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Effect of lactic acid bacteria powder on quality of fermented fish product (Pla-Som)

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Abstract Encapsulated powder was made from 10% of rice flour obtained the highest count of lactic acid bacteria (11.57 log CFU/g), whereas the microbial load in the powder made from10% of maltodextrin was 11.46 log CFU/g. Results showed that the incorporation of 3% encapsulated powder provided the best quality compared to a control group to decrease in pH and develop Pla-som flavor. The overall liking score for Pla-som containing 3% lactic acid powder was significantly higher (7.66) than the natural fermented Pla-som (6.87). Furthermore, encapsulated lactic acid bacteria was stored at 4 °C for 90 days. As a result, the viable count was 9.38 log CFU/g.

Keywords: Fermented fish, Spray dry, Lactic acid bacteria, Coating material

Introduction

Fermented fish is called “Pla-som” which is a traditional fermented fish product of Thailand. Pla-som is made of freshwater fish and mixed with the ingradients, such as cooked rice, minced garlic and salt then followed by fermentation around 3-5 days. The traditional production of Pla-som was based on spontaneous fermentation by microflora naturally in the raw material (Urso *et al*., 2006). Lactic acid bacteria (LAB) was found as main microorganisms in the Pla-som (Müller *et al*., 2002). During the fermentation, the lactic acid bacteria could breakdown carbohydrate and protein into organic acid which to created a unique characteristic, especially the aroma and flavour of product. Moreover, the pH value of the product was decreased at 4.5-5.0 which could lead to inhibit pathogenic and spoilage bacteria contributing to safety of the

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product (Saithong *et al*., 2010). However, quality of the end product depends on the microflora naturally in the raw material, which does not control the microbial contamination on spoilage of the product.

Today, starter cultures for fermented foods are created by screening and selecting microorganisms for adding in the fermentation system. The design ideas are based on an understanding of the physiology and metabolism of bacteria as well as their interactions with food products. (Hansen, 2002; Seifu *et al*., 2003; Kaban and Kaya, 2006; Ammor and Mayo, 2007; Hwanhlem *et al*., 2011).

Controlling the fermentation process normally used the starter culture for development of characteristic species in term of appearance, texture, taste nutritional value and safe product (Holzapfel, 1997). Using the starter cultures fermentation led to shorten the fermentation time and to inhibit pathogenic bacteria of the end product. LAB as *Pediococcus pentosaceus, Lactobacillus alimentarius/farciminis, Weisella confusa, L. plantarum* and *Lactococcus garviae* have been found in Pla-som product (Müller *et al*., 2002). *Lactobacillus plantarum* IFRPD P15 and *Lactobacillus reuteri* IFRPD P17 were selected from Pla-som product for using as a mixed starter culture in fermentation process that helped decrease fermention time and to inhibit the pathogenic bacteria such as *Escherichia coli* (Saithong *et al*., 2010).

Currently, the preparation of starter culture is produced by lyophilized preparations, freeze drying and spray drying. Spray drying is one of several processes for encapsulating microbial cells to prepare a starter culture for use in the fermented food industry. However, the heat transfer and water evaporation cause bacterial viability to be lost during the drying process. Several factors have been studied to improve quality of the starter culture powder. For example, encapsulation technology is a method to protect core material from hazard condition by means of coating them with wall materia (Weinbreck *et al*., 2010). The study investigated rice flour and maltodextrin DE 10 as wall material encapsulation to prevent deterioration of lactic acid bacteria starter during spray drying, and determined the effect of lactic acid bacteria starter powder for manufacturing fermented Pla-som.

Materials and methods

Preparations of lactic acid bacteria starter cultures

Lactic acid bacteria (*Lactobacillus farciminis*, *Lactococcus lactis*, *Lactococcus garvieae subsp. garvieae*, *Klebsiella pneumoniae*, *Lactobacillus sakei*, *Lactobacillus namurensis*, *Morganella* sp., *Citrobacter freundii*, and

Lactobacillus sp.) were prepared as starter from fermented Pla-som in spontaneous fermentation processes. LAB (Lactic acid bacteria) communities were used as cocktail starter cultures. The LAB was cultured in MRS (de Man Rogosa and Sharpe) broth (Merck, Darmstadt, Germany) consisted of 1% (v/v) of 10^8 CFU/ml at 37 ℃ for 24 h. The culture in the early stationary phase was collected by centrifugation (6000 g) for 10 min. The cell pellets were washed 3 times with 10 mL of sterile saline and then centrifuged. The lasting precipitate was suspended in 10 mL of sterile saline and was kept at 4 ℃ before spray drying. The cell concentration used was approximately 10^{10} CFU/mL (Zhao *et al*., 2008).

Preparation of lactic acid starter powder by spray drying

Rice flour and maltodextrin were used as coating material for lactic acid bacteria starter powder. The carrier solution was prepared by suspended the dry blends of rice flour and maltodextrin at 5, 7.5 and 10% of cell concentration. The mixtures were then stirred at 20°C for 20 min and immediately feed into spray drier. The lactic acid bacteria starter powder was dried at 140 °C of the inlet temperature and 70°C of outlet temperature. The spray dried powder was collected and put in sealed aluminum foil bag and stored at 4 ℃ for next study**.** Experimental data were carried out using completely randomized design (CRD). The physical and chemical characteristics as well as the microorganisms of the samples were analyzed in triplicate. The color value (L* a* b*) of powder samples was determined using a Hunter Lab Colorflex 4510 meter (Colorflex®, Hunter Association Laboratory, Inc., USA). Water activity (a_w) was measured using an a_w meter (Model Series 3TE, Aqua lab, Charpa Techcenter Co., Ltd., USA).

Preparation of fermented fish

The traditional fermented Pla-som was used as a control sample. Pla-som protocol was produced by freshwater fish. The freshwater fish was degutted, cleaned, and then, chopped into 10 cm x 5 cm/piece. After that, the sample was mixed with the ingredients (2% of sugar, 3 % of salt, 3% of cooked rice and 10% of minced garlic which was used as a control sample. The combination of lactic acid bacteria starter powder was studied at 1.5 and 3% of sample fermentation. Each fermented sample was determined for pH, and titratable acidity. The fermentation processes were run for 48 h and samples were drawn at 0, 4, 8, 12, 24 and 48 h.

Determination of viability of lactic acid bacteria starter powder

Lactic acid bacteria starter powder was counted by the standard plate count method on MRS agar plate. 1 g of each sample was serially diluted with 9 mL of peptone water and then plated on MRS agar (Merck, Darmstadt, Germany). The plates were incubated at 37 °C for 48 h. The viable cell counts were expressed as $\log_{10}$ CFU/g (Wattananapakasem *et al.,* 2018). Experiment was performed in triplicate.

Determination of titratable acidity

Increased in titratable acidity (TA) were measured according to the methods of AOAC, 2002. The titratable acidity was analyzed by titrating 25 g of the sample with 0.1 N NaOH using phenolphthalein as the indicator. In titratable acidity studies, observation was deterimined in triplicate. The amount of TA value was expressed as % acid equivalent to lactic acid (%LA) in the sample.

Sensory evaluation of fermented Pla-som

Pla-som in spontaneous fermentation process and fermented Pla-som with 1.5 and 3% of lactic acid bacteria starter culture, were tested with 30 semitrained panelists. Sensory evaluation was carried out using the randomized complete block design (RCBD). The samples were fried before testing. Sensory scores for color, flavor, texture, sourness and overall liking were evaluated using a 9-point hedonic scale. A 9-point hedonic scale, in which a score of 9=Like Extremely, 8=Like Very Much, 7=Like Moderately, 6=Like Slightly, 5=Neither Like nor Dislike, 4=Dislike Slightly, 3=Dislike Moderately, 2=Dislike Very Much, 1=Dislike Extremely, was used for evaluation.

Data analysis

Data obtained from the experiment were analyzed to find the mean and standard deviation. Significant difference values of data sets were analyzed by one-way ANOVA with Duncan's multiple rank tests at p ,0.05.

Results

Physical chemical and microbiological characteristics

The color value was expressed as L* (lightness), a* (red-green), b* (yellow-blue) values and the a_w of lactic acid bacteria starter powder were in the ranges 88.75-95.82, 0.05-0.23, 1.23-2.87, 0.190-0.203 respectively (Table 1).

In the present study, the color values between the rice flour and maltodextrin as coating material for lactic acid bacteria starter powder were significant difference ($p<0.05$). The higher value of L* value was found in rice flour when compared with maltodextrin. Moreover, the lactic acid bacteria starter powder samples had water activity (a_w) in the range of 0.190-0.205. Rice flour as coating material had lower water activity than maltodextrin ($p<0.05$). Increasing of carrier concentration affected the increase in the water activity of powder samples.

Table 1. Physical and chemical characteristics of lactic acid bacteria starter powder samples

Samples	color value			a_w
	L*	a*	b*	
5% of rice flour	95.82 ± 0.42^{a}	0.07 ± 0.05^{c}	2.51 ± 0.51^{a}	0.190 ± 0.001^{e}
7.5% of rice flour	95.03 ± 0.43^{a}	0.05 ± 0.04^{c}	2.77 ± 0.49^{a}	0.193 ± 0.001^{d}
10% of rice flour	95.54 ± 0.26^{a}	0.16 ± 0.07^{c}	2.87 ± 0.41^{a}	0.198 ± 0.001^{c}
5% of maltodextrin	88.75 ± 0.13^{c}	0.20 ± 0.15^{cb}	1.24 ± 0.33^{b}	0.203 ± 0.001^{b}
7.5% of maltodextrin	87.65 ± 0.15^{d}	0.32 ± 0.05^{a}	0.86 ± 0.22^{b}	0.204 ± 0.001^{b}
10% of maltodextrin	90.43 ± 0.34^{b}	0.25 ± 0.12^{ab}	1.33 ± 0.36^{b}	0.205 ± 0.001^{a}

$^{a-e}$ Different letter superscripts in the same column indicate statistical difference ($p<0.05$).

It was found that coliform bacteria (*Enterobacter and Citrobacter*) and *Escherichia coli* were not detected.

Table 2. Microbiological characteristics of lactic acid bacteria starter powder samples

Samples	Lactic acid bacteria (Log CFU/g)	coliform bacteria (MPN/100 mL)	*Escherichia coli*
5% of rice flour	11.49 ± 0.04^{abc}	< 3	ND
7.5% of rice flour	11.52 ± 0.05^{ab}	< 3	ND
10% of rice flour	11.57 ± 0.07^{a}	< 3	ND
5% of maltodextrin	11.38 ± 0.09^{c}	< 3	ND
7.5% of maltodextrin	11.53 ± 0.05^{bc}	< 3	ND
10% of maltodextrin	11.47 ± 0.04^{abc}	< 3	ND

$^{a-c}$ Different letter superscripts in the same column indicate statistical difference ($p<0.05$).
ND: Not detect

Viability of lactic acid bacteria

The viability of lactic acid bacteria was counted immediately after spray drying. The result demonstrated slightly higher viability in sample encapsulated with 10% of rice flour (11.57 Log CFU/g) than the other systems (11.38-11.52 Log CFU/g) (Table 3). Then, the high number of lactic acid bacteria starter powder with rice flour showed a better cell survival and storage stability than those with maltodextrin. All starter culture dried sample was not much different in the viability of lactic acid bacteria starter after spray drying.

During the storage at 4 ℃ for 90 days, it was found that the number of viable cells in the dried starter culture sample was more stable when 10% rice flour was used as a protective agent. The rice flour as carrier material showed a higher viability of lactia acid bacteria which indicated more enhance stability than moltodextrin. Especially, the use of 10% rice flour was showed the best protection of lactic acid bacteria starter powder would help them survive during drying process and its application in the fermentation process.

Table 3. Viability of lactic acid bacteria starter powder after spray drying and storage at 4 °C for 90 days

Samples	Viable count (Log CFU/g)		
	After spray dry	30 days	90 days
5% of rice flour	11.49 ± 0.04^{abc}	11.26 ± 0.02^{e}	8.79 ± 0.02^{e}
7.5% of rice flour	11.52 ± 0.05^{ab}	11.46 ± 0.01^{b}	8.93 ± 0.01^{cd}
10% of rice flour	11.57 ± 0.07^{a}	11.50 ± 0.03^{a}	9.38 ± 0.06^{a}
5% of maltodextrin	11.38 ± 0.09^{c}	10.88 ± 0.01^{f}	8.89 ± 0.01^{d}
7.5% of maltodextrin	11.53 ± 0.05^{bc}	11.36 ± 0.02^{d}	8.96 ± 0.02^{c}
10% of maltodextrin	11.47 ± 0.04^{abc}	11.41 ± 0.01^{c}	9.02 ± 0.04^{b}

$^{a-f}$ Different letter superscripts in the same column indicate statistical difference ($p<0.05$).

Lactic acid bacteria starter powder in fermented Pla-som

Pla-som is generally low acid food at pH around 4.5-5.2. Changes in the pH and lactic acid of Pla-som samples during fermentation are shown in Figure 1. At the beginning of the fermentation, samples had pH values approximately 6.42-6.53 and 0.45-0.53 percentages of lactic acid content. It showed that the fermented Pla-som with 1.5% and 3% of starter culture powder was more acidic and had a lower pH than the control sample (Figure 1).

Sensory evaluation of fermented Pla-som

When compared the effect of an added with 1.5 and 3% of starter culture powder, and traditional fermented Pla-som on the acceptability of color flavor, texture, sourness and overall liking of Pla-som product are presented in Table 4. The sensory acceptance in the color flavor and texture of 1.5 and 3% of lactic acid powder, and control were not significantly different ($p>0.05$). It was noted that fermented Pla-som with 3% of starter culture powder was significant higher sourness and overall liking values ($p<0.05$) when compared with natural fermented Pla-som. Interestly, acceptability of fermeted Pla-som by adding the lactic acid bcateria starter powder with a shorter fermentation period were more likely than that control group.

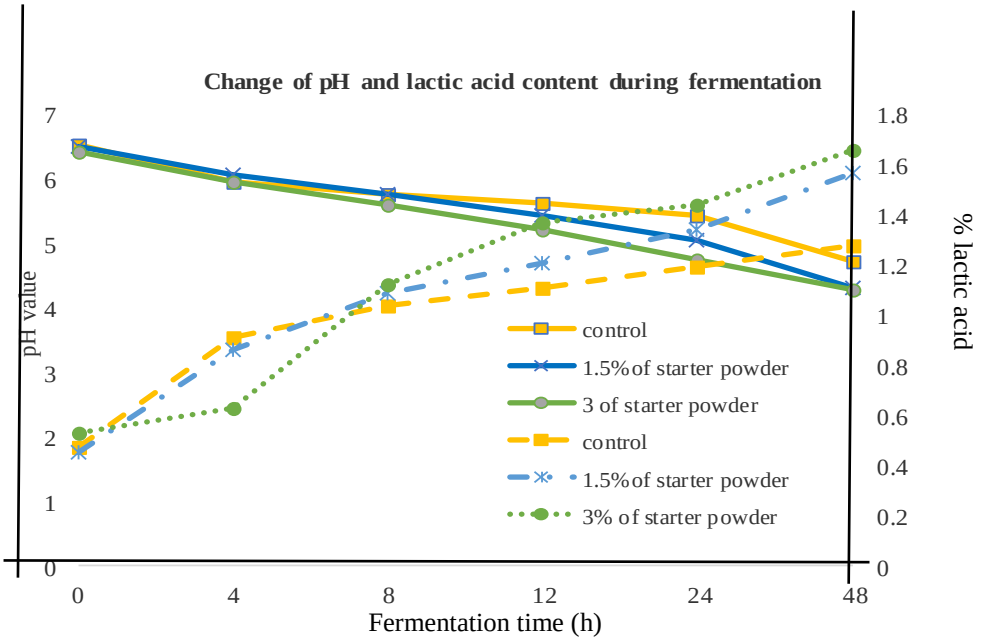

Figure 1. The changes in pH and lactic acid content during fermentation

Table 4. Sensory acceptability of panellists on fermented Pla-som

Samples	Color^{ns}	Flavor^{ns}	Texture^{ns}	Sourness	Overall liking
Control	6.83±1.15	6.63±0.97	6.53±1.32	6.80 ±1.50^{b}	6.87±1.33^{b}
1.5% of starter powder	7.20 ±1.19	6.83±1.32	6.40±1.25	7.30±1.06^{ab}	7.33±0.96^{ab}
3.0 % of starter powder	7.13±0.86	7.17±0.91	6.77±1.41	7.53±0.73^{a}	7.66±0.71^{a}

$^{a-c}$ Different letter superscripts in the same column indicate statistical difference ($p<0.05$).
ns: not significant

Discussion

As a result, rice flour-based powder samples had lower a_w values (0.190-0.198) than powder samples with maltodextrin DE 10 (0.203-0.210). These results indicated that high a_w could cause the oxidation of lipid in the cell membrane. The results were similar to those reported by Champagne *et al.* (1991) who found that the high moisture contern of powder sample increased in percentage of cell death during storage. Coating material with high glass transition temperature (Tg) is discovered to positively correlate with an increase in the number of surviving cells. The Tg of soluble starch (241.80 °C) which had Tg higher than Tg of maltodextrin DE 10 (160 °C) (Tantratian and Pradeamchai, 2020).

Ten percent of rice flour had higher viability than the other carrier material. by spray drying at 140 °C of inlet and 70 °C of outlet temperature. A higher survival rate of cell had an increase opportunity of the cell viability during storage of lactic acid bacteria starter powder. Previous research revealed that hydrolysed-HMT black rice flour as carrier material followed by spray drying at 70 ℃ of dry air outlet temperature was reploted to be able to increase on the survival of *Lactobacillus plantarum* (Wattananapakasem *et al*., 2018). The components of rice flour included the helical structure of amylose, which can prevent DNA damage and cell membranes from being damaged by heat during the spray-drying process (Ying *et al*., 2003; Ray *et al*., 2016).

The high numbers of a range of lactic acid bacteria in the dried samples would benefit a potential fermentation process in this product. In addition, all the spray dried samples were not detected in the bacterial contamination. This confirmed safety of the starter culture produced by spray drying. It could possibly be used to starter culture to the fermentation of Pla-som product in order to shorten the fermentation time.

After spray drying, these microcapsules were kept at 4 ℃ for 90 days. The result showed that lactic acid bacteria survived well in the rice flour, significantly enhanced storage stability of spray dried powders as shown by the remarkably higher viability cell (9.38 Log CFU/g) compared to all other systems (8.79-9.02 Log CFU/g). This result is supported by Yonekura *et al*. (2014). They found that no significance of *L. acidophilus* NCIMB701748 survival was shown approximately from 8.90 to 8.99 log CFU/g in sodium alginate, hydroxypropyl methylcellulose (HPMC) and chitosan as carrier after spry drying. It indicated that the carrier solution was no toxic effect level on bacteria. The relationship between the physicochemical properties of the carrier matrix was correlated with the loss or maintain of viability during spray drying and storage. Previous studies reported that the interaction of polysaccharides

with the proteins could increase the survival rate of bacteria due to prevent protein denaturation in the spray dryer (Vereyken *et al*., 2001; Sunny-Roberts *et al*., 2009).

Moreover, protection is often correlated with the high glass transition temperatures of the selected components providing stability to bacteria when enclosed in a glassy matrix (Schuck *et al*., 2007). Xing *et al*. (2015) suggested that the using the complex wall material to prepare the microencapsulated *Lactobacillus acidophilus* might be potential protection during passage through intestinal system and storage at refrigerated temperatures.

Rice flour used as a protective agent of lactic acid bacteria starter. A highest acid production was found in fermented Pla-som with 3% lactic acid bacteraia encapsulated compared to these fermented with 1.5% starter powder and spontaneous fermentation. The finish product of pla-som was found that the amount of titratable acidity produced by the fermented Pla-som with 3% lactic acid bacteraia encapsulated was slightly higher than that produced by spontaneous fermentation. A decrease in pH value of fermented Pla-som with 3% of lactic acid bacteria encapsulated had the lowest pH value of 4.27 followed by the 1.5 % of starter culture powdera added and spontaneous fermentation that had a pH of 4.34 and 4.72, respectively, within 48 h of fermentation. The rapidly decreased pH below 4.5 occurred during fermentation of Pla-som with lactic acid bacteria starter powder could be explained by the metabolic activity of lactic acid bacteria starter culture. This could be affectd by the short fermentation time. Thai community product standard, 2014, recommended that the fermented Pla-som product have a pH of 4.6 or less. These results were in agreement with those of Chaikham and Kaewjinda, 2017. They reported that pH of Pla-som complementary with *L. casei*, about 10^{10} CFU/g was lower than 4.5 within 3days. Furtermore, Pla-som fermented was rapidly increased in acidity when a starter powder of *Lactobacillus plantarum* FT 35 was added (Tantratian and Pradeamchai, 2020). So, it was found that rice flour acts as a carricer material for preventing the culture as well as a good carbon source for lactic acid bacteria during fermentation (Ruangsuwan *et al*., 2009).

Sensory evaluation in fermented Pla-som with 3% of starter powder were highest sensory score. It is possibly that the high number of lactic acid bacteria in fermented Pla-som could improve the appearance, and volatile compounds contribute to the desirable Pla-som aroma, which was accepted by the panelists. The results indicated that the lactic acid starter powder initially added to fermented Pla-som which had affected on the concentration of volatiles compound. Due to high activity of starter culture could breakdown protein into essential amino acid that as precursor for volatile organic acids during

fermentation. The changes in volatiles compound were correlated with activity of bacteria. Stefánsson and Gudmunsdóttir (1995) reported that peptides, free amino acids and these compounds gradually increased during the ripening of salted herring are believed play an important role of the sensory properties of end product.

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