Research Article | Science Technology and Engineering Journal (STEJ) Vol.8, No.1 pages 50-60

Development of crispy fried Pla-ra product using prototype from accelerated fermentation with autochthonous bacterial starter

Atchariya Suriya[1*] and Sarn Settachaimongkon[2]

[1] *Department of Food and Services, Faculty of Technology, Udon Thani Rajabhat University, Udon Thani 41000, Thailand*

[2] *Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

[*] *Corresponding author: atchariya.su@udru.ac.th*

(Received: 2nd February 2022, Revised: 31st March 2022, Accepted: 1st April 2022)

Abstract - The fermentation of Pla-ra with autochthonous starter contained *Bacillus subtilis* subsp *Subtilis* UD6-2 and *Virgibacillus halodenitrificans* NCFF-2 under solid state fermentation (SSF) condition was evaluated. Pla-ra with the starter fermented by 3.5 month had similar properties in terms of texture, color and odor to traditional Pla-ra when assessed by manufacturers. Crispy fried Pla-ra, product models from Pla-ra with starter traditional derived were made and subjected to sensory preference test. It was found that all attributes of the crispy fried Pla-ra with starter product were more accepted than the traditional Pla-ra with significant higher liking scores ($p<0.05$). The test also demonstrated that the texture of Pla-ra significantly influenced the liking of taste and overall liking ($p<0.05$). Based on crispy fried Pla-ra proposed in this study, it could be an interesting alternative Pla-ra derived product that contained high protein and also a shelf stable with low hazard risk.

Keywords: Autochthonous starter, fermented fish, Pla-ra, solid state fermentation

1. Introduction

In Thailand, demand of Thai traditional salt fermented fish (Pla-ra) in both domestic and export markets have been increased since Thai fusion foods from with interesting recipes and ingredients are widely created. Pla-ra is used as condiment, salted and savory sauce, dipping paste and crispy snack and becomes popular. Pla-ra fermentation includes the main steps of fish protein fermentation under high salt concentration. However, Pla-ra manufacturing is significantly different from many traditional salt fermented sauces and pastes, particularly, fish sauce. It is traditionally produced from variety of natural freshwater fish, locally and seasonally harvested in the local area. It was preserved, preferably with partially purified rock salt and produced with addition of roasted rice bran. Pla-ra was generally fermented for 8–24 months depending on manufacturing process. Recently, Det-Udom *et al.* (2022) demonstrated that Pla-ra could be fermented under solid-state (SSF) and in submerged (SMF). In the fermentation system, bacterial population of Pla-ra ranged from 10^2–10^6 in solid-state SSF and 10^6–10^9 CFU/g in submerged fermentation types. Through, rRNA analysis *Halanaerobium* spp. and *Lentibacillus* spp. were observed as the main genera, particularly from initial stage throughout the fermentation process. *Tetragenococcus halophillus* were dominant during the final stage in sea salt-recipe samples while *Bacillus* spp were found in those rock salt recipes. In contrast, cultural plating demonstrated that *Bacillus* spp., generally *B. amyloliquefaciens*, were the dominant genera. In addition, *B. pumilus*, *B. autrophaeus*, *B. subtilis* and *B. velezensis* shown some relations with rock salt-recipe Pla-ra. From this study, key strains associated to Pla-ra fermentation from each manufacturing process were selected and developed as Pla-ra starter for acceleration of Pla-ra fermentation. However, in use of the Pla-ra starter for commercial production, properties and sensorial property of Pla-ra and products obtained from the starter technology relative to conventional Pla-ra should be verified. Thus, in this study aimed to investigate fermentation properties of Pla-ra with starter, sensory acceptance and product properties when processed as crispy fried Pla-ra comparing to conventional Pla-ra.

2. Materials and methods

2.1 Pla-ra making process with autochthonous starter

Multi-starter powder included *Bacillus subtilis* subsp *Subtilis* UD6-2 and *Virgibacillus halodenitrificans* NCFF-2 was obtained from research group of Prakitchaiwattana (2017). Nile tilapia (*Oreochromis niloticus*) cage cultured in Mekong River in Nongkhai province with 4 fish/kg size was used for making Pla-ra. One hundred kilograms of Pla-ra made following the recipe of manufacturer, Tar-Toom community enterprise, Meung, Udon Thani. Pla-ra was made with solid state fermentation (SSF) process in glazed water jar allowing a natural fermentation process to occur for 1.5 months. The 1.5 months fermentation lot was then inoculated with 1% starter powder to have initial bacterial number

6 log CFU/g. Fermentation profile in term of microbial change with cultural plating and reverse transcriptase PCR-denaturing gradient gel electrophoresis (RevT-PCR-DGGE) and protease activity (Det-Udom *et al.*, 2022) was monitored throughout the fermentation period. The cultural dependent method was conducted by spreading serial dilutions of liquid samples onto Nutrient agar (Himedia, India) supplemented with 5% NaCl and incubated at 37 °C for 48 h. The colonies were counted and reported as CFU/g. For the RevT-PCR-DGGE, the first universal bacterial primer set was 27F and 1492R and the second was 357F with GC clamp attached and 517R. Amplification was done in a standard reaction mixture (*Taq* DNA Polymerase, Vivantis, Malaysia) following manufacturer's instruction in a DNA thermal cycler (BioRadT100TM Singapore). DGGE analysis was performed following electrophoresis technique. The identity of DNA bands was interpreted by comparing to known bacterial DNA. The characteristics and identity of Pla-ra were evaluated by manufacturers who were the recipe owner until the Pla-ra met their required quality.

2.2 Crispy fried Pla-ra making process and product composition

Two batches of crispy fried Pla-ra made from a whole batch of 100 kg of Pla-ra with starter culture and traditional Pla-ra (1 year fermentation, with the same raw material quality and manufacturing process as described in 2.1 but no starter added) were prepared, Pla-ra were washed and streamed for 15 minutes. The steamed Pla-ra were deboned and filleted before baked in oven (MEX, BS817X 70L) at 150 ^{o}C for 50 minutes. The baked Pla-ra fillets were chopped (Tefal LAMOULINETTE 1000W) and the mixed sample with seasoning, mainly sugar and tamarind juice, stir-fried with low heat until dry-crispy texture and mixed with fried crispy herbs. Eighty grams of crispy fried Pla-ra were packed into glass bottles. Oxygen absorbers were added to the enclosed bottles to help decrease the level of oxygen in the package. Product composition was analyzed through proximate analysis which measured its moisture content, ash content, crude fat, crude protein, and carbohydrate contents (AOAC, 2000).

2.3 Sensory evaluation of crispy fried Pla-ra products

Preference of crispy fried Pla-ra from Pla-ra with starter and traditional made were tested. Each sample was evaluated by 30 assessors. The sensory test was conducted at Tar-toom community enterprise office area. All volunteer panelists were screened as Pla-ra consumers. For each sample serving, 7 g of crispy fried Pla-ra topped on steam rice 20 g was served in white plastic containers covered with wrapping film and coded with a 3-digit random number. Each assessor evaluated 2 samples in the random serving order. The acceptability of the samples was evaluated using a 9-points hedonic scale for the "overall liking", the "liking of color", the "liking of texture", the "liking of flavor" and the "liking of taste".

2.4 Statistical analysis

The data were analyzed by the analysis of variance (ANOVA) using SPSS program. Duncan's multiple range test was used to compare the mean with significance level of $p<0.05$.

3. Results and discussion

3.1 Fermentation property of autochthonous starter and Pla-ra characteristic

The manufacturing process and raw materials of Pla-ra were predominantly influenced by production area and local culinary culture. In Udon Thani and Nongkhai provinces, Tilapia (*O. niloticus*) caught from Mekong river was the main large-size fish used as a raw material in the production of Pla-ra. However, a variety of small-size fishes such as catfish (*Mystus cavasius*) and Henicorhynchus (*Henicorhynchus siamensis*) were occasionally used. Pla-ra made in these two provinces was fermented in solid state for > 6–12 months with addition of around 10% of roasted rice bran, and 20–25% of rock salt from Bandung district (Det-Udom *et al.*, 2022). Fermentation period was also depended up on size of fish, particularly, Nile tilapia cage cultured in Mekong that might over 12 months to reach required quality. This long process is one of key manufacturing limit at commercial levels of production. Thus, this study evaluated efficiency of starter culture that one strains were autochthonous strains isolated from Pla-ra manufactured in these two provinces. *V. halodenitrificans* and *B. subtilis* developed as starter culture since their active growth and metabolic activity have been associated with the formation of various metabolites responsible for desirable flavor of Pla-ra as well as fermented shrimp paste, miso, fish sauce and soy sauce products (Tanasupawat *et al.*, 2002; Kobayashi *et al.*, 2016; Zang *et al.*, 2020).

The results as shown in Table 1, fermentation profile of Pla-ra with starter culture was significantly different from traditionally fermented Pla-ra (control). In traditional batch, after 1.5 months auto-fermentation, initial total bacterial count was around 7 log CFU/g and significantly reduced throughout 8 weeks. Protease activity was around 0.03 and not changed in this batch during these 8 weeks. In the batch of Pla-ra with starter, total bacteria count detected at around 8 log CFU/g and remained unchanged to the 2nd week and then reduced to around 7 log CFU/g at the 4th week of fermentation peroid. Protease activity was observed in this batch and was found higher to 0.04 throughout 8 weeks of fermentation. This demonstrated that starter added could initiate and increase fermentation activity since the first 2 weeks of fermentation. Enzymes generated could actively hydrolyzed protein throughout the fermentation. Thus, amino acids that rapidly increased through protease activity would be faster further metabolized to volatile compounds giving Pla-ra odor along with faster rates of texture softening by 8 weeks of fermentation. When Pla-ra with starter culture from 8 weeks fermentation was evaluated by manufacturers, the

characteristic of this Pla-ra was relatively similar to Pla-ra from traditional process and met their required quality (Figure 1a and 1b). On the other hand, Pla-ra from the control batch fermented for 8 weeks, fish texture was still firm with strong fishy odor (Figure 1c). This could demonstrate the efficacy of starter culture in acceleration of Pla-ra fermentation that could reduce process of production up to over 50%.

Table 1. Dynamics of bacteria, rDNA and protease activity during SSF fermentation with and without starter

Inspection times after 1.5 months of natural fermentation (week)	TPC (log CFU/mL)				Protease activity	cDNA band (sequence based on intensity)
	TPC	NCFF2	UD6-2	Other bacteria		
Pla-ra without starter (Control)						
0	7.3 ± 0.2	NO	NO	8.3 ± 0.5	0.035 ± 0.002	1. *Halanaerobium* spp. 2. *Lentibacillus* spp.
2	7.0 ± 0.6	NO	NO	7.0 ± 0.3	0.033 ± 0.021	1. *Halanaerobium* spp. 2. *Lentibacillus* spp.
4	4.7 ± 0.3	NO	NO	4.7 ± 0.3	0.037 ± 0.021	1. *Halanaerobium* spp. 2. *Lentibacillus* spp.
8	5.1 ± 0.2	NO	NO	5.1 ± 0.5	0.027 ± 0.002	1. *Halanaerobium* spp. 2. *Lentibacillus* spp.
Pla-ra with starter						
0	8.7 ± 0.4	7.1 ± 0.3	6.2 ± 0.4	6.8 ± 0.5	0.041 ± 0.006	1. *Halanaerobium* spp. 2. *Lentibacillus* spp.
2	8.4 ± 0.2	8.3 ± 0.2	6.4 ± 0.3	2.0 ± 0.4	0.044 ± 0.003	1. *Halanaerobium* spp. 2. *Lentibacillus* spp. 3. *V. halodenitrificans* 4. *B. subtilis*
4	7.4 ± 0.3	7.3 ± 0.3	6.8 ± 0.2	2.6 ± 0.5	0.046 ± 0.004	1. *Halanaerobium* spp. 2. *Lentibacillus* spp. 3. *V. halodenitrificans* 4. *B. subtilis*
8	6.4 ± 0.2	6.3 ± 0.3	5.6 ± 0.3	4.3 ± 0.4	0.035 ±0.002	1. *Halanaerobium* spp. 2. *Lentibacillus* spp. 3. *V. halodenitrificans* 4. *B. subtilis*

NO = Not observed

a b c

Figure 1. Appearance of Pla-ra with SSF; (a) One year fermented traditional Pla-ra (b) Two months fermented Pla-ra with starter (c) Two months fermented Pla-ra without starter (control)

Bacteria associated to Pla-ra fermentation were also investigated. It was found that *V. halodenitrificans* was more prevalence in Pla-ra than *B. subtilis* throughout fermentation period. Based on cultural independent technique, overall results from the rRNA analysis revealed that *Halanaerobium* spp. and *Lentibacillus* spp. as the two major bacterial population dominating in Pla-ra ecosystem (Det-Udom *et al.*, 2017) were still observed in all batch at all stages of fermentation. However, cDNA bands of starter strains were also additional observed in Pla-ra with starter batch (Figure 2). This observation demonstrated the expression of these two strains that played role in acceleration the fermentation along with the two auto-fermented strains. However, to evaluate the potential of the starter in manufacturing Pla-ra for commercial use, the quality and characteristic of product made from Pla-ra with starter including sensory quality relative to traditional made should be verified. This test was therefore conducted in the following section.

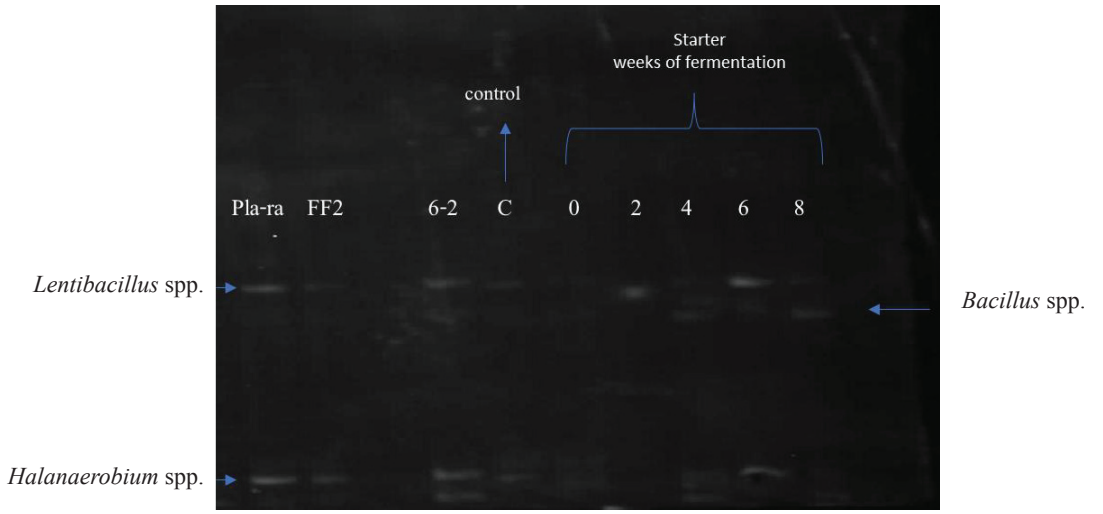

Figure 2. RevT-PCR-DGGE profiles of control and Pla-ra with starter

3.2 Product model; crispy fried Pla-ra and sensory preference

According to appearances of both Pla-ra in Figure 1 the initial odor, flavor, taste and texture of Pla-ra as evaluated by Pla-ra manufacturers were relatively similar. In the making process of crispy fried Pla-ra, when both types of fermented fish were steamed for 15 minutes, Pla-ra with starter was easier to deboning relative to traditional made Pla-ra. In addition, after steaming, Pla-ra with starter's fillet had a light brown with pink color (Figure 3a-1) while traditional Pla-ra had a dark brown color (Figure 3b-1). After baking, chopping and stir-frying, Pla-ra with starter still had light brown with much more fluffy and crispy texture. Interestingly, starter crispy Pla-ra 80 g was packed in glass bottle, the headspace observed was apparently less than the 80 g packed traditional Pla-ra. This demonstrated the fluffy character of the starter crispy Pla-ra.

Figure 3. Appearance of steamed Pla-ra fillet (1), chopped Pla-ra (2) and crispy fried Pla-ra (3); (a) 3.5 months fermented Pla-ra with starter (b) One year fermented traditional Pla-ra

The main volatile metabolites profile in Pla-ra from all manufacturing processes as reported by Det-Udom *et al.* (2022) were butanoic acid and its derivatives, associating to typical odor of Pla-ra. This research group also demonstrated that Pla-ra with starter culture fermented <50% shorter than traditional time contained similar volatile metabolites pattern to the traditional Pla-ra. This profile could be used as one of the key biomarkers in monitoring of fermentation stages. Phewpan *et al.* (2020) demonstrated that Glutamyl peptides, such as γ-Glu-Val-Gly, as the most potent kokumi substance significantly presented in mature Pla-ra. Kokumi and taste enhancing activity were clearly found in the taste profile of Pla-ra. Kokumi substances enhance salty, sweet, and umami tastes as well as the mouthfulness sensation, which is a unique property. However, glutamyl peptides in Pla-ra with stater has not been characterized and required further study to describe the taste attribute of Pla-ra relative to traditional process. The sensory preference of these 2 products in Table 2 showed that all attributes of the crispy fried Pla-ra with starter were more accepted than the traditional Pla-ra with significant higher liking scores ($p<0.05$). In addition, according to Table 3, crispy fried Pla-ra with starter was accepted by all 30 panelists while crispy Pla-ra made from traditional Pla-ra was not accepted by 7 panelists. Based on comments from the panelists, they also demonstrated that the texture of Pla-ra significantly influenced the liking of taste and overall liking ($p<0.05$). In the traditional process, Pla-ra was generally fermented over 6 months. During the first 5 months, although protein was hydrolyzed and fish texture became appropriate soften, flavor and odor of Pla-ra were not yet met the typical properties. Thus, another 6 months fermentation required to allow volatile compounds and typical odor to be more generated and developed, respectively. However, after 6 months fermentation texture of Pla-ra would be reformed and become tighter and harder than the previous stage (information obtained from manufacturers). This could be explained that during second phase of fermentation, soluble protein previously hydrolyzed might be denatured and re-bound to un-hydrolyzed protein allowing Pla-ra to have a tighter texture. On the other hand, Pla-ra with starter, after 1.5 months auto-fermented with *Halanaerobium* spp. and *Lentibacillus* spp. (Det-Udom *et al.*, 2022), the protein was already partially hydrolyzed to amino acids.Thus, after adding starter, protein was still further hydrolyzed with the previous auto-fermentative microbes along with activities of starter added. In this system, hydrolyzation of protein was accelerated along with generation of volatile compound derived from amino acids would allow Pla-ra to have or meet typical properties by 3.5 months. Based on texture, to have traditional Pla-ra with typical odor, fermentation over 6 months was required. Thus, the protein might be over hydrolyzed, denatured and reformed to obtain a hard texture. This property allowed the protein texture of typical Pla-ra to have hard, not crispy and fluffy texture relative to Pla-ra with starter. Based on the color, in the traditional fermentation, *Halanaerobium* spp could play a main role in the system

throughout the fermentation process (results as shown in Table 1 and Figure 2. This could allow *Halanaerobium* spp. to possibly convert starter thiosulfate in fish to sulfide and gave a unique taste, odor and dark color (Chhetri *et al.*, 2019) to Pla-ra. Thus, after steaming the sulfur containing agent that breakdown during heat processing released sulfide (Wei *et al.*, 2019) making the traditional Pla-ra to become significantly darker. Importantly, the sulfur containing compounds are also the key constituents produced in the Millard reaction products (Wei *et al.*, 2019). In term of taste and flavor relating to odor, the panelists gave significantly higher liking score for Pla-ra with starter because of its mild odor gone well with crispy fried Pla-ra flavor. In addition, the 1 year traditional Pla-ra released to much sulfur odor relative to the starter Pla-ra with 3.5 month fermentation. However, the properties of final Pla-ra would be also depended up on the products derived from Pla-ra that required investigation case by case. In this study, the crispy fried Pla-ra product was packaged in 80 grams glass bottles and could be stored for at least 6 months. Thus, based on crispy fried Pla-ra proposed in this study, it could be interesting alternative Pla-ra derived product that contained estimated composition as shown in Table 4, and also a shelf stable ($a_w < 0.6$) with low hazard risk.

Table 2. Preference scores for sensory characteristics of both crispy fried Pla-ra. (N=30).

Treatment	Color	Flavor	Taste	Texture	Overall acceptability
Crispy fried Pla-ra with starter	7.53 ± 0.86 [a]	7.26 ± 0.91 [a]	7.71 ± 1.02 [a]	6.57 ± 1.14 [a]	7.46 ± 0.97 [a]
Crispy fried Pla-ra without starter	4.57 ± 1.77 [b]	4.30 ± 1.80 [b]	4.90 ± 1.95 [b]	5.50 ± 1.61 [b]	5.67 ± 1.56 [b]

Values with different superscript within the same column are significantly different ($p<0.05$)

Table 3. Number of sensory panelist acceptance in crispy fried Pla-ra product from Pla-ra with starter and Pla-ra without starter. (N=30).

Treatment	Acceptance	
	Accepted	Not Accepted
Crispy fried Pla-ra with starter	30	-
Crispy fried Pla-ra without starter	23	7

Table 4. Proximate composition of crispy fried Pla-ra with starter 100 g.

Parameter	Value (g/100 g)
Moisture	4.52
Ash	11.17
Protein (N x 6.25)	27.91
Fat	17.20
Carbohydrate	39.2

4. Conclusion

From this observation, it demonstrated that starter could accelerate the fermentation process of Pla-ra to have a typical property required by manufacturers by 3.5 months. All attributes of crispy fried Pla-ra with starter were more accepted than the traditional Pla-ra with significant higher liking scores. The texture of Pla-ra significantly influenced the liking of taste and overall liking. Based on crispy fried Pla-ra proposed in this study, it could be interesting alternative Pla-ra derived product that contained relatively high protein and also a shelf stable with low hazard risk. However, the properties of final Pla-ra would be also depended up on the products derived from Pla-ra that required investigation case by case.

5. Acknowledgement

The author are grateful to the Research Program for Development of Small and Medium Enterprise, National Research Council of Thailand (NRCT) and Thailand Research Fund (TRF) of year 2017.

6. References

AOAC. (2000). *Official methods of analysis of AOAC International*. Gaithersburg, MD, USA.

Chhetri, V., Prakitchaiwattana, C., & Settachaimongkon, S. (2019). A potential protective culture; Halophilic bacillus isolates with bacteriocin encoding gene against *Staphylococcus aureus* in salt added foods. *Food Control*, *104*, 292-299.

Det-udom, R., Settachaimongkon, S., Chancharoonpong, C., Suphamityotin, P., Suriya, A., & Prakitchaiwattana, C. (2022). Factor affecting bacterial community dynamics and volatile metabolite profiles of Thai traditional salt fermented fish. *Food Science and Technology International*, DOI: 10.1177/10820132221075435.

Kobayashi, K., Shimojo, S., & Watanabe, S. (2016). Contribution of a fermentation process using *Bacillus subtilis*(natto) to high poly-amine contents of natto, a traditional Japanese fermented soy food. *Food Science and Technology Research*, *22*, 153-157.

Phewpan, A., Phuwaprisirisan, P., Takahashi, H., Ohshima, C., Ngamchuachit, P., Techaruvichit, P., Dirndorfer, S., Dawid, C., Hofmann, T., & Keeratipibul, S. (2020). Investigation of Kokumi substances and bacteria in Thai fermented freshwater fish (Pla-ra). *Journal of Agricultural and Food Chemistry, 68*(38), 10345-10351.

Prakitchaiwattana, C., & Det-udom, R. (2017). Contaminant sensors: nanosensors, an efficient alarm for food pathogen detection. In A.M., Grumezescu (Ed.), *Nanobiosensors* (pp.511-572). Academic Press.

Tanasupawat, S., Thongsanit, J., Okada, S., & Komagata, K. (2002). Lactic acid bacteria isolated from soy sauce mash in Thailand. *The Journal of General and Applied Microbiology*, *48*, 201-209.

Wei, C., Ni, Z., Thakur, K., Liao, A.-M., Huang, J.-H., & Wei, Z.-J. (2019). Color and flavor of flaxseed protein hydrolysates Maillard reaction products: effect of cysteine, initial pH, and thermal treatment. *International Journal of Food Properties*, *22*(1), 84-99.

Zang, J., Xu, Y., Xai, W., & Regenstein, J.M. (2020). Quality, functionality, and microbiology of fermented fish: A review. *Critical Reviews in Food Science and Nutrition*, *60*, 1228-1242.