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Inhibitory effects of the combined application of lauric acid and monolaurin with lactic acid against *Staphylococcus aureus* in pork

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ABSTRACT: Lauric acid and monolaurin in combination with lactic acid were evaluated for their effectiveness in reducing total plate count (TPC) and *Staphylococcus aureus* of fresh pork loin. Fresh pork loins were dipped in control (non treated), sterile distilled water, 3.2 mg/ml lauric acid, 0.4% (v/v) lactic acid, 0.2 mg/ml lauric acid + 0.1% (v/v) lactic acid, 0.1 mg/ml monolaurin, 0.05 mg/ml monolaurin + 0.1% (v/v) lactic acid solution. Total plate count, population of *S. aureus* and physical and sensory qualities were determined. TPC and *S. aureus* counts found in pork loin treated with lauric acid and monolaurin alone and in combination with lactic acid were not significantly different ($P > 0.05$). The colour, odour and overall acceptability of the pork loins were adversely affected by the treatment with lactic acid alone, but when combinations of the agents were used the sensory quality was acceptable. Furthermore, during storage, both lipids in combination with lactic acid controlled growth of *S. aureus* for 8 and 4 days of storage at 4 and 15 °C respectively, and TPC for 8 and 2 days of storage at 4 and 15 °C, respectively. The low pH of the antimicrobials caused the highest weight loss of range 4.41–5.38% drip loss, 22.56–23.35% cooking loss, and discolouration. In contrast, L^*, PV, TBARS of pork in all treatments increased but shear force and a^* decreased as storage was longer in all solution types ($P < 0.05$). However, for sensory acceptability, there was no loss of colour or adverse odour and the overall acceptability scores remained satisfactory.

KEYWORDS: antimicrobial, TBARS, food-borne diseases, food safety, contaminated meat

INTRODUCTION

Since staphylococcal foodborne intoxication is established as one of the most common bacterial foodborne diseases causing problems in the food sector in many countries, strategies to control *Staphylococcus aureus* in foods are of particular interest. Meat and meat products are regarded as one of the leading vehicles for transmission of *S. aureus*[1]. Despite this, a number of outbreaks have been attributed to contaminated meat products[2].

The problem of safe preservation in the meat industry has become more complex as today's products require greater standards of protection from pathogens. Many attempts have been made to control the growth of pathogens on the surface of meat and meat products by using of chemical antimicrobials. Considering all the organic acids that have been evaluated for their application as meat decontaminants, lactic acid is among the most widely accepted[3]. There is extensive information on the application of lactic acid to control both spoilage and pathogenic organisms in foods of animal origin. For example, significant inhibition of *S. aureus* growth was obtained by dipping or spraying meat with 1–5% lactic acid solutions[4]. However, it is difficult to stabilize preservatives on the surface of food due to evaporation, neutralization, and diffusion into the matrix[5].

Early studies[6,7] have reported the ability of lipids to inhibit bacteria. Various fatty acids and monoacylglycerols in trace amounts inhibit the growth of microorganisms[8]. Both bacteriostatic[6,7,9] and bactericidal effects[6,10,11] have been observed. Lauric acid and glycerol monolaurate ability to inhibit the growth and toxin production of *S. aureus* has been extensively characterized[12,13]. Monolaurin, a monoglycerol ester of lauric acid present in many animals and plants, possesses wide-spectrum activity against bacteria, fungi, and viruses[14,15]. It blocks the production of various exoenzymes and virulence factors,

including protein A, α-hemolysin, β-lactamase, and toxic shock syndrome toxin 1 in *S. aureus*[13,16]. Monolaurin is currently used as a "generally recognized as safe" food emulsifier, approved by the U.S. Food and Drug Administration, and is considered essentially a non-toxic compound even at relatively high dose levels. It is however insoluble in water and therefore must be dissolved in an appropriate medium before application. Its effects against both pathogenic and spoilage microorganisms in some foods and food processing surfaces are known. Furthermore, the inhibition produced by monolaurin is greatest at low pH[17–23].

The individual effectiveness of lauric acid, monolaurin, or lactic acid against foodborne pathogenic bacteria is known[24]. But the effects of the combined application of lauric acid and monolaurin with lactic acid at sub-inhibitory concentrations against *S. aureus* and some physico-chemical qualities of pork have not been reported to date.

MATERIALS AND METHODS

Test strain

Staphylococcus aureus (MSSA) was previously isolated from pig carcasses in Southern Thailand abattoirs by the standard procedure[25] and its identity was confirmed by the Department of Medical Sciences, Ministry of Public Health of Thailand. These organisms were maintained on Mueller Hinton agar (MHA) (Merck, Germany). The overnight cultures were prepared by inoculating approximately 2 ml Mueller Hinton culture medium (Merck, Germany) with 2–3 colonies taken from MHA. Broths were incubated overnight at 35 ± 2 °C. Inocula were prepared by diluting overnight culture in saline to 10^8 CFU/ml (McFarland standard of 0.5). These suspensions were further diluted with saline as required. An initial concentration of approximately 10^7 CFU/ml was used for meat models.

Antimicrobial agents

Lauric acid and monolaurin were supplied by Sigma Aldrich (Sigma, France). Lactic acid (80% (v/v), food grade) was obtained from Vichhi Enterprise Co. Ltd. (Bangkok). For meat models, the concentrations of lactic acid were assessed as % (v/v) but for lauric acid and monolaurin concentrations were measured as mg/ml.

Meat models

Fresh pork loin was purchased from a local slaughter house of Phatthalung province, Thailand. Meat pieces with a thickness of 2.5 cm, width of 5 cm, length of 8 cm and weight 100–150 g were prepared. After that, two meat pieces were divided into 6 groups. Five groups were used to determine the effects of antimicrobials on total plate count (TPC), physical and chemical analysis and sensory evaluation, for which the pieces were not inoculated with the bacterial suspension. The other group was used to determine the effect of antimicrobials on *S. aureus*. To do so, the meat pieces were inoculated with *S. aureus* suspension as follows: the pieces were individually submerged in 50 ml of the bacterial inoculum (*S. aureus* containing approximately 10^7 CFU/ml, prepared in sterile 0.85% (w/v) saline solution) for 10 min, air dried for 20 min in a bio-safety cabinet before washing them with the antimicrobials. The initial count of *S. aureus* on each piece was approximately 10^5 CFU/g. The pieces were randomly divided into seven groups and immersed for 10 min as follows: (1) control – non treated, (2) dipped in sterile distilled water, (3) dipped in 3.2 mg/ml lauric acid solution, (4) dipped in 0.4% (v/v) lactic acid solution, (5) dipped in 0.2 mg/ml lauric acid + 0.1% (v/v) lactic acid solution, (6) dipped in 0.1 mg/ml monolaurin solution, (7) dipped in 0.05 mg/ml monolaurin + 0.1% (v/v) lactic acid solution. The microbiological and physical analyses, except drip loss, and sensory evaluations were determined. The lowest concentration of antimicrobials which exhibited the most reducing microbial and lowest changing of physical and sensory qualities were chosen for further study.

For storage study, fresh pork loin was prepared similarly as described before, except for the antimicrobial solutions. The pieces were randomly divided into four groups and immersed for 10 min as follows: (1) control – non treated, (2) dipped in sterile distilled water, (3) dipped in 0.2 mg/ml lauric acid + 0.1% (v/v) lactic acid solution, (4) dipped in 0.05 mg/ml monolaurin + 0.1% (v/v) lactic acid solution. Each treated piece was packed in a polyethylene plastic bag. Then, the packages were stored in an air-circulated refrigerator at 4 and 15 °C for 0, 1, 2, 4, and 8 days of storage time. The microbiological and physico-chemical analysis and sensory evaluations were determined.

ANALYSIS METHODS

Microbiological assays

The sample meats were submitted to count for *S. aureus*[25] and TPC[26] according to standard procedures. The results were transformed to log CFU per gram of meat (log CFU/g). The plates were incubated at

35 ± 2 °C for 24–48 h before colonies were counted. *S. aureus* were enumerated on Baird-Parker agar (Merck, Germany) to which 5% (v/v) egg yolk tellurite emulsion 20% (Merck, Germany) was added. Then, presumptive colonies were examined microscopically and analysed for coagulase activity. Suspect *S. aureus* colonies were transferred into small tubes containing 0.2–0.3 ml BHI broth and emulsified thoroughly. BHI culture suspensions were incubated for 18–24 h at 35 °C, and then, 0.5 ml reconstituted coagulase plasma with EDTA (BBL and DIFCO, USA) was added and mixed thoroughly. Then, the culture was incubated at 35 °C and examined periodically over 6 h periods for clot formation. Only a firm and complete clot that stayed in place when the tube was tilted or inverted was considered positive for *S. aureus*. Enumeration of TPC was done on plate count agar (Merck, Germany). The plates were incubated at 35 ± 2 °C for 24–48 h before colonies were counted.

Physical and chemical analyses

The pH values of fresh pork were measured with a pH meter (Model SevenGo Duo pro, Mettler-Toledo, Switzerland) using a combined Cat no. 51 343 154 (InLab Solid Pro electrode, Mettler-Toledo, Switzerland). The pH values were measured in the sample for 5 mm depth and data were taken in triplicate for each sample.

The colour measurements of fresh pork were taken with a colourimeter (ColorFlex Firmware version 1.72, Hunter Associates Laboratory, USA). The colour values (CIE L^*, a^*, and b^*) were measured on the sample surfaces and data were taken in triplicate for each sample. Additionally, hue angle (H^*) was calculated as: $\tan^{-1}(b^*/a^*)$[27].

The weight losses were: exudate, drip, and cooking losses. Fresh meats were weighed before and after dipping, storage, and cooking and exudate, drip, and cooking losses for each meat was calculated as: exudate loss = (before dipped weight − dipped weight)/before dipped weight; drip loss = (dipped weight − stored weight)/dipped weight; cooking loss = (raw weight − cooked weight)/raw weight.

Cooking loss of pork meat samples was examined by a similar method to that of Wattanachant et al[28]. Meat strips, sample size 3 cm × 4 cm × 2.5 cm, were put in a tightly sealed plastic bag and cooked in a water bath (WNB22 Memmert, Germany) at 80 °C for 10 min. After being cooked, the samples were cooled in water at 10 °C. The samples were removed from the container, blotted with filter paper, and weighed to determine the cooking loss as defined above.

Meat samples were cut into sizes of 1.0 cm × 2.0 cm × 1.5 cm for shear analysis using a texture analyser (TA.XT*Plus*, Texture Technologies Corp, and Stable Micro Systems, USA) equipped with a Warner-Bratzler shear apparatus. The operating parameters consisted of a cross head speed of 2 mm/s and a 25 kg load cell. The shear force was measured perpendicular to the axis of muscle fibres. The peak of the shear force profile was regarded as the shear force value modified from Wattanachant et al[29].

The PV of all samples was determined according to AOAC method 965.33[30] and expressed as meq/kg meat of sample. For TBARS values, the distillation TBA method was performed as described by Tarladgis et al[31]. The homogenized 10 g of meat sample was transferred to a Kjeldahl flask and 97.5 ml of distilled water and 2.5 ml of 6 N HCl. The mixture was heated with steam distillation until 200 ml of distillate was collected. Then, 5 ml of distillate was added to 5 ml of thiobarbituric reactive reagent containing 0.02 M TBA in 90% glacial acetic acid and incubated for boiling water for 35 min. After cooling with tap water, the absorbance of the pink solution was read at 538 nm. The constant 7.8 was used to calculate the distillation TBA number as recommended.

Sensory evaluations

To determine sensory colour, odour, and overall acceptability scores, the meat samples were prepared approximately 1 h before sensory evaluation. Forty students conversant in food science and sensory quality of food were requested to score the colour, odour, and overall (general appearance colour, and wetness or dryness, and odour) acceptability on the basis of a nine point hedonic rating scale. The scale was as follows: 1 = extremely unacceptable, 2 = very much unacceptable, 3 = moderately unacceptable, 4 = slightly unacceptable, 5 = between acceptable and unacceptable, 6 = slightly acceptable, 7 = moderately acceptable, 8 = very much acceptable, and 9 = extremely acceptable[32,33]. All the samples were served in Petri dishes and were returned for further chemical analysis.

Statistical analysis

A completely random statistical model (7 treatments) was designed to test the effects of antimicrobials. Then, a 4×5 factorial in CRD was designed for interaction between antimicrobials and storage times on meat qualities. Data is presented as means and standard deviations. All statistical computations were performed to determine significant differences ($P < 0.05$) by ANOVA followed by Duncan's new multiple range test.

Table 1 Log-reduction of TPC and inoculated *S. aureus* onto fresh pork loin dipped in the lipid and lactic acid.

log CFU/g reduction[a]	Antimicrobials[b]					
	water	0.4% LA	Lau 3.2 mg/ml	Lau 0.2 mg/ml + 0.1% LA	ML 0.1 mg/ml	ML 0.05 mg/ml + 0.1% LA
TPC	0.13 ± 0.08^{A}	1.04 ± 0.16^{D}	0.66 ± 0.09^{BC}	0.84 ± 0.07^{CD}	0.59 ± 0.14^{B}	0.77 ± 0.15^{BC}
S. aureus	0.07 ± 0.10^{A}	1.52 ± 0.14^{C}	0.84 ± 0.16^{B}	1.03 ± 0.14^{B}	0.76 ± 0.14^{B}	1.01 ± 0.15^{B}

[a] Values correspond to mean data $\pm$ standard deviation.
[b] Lau: lauric acid, ML: monolaurin, and LA: lactic acid.
[A–D] Different letters in each row are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

TPC and *S. aureus* of fresh pork loin

In our previous study, the minimal bactericidal concentration of lauric acid, monolaurin, and lactic acid against *S. aureus* were 3.2 mg/ml, 0.1 mg/ml, and 0.4% (v/v), respectively. For synergistic effects, fractional bactericidal concentration index of the combined action of lauric acid and monolaurin with lactic acid were 0.3125 (0.2 mg/ml lauric acid + 0.1%lactic acid) and 0.7500 (0.05 mg/ml monolaurin + 0.1% lactic acid) for strain again suggesting synergy and partial synergy, respectively (data not shown).

Table 1 shows the log-reduction of TPC and *S. aureus* inoculated onto fresh pork loin and dipped in different antimicrobials. Initial counts of TPC and inoculated *S. aureus* on loin were 3.87 ± 0.14 and 5.36 ± 0.24 log CFU/g, respectively. Lactic acid was more effective in reducing initial of TPC and *S. aureus* counts than lipid alone and in combinations. This could be due to undissociated forms of organic acid, which penetrate the lipid membrane of the bacterial cell and dissociate within the cell. As the bacterial cytoplasm needs to be maintained at neutral pH, the excess export of protons results in consumption of cellular ATP and subsequent depletion of energy, with the intracellular pH becoming more acidic. This results in loss and change of the cytoplasm, a loss of membrane integrity and concomitant cell injury and death[34,35]. For lauric acid and monolaurin alone and in combinations at sub-inhibitory concentrations, there were no significant differences ($P > 0.05$) in the log-reduction of bacterial counts on pork loin. This could be due to the high hydrophobicity that lauric acid presents and its accumulation into the membrane bilayer. This causes a change in the hydrogen bonding and the dipole-dipole interaction between acyl chains and, at high concentrations, cell inactivation is achieved due to the disruption of the glycerophospholipid organization within the membrane[36]. Monolaurin is known to produce highly ordered membranes, which is thought to disrupt membrane function by affecting signal transduction due to uncoupling of energy systems, altered respiration and altered amino acid uptake[21]. A previous study demonstrated that monolaurin caused a constant increase in leakage of *S. aureus* CMCC(B) 26 003 membrane to 91.6% over a period of 60 min[23]. Furthermore, the presence of lactic acid improving the uptake of lauric acid into the membrane, which probably affects membrane function and furthermore, leads to measurable synergism of the combined antimicrobial treatment[37]. Moreover, the antimicrobial synergy[38] between monolaurin and lactic acid might be related to changes in both membrane function and fluidity[39]. However, the activity of monolaurin is reduced or affected by the presence of carbohydrate and protein materials[40,41]. These may partly explain the reduced effect of monolaurin in pork loin which is a high protein food.

Physical and sensory qualities of fresh pork loin

The use of lactic acid alone reduced the pH value of pork from 5.86–5.66. This would result in an increasing of exudate and cooking losses in pork dipped in 0.4% (v/v) lactic acid. Sawyer et al[42] suggested that the 0.5–1.0% (v/v) lactic acid may be attributed to myofibrillar and/or sarcoplasmic protein denaturation caused by the acidity of the lactic acid solution. In the present study, the highest value of shear force was found in pork dipped in lactic acid alone. Tenderness of meat is considered to be strongly related to pH value[43,44]. Moreover, the fresh pork dipped in lactic acid alone was much lighter (higher L^* values; $P < 0.05$) and less red (lower a^* and higher H^* values, $P < 0.05$) than control. The L^* value of the sample depends upon the moist surface which has higher reflecting property. The increase or decrease in the H^* value depends upon change of a^* and b^* values. Increment of H^* value shows the decrement of redness[45]. Similarly, sensory analysis of fresh pork revealed that the use of lactic acid alone or in combinations reduced the colour and overall

Table 2 Effects of the lipid and lactic acid on physical qualities and sensory attributes of fresh pork loin.

	Antimicrobials[a]						
	control	water	0.4% LA	Lau 3.2 mg/ml	Lau 0.2 mg/ml + 0.1% LA	ML 0.1 mg/ml	ML 0.05 mg/ml + 0.1% LA
Physical qualities:[b]							
pH value	5.86 ± 0.02^{A}	5.91 ± 0.05^{A}	5.66 ± 0.02^{C}	5.74 ± 0.01^{AB}	5.72 ± 0.01^{AB}	5.79 ± 0.05^{AB}	5.72 ± 0.02^{AB}
colour measurement:							
L^*	53.45 ± 0.66^{A}	55.55 ± 0.35^{B}	61.86 ± 0.12^{E}	58.30 ± 0.17^{C}	60.13 ± 0.81^{D}	58.15 ± 0.08^{C}	59.99 ± 0.81^{D}
a^*	8.54 ± 0.29^{A}	7.35 ± 0.09^{B}	6.14 ± 0.13^{D}	6.50 ± 0.09^{C}	6.32 ± 0.13^{CD}	6.48 ± 0.09^{C}	6.41 ± 0.04^{CD}
b^*	4.69 ± 0.95^{A}	3.03 ± 0.64^{B}	2.26 ± 0.46^{B}	3.00 ± 0.63^{B}	2.83 ± 0.61^{B}	3.11 ± 0.66^{B}	2.95 ± 0.63^{B}
H^*	31.54 ± 0.76^{A}	28.72 ± 0.39^{B}	21.98 ± 0.64^{D}	27.49 ± 0.25^{BC}	26.57 ± 0.49^{C}	28.53 ± 0.36^{B}	27.65 ± 0.40^{BC}
weight loss (%):							
exudates loss	0.26 ± 0.06^{A}	0.54 ± 0.22^{AB}	1.24 ± 0.05^{C}	0.57 ± 0.10^{AB}	0.88 ± 0.10^{BC}	0.52 ± 0.09^{AB}	0.76 ± 0.06^{BC}
cooking loss	11.20 ± 0.61^{A}	13.88 ± 0.95^{AB}	18.41 ± 1.89^{C}	14.75 ± 1.34^{AB}	16.51 ± 1.54^{BC}	14.45 ± 1.14^{AB}	15.86 ± 0.85^{BC}
shear force (kg)	3.00 ± 0.15^{A}	3.82 ± 0.07^{B}	4.57 ± 0.08^{D}	3.79 ± 0.08^{B}	4.08 ± 0.06^{C}	3.63 ± 0.06^{B}	3.68 ± 0.06^{B}
Sensory attributes:[b]							
Colour	7.87 ± 0.15^{A}	7.83 ± 0.15^{A}	7.38 ± 0.28^{D}	7.77 ± 0.15^{A}	7.53 ± 0.12^{A}	7.80 ± 0.10^{A}	7.67 ± 0.12^{A}
Odour[†]	7.97 ± 0.15	7.93 ± 0.25	7.90 ± 0.10	7.87 ± 0.06	7.83 ± 0.06	7.90 ± 0.17	7.87 ± 0.21
Overall	7.97 ± 0.12^{A}	7.90 ± 0.10^{AB}	7.50 ± 0.17^{C}	7.83 ± 0.12^{AB}	7.77 ± 0.15^{B}	7.83 ± 0.12^{AB}	7.80 ± 0.17^{B}

[a] Lau: lauric acid, ML: monolaurin, and LA: lactic acid.
[b] Values correspond to mean data ± standard deviation.
[A–E] Different letters in each row are significantly different ($P < 0.05$).
[†] Odour scores are not significantly different ($P > 0.05$).

acceptability ($P < 0.05$). However, the colour, odour, and overall acceptability of all samples were satisfactory; exhibiting scores in a range of 7.38–7.87, 7.83–7.97, and 7.50–7.97, respectively (Table 2). Thus antimicrobials in combination were optimal because they were effective in reducing microbial at lower concentration but less effective in physical and sensory qualities of loin. Antimicrobial compounds, used as food preservatives, often alter flavour of products. Therefore, it is advisable to determine antimicrobial efficacy at sufficiently low concentrations so as not to decrease the organoleptic acceptability of food[17]. However, the colour and overall acceptability scores of the lactic acid treated samples were lower than those of other treated samples (Table 2) but scores for all treated samples were higher than 7 (moderately acceptable). At low lactic acid concentrations, the discolouration effect is small[46] and 2% (v/v) lactic acid sprayed on deboned meat does not produce any noticeable bleaching effect[47].

TPC and *S. aureus* on pork loin stored at 4 and 15 °C

The results revealed that the use of lauric acid and monolaurin in combinations with lactic acid reduced TPC and *S. aureus* count on pork and the effect is higher at low storage temperatures (Fig. 1). TPC of pork dipped in both lipids in combination with lactic acid decrease by 0.59–1.00 log CFU/g before storage and growth was retarded throughout the 8 and 1-d storage time at 4 and 15 °C, respectively. At the end of the 8-d storage time at 4 and 15 °C, TPC on pork treated with non-treated and water (control) were in the range of 1.19–1.48 and 1.43–1.75 log CFU/g, respectively, higher than the TPC counts on pork treated with both lipids in combination with lactic acid. On the other hand, the number of TPC increased significantly in non-treated and water treatments with increasing days for storage from 2nd to 8th day at 4 °C and 0th to 8th day at 15 °C (Fig. 1a). *S. aureus* of pork dipped in lauric acid and monolaurin in combination with lactic acid decreased by 1.25–1.31 log CFU/g before storage and growth was retarded throughout the 8 and 4-d storage time at 4 and 15 °C, respectively. At the end of the 8-d storage time at 4 and 15 °C, TPC on pork treated with non-treated and water (control) were in the range of 1.76–2.36 and 1.46–2.02 log CFU/g, respectively, higher than the TPC counts on pork treated with both lipids in combination with lactic acid. On the other hand, the number of TPC increased significantly in non-treated and water treatments with increasing days for storage from 2nd to 8th day at 4 °C and 0th to 8th day at 15 °C (Fig. 1b).

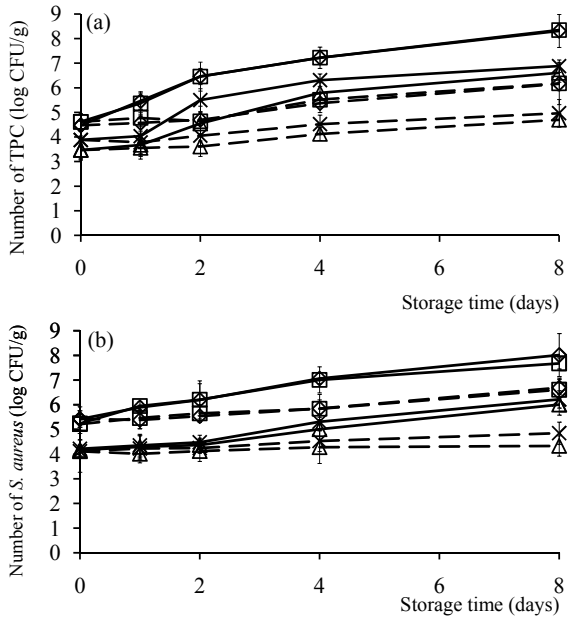

Fig. 1 Effects of the lauric acid and monolaurin in combinations with lactic acid on the (a) TPC and (b) *S. aureus* value of fresh pork loin stored at 4 °C (solid line) and 15 °C (dashed line); non-treat (lozenge), water (square), 0.2 mg/ml lauric acid + 0.1%(v/v) lactic acid (triangle) and 0.05 mg/ml monolaurin + 0.1%(v/v) lactic acid (cross).

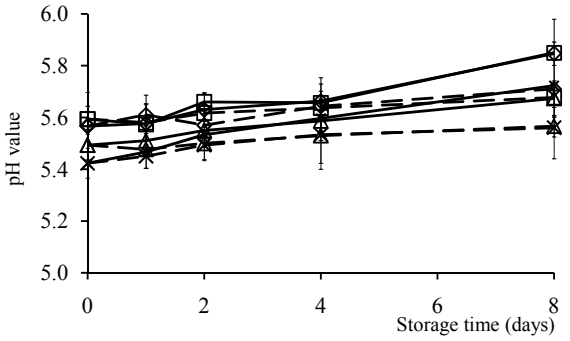

Fig. 2 Effect of the lauric acid and monolaurin in combinations with lactic acid on the pH value of fresh pork loin stored at 4 °C (solid line) and 15 °C (dashed line); non-treat (lozenge), water (square), 0.2 mg/ml lauric acid + 0.1%(v/v) lactic acid (triangle) and 0.05 mg/ml monolaurin + 0.1%(v/v) lactic acid (cross).

Physical and sensory qualities and lipid oxidations of pork loin stored at 4 and 15 °C

The pH values of fresh pork and the water used for control samples were 5.57 and 5.59, respectively. The pH values of lauric acid and monolaurin in combinations with lactic acid solution were 4.38 and 4.44, respectively. The pH values of fresh pork dipped in both lipids in combinations with lactic acid stored at 4 and 15 °C for 8 days are presented in Fig. 2. The pH values of pork dipped in both lipids in combinations and non-treated were not significantly different ($P > 0.05$) before storage. The pH of all pork increased throughout the storage time, but remained below pH 6.0 after 8 days at both storage temperatures. However, at the end of the 8-d storage time, pH values of pork stored at 15 °C were higher than that of the pork stored at 4 °C.

The L^*, a^*, b^*, and H^* values for fresh pork loin were in the range of 49.67–50.38, 6.32–7.20, 3.38–4.03, and 15.02–20.48, respectively. Changes in L^*, a^*, b^*, and H^* values of pork loin dipped in antimicrobials and stored at 4 and 15 °C for 8 days are shown in Fig. 3. The colour of pork dipped in both lipids in combinations with lactic acid and water was much lighter ($P < 0.05$) than non-treated pork. The lighter colour may be due to the decreased pH value, which results in an increase in drip loss (Fig. 4a) and higher reflecting property. Even though the L^* values of all pork increased during storage, they were not significant different ($P > 0.05$) until the end of storage at each temperature storage (Fig. 3a). The a^* values (redness) of pork dipped in both lipids in combinations with lactic acid were significantly ($P < 0.05$) lower (> 1.5 units) compared to those of non-treated and dipped in water. At the end of storage time, at 4 °C, a^* values of non-treated pork, pork dipped in water, and lauric acid in combination with lactic acid were less than 1 unit. But a^* values of pork dipped in monolaurin in combination with lactic acid were constant until the end of the 8-d storage time. At 15 °C, a^* values of control pork were less than 2 units. But a^* values of pork dipped in both lipids in combination with lactic acid were less than 1 unit until the end of the 8-d storage time (Fig. 3b). The decrease in a^* value after treat is reported to be associated with the effect of pH on the myoglobin proportion. Whereas the decrease in a^* value during storage is attributed to the oxidation of oxymyoglobin to metmyoglobin[48]. From the previous studies, there was a decrement of redness with dipped in lactic acid and the increasing of redness during storage[33,49]. For b^* value, all pork was constant until the end of the 8-d storage time (Fig. 3c). Fig. 3d shows that there was an increment of the hue angle with the storage period of the pork dipped in lipids in combination with lactic acid and stored at 4, 15 °C. It also indicates that the H^* value given by $\tan^{-1}(b^*/a^*)$, increased with the

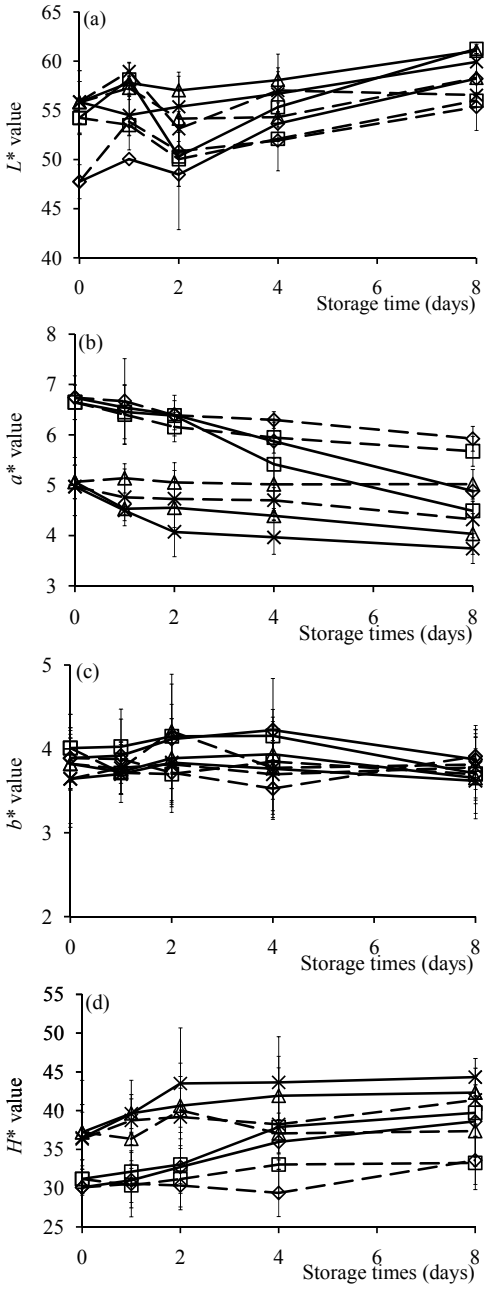

Fig. 3 Effect of the lauric acid and monolaurin in combinations with lactic acid on the (a) L^*, (b) a^*, (c) b^*, and (d) C^* values of fresh pork loin stored at 4 °C (solid line) and 15 °C (dashed line); non-treat (lozenge), water (square), 0.2 mg/ml lauric acid + 0.1%(v/v) lactic acid (triangle) and 0.05 mg/ml monolaurin + 0.1%(v/v) lactic acid (cross).

increasing storage temperature and period.

Changes in drip and cooking loss of pork loin dipped in antimicrobials and stored at 4 and 15 °C for 8 days are shown in Fig. 4. The drip loss of

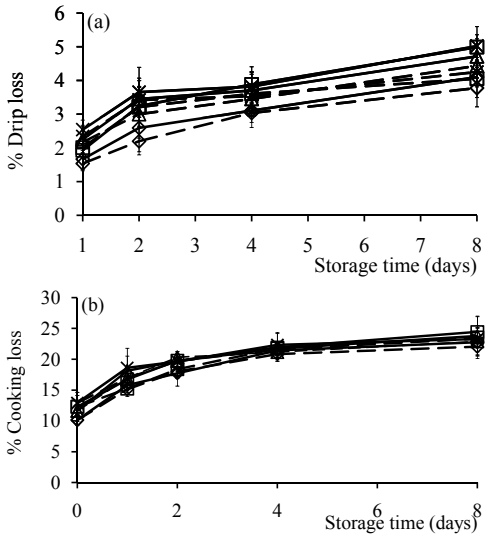

Fig. 4 Effect of the lauric acid and monolaurin in combinations with lactic acid on the percentage of (a) drip and (b) cooking loss of fresh pork loin stored at 4 °C (solid line) and 15 °C (dashed line); non-treat (lozenge), water (square), 0.2 mg/ml lauric acid + 0.1%(v/v) lactic acid (triangle) and 0.05 mg/ml monolaurin + 0.1%(v/v) lactic acid (cross).

pork dipped in both lipids in combinations with lactic acid and water was lighter ($P < 0.05$) than non-treated. After storage at 4 and 15 °C, drip loss of all pork increased with the increasing storage period ($P < 0.05$). However, cooking loss of all pork was no significant difference though out storage time ($P > 0.05$).

For Fig. 5, there was small change in shear force during storage at 4 °C but large changes during storage at 15 °C. Changes in shear force represent a balance between proteolysis and connective tissue development[50]. This would correspond to protein degradation due to microbial and enzymatic activity[51]. In the present study the trend for shear force to decrease with amount of TPC is opposite to what might be expected if proteolysis and increase with increasing drip loss were the major influence.

Peroxide values (PVs) are a measure of lipid oxidation assessed by the production of hydroperoxides, primary oxidation products[52]. The PVs of the fresh loin pork are shown in Fig. 6a, and ranged from 1.14–2.75 meq of peroxides of kg of meat during storage. At both storage temperatures, PVs of all pork increased with the increasing storage period. Similarly, TBARS values of all pork increased with the increasing storage period at both 4 and 15 °C (Fig. 6b). TBARS values measure secondary lipid

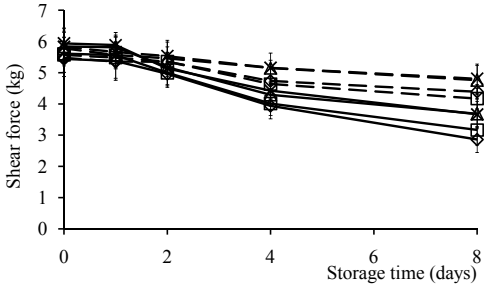

Fig. 5 Effect of the lauric acid and monolaurin in combinations with lactic acid on the shear force of fresh pork loin stored at 4 °C (solid line) and 15 °C (dashed line); non-treat (lozenge), water (square), 0.2 mg/ml lauric acid + 0.1%(v/v) lactic acid (triangle) and 0.05 mg/ml monolaurin + 0.1%(v/v) lactic acid (cross).

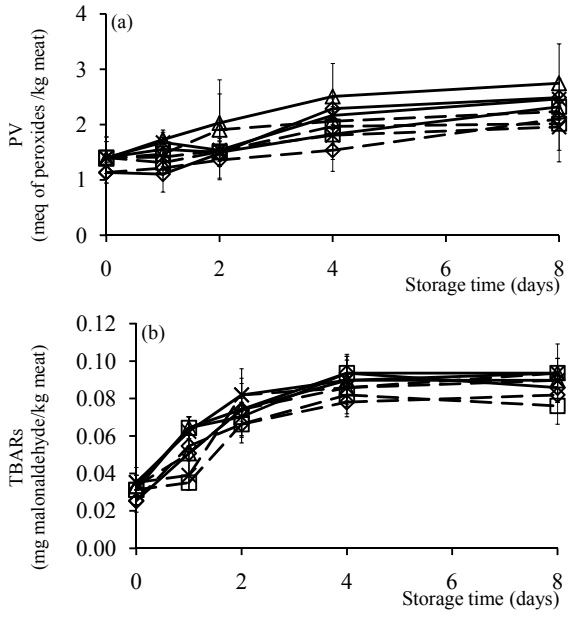

Fig. 6 Effect of the lauric acid and monolaurin in combinations with lactic acid on the (a) PV and (b) TBARs of fresh pork loin stored at 4 °C (solid line) and 15 °C (dashed line); non-treat (lozenge), water (square), 0.2 mg/ml lauric acid + 0.1%(v/v) lactic acid (triangle) and 0.05 mg/ml monolaurin + 0.1%(v/v) lactic acid (cross).

oxidation products such as aldehydes, carbonyls, and hydrocarbons, which cause off-aromas in meat. In general, TBARS values increased with increasing storage time[53]. These results of lipid oxidation analysis suggest that the antimicrobials under both temperature conditions had no effect on PVs or TBARS values.

The contribution of sensory attributes to overall

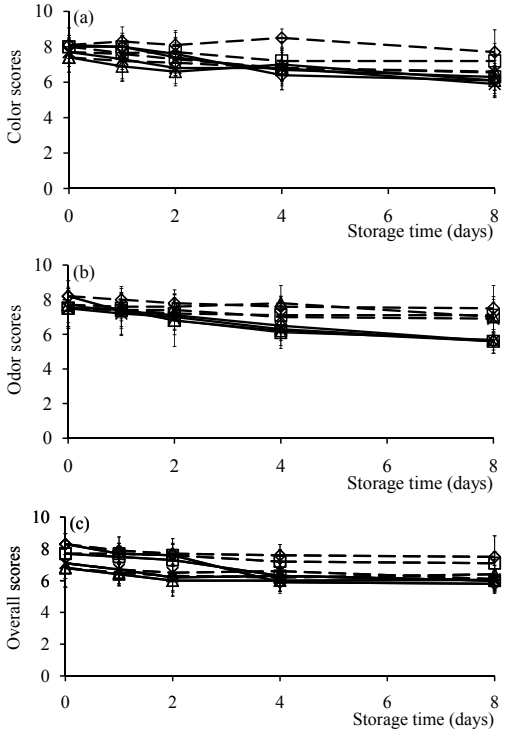

Fig. 7 Effect of the lauric acid and monolaurin in combinations with lactic acid on the (a) colour, (b) odour, and (c) overall scores cooking of fresh pork loin stored at 4 °C (solid line) and 15 °C (dashed line); non-treat (lozenge), water (square), 0.2 mg/ml lauric acid + 0.1%(v/v) lactic acid (triangle) and 0.05 mg/ml monolaurin + 0.1%(v/v) lactic acid (cross).

acceptance was studied. Fig. 7 shows the colour, odour and overall scores for fresh loin pork stored at 4 and 15 °C for 8 days. There were no significant differences in colour, odour, or overall appearance at same storage temperature. However, the pork stored at 4 °C had higher odour score than that stored at 15 °C ($P < 0.05$).

From the above results and discussions it can be concluded that lauric acid and monolaurin in combinations with lactic acid application in the fresh meat could reduce the TPC and *S. aureus* loads, but a better effects could be observed with 0.05 mg/ml monolaurin in combination with 0.1%(v/v) lactic acid at 4 °C. However, there was a significant loss of drip and cooking weight, lightness, and redness colour. Moreover, lipid oxidation values increased with increasing storage period. Therefore, to enhance the effect of lauric acid and monolaurin in combinations with lactic acid application in fresh meat packed in MAP, suitable weight loss, colour and lipid stabilizer need to be

sought.

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