural antimicrobial compounds that can properly serve the needs of food manufacturers and consumers. Various herbs and spices have been reported for their medicinal value, in particular antimicrobial activity, and used throughout the past as an alternative approach to preserve foods. They contain antimicrobial compounds that may prove useful as natural preservatives [1]. In Thailand, there are many herbs and spices that exhibit antimicrobial activity and may be used for food preservation [2]. In addition, bio-preservative ingredients produced by microorganisms such as bacteriocins have been investigated and shown to be effective in controlling growth of pathogenic and spoilage bacteria in food products [3]. Bacteriocins are ribosomally synthesized, extracellularly released bioactive peptides or peptide complexes which have a bactericidal or bacteriostatic effect on other species (usually closely related). Lactic acid bacteria (LAB) are known to be the most important microorganisms producing bacteriocins. Nisin is a well-known pure commercial bacteriocin produced from LAB which is normally used as food preservative. Nisin has a broad spectrum of inhibition activity against Gram-positive bacteria and been used to preserve meat products [4]. However, combined use of bacteriocins with other ingredients or treatments such as herb and spice extracts and high hydrostatic pressure (HHP) have also been investigated to prevent growth of pathogenic and spoilage bacteria, especially in situations where contamination could occur after production [5]. HHP has been used with a variety of products over the years and gained popularity in meat products because of its ability to reduce food pathogens [6]. This technique is considered as a non-thermal process to preserve the product appearance and flavor [7]. The efficacy of treatment also depends on the achieved pressure, treatment temperature and exposure time. Therefore, the objectives of this experiment were to investigate the effects of two different low pressure conditions of HHP in combination with two selected local Thai herbs and crude bacteriocins produced from *Lactococcus lactis* TISTR 1401 on microbial growth and to observe changes of some physical quality, i.e., weight loss, tenderness and color of aerobically packaged sliced pork loins during storage at 4 ± 1 ºC for 9 days.

II. MATERIALS AND METHODS

Preparation of herb extracts and bacteriocins

Two Thai herbs (*Spondias pinnata* (L.f.) Kurz: E1 and *Schinus terebinthifolius*: E2) were screened and selected from 15 local Thai herbs according to their ability to inhibit growth of indicator bacteria and predominant bacteria obtained from fresh pork samples. The herb freeze-dried powder was extracted using ethanol. The ethanolic extract was freeze-dried and resolubilized in 1% DMSO for using. Freeze-dried powder of crude bacteriocins (B) was prepared from the fermentation product of *Lactococcus lactis* TISTR 1401 according to the method of Intarapichet *et al*. [8].

Sample preparation and high pressure processing

Fresh postmortem pork loins (*M. Longissimus dorsi*) were obtained from Max Rubner-Institut, Kulmbach, Germany. Visible fat was trimmed off and the loin was transversally cut in slices of about 2.54 cm in thickness. The randomly chosen pork slice was dipped in 0.2% crude bacteriocins or 1.0% of each extract solution, then aerobically packaged in a plastic bag (PA/PE) and kept in a freezing room before being pressurized at 200 and 300 MPa, respectively at 10 ºC for 10 min. The HHP treatment was carried out in a high pressure lab unit (EPSI N.V. Belgium). Treated pork samples were stored at 4 ± 1 ºC for 9 days and sampled for aerobic plate counts, weight loss, tenderness and color measurement every 3 days.

Microbial analysis

Microbial enumeration was performed every 3 days during storage. Total plate counts were performed by spread-plate technique and incubated at 30 ºC for 72 h.

Physical quality

Percent weight loss was determined by calculating weight difference of the pork sample before and after cooking to an internal temperature of 72 ºC. Color of sample was measured in terms of CIE L*, a*, b* values using a colorimeter with the Minolta Chromameter CR 300 (Konica Minolta, Munich, Germany). Texture of the sample was measured using a texture analyzer (Instron 3369, Pfungstadt, Germany).

Statistical analysis

Statistical analysis was evaluated by randomized complete block design. Analysis of variance was analyzed and comparison of means was done by Duncan's Multiple Range Test. Significant difference was defined at $p<0.05$. Two replications of the experiment were performed with ten measurements for texture and color.

III. RESULTS AND DISCUSSION

Total microbial counts of sliced pork loin samples during storage at 4 ºC for 9 days are shown in Figure 1. Initially, it was observed that total plate counts of all pork samples pressurized at 300 MPa were about 0.62-2.03 log cycles lower than those of samples obtained from 200 MPa treatments. The total microbial counts of pork samples pressurized at 200 MPa slowly decreased to day 6 and slightly recovered thereafter except for those obtained from HHP combined with crude bacteriocins. Slight increase in microbial growth towards the end of storage could be expected in an aerobically packaged meat sample due to the recovery of sub-lethally injured bacterial cells.

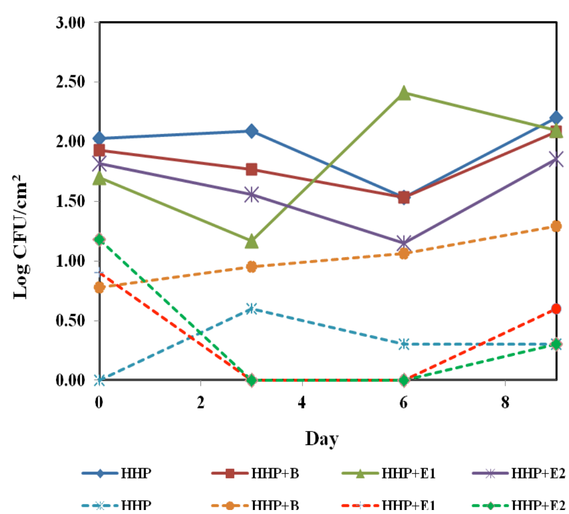

Figure 1. Total microbial counts of sliced pork loins during storage at 4 ºC; solid line for 200 MPa, broken line for 300 MPa.

Pressurization of HHP at 300 MPa and with combination with both herb extracts resulted in a reduction in total plate counts of pork samples of about 0.09-1.18 log cycles to day 6 and similar number of counts was observed at the end of storage. However, microbial growth of the samples from 300 MPa HHP combined with crude bacteriocins gradually increased toward the end of storage but was still lower than those pressurized at 200MPa.

High pressure treatment of sliced pork loins at these medium pressure levels of 200 and 300 MPas and with combination of crude bacteriocins and both herb extracts did not make any significant differences ($p>0.05$) in weight loss as shown in Table 1. This could be due to the fact that no significant difference ($p>0.05$) in pH values of all pork samples was observed (data not shown).

Table 1 Weight Loss of sliced pork loins during storage at 4 ºC, (% mean ± SD)

Storage time		HHP	HHP+B	HHP+E1	HHP+E2
200 MPa	Day 0	30.0±1.4	31.1±1.7	28.4±4.2	29.7±1.7
	Day 3	27.9±0.4	30.1±2.1	29.7±1.6	29.8±2.8
	Day 6	29.7±0.1	29.4±4.0	25.3±2.3	25.7±3.3
	Day 9	23.0±1.8	25.3±0.5	26.8±4.4	24.2±4.0
300 MPa	Day 0	29.1±1.41	31.8±4.8	26.5±4.5	24.1±9.7
	Day 3	27.7±4.7	26.5±1.1	27.2±7.4	27.2±1.0
	Day 6	29.9±0.8	29.5±5.7	27.1±4.4	27.0±1.5
	Day 9	28.5±0.9	28.0±0.6	25.4±0.4	25.8±0.8

HHP = High hydrostatic pressure, HHP+B = HHP + 0.2% crude bacteriocins, HHP+E1 = HHP + 1% extract 1 and HHP+E2 = HHP + 1% E2.

During storage, all pork samples became more tender as the hardness values decreased ($p<0.05$) for both HHP conditions and all combination treatments while the hardness values of HHP+B and HHP+E2 samples at 300 MPa were slightly increased with storage time as shown in Table 2. This was in agreement with the results of Iwasaki et al. [9] who confirmed that moderate pressure at 100 to 300 MPa caused denaturation of protein (swelling, aggregation, gelation), resulting in an increase in hardness of the whole meat.

Application of increasing pressure to meat could cause a lightening effect on the meat surface. It was observed that higher HHP at 300 MPa caused lighter and less redness of pork color as shown in Table 3 and 4 for L* and a* values, respectively. When crude bacteriocins were combined with HHP the color of pork samples was lighter than that from other treatments ($p<0.05$) and redness was less intense while yellowness of pork samples was not affected (Table 5). The effect of HHP on pork color in our experiment was in agreement with the results of Carlez *et al*. [10] who reported that L* values increased from 200 to 350 MPa and a* values decreased with increasing pressure, especially above 400 MPa, while b* values remained constant for minced beef.

Table 2 Hardness (N) of sliced pork loins during storage at 4 ºC, (% mean ± SD)

Storage time		HHP	HHP+B	HHP+E1	HHP+E2
200 MPa	Day 0	51.0 ± 6.2^{aA}	49.44 ± 4.0^{aA}	37.6 ± 1.6^{aB}	44.8 ± 2.4^{aC}
	Day 3	35.4 ± 2.7^{cB}	26.8 ± 2.0^{dA}	38.4 ± 3.9^{aA}	38.6 ± 3.8^{bA}
	Day 6	39.9 ± 4.3^{bA}	33.0 ± 5.6^{cB}	37.4 ± 2.8^{aAB}	35.4 ± 1.8^{bB}
	Day 9	36.2 ± 3.2^{cBC}	41.0 ± 5.3^{bA}	33.5 ± 5.2^{bC}	37.4 ± 3.8^{bB}
300 MPa	Day 0	53.7 ± 3.5^{aA}	32.3 ± 2.9^{B}	46.9 ± 3.3^{aB}	37.3 ± 4.8^{bC}
	Day 3	38.3 ± 4.7^{b}	38.3±6.8	36.7 ± 4.6^{c}	41.2 ± 4.9^{a}
	Day 6	40.6 ± 6.4^{bA}	36.9 ± 3.7^{B}	38.9 ± 3.1^{cB}	41.5 ± 4.8^{aA}
	Day 9	39.1 ± 3.6^{b}	37.1±3.8	40.8 ± 3.5^{b}	37.8 ± 3.2^{b}

Upper case letters indicate significant difference in the same row. Lower letters indicate significant difference in the same column in each HHP condition.

Table 3 Lightness (L*) of sliced pork loins during storage at 4 ºC, (% mean ± SD)

Storage time		HHP	HHP+B	HHP+E1	HHP+E2
200 MPa	Day 0	68.9±2.1	68.8 ± 0.1^{b}	66.8 ± 2.9^{a}	61.5±2.7
	Day 3	66.6 ± 2.7^{B}	72.7 ± 1.9^{aA}	64.0 ± 2.0^{abC}	67.2 ± 1.8^{B}
	Day 6	66.2±2.1	71.2 ± 2.0^{a}	67.3 ± 1.9^{a}	63.7±1.5
	Day 9	67.9±2.3	65.3 ± 3.0^{b}	60.9 ± 3.5^{b}	63.9±0.7
300 MPa	Day 0	69.0 ± 2.9^{B}	73.7 ± 2.5^{A}	66.8 ± 2.9^{a}	61.5±2.7
	Day 3	70.0 ± 2.2^{A}	70.4 ± 3.2^{A}	71.2 ± 2.4^{aA}	64.8 ± 3.0^{bB}
	Day 6	72.5±2.6	72.7±1.9	69.2 ± 2.3^{ab}	66.2 ± 2.6^{b}
	Day 9	71.7 ± 1.9^{A}	71.7 ± 3.3^{A}	65.8 ± 2.2^{bC}	68.5 ± 1.9^{bB}

Upper case letters indicate significant difference in the same row. Lower letters indicate significant difference in the same column in each HHP condition.

Table 4 Redness (a*) of sliced pork loins during storage at 4 ºC, (% mean ± SD)

Storage time		HHP	HHP+B	HHP+E1	HHP+E2
200 MPa	Day 0	10.20±1.3	8.59 ± 1.7^{b}	9.26±1.0	10.08±1.3
	Day 3	10.27 ± 1.4^{B}	8.19 ± 0.9^{aC}	11.54 ± 0.8^{A}	8.12 ± 1.0^{C}
	Day 6	9.67±1.1	8.89 ± 1.1^{b}	9.44±0.8	9.14±1.3
	Day 9	8.71±1.1	9.36 ± 0.9^{ab}	10.92±1.7	10.27±1.5
300 MPa	Day 0	8.45 ± 1.4^{B}	5.93 ± 0.7^{C}	8.29 ± 1.3^{A}	7.25 ± 1.2^{B}
	Day 3	8.16 ± 1.1^{B}	7.09 ± 1.5^{C}	9.45 ± 1.0^{A}	7.90 ± 0.6^{B}
	Day 6	6.93 ± 1.5^{B}	6.43 ± 1.2^{C}	9.21 ± 1.1^{A}	8.53 ± 1.8^{B}
	Day 9	7.73 ± 1.9^{B}	4.77 ± 1.9^{C}	10.66 ± 1.5^{A}	8.85 ± 0.9^{B}

Upper case letters indicate significant difference in the same row. Lower letters indicate significant difference in the same column in each HHP condition.

Table 5 Yellowness (b*) of sliced pork loins during storage at 4 ºC, (% mean ± SD)

Storage time		HHP	HHP+B	HHP+E1	HHP+E2
	Day 0	8.67±1.1	8.44±1.5	9.12±1.2	7.44±1.3
200	Day 3	7.8±1.5	8.65±1.6	8.45±1.8	9.22±1.6
MPa	Day 6	6.37±2.1	8.16±1.8	8.19±1.0	7.02±1.6
	Day 9	6.78±2.1	6.31±1.5	8.49±2.6	8.85±2.0
	Day 0	9.79±0.8	9.14±0.4	9.88±0.9	9.33±0.5
300	Day 3	7.55±1.5	8.44±1.8	9.64±1.5	9.55±1.2
MPa	Day 6	7.86±1.5	8.38±1.6	8.57±1.1	6.46±1.2
	Day 9	8.59±1.2	5.31±1.2	8.67±1.0	8.84±1.3

Upper case letters indicate significant difference in the same row. Lower letters indicate significant difference in the same column in each HHP condition.

IV. CONCLUSION

Our results indicated that both Thai herb extracts combined with HHP at 300 MPa had a better effect to inhibit microbial growth than when crude bacteriocins were combined. At the pressure of 200 MPa, microbial contents of the pork samples from all treatments were not different at the end of storage time. HHP and its combination with crude bacteriocins and herb extracts did not affect the weight loss of pork while higher pressure resulted in higher more meat tenderness. The color of pork samples was lighter when higher pressure was applied except when crude bacteriocins were combined.

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