Effects of Thai Herb Extracts in Combination with High Pressure Treatment on the Microbial and Physicochemical Quality of Fresh Pork

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Abstract

The objective of this study was to investigate the antibacterial activity of ethanolic extracts of 15 local Thai herbs against twelve different species of bacteria. The ethanolic extracts of *Schinus terebinthifolius*, *Spondias pinnata* (L.f.) Kurz and *Garcinia mangostana* Linn had a greater potential as antimicrobial agents against Gram positive and Gram negative bacterias and *Spondias pinnata* (L.f.) Kurz showed the most effective activity against all dominant floras. This study also evaluated the impact of high hydrostatic pressure processing (HHP at 200 and 300 MPa) in combination with crude bacteriocins (B), *Spondias pinnata* (L.f., Kurz (E1) or *Schinus terebinthifolius* (E3) on the microbial, physicochemical quality and shelf life of vacuum packed pork loins, stored at 4 ^{o}C for 9 days. At 200 MPa, total plate count (TPCs) of samples from all treatments in day 0, 6 and 9 were not different. However, the HHP samples had a different count from the others on day 3. At 200 MPa, the TPCs of HHP + E1 sample was higher ($p < 0.05$) than those of HHP + E3 and HHP + B. Increasing pressure for HHP had no impact on the pH, a_w and weight loss (%) of the pork samples for all treatments. L* and a* values of all treated samples increased while the b* value decreased slightly and then remained constant. Pork samples from all treatments became tender ($p < 0.05$)

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as storage time progressed each day. This finding suggested that HHP in combination with herb extracts could improve the microbiological quality and tenderness of fresh pork during storage and had a minimal effect on the color of the fresh pork.

Keywords: Thai Herb Extracts; High Hydrostatic Pressure (HHP); Microbial and Physicochemical Quality; Fresh Pork

Introduction

Pork is currently the most widely consumed meat in the world followed by poultry, beef, and mutton [1] and the global demand for pork meat continues to rise [2]. Pork meat offers a rich nutrient matrix that provides a suitable environment for the proliferation of pork meat spoilage microorganisms and common foodborne pathogens. Therefore, adequate preservation technologies must be applied in order to preserve its safety and quality. Food safety is the top priority for authorities and consumers worldwide. Consumers demand high quality, convenient, innovative, regular and safe pork meat products with natural flavors and tastes and an extended shelf life.

High pressure processing (HPP), a non - thermal technology, is being increasingly used in the meat industry to extend the shelf life of its products and to improve its quality characteristics [3] - [5]. High hydrostatic pressure (HHP) processing is carried out with intense pressure in the range of 100 - 1000 MPa, with or without heat, allowing most foods to be preserved with minimal effect on taste, texture or nutritional characteristics. The main advantage of HHP processing, compared with thermal sterilization and pasteurization, is the maintenance of the sensory and nutritional characteristics of the treated food products [6]. HHP technology has been successfully applied to the processing of cured meat products - cooked or dried and cooked ready to - eat meats [7]. However, it has been reported that HHP can impact structural, physiochemical, morphological and textural characteristics of the meat, and can cause partial discoloration of fresh red meat [8] - [9]. Thus, the physicochemical properties of HHP treated meat should be evaluated. High pressure processing may be combined with other preservation methods to increase its efficacy and commercial feasibility. Current and potential food additives, such as bacteriocins, have been tested in combination with high pressure processing. Combining phenolic compounds, which are hydrophobic, with high pressure may be useful in controlling pathogens, particularly in fat - rich food such as salad dressings and sausage [10]. To further increase the shelf life and safety of pressurized products, HHP has been investigated in combination with other technologies such as bacteriocins or other natural antimicrobials [11].

For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties [12]. Some herb extracts have been reported as antimicrobial such as *Momordica charantia* L. [13], *Sauropus androgynus* (L.) Merr. [14], *Schinus terebinthifolius* [15], *Anacardium occidentale* [16], *Centella asiatica* [17], *Spondias pinnata* (Linn. F.) kurz [18], *Garcinia mangostana* Linn [19], *Senna siamea* Lam. [20], and *Barringtonia acutangula* (L.) Gaertn [21]. Thai plants have been used as medicines for many centuries because they contain active phytochemicals including phenolic compounds. These components function as antibiotics, help to make cell walls impermeable to gas and water, act as structural materials to give plants stability and provide protection against ultraviolet (UV) light. Hence, plants in the tropical zone including Thailand contain a high concentration of phenolic compounds formed as secondary metabolites in plants [22].

Therefore, the objectives of this study were to evaluate and screen antimicrobial efficiency of 15 local Thai herbs ethanolic extracts with 12 pathogenic and dominant spoilage bacteria isolated from fresh pork by the paper disc diffusion technique. The objectives also included an investigation of the effectiveness of HHP in combination with the most active antibacterial herb extract or bacteriocins on inhibition growth of normal bacterial flora on fresh pork, and to evaluate physicochemical properties of treated pork packed under vacuum conditions and stored at 4 ^{o}C.

Materials and Methods

1. Preparation of Herb Extracts

The fifteen indigenous herbs used for this study as shown in Table 1 were purchased from local markets in Sakon Nakhon Province and the Thai herbs collection division of the Sakon Nakhon Campus of Rajamangala University of Technology Isan in July 2010. The herbs were cut into small pieces, freeze dried (Heto FD8 Thermo Electron Corporation, USA) and finely ground (IKA-Werke GmbH & Co., Germany) and stored at -20 ^{o}C for further application. For the ethanolic extracts, the dried herbs were mixed at 50 g each of dried herbs with 400 ml of 95 % (v/v) ethanol and constantly shaken at 100 rpm for 12 h (New Brunswick Scientific, UK). The mixture was filtered through a Whatman No. 1. The residue was re-extracted with 200 ml of 95 % ethanol, the filtrates combined and concentrated on a rotary evaporator (Rotary evaporator rotavapor R-114, Buchi, Switzerland) at 40 ^{o}C. The extract was freeze dried (Heto FD8, Thermo Electron Corporation, USA), stored in a sealed bottle and kept at -20 ^{o}C until use. The dried herb powder was

solubilized with 1 % Dimethyl sulfoxide to prepare a herb solution at concentrations of 20, 60 and 100 ppm for testing antimicrobial activity against the indicator bacteria.

Table 1 List of collected Thai indigenous herbs used for the study

Common name/Thai name	Scientific name	Parts of herbs used
1. Bitter cucumber/ma ra khee nok	*Momordica charantia*	Leaves / branches
2. Horse tamarind/gra tin	*Leucaena leucocephala (*Lamk.) de Wit	Leaves / branches
3. Cassod tree/khee lek	*Senna siamea* Lam	Leaves / branches
4. Sweet vegetable/phuk-wan	*Sauropus androgynus*	Leaves / branches
5. Brazillian pepper tree/ma toom khaek	*Schinus terebinthifolius*	Leaves / branches
6. Horse radish tree/ma room	*Moringa oleifera* Lam	Leaves / branches
7. Cashew nut tree/ma maung himmapaan	*Anacardium occidentale*	Leaves / branches
8. Asiatic pennywort/bua bok	*Centella asiatica*	Leaves / branches
9. Wood apple/ma sung	*Feroniella lucida (*Scheff.*)*	Leaves / branches
10. Hog plum/ma gok paa	*Spondias pinnata* (L.f.) Kurz	Leaves / branches
11. Mangosteen/mungkoot	*Garcinia mangostana* Linn	Peel or pericarp
12. Tummy-wood, Patana oak	*Careya sphaerica Roxb.*	Leaves / branches
13. Sea holly/ngeak pla mhoo	*Acanthus ebracteatus* Vahl	Leaves / branches
14. Phuk mek	*Syzygium gratum (*Wight*)* S.N.Mitra var. gratum	Leaves / branches
15. Sesban/khae baan	*Sesban sesbania*	Leaves / branches

2. Determination of Antimicrobial Activity: Paper Disk Diffusion Assay

The selected indicator bacteria obtained from the Thailand Institute of Scientific and Technological Research (TISTR) were used for testing antibacterial activity of herb extracts. Twelve bacterial strains, namely *Pseudomonas fluorescens* TISTR 358, *Staphylococcus aureus* TISTR 029, *Escherichia coli* TISTR 887, *Acinetobacter calcoaceticus* TISTR 1264, *Enterobacter aerogenes* TISTR 1540, *Enterococcus faecalis* TISTR 379, *Micrococcus luteus* TISTR745, *Listeria monocytogenes* TISTR17303 and dominant spoilage bacteria isolated from fresh pork; PM20 (96.0 % similar to *Serratia liquefaciens*), MT35 (74 % similar to *Klebsiella oxytoca*), PM23 (99.0 % similar to *Enterobacter* sp.) and MT4 (99.9 % similar to *Hafnia alvei*) were used for antibacterial testing. Stock bacterial cultures were maintained at 4 °C on agar slant. The cultivation/assay medium for *L. monocytogenes* and *S. aureus* was tryptone soy broth or agar (TSB, TSA, Oxoid, Hampshire, UK), for other strains nutrient broth or agar (Muller Hilton) were used. Active culture for the experiment

was prepared by streaking a loop full of bacterial culture on agar petri dishes and then incubating them at 30 or 37 °C for 24 h. The antibacterial action of the extract was tested on different bacterial strains using the paper disk diffusion method [23]. For the cultivation/ assay, the medium used was the same as above. The bacterial cultures for antimicrobial testing were prepared by picking a colony from 24-h-old TSA/NA plates and suspended in an appropriate medium. The cultures were grown aerobically for 18 - 20 h and continuously centrifuged at 9,000 rpm at 4 °C. The bacterial suspension was adjusted by diluting the inoculated bacteria with sterile 0.85 % NaCl until it reached the desirable level of about 10^6 - 10^8 CFU/ml. The cultures were subsequently transferred into a flask containing 25 ml of sterile nutrient agar at 43 - 45 °C, and poured into a Petri dish plate (8 cm diameter). Ten sterile paper disks (6 mm in diameter; Becton, Dickinson & Co.) were impregnated with known amounts of the test substances. Then, the disks containing the test materials were placed onto the surface of each plate uniformly seeded with the test microorganisms. The plates were incubated at 37 °C for 24 h. The paper disks impregnated with 1 % DMSO were used as a negative control and a disk with 200 ppm of nisin served as a positive control. Experiments were performed in triplicate. The inhibition zone diameter was measured (including the filter paper disk, 6 mm in diameter) using vernier caliper and expressed a clear zone in millimetres.

3. Preparation of Herb Extracts and Bacteriocins for High Pressure Processing

Two Thai herbs; *Spondias pinnata* (L.f.) Kurz (E1) and *Schinus terebinthifolius* (E3) 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 extract powder was resolubilized in 1 % DMSO at concentration of 1 % w/v. Then, the extract solution was shaken at 150 rpm, 20 min, 40 °C, centrifuged at 9,000 rpm, at 4 °C for 15 min then filtered through a Whatman No 1 filter paper. The filtrate was again filtered through a sterile cellulose acetate filter (0.45 micron) and the final filtrate was kept at 4 °C before use. The freeze-dried powder of crude bacteriocins (B) was prepared from the fermentation of *Lactococcus lactis* TISTR 1401 according the method of Intarapichet, K. and Gosaarak, S. [24].

4. Sample Preparation and High Pressure Processing

Fresh postmortem pork loins (*Longissimus dorsi*) were obtained from the Max Rubner-Institute, Kulmbach, Germany. Visible fat was trimmed off the loin and then they were transversally cut at about 2.54 cm thick per slice. The pork slices were randomly dipped in 0.2 % crude bacteriocins or 1.0 % of each extract solution, then vacuum packaged in plastic bags (PA/PE:O_2 30 cm^3 per m^2 and CO_2 150 cm^3) and kept in a freezing room before being pressurized at 200 or 300 MPa at 10 °C for 10 min. The HHP treatment

was carried out in an industrial hydrostatic pressurization unit (EPSI N.V. Belgium). The treated pork samples were stored at 4±1 °C for 9 days and sampled for aerobic plate counts, weight loss, tenderness, pH, a_w and color measurement every 3 days.

4.1 Microbial Enumeration

At each selected time, a piece of meat was extracted aseptically using a sterilized template and placed in a sterile plastic bag with 1 ml of sterile 0.85 % NaCl solution and finally diluted with 9 ml of 0.85 % NaCl solution. After homogenization for 90 s in a Stomacher blender dilutions were made to determine the microbial count using the CFU (colony forming unit) method. All counts were evaluated by pour plate technique with 1 ml of appropriate dilutions. Aerobic mesophilic counts were determined on plate count agar (PCA, Biokar) incubated at 30 °C for 72 h. The plates were prepared in duplicate for each dilution. The detection limit was 5 CFU/g for meat, and the results are expressed in log CFU/cm^2. Microbial enumeration was performed every 3 days during storage at 4 °C (9 days total).

4.2 Physical Quality

The weight loss percentage was determined by calculating the weight difference of the pork sample before and after cooking to an internal temperature of 72 °C.

The 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).

The texture of the sample was measured in Hardness, the Warner-Bratzler shear force value was determined using a texture analyzer (3369 Instron, Pfungstadt, Germany).

The pH values were determined by Knick Portamess 912 pH; Electrode SE 104 C (Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany).

Water activity was measured using the following device: A Type SE a_w Lab (company: SE-Schulz Electronic, Höhenkirchen, Germany). A high pressure food processor EPSI N.V. (Belgium): Meat temp = 0 °C, Temperature medium = -5 - -10 °C, Pressure medium : H_2O : Glycol 50 : 50, Finish temp = 10 °C

5. Statistical analysis

A statistical analysis was evaluated by a randomized complete block design. The variance was analyzed and a comparison of means was done using Duncan's Multiple Range Test. The significant difference was defined at $p < 0.05$.

Results and Discussion

1. Inhibition of Selected Herbs Extracts against Indicator Bacteria

Preliminary screening of the antimicrobial activity of the fifteen local Thai herbs against 12 pathogens microorganisms using the filter paper disk agar diffusion technique is shown in Table 2. The testing capability of the antimicrobial efficacy for the selected Thai herb extracts tested against indicators are shown in Table 2. The results from the clear zone showed that only *Spondias pinnata* (L.f.) Kurz extract could inhibit all of 12 indicator bacteria while *Momordica charantia*, *Leucaena leucocephala* (Lamk.) de Wit, *Sauropus androgynus*, *Moringa oleifera* Lam, *Centella asiatica*, *Feroniella lucida* (Scheff.), *Acanthus ebracteatus* Vahl and *Sesban sesbania* showed no inhibition. Four ethanolic extracts from *Senna siamea* Lam, *Schinus terebinthifolius*, *Anacardium occidentale* and *Garcinia mangostana* Linn could inhibit the same species of bacteria both Gram - positive and Gram - negative. At a high concentration of ethanolic extract from *Careya sphaerica* Roxb. and *Syzygium gratum* (Wight) S.N.Mitra var. gratum, the extract could inhibit the same species of bacteria and the same gram strain too. The ethanolic extracts of *Schinus terebinthifolius*, *Spondias pinnata* (L.f.) Kurz and *Garcinia mangostana* Linn had a greater potential as an antimicrobial agent against Gram positive and Gram negative bacterias. The results in antibacterial activity of some Thai local herbs are in agreement with the results of some earlier studies. Bukar, A. et al. reported the ethanolic extract of *Senna siamea* Lam against *Pseudomonas aeruginosa* at high concentration levels of 500 μg/disk and 1,000 μg/disk had zones of inhibition of 10 mm and 16 mm, respectively [20]. Torrungruang, K. et al. reported the antibacterial activity of mangosteen pericarp extract against cariogenic *Streptococcus mutans* [25]. The studies of De Lima, M. R. F. et al. using extracts of *Schinus terebinfolius* showed activity against *S. aureus* but were not active against *Escherichia coli* [26]. These activities may be attributed to the presence of m-cymene, 1-ß-pinene, α-pinene, α-terpinene, γ-terpinene and camphene found in *S. terebinthifolius* essential oil. It has been demonstrated that α-pinene and ß-pinene are able to destroy cellular integrity and thereby inhibit the respiration and ion transport processes. They also increase the membrane permeability in yeast cells and in isolated mitochondria [27]. Our results are in agreement with the findings of Mayachiew, P. and Devahastin, S. [28] who reported good antimicrobial activity for local Thai herbs and spices (*Phyllanthus emblica* Linn (Makham Pom) and *Alpinia galangal* (Kha)). The main reason for the differences in bacterial susceptibility could be the outer membrane surrounding the cell wall in gram - negative bacteria, which restricts the diffusion of compounds through its lipopolysaccharide covering [29]. In addition, the periplasmatic space contains enzymes which are capable of breaking

down foreign molecules introduced from the outside [29]. Our results showed remarkable antimicrobial activity for the ethanol extract of *Spondias pinnata* (L.f.) Kurz against all microorganisms tested, both Gram positive and Gram negative bacteria, followed by *Schinus terebinthifolius*. Therefore, the ethanol extract of these two Thai herb extracts were selected to study the effectiveness of HHP in combination with these two Thai herb extracts on the growth inhibition for normal bacterial flora on fresh pork.

Table 2 Antimicrobial activity of local Thai herbs extracts by paper disc diffusion test

Local Thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	***S.aureus***	***P.fluorescens***	***E. faecalis***	***M. luteus***	***L.monocytogenes***	***E. coli***	***S.liquefaciens* (PM20)**	***K.oxytoca* (MT35)**	***Enterobacter sp.* (PM23)**	***A.calcoaceticus***	***H. alvei* (MT4)**
Momordica charantia	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Leucaena leucocephala (Lamk.) de Wit	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Senna siamea Lam	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	+	-	-	+	++	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	

Table 2 Antimicrobial activity of local Thai herbs extracts by paper disc diffusion test (Cont.)

Local Thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	***S.aureus***	***P.fluorescens***	***E. faecalis***	***M. luteus***	***L.monocytogenes***	***E. coli***	***S.liquefaciens* (PM20)**	***K.oxytoca* (MT35)**	***Enterobacter sp.* (PM23)**	***A.calcoaceticus***	***H. alvei* (MT4)**
Sauropus androgynus	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Schinus terebinthifolius	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	+	-	-	-	-	-	-	-	-	-	-
	100	-	++	++	++	++	+	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Moringa oleifera Lam	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Anacardium occidentale	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	+	-	-	-	-	-	-	-	-	-	-
	100	-	+	-	+	+	+	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Centella asiatica	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++

Table 2 Antimicrobial activity of local Thai herbs extracts by paper disc diffusion test (Cont.)

Local Thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	***S.aureus***	***P.fluorescens***	***E. faecalis***	***M. luteus***	***L.monocytogenes***	***E. coli***	***S.liquefaciens* (PM20)**	***K.oxytoca* (MT35)**	***Enterobacter sp.* (PM23)**	***A.calcoaceticus***	***H. alvei* (MT4)**
Feroniella lucida (Scheff.)	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
***Spondias pinnata (L.f.)* Kurz**	20	-	++	-	+	+++	-	-	-	-	+	-	-
	60	+	++	++	+++	+++	-	++	++	+	++	++	-
	100	++	+++	+++	+++	+++	+	++	++	++	+++	+++	++
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Garcinia mangostana Linn.	20	-	+	-	+	++	-	-	-	-	-	-	-
	60	-	++	-	++	++	-	-	-	-	-	-	-
	100	-	++	-	++	+++	+	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Careya sphaerica Roxb.	20	-	-	-	-	-	-	-	-	-	-	-	-
	60		+	-	-	-	-	-	-	-	-	-	-
	100	-	+	-	-	++	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++

Table 2 Antimicrobial activity of local Thai herbs extracts by paper disc diffusion test (Cont.)

Local Thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	*S.aureus*	*P.fluorescens*	*E. faecalis*	*M. luteus*	*L.monocytogenes*	*E. coli*	*S.liquefaciens* (PM20)	*K.oxytoca* (MT35)	*Enterobacter sp.* (PM23)	*A.calcoaceticus*	*H. alvei* (MT4)
Acanthus ebracteatus Vahl	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Syzygium gratum (Wight) S.N.Mitra var. gratum	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	+++	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++

Note; -, No inhibition; +, <10 mm.; ++, 10-15 mm.; +++, >15 mm.; Nisin 200 ppm for positive control; DMSO 1 % for negative control

2. Total Plate Counts of Vacuum Packed Pork

Total plate counts of pork chops in vacuum packed treated by HHP at 200 MPa and in combination with herb extracts and crude bacteriocins and control samples (without HHP) are shown in Figure 1. Compared with a control without high pressure at the beginning, it was observed that only HHP could reduce total counts of 0.74 log CFU/cm^2 which is better than all combined treatments. From day 3 to 6, HHP combined with B and with E1 and E3 gave the same results for a total count reduction (0.37 - 0.95 log CFU/cm^2). For the last day of storage, HHP, HHP combined with E1 and E3 gave no difference in TPCs (1.53, 1.39, 1.26 log CFU/cm^2, respectively) and a better reduction in TPC than the treatment of HHP combined with B (0.5 log CFU/cm^2). When compared with the combined treatments at day 0, 6 and 9, there was no significant difference ($p > 0.05$) of TPCs except for day 3 when the treatment of HHP and HHP combined with E1 showed higher TPCs ($p < 0.05$)

than the HHP combined with E3 and HHP combined with B. These results indicate that HHP, HHP in combination with herb extract and bacteriocins can inhibit the growth of normal bacterial flora on fresh pork.

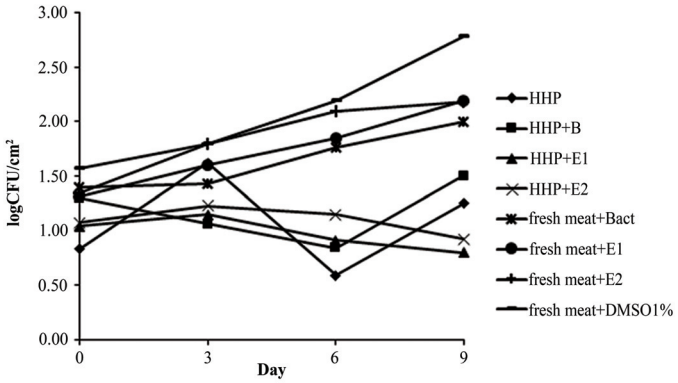

Figure 1 Total plate count of vacuum packed pork chops treated by HHP at 200 MPa and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (n = 4).

3. Physical Quality of Vacuum Packed Pork

3.1 Color

Color is one of the most important factors that can influence consumers' purchase preferences of fresh meat [30] - [31]. Regarding chromatic parameters (Table 3), for samples treated at 200 MPa HHP, it could be seen that L* increased significantly ($p < 0.05$) in HHP sample compared with other treated samples. On applying pressure at 300 MPa for all samples, it could be seen that L* increased in comparison with a lower pressure. The increment of the L* value could be associated with globin denaturation and/or with heme displacement at pressures above 300 MPa [32]. These results are in agreement with those of Cheftel, J. C, and Culioli, J. [33] who reported an increase in L* values or "whitening/brightening" effect of pressure which can be attributed to globin denaturation, heme displacement or release, and ferrous ion oxidation. The increase in L* values started from 200 MPa and become stabilized for pressures around 300 - 400 MPa, as shown by Carlez, A. et al. in beef mince [32] and Shigehisa, T. et al. in pork slurries [34]. It was reported that there was an increase in L* value between treatments at 200 and 300 MPa, but no significant difference between 300 and 400 MPa [35]. In addition, Sun, S. et al. found that 600 MPa HPP treated steaks were lighter than non-HPP treated steaks [31]. Additionally, Ledward reported that protein precipitation that could lead to loss of solubility is the main reason for higher L* value of meat color [36].

For the pressurization of pork at 200 MPa, the HHP and HHP+B treated samples had a* value with significantly lower values ($p < 0.05$) than HHP+E1 and HHP+E3 treated ones throughout the storage time (Table 4). When the pressure at 300 MPa was used, a*values of all treated samples remained almost constant but slightly higher than all samples treated at 200 MPa. An increase in the redness of the pork samples correlated with a lower accumulation of metmyoglobin during pressurized sample storage [37]. At pressures up to 300 MPa, with a liquid pressurization of 10 ^{o}C, the production of metmyoglobin was decreased leading to an increase in a* value [38]. An increase in the a* value is likely due to mechanism - alteration of the redox chemistry of myoglobin [31].

For modification of b* parameter (Table 5), pressurized at 200 and 300 MPa of HHP, HHP+E3 and HHP+E1 tended to stay constant while the HHP+B gradually decreased with storage time. It could be seen that the application of higher pressures led to a slight increase in b*. The increase in b* values could be due to metmyoglobin formation initiated by HHP [32].

Table 3 The color L* of pork chops in vacuum packed treated by combined HHP (200 and 300 MPa) with extracts (E1, E3), crude bacteriocins (B) (Mean ± SD).

Pressure	Day of storage	L* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	69.11 ± 0.7^{A}	68.30 ± 1.9^{bcA}	68.57 ± 2.0^{aA}	66.12 ± 0.2^{aB}
	3	67.39 ± 1.2^{B}	71.70 ± 4.0^{aA}	67.64 ± 4.2^{aB}	62.34 ± 6.8^{aB}
	6	67.39 ± 2.7^{A}	69.68 ± 0.7^{bA}	64.87 ± 1.8^{bB}	56.70 ± 4.1^{bC}
	9	70.36 ± 6.4^{A}	66.68 ± 2.0^{cB}	66.705 ± 1.8^{abB}	67.15 ± 2.8^{aB}
300 MPa	0	71.49 ± 3.1^{aB}	74.76 ± 2.5^{aA}	73.36 ± 0.3^{aAB}	72.01 ± 4.7^{aB}
	3	68.51 ± 1.2^{bB}	69.30 ± 2.7^{bB}	68.94 ± 5.6^{bB}	71.80 ± 1.2^{aA}
	6	68.14 ± 1.4^{bB}	70.43 ± 2.3^{bA}	66.13 ± 1.0^{cC}	64.73 ± 1.7^{cC}
	9	71.61 ± 5.1^{aA}	71.23 ± 5.4^{bA}	63.29 ± 3.4^{cB}	67.79 ± 2.1^{bB}

Upper case letters are significantly different in the same row ($p < 0.05$).

Lower case letters are significantly different in the same column ($p < 0.05$) ($n = 20$).

Table 4 The color a* of vacuum packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ± SD).

Pressure	Day of storage	a* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	7.80 ± 0.7^{C}	9.97 ± 0.9^{aA}	8.63 ± 1.3^{cBC}	9.49 ± 0.3^{bAB}
	3	8.48 ± 0.3^{B}	9.92 ± 0.9^{aA}	9.52 ± 1.2^{bA}	9.23 ± 1.6^{abA}
	6	9.01 ± 1.5^{BC}	9.55 ± 0.4^{aB}	11.60 ± 0.5^{aA}	8.50 ± 0.8^{cC}
	9	7.93 ± 1.3^{C}	8.08 ± 0.7^{bC}	10.83 ± 0.1^{aA}	9.86 ± 0.7^{aB}
300 MPa	0	10.55 ± 0.1^{bA}	8.35 ± 0.3^{bC}	9.56 ± 1.1^{cB}	9.87 ± 0.8^{B}
	3	12.62 ± 1.5^{aA}	11.08 ± 0.5^{B}	11.39 ± 0.9^{bB}	11.10 ± 0.7^{B}
	6	10.39 ± 1.4^{b}	11.47 ± 1.2^{a}	11.51 ± 0.7^{b}	11.11 ± 0.7
	9	10.82 ± 0.2^{b}	10.74 ± 0.1^{a}	12.55 ± 0.7^{a}	12.70 ± 1.7

Upper case letters are significantly different in the same row ($p < 0.05$).

Lower case letters are significantly different in the same column ($p < 0.05$) ($n = 20$).

Table 5 The color b* of vacuum packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ± SD).

Pressure	Day of storage	b* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	6.50 ± 0.5^{aB}	7.43 ± 0.7^{aA}	7.22 ± 1.2^{A}	$5.630.7^{aC}$
	3	5.44 ± 0.9^{bC}	6.65 ± 0.4^{bAB}	7.06 ± 0.3^{A}	4.50 ± 2.2^{aC}
	6	5.30 ± 1.2^{bB}	5.88 ± 0.5^{cB}	7.28 ± 0.5^{A}	3.17 ± 1.1^{bC}
	9	6.07 ± 2.0^{abB}	5.36 ± 0.4^{dC}	7.49 ± 1.0^{A}	6.29 ± 0.6^{aB}
300 MPa	0	7.39 ± 1.0^{aB}	7.15 ± 0.9^{aBC}	8.14 ± 0.3^{aA}	6.55 ± 1.4^{aC}
	3	6.19 ± 0.9^{bB}	6.56 ± 0.1^{bB}	7.48 ± 2.1^{aA}	7.26 ± 0.3^{aA}
	6	4.61 ± 0.5^{cC}	6.22 ± 0.5^{bA}	6.17 ± 0.9^{bA}	5.54 ± 0.5^{bB}
	9	6.53 ± 1.6^{bBC}	6.15 ± 1.0^{bC}	7.44 ± 2.0^{aA}	7.21 ± 1.7^{aAB}

Upper case letters are significantly different in the same row ($p < 0.05$).

Lower case letters are significantly different in the same column ($p < 0.05$) ($n = 20$).

3.2 Hardness, Weight Loss (%), Aw and pH

Consumer perception of hardness is considered the most important texture attribute and dictates the commercial value of meat [39]. The hardness of pork samples for all treatments pressurized at 200 MPa, HHP tended to be reduced except HHP+E1 during storage (Table 6). In addition when compared among treatments on the same day of storage, the hardness of HHP only was much higher than for the other treatments but not different from HHP+B and HHP+E3 for day 0 of storage, and HHP+E1 for day 6 of storage. These results suggest that HHP (at 200 MPa) in combination with two Thai herbs extracts could improve the tenderness of meat. The application of pressure at 300 MPa HHP, HHP+B and HHP+E3 led to an increase in the hardness of the pork samples at 3 days of storage but was not significantly different at the end of storage while the hardness of HHP+E1 was continuously reduced. This indicates that HHP (at 300 MPa) in combination with E1 could improve the tenderness of meat during storage. Normally, for pressures ranging from 100 to 300 MPa, changes are normally reversible, whereas at higher pressures they are normally non-reversible [11]. In addition, hardness increased at a relatively constant rate with a high-pressure processing treatment between 0 and 400 MPa [40]. The hardness increased as a result of high pressure processing at 200 and 400 MPa and during storage only minor changes in the hardness occurred. Even the non-pressurised sample showed only a small increase in the hardness during storage [41].

A combination of treatments of HHP with herb extracts and crude bacteriocins did not make any significant differences ($p > 0.05$) in weight loss (%) except at the pressure at 300 MPa which differed for the last day of storage (Table 7).

High pressure treatment of sliced pork loins at these medium pressure levels of 200 and 300 MPas and with a combination of crude bacteriocins and both herb extracts did not make any significant differences ($p > 0.05$) in a_w (95.9±0.1 - 97.4±0.5 %) and pH (5.6±0.0 - 6.0±0.0) (data not shown).

Table 6 The hardness of vacuum packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ± SD).

Pressure	Day of storage	Hardness (N)			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	53.29±3.1^{aA}	50.12±3.6^{aA}	39.89±2.8^{B}	50.91±2.0^{aA}
	3	52.68±5.6^{aA}	42.67±4.2^{aB}	37.01±3.7^{C}	41.93±4.2^{bBC}
	6	40.22±5.2^{bA}	35.25±3.4^{bB}	39.03±5.5^{A}	32.26±4.6^{cB}
	9	42.27±4.5^{bA}	37.59±3.8^{bB}	36.97±3.4^{B}	31.86±4.3^{cC}
300 MPa	0	36.88±1.2^{bBC}	34.08±2.6^{bcC}	51.70±4.2^{aA}	38.99±4.6^{bB}
	3	48.81±5.5^{a}	46.21±3.7^{a}	45.06±2.2^{b}	43.17±3.0^{a}
	6	40.43±2.3^{bA}	31.48±5.2^{cB}	40.25±3.1^{cA}	33.39±2.9^{cB}
	9	41.19±6.5^{bA}	36.25±2.7^{bB}	37.57±4.2^{cAB}	35.35±3.4^{bcB}

Upper case letters are significantly different in the same row ($p < 0.05$).

Lower case letters are significantly different in the same column ($p < 0.05$) ($n = 20$).

Table 7 Weight Loss (%) of vacuum packed pork chops treated by HHP (200 and 300 MPa) in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ± SD) (n=4).

Pressure	Day of storage	Weight loss (%)			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	27.1±1.8	32.1±3.1	29.3±2.8	31.1±0.2
	3	28.7±2.5	27.3±4.8	25.1±4.3	30.8±0.5
	6	24.9±4.2	28.2±0.9	25.4±8.0	26.2±1.1
	9	25.9±2.0	25.2±0.4	22.7±3.8	27.7±1.3
300 MPa	0	30.02±1.4^{a}	31.09±1.7	28.35±4.1	30.65±3.2
	3	27.92±0.4^{a}	30.05±2.1	29.65±1.6	29.77±2.9
	6	29.70±0.2^{a}	29.36±3.9	25.29±2.3	25.67±3.3
	9	22.94±1.8^{b}	25.25±0.5	26.84±4.4	24.18±4.0

Lower case letters are significantly different in the same column ($p < 0.05$).

Conclusion

The ethanolic extracts of *Schinus terebinthifolius*, *Spondias pinnata* (L.f.) Kurz and *Garcinia mangostana* Linn had greater potential as antimicrobial agent against Gram positive and Gram negative bacteria and *Spondias pinnata* (L.f.) Kurz had the most effective activity against all dominant floras. The moderate pressure of 200 MPa in combination with herb extracts or bacteriocins did not ensure complete inactivation of bacteria growth. However, it was obvious that it could allow for an improvement in the microbiological quality and extended shelf life of sliced pork in the stage dependent on the level of initial contamination of the raw material. HHP could affect the physicochemical qualities of fresh pork meat, particularly depending on the pressure level applied, and thus the typical characteristics associated with fresh meat, particularly texture and color, could be remarkably modified. The HHP treatment caused a lighter color for pork meat products. In addition, the tenderness of fresh pork was improved. This finding suggests that HHP (200 MPa) in combination with herb extracts might be an effective technology in reducing microbial spoilage and extending shelf-life of fresh pork meat. However, HHP may have a negative impact on sensory characteristics. Further work should be conducted to evaluate sensory properties of fresh pork treated by HHP.

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