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Potential use of lactic acid bacteria with bacteriocin-like activity against *Staphylococcus aureus* as dual starter cultures in Thai fermented sausage "Sai Krok Prew"

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Abstract: Three strains of lactic acid bacteria (LAB), *Pediococcus acidilactici* (CP7-3) and *Lactobacillus plantarum* (CP1-15 and CP2-11) isolated from Thai fermented sausage "Sai-Krok-Prew" were tested for their bacteriocin-like activity against foodborne pathogen, *Staphylococcus aureus*. The strains CP7-3 and CP1-15 showed the inhibitory activity against the test organism, while CP2-11 had no inhibition. The three LAB strains were further investigated for use as single and dual starter culture in Sai-Krok-Prew. With a single inoculum of either CP2-11 or CP1-15, only the number of total lactic acid bacteria in the sausage with CP 2-11 was higher than that in the other samples. However, the colony count of fungi, *Escherichia coli* and *S. aureus* were not significantly different. With the dual starters of CP7-3 and CP2-11 and of CP7-3 and CP1-15, the numbers of *S. aureus* reduced almost 1 log CFU/g. Furthermore, the counts of *Salmonella* in both inoculated sausages were reduced to an undetectable level. The reduction was 3 log CFU/g, which was significantly different from that in the control ($P \leq 0.05$). Sensory assessment indicated that most panelists gave higher scores for characteristic, taste, texture and overall likeness on the sausage with the dual starter culture CP7-3 and CP1-15 than the uninoculated sausage. Therefore, uses of the mixed cultures of CP1-15 and CP7-3 may be beneficial to both safety and characteristics of Sai Krok Prew.

Keywords: Lactic acid bacteria, bacteriocin-like activity, Sai Krok Prew, single starter culture, dual starter cultures

Introduction

Two popular traditional Thai fermented sausages from red meat are "Nham" and "Sai Krok Prew". The ingredients of the two sausages are quite similar with some differences that make each product unique. Nham contains pork rind, but Sai Krok Prew contains pork lard. In addition, the packages are different: air-tight plastic for Nham and edible casing for Sai Krok Prew (Chokesajjawatee *et al*., 2009). The fermentation period differs slightly: 3-5 days for Nham, but only 1-2 days for Sai Krok Prew (Phromraksa *et al*., 2004). Nham is usually consumed without cooking (Swetwiwatana *et al*., 2007), while Sai Krok Prew is typically grilled or fried prior to consumption (Phromraksa *et al*., 2003). Intensive researches about Nham have been conducted (Visessanguan *et al*., 2004, 2006; Swetwiwatana *et al*, 2007; Luxananil *et al*., 2009). The starter cultures for Nham production have become commercially available but relatively few manufacturers are actively using the culture to date (Valyasevi and Rolle, 2002). In contrast, only few researches about Sai Krok Prew have been published (Phromraksa *et al*., 2003, 2004). The literature focused on the sensory quality and the optimum fermentation time. The production of Sai Krok Prew is typically on a small-scale and relies on spontaneous fermentation. The food poisoning bacteria that have been stated to be dominant in Thai fermented sausages are *Staphylococcus aureus* and *Salmonella* (Valyasevi and Rolle, 2002; Chokesajjawatee *et al*., 2009). In particular, the tropical climate in Thailand is an optimum environment for bacterial growth leading to an awareness of the food safety. Thus the study on an incidence of foodborne bacteria during fermentation process of Sai Krok Prew is rather limited.

Application of bioprotective cultures and/or their bacteriocins has been considered during the past few years to enhance preservation by natural means. Several publications have reported that bacteriocinogenic lactic acid bacteria can be used as bioprotective cultures in sausage and meat manufacturing processes. Albano *et al*. (2009) reported the ability of *Pediococcus acidilactici* HA-6111-2, a PA-1 bacteriocin-producing LAB, isolated from a traditional Portugal sausage "Alheira" in inhibiting a cocktail of *Listeria innocua* strains during production and shelf-life of these products. Ruiz-Moyano *et al*. (2008) reported the potential of *P. acidilactici* and *Enterococcus faecium* isolated from Iberian dry fermented sausages, the traditional

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meat products produced in the central-west of Spain, as promising probiotic meat cultures. Dalmiş and Soyer (2008) reported that the use of a mixture of *S. xylosus* and *P. pentosaceus* as starter culture resulted in safer Turkish sausages (sucuk) due to lower pH, moisture content and water activity than non-inoculated sausages. In addition to biopreservative characteristics, bioprotective cultures may also provide the desired sensory properties and color of sausage products. Zdolec *et al*. (2008) reported that the addition of *Lactobacillus sakei* to traditional Croatian fermented sausages not only resulted in significantly higher lactic acid bacteria count and content of lactic acid, lower pH and reduced microbial counts in the final products, but also enhanced their sensory properties.

Our previous study has reported the isolation and identification of 133 LAB as well as screening for bacteriocin-like activity (BLA) from Sai Krok Prew (Phalakornkule and Tanasupawat, 2006). Only four out of 133 isolates were found to have BLA against *S. aureus* (Chavasirikunton *et al*., 2006). In this paper, the strains of homofermentative lactic acid bacteria that showed significant inhibition against *S. aureus* were further investigated. This work aims to compare the use of single and dual starter cultures isolated from the similar product of origin in controlling the population of foodborne bacteria which present during short fermentation process as well as the sensory preference of Sai Krok Prew.

Materials and Methods

Starter and indicator strains

Three starter culture strains, *L. plantarum* CP1-15, *L. plantarum* CP2-11 and *P. acidilactici* CP7-3, were previously isolated from Sai Krok Prew. The strains were identified by conventional methods and 16S rDNA sequence analysis (Phalakornkule and Tanasupawat, 2006). The strains CP1-15 and CP7-3 showed high BLA against *S. aureus* when they were cultured in tryptic soy broth with no glucose plus 2% yeast extract (TSBYE). Whereas the strain CP2-11 had no BLA on *S. aureus* (Chavasirikunton *et al*., 2006). All strains were grown on MRS agar (Himedia) at 37°C for 24 h and sub-cultured for two consecutive times. The colonies were suspended in normal saline solution (NSS), centrifuged and the settled cells were washed with phosphate buffer saline (pH 7). This washing step was repeated twice and the cells were resuspended in NSS to obtain an optical density ($OD_{600\ nm}$) of 0.5, resulting in viable cell numbers of $8 - 9 \times 10^8$ CFU/ml. The suspended cells were used as the inoculum in the inhibitory activity tests and as the starter cultures in the sausage formulation. The indicator strain used in this study was *S. aureus* TISTR 029. The overnight culture of *S. aureus* TISTR 029 on nutrient agar was suspended in NSS to obtain an $OD_{600\ nm}$ of 0.5, corresponding to viable cell numbers in the order of 1×10^9 CFU/ml.

Tests for inhibitory activity and effect of protease

The cell suspension of each lactic acid bacteria (1% v/v) was inoculated in 200 ml of MRS broth normal formula (De Man *et al*., 1960), MRS without glucose (MRS-NG) and TSBYE. The cultures were gently shaken at 40 rpm, 37°C for 24 h. The media was then centrifuged at 5000 rpm (TOMY MX301, Japan) for 15 min. The supernatants were collected and pH was measured. Thereafter these supernatants were filtered through a 0.45 µm pore-size membrane (Whatman). The inhibitory activity tests of the filtrates followed the modified methods from Parente *et al*. (1995) and Cuozzo *et al*. (2001). Briefly, half volume of each indicator cell suspension was added to the tube containing one volume of the filtrate and one volume of Mueller Hinton broth (MHB, Difco). The same indicator cell suspension was added to a control tube containing one volume of sterile MRS broth and one volume of MHB. The mixtures were incubated at 37°C. Optical density at 600 nm (OD_{600nm}) of each mixture was recorded at 24 h and 48 h after the incubation. One bacteriocin unit (BU) was defined as the amount of bacteriocin that inhibited growth of the test organism by 50% relative to the control tube (Cuozzo *et al*, 2001). The BU divided by the dilution factor of the filtrate gives BU ml^{-1} as the final unit of inhibition activity.

To analyze sensitivity to protease enzyme, the filtrates were treated with proteinase K (Sigma Chemical Co.) at final concentration of 1 mg/ml, at 37°C for 2 h, prior to mix with the indicator organisms as mentioned above. Antagonistic activity among the three LAB strains was also tested by streaking one strain on a plate poured with another strain.

Formulation of sausages

Sai Krok Prew was prepared by thoroughly mixing the following ingredients: 42% minced lean pork, 15% minced pork lard, 34% steamed rice, 6% finely chopped garlic, 1% pepper powder, 0.3% sugar and 1.5% salt. Four Sai Krok Prew samples were prepared from the same batch of ingredients: 1) only the ingredients (the control) 2) the ingredients with 125 ppm of sodium nitrite ($SKP_{nitrite}$) 3) the ingredients with an inoculation of *L. plantarum* CP2-11 cell suspension ($SKP_{CP2\text{-}11}$) and 4) the ingredients with an inoculation of *L. plantarum* CP1-15 cell

Table 1. Inhibitory activity of the LAB filtrates grown from different media against *S. aureus* TISTR 029

Sources of filtrates	BU/ml at 24 h			BU/ml at 48 h		
	MRS (pH 4.5)	MRS-NG (pH 6.5)	TSBYE (pH 6.5)	MRS (pH 4.5)	MRS-NG (pH 6.5)	TSBYE (pH 6.5)
P. acidilactici CP7-3	3.95 ± 0.03	1.52 ± 0.06	3.39 ± 0.04	4.01 ± 0.05	0.54 ± 0.02	3.66 ± 0.08
L. plantarum CP1-15	4.31 ± 0.22	0.53 ± 0.24	0.54 ± 0.13	4.00 ± 039	1.32 ± 0.93	0.18 ± 0.02
L. plantarum CP2-11	4.54 ± 0.01	0	0	4.56 ± 0.01	0	0

Reported values are the mean ± standard deviation, n = 4.
MRS-NG = MRS without glucose, TSBYE = Tryptic soy broth with no glucose + 2% Yeast extract.

suspension (SKP_{CP1-15}). To study the effect of dual starter cultures, the newly prepared batch was divided into three Sai Krok Prew samples: 1) only the ingredients (the control) 2) the ingredients with dual starters of *P. acidilactici* CP7-3 and *L. plantarum* CP2-11 ($SKP_{CP7-3+CP2-11}$) and 3) the ingredients with dual starters of CP7-3 and CP1-15 ($SKP_{CP7-3+CP1-15}$). The inoculation dose was 1 µl cell suspension/g sausage which corresponding to the final cell number of about 10^6 CFU/g sausages. The Sai Krok Prew samples were stuffed in collagen casing of diameter 3.0 cm. The stuffed casing was tied with thread every 3 cm long. Thereafter, the sausages were kept in a close container and left to ferment at room temperature (28-32°C) before being sampling for analysis everyday up to 2 days.

Analyses

Microbial analysis

Twenty-five grams of each sausage sample were homogenized in 225 ml of sterile maximum-recovery diluent (MRD, Difco) for 1 min using a Stomacher (Lab blender 400, Seward, England). Serial ten fold dilutions were prepared in sterile MRD. Each dilution was transferred to a selective agar plate or Compact Dry plate (CD, NISSUI Pharmaceutical). The number of lactic acid bacteria was determined on MRS agar supplemented with 0.02% sodium azide and 0.004% bromcresol purple after anaerobic incubation. The numbers of pathogens were determined as follows: total *Staphylococci* on manitol salt agar (MSA, Himedia Lab); *Salmonella* on xylose lysine deoxycholate agar (XLD, Difco); *S. aureus* on Compact Dry (CD X-SA); *Escherichia coli* on CD EC; yeast and mold on CD YM. All plates were incubated at 37°C for 24 – 48 h except CD YM at 30°C for 3-5 days.

pH and available water (a_w)

Ten grams of each sausage sample were homogenized with 90 ml distilled water and the pH of the suspension was measured by a pH meter (Cyberscan 510, Singapore). The ingredients that were removed from their casings and mixed thoroughly were placed in a small plastic plate (one-half full). The plate was then inserted into the Aqualab CX-2 machine (USA) to measure the available water.

Preliminary sensory assessment

The sausages selected for sensory assessment were prepared by oven grill at 200°C for 15 min. All samples were coded with random number and served to a group of untrained panelists (n=45) along with the questionnaire of liking score based on 9-point hedonic scale (9 = extremely like, 5 = neither like nor dislike, 1 extremely dislike). The samples were scored for appearance, color, odor, taste, texture and overall acceptance.

Statistical analysis

Each treatment was repeated at least twice and the measurement of optical density was of triplicate performance. The average values were reported along with standard deviations. Test of significant difference on microbial count was based on analysis of variance (ANOVA). For sensory evaluation, differences between means were compared by Duncan's multiple range test using SPSS software (USA).

Results and Discussion

Inhibitory activity of cell free filtrates in different media

The inhibition activities against *S. aureus* of the cell free filtrates are shown in Table 1. The comparisons were made between the filtrate derived from MRS broth (contained glucose), MRS-NG (no glucose) and TSBYE (no glucose). It was clear that in MRS where glucose was present the final pH dropped down to 4.5 and the inhibitory activities expressed as BU/ml remained high for all filtrates at both 24 h and 48 h of incubation. In comparison the final pH of MRS-NG and TSBYE was 6.5, while the inhibition activities only appeared in the filtrates of *P. acidilactici* CP7-3 and *L. plantarum* CP1-15. No suppression was found in the strain CP2-11. This result partly agreed with the previous finding that the BLA against *S. aureus* was detected in CP1-15 and CP7-3 but absent in CP2-11 when the isolates were grown in TSBYE

(Chavasirikunton *et al*., 2006). The neutral filtrates treated with proteinase K eliminated inhibitory effect against the indicator organism, these was to confirm that the bacteriocin in the filtrates of the strain CP7-3 and CP1-15 contribute to the suppression of *S. aureus*. It was also noticed that the filtrates from MRS-NG mostly showed lower inhibition activities than those from TSBYE, especially in CP7-3. The differences in final pH between the media (4.5 and 6.5) were due to lactic acid production via glucose catabolism in MRS, thus the detection of BU/ml in this medium was not solely due to bacteriocin production. The MRS medium is the general maintenance medium for LAB and the cell growth at 24 h was higher in MRS ($1.0 – 1.5 \times 10^{10}$ CFU/ml) than that in MRS-NG (5.0×10^7 CFU/ml) and TSBYE (1.3×10^8 CFU/ml). In addition, the types and amount of nitrogen source in MRS and TSBYE are different. MRS contains peptone 10 g/l, yeast extract 5 g/l and beef extract 10 g/l; TSBYE comprises soya peptone 3 g/l, tryptone 17 g/l, yeast extract 20 g/l. It has been reported that the degree of bacteriocin activities relate to the amount and types of nitrogen source. Parente and Hill (1992) found that the activities of enterocin 1146 and lactocin D increased with increasing level of yeast extract, but low level of tryptone (2.5 g/l). Todorov and Dicks (2006) observed the bacteriocin production of two *L. plantarum* strains and found that tryptone stimulated bacteriocin production of strain ST341D whereas a combination of yeast extract and tryptone at 1:1 ratio was required for strain ST23LD. Another study reported that *Micrococcus* sp. showed the maximum activity of micrococcocin GO5 in MRS medium containing 0.5% tryptone and 1% yeast extract as nitrogen sources (Kim *et al*., 2006). Five strains of *Enterococcus mundii* showed low or no bacteriocin activity in the medium without nitrogen sources (Settanni *et al*., 2008). The use of media without glucose in this study limited the production of organic acids in order to disintegrate the effect of pH and BLA. The production of bacteriocin by CP7-3 and CP1-15 may also be affected by the different nitrogen sources.

The use of single starter culture in Sai Krok Prew

Inoculation of single starter culture in Sai Krok Prew was compared between *L. plantarum* strains with BLA (CP 1-15) and without BLA (CP2-11). The pH changes during 2 day fermentation in Sai Krok Prew with the single starter culture (SKP_{CP2-11} and SKP_{CP1-15}), in the control and the sausage with nitrite addition ($SKP_{nitrite}$) was illustrated in Figure1. The pH of all samples dropped significantly in the first day. After 2 day fermentation, the pH of the control and of the inoculated sausages reached similar values (4.66 ± 0.06 with $P \geq 0.05$). However, the sausage with nitrite addition had significantly higher pH than the others ($P \leq 0.05$). It was explained that the lower pH in the control and in the inoculated sausages were due to the higher activities of lactic acid bacteria in these samples than those in the sausage with nitrite addition. The final pH value was similar to that in Nham, in which lactobacilli were responsible for the pH decrease to 4.6 at an early stationary phase of fermentation (Visessanguan *et al*., 2004).

The alteration of microbial numbers during fermentation is presented in Figures 2a-2e. The initial cell numbers of lactic acid bacteria in the inoculated sausages (SKP_{CP2-11} and SKP_{CP1-15}) were in the order of 10^6 CFU/g as same as the inoculation size. While the population of lactic acid bacteria in control and $SKP_{nitrite}$ were only in the order of 10^4 CFU/g, then the LAB numbers rose up to 4 log cycle within 24h and were unchanged at 48h. However, the sausage inoculated with CP2-11 showed significantly higher LAB numbers than the other three samples ($P \leq 0.05$; Figure 2a). The main fungi in the sausages was yeast, which increased by 2 log CFU/g within 24 h (Fig. 2b). Similarly, the population of *E. coli* multiplied nearly to 2 log CFU/g in the first day of fermentation (Figure 2c). The numbers of both yeast and *E. coli* in all samples did not differ significantly ($P \geq 0.05$). The total staphylococci remained unchanged during the period of fermentation (Figure 2d), whereas the count of *S. aureus* was distinctive between each sausage sample particularly at 1 day fermentation (Figure 2e). The sausages inoculated with either starter culture indicated the slow growth of *S. aureus* since the numbers were significantly lower ($p \leq 0.05$) than those found in the control and the nitrite added sausages. In contrast, the population of *S. aureus* in the control and $SKP_{nitrite}$ slightly went up in the first day and dropped down in the second day. This was due to the decrease of pH to 4.67 and 4.85, respectively, because *S. aureus* has minimum pH of 4.0 (Jay, 1996) and that the more pH drop near to the minimum pH, the more injured cells occur. Even though the number of *S. aureus* in the sausages inoculated with the BLA starter (SKP_{CP1-15}) and non-BLA starter (SKP_{CP2-11}) was not significantly different, inoculation of the single starters created the competitive conditions which may contribute to slow down the growth of *S. aureus* as compare to the control and $SKP_{nitrite}$. Furthermore nitrite has long been applied to curing meat products as well as for the anti-microbial purpose (Honikel, 2008), thus it usually exists in the recipe of fermented sausages. Our results indicated that an addition of sodium nitrite at the quantity regulated by TISI (Thai

Industrial Standards Institute, 1994) did not retard the number of any organisms. Specifically, the number of *S. aureus* found in $SKP_{nitrite}$ was not different from that in the control and in fact was the highest among all samples in the first day.

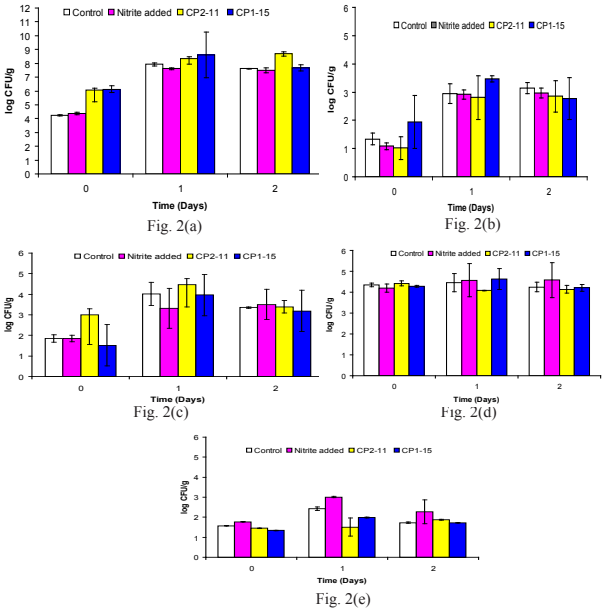

Fig. 2(a) Fig. 2(b) Fig. 2(c) Fig. 2(d) Fig. 2(e)

Fig. 2 Microbial count of Sai Krok Prew with single starter culture, the control and the sausage with nitrite addition: (a) total lactic acid bacteria; (b) Yeast; (c) *E. coli*; (d) Total staphylococci and (e) *S. aureus*. Error bars indicate standard deviation of the mean

Inhibition to pathogens in Sai Krok Prew by dual starter cultures

The antagonistic effect among the LAB strains CP2-11, CP1-15 and CP7-3 was investigated prior to use as the dual starters. No cross-reaction was obtained between the three strains. The pH changes during 2 day fermentation in Sai Krok Prew with the dual starter culture ($SKP_{CP7-3+CP2-11}$ and $SKP_{CP7-3+CP1-15}$) and in the control shows in Figure 3. The pH in both inoculated sausages was significantly lower than that in the control ($P \leq 0.05$). It also decreased faster during the period of fermentation when compared to the single culture (Figure 1). The result suggested that *Pediococcus* was the secondary acid producer. After the lactobacilli reached their late logarithmic phase, *Pediococcus* was responsible for the further pH reduction to approximately 4.2 in the second day. The values of available water (a_w) were not different among all samples; 0.95 ± 0.03 in the control, in $SKP_{CP7-3+CP2-11}$, and 0.97 ± 0.03 in $SKP_{CP7-3+CP1-15}$. The a_w in all Sai Krok Prew samples was close to that in raw meat since the fermentation period was short and was without ripening period. Ripening process that was allowed in other dry fermented sausages such as Sucuk (Turkish sausages), Varzi, Brianza and Piacentino (Italian sausages), typically resulted in a_w between 0.87 – 0.90 (Kaban and Kaya, 2006; Di Cagno *et al*., 2008).

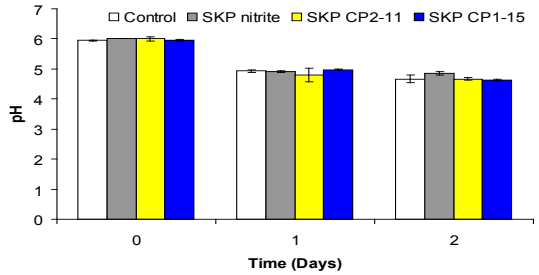

Fig. 1 Changes of pH in Sai Krok Prew with and without single culture inoculation during 2 day fermentation. Error bars indicate standard deviation of four samples

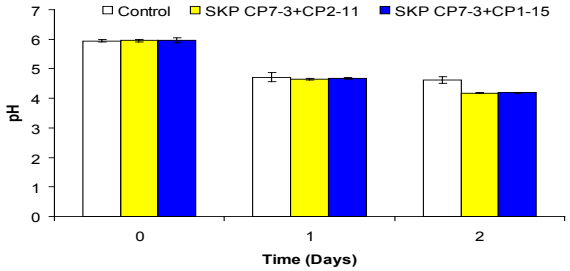

Fig. 3 Changes of pH in Sai Krok Prew with and without dual culture inoculation during 2 day fermentation. Error bars are standard deviation of 4 samples

Bacterial population changes in these sausages were illustrated in Figurs 4a-e. The LAB number in $SKP_{CP7-3+CP2-11}$ was the highest in the first day. In the second day, the LAB number in the control, $SKP_{CP7-3+CP2-11}$ and $SKP_{CP7-3+CP1-15}$ was 8.91 ± 0.07, 9.12 ± 0.07 and 9.26 ± 0.13, respectively. The sausages inoculated with the dual cultures showed significantly higher LAB ($P \leq 0.05$) than the sausage spontaneously fermented (Figure 4a) and this was concurrent to the lower pH presented earlier. The populations of *E. coli* were demonstrated in Figure 4b. The level of *E. coli* was maintained in $SKP_{CP7-3+CP2-11}$ and $SKP_{CP7-3+CP1-15}$ during 2 fermentation days, whereas a slight increase was observed in the control. This organism generally presents in fermented meat products, even persists in long ripening period, due to its ability to survive acidic condition as its habitat in the gastrointestinal tracts (Fontán *et al*., 2007). For *Salmonella* count the sausages inoculated with the dual culture showed quite promising reduction to an undetectable level within 2 days of fermentation, while it was countable in the control (Figure 4c). Furthermore the numbers of total staphylococci (Figure 4d) and *S. aureus* (Figure 4e) also clearly reduced more significantly ($P \leq 0.05$) in the sausages with dual starters than the control. Our former work published that the strains CP7-3, CP2-11 and CP1-15 did not show BLA on *Salmonella* (Chavasirikunton *et al*., 2006). Therefore the observed *Salmonella* inhibition in this case may be due to the synergistic effect of pH, lactic acid and other organic acids which were the products from

Table 2. Sensory attributes of Sai Krok Prew with and without dual starters

Fermented sausages Formulae	Appearance	Color ^{NS}	Odor ^{NS}	Taste	Texture	Overall
Control	5.6 ± 1.3^{ab}	5.8 ± 1.2	6.2 ± 1.2	5.3 ± 1.7^{ab}	5.6 ± 1.6^{ab}	5.9 ± 1.5^{ab}
$SKP_{CP7-3+CP2-11}$	5.2 ± 1.4^{a}	5.6 ± 1.3	5.9 ± 1.2	4.9 ± 1.8^{a}	4.0 ± 1.6^{a}	5.5 ± 1.3^{a}
$SKP_{CP7-3+CP1-15}$	5.9 ± 1.2^{b}	6.0 ± 1.2	6.4 ± 1.2	5.8 ± 1.7^{b}	5.8 ± 1.6^{b}	6.3 ± 1.5^{b}

Data are means ± standard deviation, n = 45
^{NS} Mean values in the same column are not significantly different ($P \geq 0.05$).
^{ab} Means with different letters in the same column indicate significant differences ($P \leq 0.05$).

the fermentation process. According to Adam and Nicolaides (1997), weak acids including lactic acid have substantial lipid solubility. The undissociated forms of the acids readily penetrate cell envelope and subsequently dissociate inside the cells. The dissociated forms can in turn cause pH imbalance. Furthermore, a large LAB population can compete with *Salmonella* for nutrients resulting in a limited growth of the latter. This observation was also supported by the study of Swetwiwathana *et al*. (2007) who reported that the inhibition of *Salmonella* in Nham model broth inoculated with *P. pentosaceus* TISTR 536, a pediocin PA-1 producing strain, caused by rapid production of lactic acid. Consequently, the significant amount of lactic acid was associated with a large pH drop that resulted in a sub-injury to *S. anatum* in the first 12 h. Thereafter, the interaction effects of antagonistic products, nitrite and fresh garlic played a role to cease the stress cells of *S. anatum*. In a study of sucuk, a Turkish sausage, the use of mixed commercial starters of LAB and either *S. xylosus* or *S. carnosus* reduced the population of *S. aureus* by 1 log cycle in 14 days during a ripening period. The population of *S. aureus* was increased in the control (Kaban and Kaya, 2006). The authors suggested that pH was an important factor in limiting the growth of this foodborne disease. Whilst acid production is dependent on the initial number of LAB, fermentation temperature and the use of sugar (Geisen *et al*., 1992).

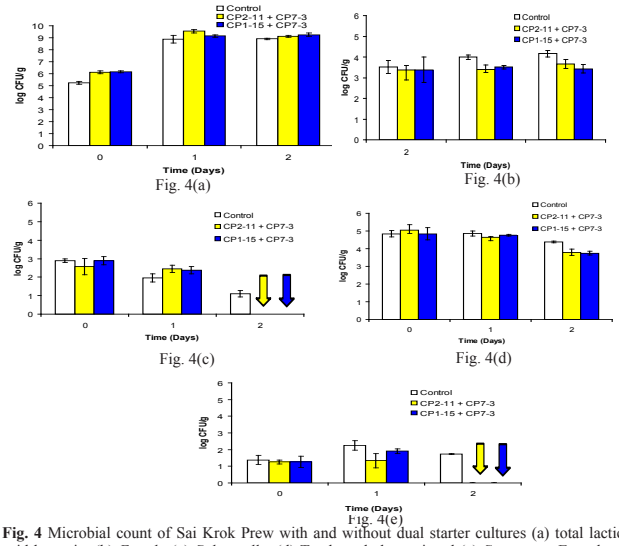

Fig. 4(a) Fig. 4(b) Fig. 4(c) Fig. 4(d) Fig. 4(e)

Fig. 4 Microbial count of Sai Krok Prew with and without dual starter cultures (a) total lactic acid bacteria; (b) *E. coli*; (c) *Salmonella*; (d) Total staphylococci and (e) *S. aureus*. Error bars are standard deviation of the mean. The arrows indicate that no colony appeared in the lowest dilution count

Liking score evaluation

The liking scores of grilled sausages evaluated by the 9 point hedonic scale method are presented in Table 2. The scores on the color and odor of the samples with and without the starters were not significantly different. However, $SKP_{CP1-15+CP7-3}$ obtained significant higher scores on appearance, taste, texture and overall likeness than those scores from the other two sausages. According to most panelists, $SKP_{CP1-15+CP7-3}$ had smooth sour taste and firmness of texture. The dual starter of CP1-15 and CP7-3 may help improve the overall characteristics of Sai Krok Prew by enhancing the acidification and proteolysis activities, in a similar fashion to *L. sakei* in the traditional Croatian fermented sausages (Zdolec *et al*., 2008).

Conclusion

The BLA of *L. plantarum* CP1-15 and P. *acidilactici* CP7-3 grown in MRS-NG and TSBYE was found to provide a restriction to the growth of *S. aureus*. Although the effect to BLA was not apparent in the system of fermented sausages, inoculation with dual starter cultures play a distinctive role in controlling the number of *Salmonella* and *S. aureus*. As the food industry does not always employ purified bacteriocins due to the requirement for a regulation approval, fermented ingredient or bacteriocin-producing culture becomes more appealing. Sai Krok Prew is an indigenous fermented food that need to be cooked before consuming, thus the uses of the mixed starters, CP1-15, CP2-11 and CP7-3, can help fasten the fermentation process (in accordance to the lower pH in 2 days), help control the number of undesired bacteria as well as help maintain the product quality of each processing batch.

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