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Fermented Fish Product (Pla-ra) from Marine Fish and Preservation

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ABSTRACT

Fermented fish products (Pla-ra) were produced from 4 species of marine fish. The results showed that Pla-ra from *Rastrelliger neglectus* and *Rachycentron canadus* with 25 percent and 30 percent salt added followed by fermenting at room temperature (28°C-30°C) for 18 months had the highest acceptability scores on color, odor and appearance by the organoleptic test. Preservation of those Pla-ra was studied by sterilizing the products, then packing into glass bottles, tin cans and retort pouches. The samples were determined by organoleptic test and compared with normal Pla-ra after being preserved for 6 months. The results showed that Pla-ra preserved in polyethylene plastic jar at room temperature had the highest acceptability. However, Pla-ra kept in a glass-bottles were more popular than Pla-ra kept in a tin cans and retort pouches.

Key words: Pla-ra, marine fish, traditional food

INTRODUCTION

Pla-ra is the traditional food which was popular among the people who live in every region of Thailand especially in the northern and north-eastern parts of Thailand. Mostly Pla-ra is made from fresh water fish such as *Channa striata* (Striped snake head fish, Chon), *Trichogaster trichopterus* (Gourami, Kra-dee), *T. leeri* (Kra-dee-nang), *Cyclocheilichthys repasson* (Silver Carp, Soi), *Puntius gonionotus* (Barb, Ta-pien). (National Research Journal of Thailand, 1981-1982). Marine fish namely *Johnius argentatus* (Silver johnfish), *Rastrelliger neglectus* (Short-bodied mackerel), *Rachycentron canadus* (Cobia), and *Caranx leptolepis* (Slender trevally) were used for making Pla-ra and were famous among people who live in the north-eastern part of Thailand (Sangjindavong, 2005). Pla-ra were popular among ASEAN countries such as Myanma, Laos, Vietnam and Cambodia. Pla-ra is an ingredient in Thai foods such as : Namprik Pla-ra, Som Tam Pla-ra, Kang Lao, Namya, Pla-ra Sub, and Pla-ra Song Kraung (Poosereepab, 1996; Yamprayoon and Sukkho, 1999)

Pla-ra was classified into 2 types according to the ingredients. Pla-ra which was processed by adding roasted rice was called Pla-ra Khao-kuo and Pla-ra which bran added was called Pla-ra Ram (Pooscreepab, 1996).

The production of Pla-ra in Thailand was 20,000-40,000 tons/year and the value of Pla-ra was about 800 million baht/year but the value of Pla-ra for exporting was more than 20 million baht/

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year (Anonymous, 2000). So suitable preservation of Pla-ra is important for extending shelf-life of Pla-ra.

The objective of this project was to study the processing of Pla-ra by using four species of marine fish namely : *Rastrelliger neglectus* (Short-bodied mackerel), *Rachycentron canadus* (Cobia), *Otolithes ruber* (Tiger-toothed croaker) and *Nemipterus hexodon* (Ornated threadfin bream). Shelf-life extension of Pla-ra was also studied. This research concerned about marine fine especially *Rastrelliger neglectus* because in some season there are a lot of raw materials, so preparing Pla-ra was one method to preserve them and fishermen can use as food during working in the sea.

MATERIALS AND METHODS

Pla-ra from four species of marine fish : *Rastrelliger neglectus*, *Rachycentron canadus, Otolithes ruber* and *Nemipterus hexodon* were prepared by using 13, 18, 22, 25 and 30 percent of salt by weight and adding 10 percent of roasted rice powder in every sample. The samples were left at room temperature (28°C-30°C) for 18 months and were examined every months which as follows :

Chemical analysis

The Pla-ra samples were taken to determine for pH, % NaCl, % lactic acid, amino-nitrogen, and histamine according to AOAC Official Method (AOAC, 1995).

Microbiological examination

Each Pla-ra samples was determined for Total Plate Count, Lactic Acid Bacteria, Coliform Bacteria, Faecal Coliform Bacteria, (*Escherichia coli), Staphylococcus aureus, Vibrio parahaemolyticus* and *Clostridium perfringens* by following the AOAC Official Method (AOAC, 1995).

Statistics

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

Organoleptic test

Sensory evaluation of Pla-ra were studied using 14 panelists. They were lecturers and students from Department of Fishery Products, Kasetsart University. A five-point hedonic scale (5 =extremely like, 4 = like, 3 = neither like nor dislike, 2 = dislike, 1 = extremely dislike) was used for the appearance, color flavor, and overall liking for each of the Pla-ra samples.

The Pla-ra samples which had the highest scores in sensory evaluation after 18 months of fermentation were further studied for shelf-life extension by using four different types of packaging with the process condition as follows :

1. Pla-ra was kept in polyethylene plastic jars and left at room temperature (28°C-30°C) without sterilization.
2. Pla-ra was kept in glass bottles and sterilized at 100°C for 20 minutes and then left at room temperature.
3. Pla-ra were kept in tin cans and sterilized at 116°C for 15 minutes and left at room temperature.
4. Pla-ra was kept in retort pouches and sterilized at 116°C for 15 minutes and left at room temperature. Pla-ra with every storage condition was kept for 6 months after which the samples were studied by the organoleptic test, chemical analysis and microbiological characteristics.

RESULTS AND DISCUSSION

Microbiological and chemical studies of Pla-ra were conducted according to AOAC (1995). Sensory evaluation of Pla-ra used the method of 5 point Hedonic scale and statistical analysis by Duncan's multiple range test. The results are

shown in Table 1- Table 8 as follows :

Table 1 showed that Pla-ra prepared from *Rastrelliger neglectus* and *Rachycentron canadus* by using 25 percent and 30 percent of salt received the highest acceptability scores using 5 point Hedonic scale within 6 months of storage.

Table 2 - table 4 showed the results of the highest acceptabiligy scores of uncooked Pla-ra at different months.

From Table 2, the sensory scores (appearance) of Pla-ra prepared from *Rachycentron canadus* with 25% NaCl added received the most highest acceptability scores at 18 months of fermentation. However, all average were not significantly different during 18 months of fermentation.

From Table 3, the sensory scores (color) of Pla-ra which prepared from *Rastrelliger neglectus* added 30% as well as Pla-ra which prepared from *Rachycentron canadus* with 25% NaCl added received the highest scores at 18 months of fermentation.

Table 4 showed that Pla-ra prepared from *Rachycentron canadus* with 25% NaCl added received the highest scores (flavor) at 18 months of fermentation.

Table 5 showed that uncooked Pla-ra made from *Rachycentron canadus* with 25% NaCl added preserved in plastic jar at room temperature (28°C-30°C) received the highest acceptability scores.

Table 6 showed that cooked Pla-ra kept in glass bottles was popular among Pla-ra kept in tin cans and retort pouches.

Table 1 Sensory evaluation for appearance of Pla-ra made from four types of fish and five levels of salt concentrations during six months period of fermentation.

Fish samples	NaCl	Fermented Time (Month)						
	(%)	1	2	3	4	5	6	Average
Otolithes ruber	13%	3.14	2.61	2.64	3.14	2.54	3.28	2.89d
Otolithes ruber	18%	2.85	2.82	2.32	3.00	2.96	3.21	2.86d
Otolithes ruber	22%	2.21	2.86	2.89	2.61	2.96	3.25	2.80cd
Otolithes ruber	25%	3.00	3.11	2.79	3.17	3.50	3.36	3.16bcd
Otolithes ruber	30%	2.86	2.75	3.00	3.14	3.43	3.54	3.12cd
Rachycentron canadus	13%	3.00	3.07	2.67	3.00	2.57	3.28	2.93cd
Rachycentron canadus	18%	3.07	3.29	2.82	2.68	3.14	3.28	3.05cd
Rachycentron canadus	22%	2.86	3.07	2.75	3.07	3.36	2.93	3.01cd
Rachycentron canadus	25%	2.96	3.68	3.25	3.64	3.68	4.07	3.55ab
Rachycentron canadus	30%	3.07	3.61	3.50	3.28	4.14	3.79	3.57a
Rastrelliger neglectus	13%	2.89	2.79	2.96	2.54	3.11	3.07	2.89d
Rastrelliger neglectus	18%	2.96	2.93	3.25	2.75	3.18	2.86	2.99cd
Rastrelliger neglectus	22%	3.04	2.86	2.86	3.03	2.93	2.78	2.92cd
Rastrelliger neglectus	25%	2.14	3.00	3.14	3.57	3.82	4.29	3.33abc
Rastrelliger neglectus	30%	2.50	3.21	2.93	3.21	3.36	4.29	3.25abcd
Nemipterus hexodon	13%	3.04	2.79	2.64	2.39	2.86	3.28	2.83d
Nemipterus hexodon	18%	2.68	2.64	2.93	2.46	3.07	3.14	2.82d
Nemipterus hexodon	22%	2.68	2.71	2.82	2.86	3.18	3.00	2.88d
Nemipterus hexodon	25%	2.86	3.29	3.25	3.14	3.29	3.07	3.15bcd
Nemipterus hexodon	30%	2.14	3.36	3.21	3.21	3.50	3.21	3.11cd

Means follow by different letters in the same column are significantly different ($P<0.05$)

Table 2 Average appearance scores of Pla-ra at 18 months of fermentation.

Type of Pla-ra	Months								
	1	2	3	4	5	6	12	18	Average[1]
Rachycentron canadus + 25% NaCl	2.96	3.68	3.25	3.64	3.68	4.29	4.14	4.42	3.76[a]
Rachycentron canadus + 30% NaCl	3.07	3.61	3.50	3.28	4.14	4.29	4.29	3.07	3.66[a]
Rastrelliger neglectus + 25% NaCl	2.14	3.00	3.14	3.57	3.82	4.07	4.43	3.60	3.47[a]
Rastrelliger neglectus + 30% NaCl	2.50	3.21	2.93	3.21	3.36	3.79	4.43	3.82	3.41[a]
Average[2]	2.67[d]	3.38[c]	3.21[c]	3.43[bc]	3.75[b]	4.11[a]	4.32[a]	3.73[a]	

Average[1] Means followed by same letters in the same column are significantly different (P<0.05)
Average[2] Means followed by different letters in the same row are significantly different (P<0.05)

Table 3 Average color scores of Pla-ra at 18 months of fermentation.

Type of Pla-ra	Months								
	1	2	3	4	5	6	12	18	Average[1]
Rachycentron canadus + 25% NaCl	3.21	3.82	3.93	3.64	4	4.21	4.43	4.28	3.94[a]
Rachycentron canadus + 30% NaCl	3.29	3.75	3.86	3.64	4.36	4.29	4.36	3.21	3.85[a]
Rastrelliger neglectus + 25% NaCl	2.39	2.86	4	3.36	4.21	4.14	4.57	3.57	3.64[ab]
Rastrelliger neglectus + 30% NaCl	2.39	3.82	3.93	3.64	4	4.21	4.43	4.28	3.94[a]
Average[2]	2.82[d]	3.42[c]	3.92[b]	3.41[c]	4.07[ab]	4.14[ab]	4.43[a]	3.68[ab]	

Average[1] Means followed by different letters in the same column are significantly different (P<0.05)
Average[2] Means followed by different letters in the same row are significantly different (P<0.05)

Table 4 Average flavor scores of Pla-ra at 18 months of fermentation.

Type of Pla-ra	Fermentation time (month)								
	1	2	3	4	5	6	12	18	Average[1]
Rachycentron canadus + 25% NaCl	3.07	3.21	3.89	2.93	3.14	3.32	4.21	3.57	3.42[a]
Rachycentron canadus + 30% NaCl	3	3.28	3.86	3.86	3.68	3.71	3.93	3.00	3.54[a]
Rastrelliger neglectus + 25% NaCl	2.5	3.21	3.04	3.28	3.75	3.36	4	3.28	3.30[a]
Rastrelliger neglectus + 30% NaCl	2.64	3.14	2.68	3.14	3.39	3.54	4.14	3.07	3.22[a]
Average[2]	2.80[a]	3.21[bc]	3.37[b]	3.30[b]	3.49[b]	3.48[b]	4.07[a]	3.23[b]	

Average[1] Means followed by same letters in the same column are significantly different (P<0.05)
Average[2] Means followed by different letters in the same row are significantly different (P<0.05)

Table 5 Average overall acceptability scores of uncooked Pla-ra preserved in different packaging.

Type of Pla-ra \ Packaging	Glass Bottle	Retort Pouch	Tin Can	Plastic Jar	Average[1]
Rachycentron canadus +25% NaCl	3.28	3.25	3.14	4.10	3.45[a]
Rachycentron canadus +30% NaCl	3.00	3.21	2.67	3.00	2.97[b]
Rastrelliger neglectus +25% NaCl	3.28	3.17	2.89	3.42	3.19[ab]
Rastrelliger neglectus +30% NaCl	3.03	3.00	3.14	3.46	3.19[ab]
Average[2]	3.15[b]	3.16[b]	3.00[b]	3.50[a]	

Average[1] Means followed by different letters in the same column are significantly different (P<0.05)
Average[2] Means followed by different letters in the same row are significantly different (P<0.05)

Table 6 Comparative sensory scores of cooked Pla-ra in different packaging.

Type of Pla-ra \ Packaging	Glass bottle	Retort pouch	Tin can	Average[1]
Rachycentron canadus + 25% NaCl	4.21	4.21	4.17	4.15[a]
Rachycentron canadus + 30% NaCl	4.14	4.14	4.17	4.09[a]
Rastrelliger neglectus + 25% NaCl	4.03	3.71	4.00	3.17[b]
Rastrelliger neglectus + 30% NaCl	4.03	3.64	3.67	3.75[b]
Average[2]	4.11[a]	3.93[ab]	4.01[a]	

Average[1] Means followed by different letters in the same column are significantly different (P<0.05)
Average[2] Means followed by different letters in the same row are significantly different (P<0.05)

Table 7 showed that the bacteriological examination of uncooked Pla-ra fermented for 12 months and no pathogenic bacteria were detected.

Table 8 showed the chemical composition of cooked Pla-ra in different packaging.

Chemical compositions of Pla-ra depended on freshness of raw materials. From this result it was found that Pla-ra had pH between 4.55-7.48, NaCl between 8.24-21.09%, lactic acid between 1.25 - 3.53%, nitrogen between 18.13 – 32.19 g/l and histamine content between 15.88 – 91.47 mg/100 g. Pla-ra made from *Rastrelliger neglectus* with 30% NaCl added and packaged in retort pouch has the highest value of pH at 7.48. Pla-ra made from *Rastrelliger neglectus* with 30% NaCl added and packaged in plastic jar has the highest value of NaCl content at 21.09%. The highest value of lactic acid content at 3.53% was belonged to Pla-ra which was made from *Rachycentron canadus* with 30% NaCl added and packaged in retort pouch. Total nitrogen of Pla-ra made from *Rastrelliger neglectus* with 30% NaCl added and packaged in plastic jar was the highest value at 32.19 g/l. The highest value of histamine at the level 91.47 mg/100 g was belonged to Pla-ra made from *Rastrelliger neglectus* added 25% NaCl and packaged in glass bottle.

According to the results, Pla-ra which were prepared from *Rastrelliger neglectus* and *Rachycentron canadus* by added 25% and 30% NaCl had the highest acceptability scores. Pla-ra which were preserved in plastic jars at room temperature (28°C-30°C) had the most acceptability scores. However, Pla-ra kept in glass-bottles was popular among Pla-ra kept in tin cans and retort pouches.

Only three samples of Pla-ra had a high level of histamine. Pla-ra made from *Rastrelliger neglectus* with 25% NaCl added in glass bottles had the highest histamine content (91.47 mg/100 g). Pla-ra made from *Rastrelliger neglectus* with 25% NaCl added in tin cans had histamine content 74.2 mg/100 g. The last one which had a higher histamine content of 60.47 mg/100 g was Pla-ra made from *Rastrelliger neglectus* with 25% NaCl added in plastic jar. All of the samples made from *Rastrelliger neglectus* showed higher histamine content than those of the samples made from *Rachycentron canadus*. Higher histamine content more than 200 mg/100 g. was one of the indicators of spoilage (Brilliantes and Samosorn, 1998). Microorganisms and the environment were an important factors for spoilage of fish (Shewan *et al*.,1960; Shewan, 1971; Longrâe; Armbruster, 1996). Some bacteria which were found in solar salt caused the spoilage of protein (Amano, 1962). Most of bacteria which were found in solar salt are *Bacillus* (Horie and Hinago, 1924; Sangjindavong, 1982). Virulhakul *et al*., (2000) studied quality and safety of Pla-ra by sampling 26 products from 8 places. The study found that organoleptic test, chemical analysis and microbiological characteristics were shown good quality and safety for the human consumer. The products had no harmful pathogenic bacteria such as *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Clostridium perfringens*.

Chemical analysis of Pla-ra (table 8) made from *Rastrelliger neglectus* and *Rachycentron canadus* was accepted. Although pH 6.5 was suitable for histamine production (Eitenmiller *et al*., 1982), in this study histamine content was not higher than that of fish sauce. The average histamine content from 200 samples of fish sauce grade 1 and grade 2 were 200-800 mg/ 1000 ml. Most of fish sauce had a histamine content average of 200-600 mg/1000 ml (Brillantes and Samosorn., 1998). Because Pla-ra and fish sauce are fermented fish products and there is no standard for Pla-ra, so some data of fish sauce such as histamine content was picked up for comparing the quality of Pla-ra. From previous research, Pla-ra which was made from marine fish was safe and could be processed for instant fermented fish products (Rattagool *et al*., 1985)

Table 7 Bacteriological examination of Pla-ra fermented for12 months.

Type of Pla-ra	Total Plate Count (CFU/g)	Lactic Acid Bacteria (CFU/g)	Coliform Bacteria (MPN/g)	Faecal Coliform Bacteria (MPN/g)	*E. coli* (MPN/g)	*S. aureus*	*V.parahaemolyticus*	*Cl.perfringens*
Rachycentron canadus +25% NaCl	1.0×10^6	8.0×10^2	150	<3	<3	-ve	-ve	-ve
Rachycentron canadus +30% NaCl	6.3×10^6	5.2×10^3	150	<3	<3	-ve	-ve	-ve
Rastrelliger neglectus +25% NaCl	8.0×10^4	None	<3	<3	<3	-ve	-ve	-ve
Rastrelliger neglectus +30% NaCl	1.0×10^6	1.0×10^4	<3	<3	<3	-ve	-ve	-ve

- ve = negative test

Table 8 Chemical composition of cooked Pla-ra in different packaging.

Samples	pH	% NaCl	% Lactic acid	Nitrogen (g/l)	Histamine (mg/100 g)
Plastic jar					
Rastrelliger neglectus +25% NaCl	4.86	19.13	1.34	31.45	60.47
Rastrelliger neglectus +30% NaCl	5.12	21.09	1.89	30.09	29.68
Rachycentron canadus +25% NaCl	4.55	16.88	1.83	32.19	15.88
Rachycentron canadus +30% NaCl	4.79	17.11	2.05	30.84	21.10
Tin can					
Rastrelliger neglectus +25% NaCl	4.91	10.79	2.76	27.85	74.2
Rastrelliger neglectus +30% NaCl	5.13	13.26	1.83	22.37	37.66
Rachycentron canadus +25% NaCl	4.75	9.04	2.99	19.56	46.0
Rachycentron canadus +30% NaCl	4.84	11.93	3.45	18.13	37.31
Glass bottle					
Rastrelliger neglectus +25% NaCl	4.96	8.98	1.92	26.45	91.47
Rastrelliger neglectus +30% NaCl	5.28	9.33	1.6	24.52	36.58
Rachycentron canadus +25% NaCl	6.39	8.24	2.15	23.91	29.16
Rachycentron canadus +30% NaCl	6.74	11.18	2.07	23.30	27.36
Retort pouch					
Rastrelliger neglectus +25% NaCl	7.13	9.33	1.25	24.72	49.24
Rastrelliger neglectus +30% NaCl	7.48	16.22	1.37	24.06	27.42
Rachycentron canadus +25% NaCl	6.34	11.06	1.86	25.27	24.43
Rachycentron canadus +30% NaCl	4.84	10.37	3.53	23.03	21.14

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