International Food Research Journal 27(6): 1067 - 1075 (December 2020)
Journal homepage: *http://www.ifrj.upm.edu.my*

Application of probiotic strains to extend shelf-life of marinated beef and pork meats

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Article history

Received: 11 March 2020
Received in revised form: 24 June 2020
Accepted: 18 August 2020

Keywords

probiotic strains, mesophilic, psychrotrophic, marinade, inoculation, spoilage

Abstract

The present work was conducted to determine the antimicrobial performance of three commercial probiotic strains: *Lactobacillus curvatus* TISTR 938, *L. sakei* TISTR 890, and *L. delbrueckii* TISTR 892, at log 8 CFU/mL, in comparison with nisin, to extend the shelf-life of marinated beef and pork meats stored for 10 d at 4°C. The spoilage bacteria in the meat samples were determined on storage days 0, 3, 6, and 10. The obtained results revealed that *L. sakei* and nisin suppressed mesophilic bacteria in marinated beef by an average of log 1.7 and 1.5 CFU/g, and in pork by log 1.5 CFU/g, as compared to the control (non-marinated sample) at the end of storage period. Nisin and *L. sakei* inhibited psychrotrophic bacteria at an equivalent level by an average of log 1.5 CFU/g as compared to the control (non-marinated sample). Throughout the storage period, there were no significant differences in pH, colour, and a_w parameters amongst the probiotic strains inoculated samples and marinated sample. Results indicate that the utilisation of *L. sakei* probiotic culture in marinated beef and pork meats may be an important intervention to extend the shelf-life of marinated meat and an alternative bio-protective culture to nisin.

Introduction

For decades, beef and pork meats are among the most consumed meat globally. The demand is growing steadily as a result of the increase in population. To satisfy the demand, countless meat industries are being established across the world by private and public enterprises. However, it has also ensued in the prevalence of adverse effects of food contamination as a result of poor preparation, handling, and preservation methods which has increased the risk of opportunistic harmful microorganisms to inhibit the food, thereby, adversely affecting the shelf-life and quality of meats and meat products (Patsias *et al.,* 2006).

In relation to the numerous factors that affect the shelf-life of raw meats, the growth of microorganisms and metabolic activities are regarded as the major causes of spoilage which are normally manifested as visible growth (colonies, slime), off-flavours, and textural changes (Gram *et al.,* 2002). The rapid proliferation of microorganisms on refrigerated fresh meats triggers spoilage and the shelf-life can be further reduced by the use of inappropriate temperature conditions mainly during distribution and storage (temperature abuse). Such situations can cause substantial economic losses to the meat industries and reduced consumer confidence. For instance, according to 2017 report by the National Cattlemen's Beef Association of United States, annually, beef spoilage results in an economic loss of approximately 1 billion dollars over there. In order to lessen such economic losses, both small- and large-scale meat industries are searching for natural preservation methods that can effectively provide meat products with a longer shelf-life, and at the same time satisfy the consumers' needs for meats of high quality, improved flavour, and health benefits.

To meet such consumers' demands, many meat enterprises are marinating fresh meats to enhance their quality traits such as tenderness, flavour, and water-holding capacity. The marination technique of fresh meat is done by either tumbling, injecting, or soaking the meat with an aqueous solution composed of different ingredients (Gamage *et al.,* 2017). Meat enterprises are focussing in products with longer shelf-life, quality organoleptic properties, improved tenderness, and containing explicit nutrients to meet consumer' preference (Fadda *et al.,* 2010). A food acidulates, such as citric acid, are usually used in meat marination to improve the water holding capacity and the tenderness of the meat muscle (Ke *et al.,* 2009). However, the application of marination technique alone is not sufficient to extend meat shelf-life. Therefore, the addition of other natural bio-preservatives such as lactic acid bacteria (LAB) is imperative to increase the meat shelf-life. The capacity of probiotic

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bacteria or LAB to preserve foods is accredited to their characteristics of producing anti-microbial metabolites like bacteriocins and organic acids (Fadda *et al.,* 2010). The mechanisms used by LAB to inhibit spoilage and pathogenic microorganisms are interconnected. LAB release antimicrobial compounds that have a direct inhibition on several target pathogens. They have the ability to secrete organic acids such as acetic and lactic acids which lead to a decrease in the pH of the environment, making the microenvironment acidic; thus, eliminating pathogens that cannot survive acidic conditions. LAB also produce other metabolites with antibacterial properties such as bacteriocins, which are small antimicrobial peptides secreted to compete with other bacteria in a natural ecosystem. Bacteriocins act as colonising peptides that facilitate the penetration of probiotics into an occupied niche; hence, inhibiting the interaction of harmful bacteria. They also act as killing peptides, by directly affecting the pathogens.

The fermentative metabolism of LAB inhibits the growth of spoilage and pathogenic microbes by acidifying the product, as well as, enhancing the texture and colour stabilisation of the fresh meat (Ercolini *et al.,* 2009). Numerous studies have reported the effectiveness of marination in improving palatability and tenderness of the meat. However, limited data are available on the utilisation of LAB to enhance the microbiological quality of marinated raw meat to extend its shelf-life. Through numerous previous studies, *Lactobacillus sakei* and *L. curvatus* have been associated with inhibition of spoilage and pathogenic bacteria in fresh meats. Additionally, *L. delbrueckii* subs. *bulgaricus* has been extensively applied in fermented milk as a starter culture. However, no research information is available on application of these specific probiotic strains in marinated meat and comparison of their inhibitory effect on spoilage bacteria with the commercial nisin that is approved by the Food and Drug Administration (FDA).

The scope of the present work was therefore to conduct an *in vitro* study on the antimicrobial effects of three commercial probiotic strains namely *L. curvatus* TISTR 938, **L. sakei** TISTR 890, and *L. delbrueckii* TISTR 892 in comparison with the commercial nisin to extend the shelf-life of marinated beef and pork meats.

Materials and methods

Preparation of meat sample

Fresh beef steak and pork loin meats (< 48 h post-mortem) were purchased from a commercial slaughterhouse (Khon Kaen slaughterhouse, Thailand), immediately stored in the icebox, transported to the microbiology laboratory of Faculty of Technology, Khon Kaen University, and refrigerated at 4°C. Prior to slicing, the dissection area and knives were cleaned using detergents and disinfected with ethanol to prevent microbial contamination. The meats were cut into 2 cm^3 dimension (each 10 g), and trimmed off excess fat and membranes.

Meat marination process

Eight samples of each type of meat (beef or pork) were sealed independently in plastic bags, and then stored at 4°C for use as a negative control (without marinade or any treatment) and labelled (S1). Forty samples of each type of meat (beef or pork) were marinated by immersing them into a marinade that was prepared in a disinfected plastic bowl comprising 100 mL of commercial thin soy sauce (11% salt content), and mixed with pepper, garlic, oregano, rosemary, and chili (each 0.8 g). The samples were marinated in a single bowl to ensure homogeneity of the marinade on all the meat sample surfaces. Afterward, the bowl with the marinated samples was covered with a lid and stored in a refrigerator at 4°C for 5 h for the marinade to penetrate into the meat matrix.

Preparation of nisin

Nisin solution was prepared from nisin powder containing 2.5% purified nisin (AmbicinTM, Applied Microbiology, New York, USA). It was solubilised in sterilised distilled water at 1 mg/mL (pH 6.0), and stored at 4°C. Prior to experiments, the stock solution was thawed to 25°C (Gálvez *et al.,* 2008).

Preparation of probiotic culture

Commercial probiotic strains *L. curvatus* TISTR 938, *L. sakei* TISTR 890, and *L. delbrueckii* TISTR 892 were re-grown in a 5 mL of selective culture medium (De Man, Rogosa, and Sharpe broth). The strains were incubated at 37°C for 24 ± 1 h, then a loopful of culture was streaked on a plate with MRS agar and 1% $CaCO_3$. Afterward, the plates were incubated at 37°C for 24 ± 1 h, and single colonies were identified proving the probiotic culture was pure.

A single colony from the plate of each strain was inoculated into the tubes containing 10 mL of MRS broth, and incubated at 37°C for 24 ± 1 h. After incubation, each culture was poured into 90 mL MRS broth and incubated at 37°C until their respective stationary phases (identified after determining the growth curve of each strain to get the desired population of 10^8 CFU/mL). Prior to the addition of

the strains into meat samples, 1 mL of each culture was centrifuged at 10,000 *g* for 1 min to harvest the cells. Collected cell pellets were washed twice, and re-suspended into 0.1 mL in 0.85% NaCl (Goswami *et al.,* 2017).

Preparation and inoculation of marinated meat

After the marination period, each sample was transferred into an individual plastic bag. The samples were sub-divided into three groups, one group with marinade only (positive control) labelled (S2). The second group was mixed with 0.1 mL of nisin solution layered on the meat surface (S3). The other group was incorporated with 0.1 mL of each probiotic culture containing a population of 10^8 CFU/mL layered on the meat surface, and labelled (Ls, Lc, and Ld). All samples were sealed in plastic bags and stored at 4°C for 10 d. The biological and physicochemical analyses were done on storage day 0, 3, 6, and 10 of the samples. Analysis for day 0 was done after overnight storage of samples, to ensure sufficient time for probiotic culture and nisin to interact and have effects on spoilage microbes found in the meat samples.

Physicochemical analyses

pH measurement

The pH values of the samples were measured using a glass electrode pH meter (Model HI-9125, HANNA instruments, Cluj-Napoca, Romania). Each stored meat sample was blended, and 5 g of the sample was weighed and mixed with 95 mL of distilled water before inserting the electrode. For precise pH readings, the pH meter was calibrated at 7 ± 0.5 pH level prior to use, and was adjusted to the temperature of the tissues to be measured. After every measurement, the electrode was rinsed with distilled water. pH was analysed during the storage day 0, 3, 6, and 10 for each individual sample in duplicate (Saldaña *et al.,* 2009).

Colour measurement

Colour was determined using a calorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA; Illuminant D65, 2.54 cm diameter aperture, 10° standard observer) during storage day 0, 3, 6, and 10 at 4°C, and stated as a*, b*, and L* values. a* refers to the chromatic scale from green to red, b* to the chromatic scale from blue to yellow, and L* to the measure of lightness. The equipment was standardised using a white tile and black glass. The total colour change (ΔE*) was calculated using the following formula (Karamucki *et al.,* 2013):

$$\Delta E^* = \sqrt{\Delta L * 2 + \Delta a * 2 + \Delta b * 2}$$

The differences in *ΔE** were subdivided into six levels based on the colour difference classification by the National Bureau of Standards (NBS) (Jeong et al., 2018).The differences in ΔE* among the samples were classified as 0 - 0.5 (trace), 0.5 - 1.5 (slight), 1.5 - 3.0 (noticeable), 3.0 - 6.0 (appreciable), 6.0 - 12.0 (much), and 12.0 or more (very much).

Water activity (a_w) measurement

The a_w was measured at 25°C using Lab Master a_w (Novasina; Lachen, Switzerland). The meat sample was placed inside a hermetically closed small can-like container. Through the evaporation process, an equilibrium of humidity in the small airspace above the product and the humidity of the sample was directly measured by means of a hygrometer built in the lid of the instrument. Pure water (representing 100% free water) is equivalent to a_w-value of 1, all other food samples have lower a_w values than 1 depending on their free water content to give the a_w value in the meat sample. The a_w values were measured during the storage day 0, 3, 6, and 10 for each individual sample in duplicate (Yang *et al.,* 2009).

Microbiological analysis

Ten grams of each meat sample (S1, S2, S3, Ls, Lc, and Ld) was used for the viable counts and isolation after storage on day 0, 3, 6, and 10 at 4°C. Each sample was aseptically weighed and homogenised in 90 mL of buffered peptone water (Oxoid, UK) at a concentration of 1% (w/v) for 2 min in a stomacher (LAB blender 400; PBI, Italy) at room temperature using a speed of 300 rpm. Then decimal dilutions were prepared in the same solution, and aliquots of 0.1 mL of the appropriate dilutions were plated in triplicate on plate count agar (PCA; Oxoid, UK), and incubated aerobically at 30°C for 48 ± 3 h for mesophilic microorganism counts, and 7 ± 1°C for 10 d for psychotropic microorganism counts. Colonies were recorded in duplicates.

Survival of probiotic strains

Ten grams of each marinated beef and pork meat samples treated with the probiotic strain (Lc, Ls, and Ld) were used for determining the survival of probiotic strain by viable counts during sample storage on day 0, 3, 6, and 10 at 4°C. Each sample was aseptically weighed and homogenised in 90 mL of buffered peptone water (Oxoid, UK) at a concentration of 1% (w/v) for 2 min in a stomacher (LAB blender 400; PBI, Italy) at room temperature using a speed of 300 rpm. Decimal dilutions were prepared in the same solution, and aliquots of 0.1 mL

of the appropriate dilutions were spread plated in triplicate on MRS agar and 1% CaCO3. The plates were incubated at 37°C for 48 ± 3 h, and colonies were recorded in duplicates.

Statistical analysis

A one-way analysis of variance (ANOVA) using split-plot experimental design was used to analyse the data (sample treatments was the main plot and storage day was the subplot) using the SPSS program (version 17.0). Means and standard errors were calculated and a probability level of $p < 0.05$ was used in testing the statistical significance of all experimental data. Duncan multiple range tests were used to determine the significance of mean values for multiple comparisons at $p < 0.05$.

Results and discussions

Microbiological analysis

Bacteria related to meat and meat products spoilage are primarily mesophilic and psychrotrophic. They normally cause defects on meat quality such as discoloration, sour off-flavours, slime production, gas production, and decrease in pH. The inhibition of spoilage bacteria is imperative to prolong the shelf-life of meat especially raw meats, which are predominantly susceptible to bacterial spoilage. The present work analysed the mesophilic and psychrotrophic in marinated beef and pork meat samples stored for 10 d at 4°C inoculated with different treatments, and using the non-marinated meat sample as a control.

Figures 1A and 1B show the effects of marinade and treatments on mesophilic bacteria during the 10-d storage of beef and pork meat samples at 4°C. In both beef and pork meat samples, the control (non-marinated) sample showed the highest number of mesophilic bacteria count in all days, followed by marinated sample (positive control). There was a significant difference ($p < 0.05$) in number of mesophilic bacteria between non-marinated and marinated meat samples throughout the storage period in both beef and pork, except on storage day 0 for pork that showed no significant difference on the marinated and non-marinated samples. Sample inoculated with *L. sakei* probiotic culture was able to suppress mesophilic bacteria significantly higher ($p < 0.05$) at the end of storage period when compared with *L. curvatus* and *L. delbrueckii*. Nisin exhibited stronger inhibitory effects on mesophilic bacteria at the end of the storage period as compared to the other treated samples, except in *L. sakei* marinated beef meat

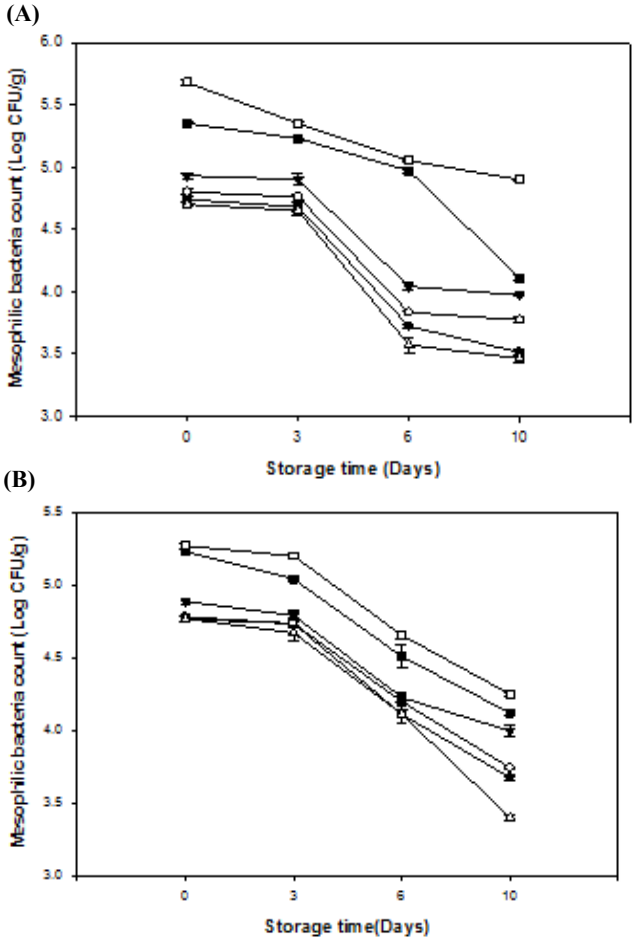

Figure 1. Antimicrobial effects of nisin, *L. sakei, L. curvatus, L. delbrueckii,* marinated, and non-marinated (control) samples on growth of mesophilic spoilage bacteria on (A) fresh beef and (B) pork meat samples, under normal packaging stored at 4°C for 0 to 10 d.

sample ($p < 0.05$). From Figure 1A, it can be seen that *L. sakei* had a stronger inhibitory effect on mesophilic spoilage bacteria on beef meats than *L. delbrueckii* at the end of the storage period ($p < 0.05$). At the end of storage period, both nisin and *L. sakei* reduced mesophilic bacteria in pork meat samples at the same rate ($p > 0.05$) (Figure 1B). Similar results were reported by Ercolini *et al.* (2009) and Chaillou *et al.* (2014) that LAB could inhibit the growth of spoilage-related bacteria in raw pork loin meat, cooked ham, raw beef, and other meat products because of its ability to generate a wide array of antimicrobial metabolites such as organic acids.

Likewise, Alvarado and McKee (2007) reported that *Lactobacillus* strains isolated from traditional Mexican foods are able to inhibit the spoilage microorganism, while working on food-related lLAB containing antimicrobial potential from traditional foods. The primary antimicrobial effects exerted by LAB is due to a combination of many factors, e.g., production of lactic acid with

reduced pH, and also production of numerous antimicrobial substances that can be categorised either as high-molecular-mass compounds like bacteriocins, which are responsible for the most antimicrobial activities or low-molecular-mass compounds, for instance, hydrogen peroxide and carbon dioxide (Ahmadi and Moreno, 2013). Bacteriocin's antimicrobial activity happens in stepwise-adsorption of the bacteriocin on the cell wall, it then moves through the cell membrane, and at the end of its action the effect occurs within the cytoplasm (Garcha and Sharma, 2013).

From Figures 2A and 2B, it can be seen that after marinated beef and pork meat samples were inoculated with *L. delbrueckii, L. sakei,* and *L. curvatus* for 10 d at 4°C, there was no significant difference in the survival of the three probiotic strains on storage day 0 ($p > 0.05$). In beef meat samples, the three strains also had no significant difference on storage day 3. On storage day 6, *L. sakei* and *L. curvatus* survival had no significant difference ($p > 0.05$). In all meat samples, *L. sakei* survived significantly at a higher level on storage day 10 when compared with *L. curvatus* and *L. delbrueckii* ($p < 0.05$). On storage days 6 and 10, *L. curvatus* significantly revealed a higher number of the surviving cells than *L. delbrueckii* on both beef and pork samples ($p < 0.05$).

Correspondingly, in pork treated meat samples, there was no significant reduction in the number of the surviving cells for both *L. sakei* and *L. curvatus* between storage days 3 and 6. There was a significant decrease in *L. delbrueckii* throughout the entire storage period of beef and pork meats. Similar results were reported by Chaillou *et al.* (2014) who revealed that *L. sakei* had the capability to lower the arginine to avoid cell death after the depletion of glucose, which plays a crucial role in the survival of *L. sakei* on meat as compared to *L. curvatus*.

The ability of *L. sakei* to display a stronger survival traits in the meat samples than *L. curvatus* and *L. delbrueckii* is in line with Champomier-Vergès *et al.* (2001) who pointed out that the predominance of *L. sakei* in diverse surroundings indicates its potential to adapt to different environments. For instance, it is able to proliferate in the presence of high salt concentrations (up to 9% sodium chloride) and at refrigeration temperatures.

Microorganisms growing on the meat stored at chill temperatures are considered as psychrotrophic. They belong to both Gram-negative bacteria, such as *Enterobacteriaceae* and *Pseudomonas* species, and Gram-positive, mainly LAB. *Pseudomonas* species are predominantly spoilage bacteria of meat

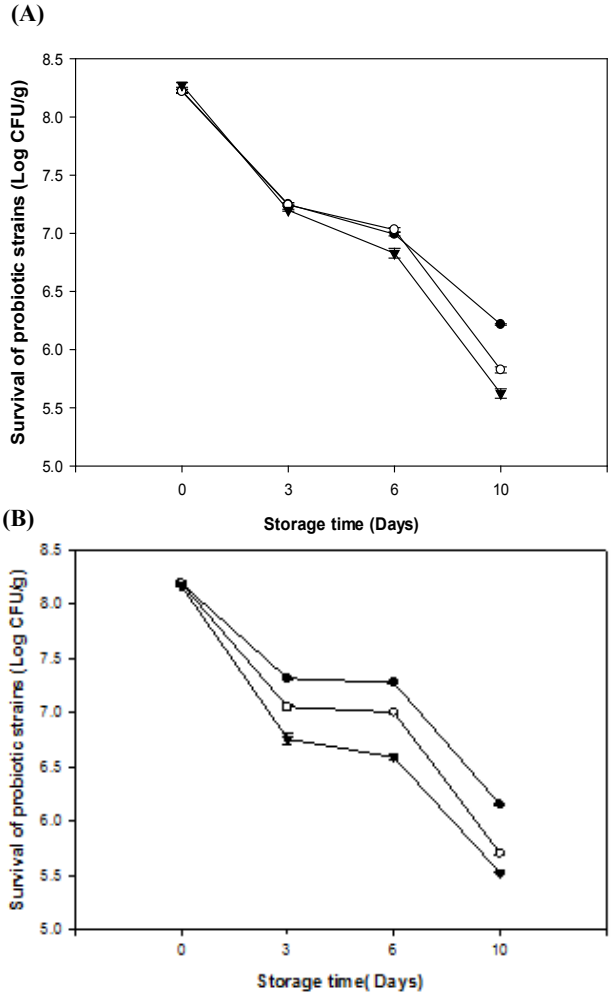

Figure 2. Survival of probiotic strains (*L. sakei, L. curvatus,* and *L. delbrueckii*) inoculated into (A) marinated beef and (B) pork meat samples under normal packaging stored at 4°C for 0 to 10 d.

stored at chill temperatures (Ercolini *et al.,* 2009).

From Figures 3A and 3B, in both beef and pork meat samples, nisin, *L. sakei*, and *L. curvatus* had no significant inhibitory effects on psychrotrophic bacteria on storage day 0 ($p > 0.05$). At the end of the storage period in beef meat treated samples, nisin and *L. sakei* presented the highest reduction in number of psychrotrophic bacteria at an average of log 1.5 CFU/g ($p < 0.05$) when compared with the non-marinated (control) sample.

In pork meat, nisin, *L. sakei*, and *L. curvatus* exhibited the highest reduction of psychrotrophic bacteria at an average of log 1.5 CFU/g ($p < 0.05$) when compared with the non-marinated (control) sample. Also, *L. delbrueckii* suppressed a significantly fewer number of microorganisms than nisin, *L. sakei,* and *L. curvatus*. The marinated meat samples showed a significant stronger antimicrobial effect than non-marinated samples during the entire storage period. The inhibition of microorganisms' development on raw marinated pork meats during the

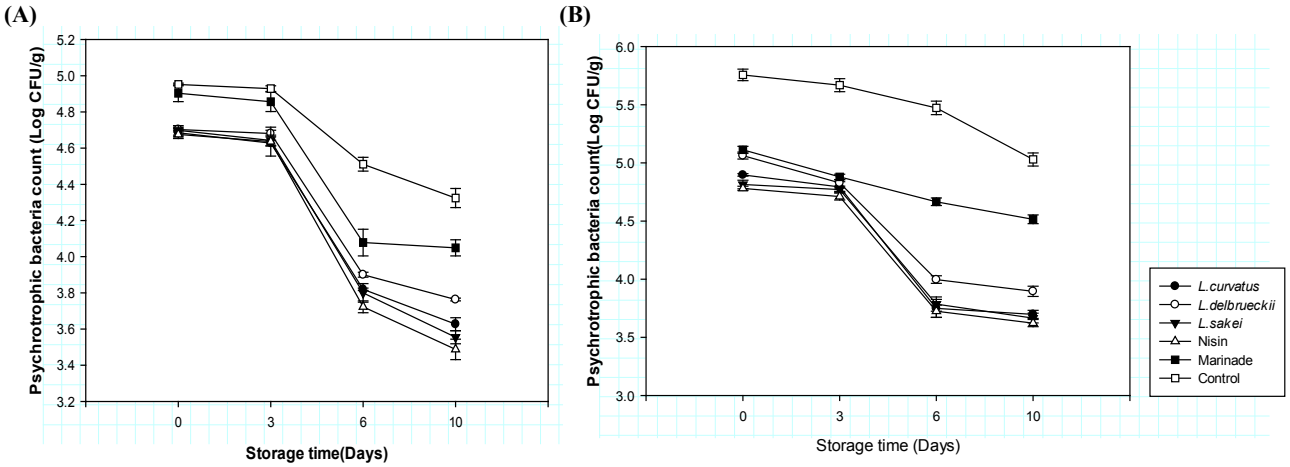

Figure 3. Antimicrobial effects of nisin, *L. sakei, L. curvatus, L. delbrueckii,* marinated, and non-marinated (control) samples on the growth of psychrotrophic spoilage bacteria on (A) fresh beef and (B) pork meat samples under normal packaging during storage at 4°C for 0 to 10 d.

storage period could be due to the marinade sipping into the meat matrix (Saldaña *et al.,* 2009). The content of the marinade such as fermented soy sauce contains tartaric isoflavone derivatives, known as shoyuflavones, which have antimicrobial property that contributes to the suppression of the spoilage bacteria. Additionally, the use of pepper and garlic in the marinade formulation also enhances the inhibitory activity due to the antimicrobial properties of piperine in black pepper and allicin in garlic (Kargiotou *et al.,* 2011).

Physiochemical analysis

There was a significant difference in the pH values between non-marinated samples and marinated samples during the storage period of beef and pork meat samples ($p < 0.05$). The control samples (non-marinated) retained a higher pH of 6.12 in beef, and 5.22 in pork meat at the end of the storage period (Table 1). In beef meat samples, all samples displayed a significant decrease in pH value between storage day 0 and 10 ($p < 0.05$). Throughout the storage days, all treated samples showed lower pH values than the control sample (non-marinated sample). The pH of the non-marinated pork meat sample was lower than the normal pH of pork meat, which is usually in the range of 5.4 to 5.8 pH value within 6 - 10 h after slaughter.

The final pH values of treated samples (marinated, nisin, *L. sakei*, *L. curvatus*, and *L. delbrueckii*) were lower than the control sample. This finding is in agreement with the previous study by Zhang *et al.* (2018) which reported that the inoculation of fresh meat with probiotic bacteria caused a slight decrease in pH for beef, pork, and lamb meat slurry. The study claimed that it is likely that the decline in pH was due to the marinade and probiotic bacterial inoculum. Correspondingly, Mitropoulou *et al.* (2013) reported that for probiotic culture to be efficacious in bio-preservation, it should be able to survive in the meat stored at refrigeration temperatures. The culture also needs to effectively compete with the high number of native microorganisms existing in raw meat to actively inhibit them, and maintain the acceptable sensory properties of meat.

One of the major challenges during meat processing is water loss which is normally expressed as either cooking loss, cooling loss, or drip loss based on the processing stage during measurement

Table 1. pH values of beef and pork meat samples at different conditions and stored at 4°C under normal packaging for 10 d.

Storage (day)	Control (non-marinated)	Marinated	Nisin	*L. curvatus*	*L. sakei*	*L. delbrueckii*
Beef						
0	6.65 ± 0.11^{A}	5.42 ± 0.02^{C}	5.40 ± 0.24^{C}	5.43 ± 0.08^{C}	5.42 ± 0.16^{C}	5.40 ± 0.03^{C}
10	6.12 ± 0.19^{B}	5.22 ± 0.06^{D}	5.18 ± 0.32^{E}	5.17 ± 0.06^{E}	5.17 ± 0.09^{E}	5.18 ± 0.07^{E}
Pork						
0	5.36 ± 0.21^{A}	5.18 ± 0.1^{C}	5.20 ± 0.35^{BC}	5.19 ± 0.07^{BC}	5.20 ± 0.14^{BC}	5.18 ± 0.01^{BC}
10	5.22 ± 0.14^{B}	4.99 ± 0.00^{D}	4.90 ± 0.00^{E}	4.91 ± 0.07^{E}	4.90 ± 0.07^{E}	4.90 ± 0.07^{E}

Means with different superscripted letters ($^{A-E}$) indicate significant differences between treatments ($p < 0.05$).

(Gao *et al.,* 2015). From the results in Table 2, the water activity (a_w) mean values measured from beef and pork meat samples treated with marinade, nisin, *L. curvatus, L. sakei,* and *L. delbrueckii* varied significantly ($p < 0.05$) from that of the control sample. Control sample showed a higher amount during the 10 d of storage with an average of 0.965 a_w for beef, and 0.955 a_w for pork at the end of storage. The five treated samples exhibited approximately an average of 0.953 a_w for beef, and 0.948 a_w for pork on day 10. Beef meat samples treated with nisin, *L. sakei, L. curvatus*, and *L. delbrueckii* revealed no significant change in a_w from storage on day 3 until the end of storage period. All pork meat samples displayed a significant decrease in a_w between storage days 0 and 3 ($p < 0.05$). Excluding the marinated sample, all other samples revealed no significant change in a_w between storage days 6 and 10.

These results are in agreement with the previous study by Mauriello *et al.* (2005) who reported that a slight decrease in a_w of the treated samples was correlated to the decrease in pH level. Once the pH level approaches the isoelectric point of proteins for pork and beef meat, which ranges between 5.1- and 5-4, a decline in water holding capacity ensues, thus enabling dehydration and subsequently resulting in a decrease of a_w in raw pork meats. Also, Alvarado and McKee (2007) pointed out that the cause of a decrease in a_w is assumed to be protein constituents' denaturation during marination.

Meat colour is one of the key traits used to define the quality of meat (Jeong *et al.,* 2018). Based on the scale used, colour measurement is normally presented in three different colour components such as L*, a*, and b* on the CIELAB scale. These colour parameters define various physical and chemical quality traits of the meat.

The results of the colour assessment for beef and pork samples revealed that all the colour values were affected by the marinade. The treated meat samples (marinade, *L. sakei, L. delbrueckii, L. curvatus*, and nisin samples) showed lower CIE L* value (lightness) than the control (non-marinated), throughout the 10 d of storage. There was no significant difference in L* (lightness) for individual treatments during the entire storage period ($p > 0.05$). It is widely recognised that variations in muscle structure can modify light reflectance and the extent of denaturation of which the muscle proteins differ on normal and in pale meat colour. In relation to the decreased lightness in marinated beef and pork meat samples, the surface darkening of meat was primarily due to marinade ingredients, particularly garlic, which might have enhanced the binding reaction of myofibrillar and myoglobin protein. Marinated meat samples treated with nisin, *L. curvatus, L. sakei,* and *L. delbrueckii* exhibited no significant difference in L*, a*, and b* value of colour variations ($p > 0.05$) at the end of storage time. However, in all beef and pork meat samples, L* value increased at the end of the storage period.

The decrease in a* value during storage time in all samples could be due to myoglobin denaturation by interaction of marinade ingredients in the meat, and exposure to air during meat preparation. These observations are similar to the results obtained by Ke *et al.* (2009) who reported that CIE a* value (redness) is associated with the concentration of myoglobin and the degree of myoglobin denaturation. The control (non-marinated) samples had an intense red colour (higher CIE a* value) as compared to treated samples ($p < 0.05$) because there was less denaturation of myoglobin as compared to the marinated samples. Throughout the storage period, the control sample exhibited significantly higher L* and a* value as compared to treated samples ($p < 0.05$). On the contrary, the control sample showed significantly lower b* value than treated samples throughout the storage period ($p < 0.05$). Similarly, the previous study by Gao *et al.* (2015) reported that with exposure to air, the 'bright red' oxymyoglobin pigment oxidises to the 'brownish-green' metmyoglobin. Oxidation to metmyoglobin results in the decrease of a* value

Table 2. a_w values of beef and pork meat samples at different conditions and stored at 4°C under normal packaging for 10 d.

Storage (day)	Control (non-marinated)	Marinated	Nisin	*L. curvatus*	*L. sakei*	*L. delbrueckii*
Beef						
0	0.972 ± 0.002^{A}	0.963 ± 0.021^{A}	0.962 ± 0.012^{A}	0.963 ± 0.011^{A}	0.962 ± 0.004^{A}	0.962 ± 0.012^{A}
10	0.965 ± 0.081^{A}	0.954 ± 0.031^{B}	0.953 ± 0.002^{B}	0.953 ± 0.040^{B}	0.953 ± 0.013^{B}	0.953 ± 0.000^{B}
Pork						
0	0.962 ± 0.000^{DE}	0.959 ± 0.010^{A}	0.959 ± 0.002^{AB}	0.960 ± 0.010^{AB}	0.959 ± 0.011^{B}	0.961 ± 0.000^{B}
10	0.955 ± 0.013^{C}	0.948 ± 0.014^{D}	0.947 ± 0.011^{D}	0.949 ± 0.010^{D}	0.948 ± 0.003^{D}	0.948 ± 0.002^{D}

Means with different superscripted letters ($^{A-E}$) indicate significant differences between treatments ($p < 0.05$).

(redness) and the increase in b* value (yellowness), with small changes in lightness. This was an implication that marinade exerted a stronger effect on meat myoglobin denaturation that resulted in an increase in yellowness and lipid oxidation during storage.

The value of *ΔE** (total colour difference) was found to increase significantly during the storage period in all meat samples. At the end of the storage period of beef and pork meat samples, marinated samples displayed the highest *ΔE** of 10.16 and 14.60, followed by nisin of 10.03 and 12.53, respectively. Beef and pork meat samples treated with probiotic strains (*L. curvatus, L. sakei,* and *L. delbrueckii* revealed *ΔE** at an average of 10.02 and 10.41, respectively ($p > 0.05$). Non-marinated samples displayed the lowest *ΔE** in both beef and pork meats at an average of 8.49 and 7.94, respectively, at the end of the storage period. In accordance with the colour difference classification of the National Bureau of Standards (NBS) as reported by Jeong *et al.* (2018), the difference in ΔE* among the samples was classified as 0 - 0.5 (trace), 0.5 - 1.5 (slight), 1.5 - 3.0 (noticeable), 3.0 - 6.0 (appreciable), 6.0 - 12.0 (much), and 12.0 or more (very much). The total colour change column showed that beef and pork meat samples exhibited a *ΔE** > 3 at the end of the storage period. Related results were reported by Jeong *et al.* (2018) who pointed out that once ΔE* > 3, the colour differences are observable by the human eye.

The L*, a*, and b* values are only used to define or describe meat colour; however, these parameters do not disclose the origin of the observed colour in relation to the myoglobin species since the meat myoglobin species is a key determiner of changes in colour parameters. Hence, it is imperative to assess the myoglobin species as a complement of L*, a*, and b* values (O'Grady *et al.,* 2001). Thus, it can be deduced that probiotic strains and nisin had no significant effect on colour changes of marinated pork meat. The variations in colour parameters between the non-marinated and marinated samples were the result of the marinade ingredients.

Conclusion

The present work showed that the addition of probiotic strains namely *L. curvatus* (TISTR 938), *L. sakei* (TISTR 890), and *L. delbrueckii* (TISTR 892) in marinated beef and pork meats suppressed mesophilic and psychrotrophic spoilage bacteria during the storage of meat at 4°C for 10 d. But as the storage days extended, the antimicrobial performance of the strains and their survival reduced differently. Equally, *L. sakei* displayed a stronger antimicrobial effect similar to commercial nisin that was used as a standard, and also, it maintained a higher survival in marinated beef and pork meats at the end of storage time. The efficacy of *L. sakei* to inhibit spoilage bacteria and maintain higher survival proved its capacity to extend shelf-life of marinated beef and pork meats than *L. curvatus* and *L. delbrueckii*. The spoilage bacteria inhibitory capacity of *L. sakei* in marinated meat presented that the use of bacteriocin-producing lactic acid bacteria can be an imperative way to promote meat shelf-life.

The current increase in consumer preferences, particularly on safety of marinated meat, and the use of preservation methods that contain minimal or no chemical, strengthens the fact that the utilisation of lactic acid bacteria can be an indispensable method for marinated meat preservation. Therefore, to meet such consumer needs, the application of *L. sakei* in the meat industry to extend shelf-life of marinated meat is vital and as an alternative bio-protective culture to nisin produced by *L. lactis*.

The similarity of obtained results from colour, pH, and a_w parameters of the marinated beef and pork meats treated with different probiotic strains or nisin during storage indicated that the treated samples had minimal or no effects on the chemical and physical properties of the marinated meat. Nevertheless, these effects can be detected in non-marinated meats since the physiochemical variant between the control and the treated samples was primarily due to marinade ingredients. However, to approve the organoleptic properties of the meat after storage, further sensorial evaluation is recommended.

Acknowledgement

The present work was financially supported by the Faculty of Technology, Department of Food Technology, Khon Kaen University, and Thailand International Cooperation Agency 2019.

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