

Effect of unripe papaya and ripe pineapple supplementation on the quality characteristics of rotten fermented fish (Plara Hnong) from tilapia frames

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ABSTRACT: Rotten fermented fish (RFF) produced from tilapia frames was explored. Addition of unripe papaya and ripe pineapple, containing proteases, to increase hydrolysis of the tough frame meat was investigated and compared to a control (distilled water). Yeast and mold (YM), formol nitrogen (FN) and CIE color values (L^* , a^* , and b^*) were determined in months 0, 2, 4, and 6. The results showed that papaya reduced YM to an undetectable level after month 0, but pineapple increased YM to $> 3 \log \text{CFU/g}$, causing quality failure. Papaya and pineapple significantly increased FN compared to the control. However, the level of FN did not improve after month 2. A high salt content may have inhibited the proteases in the fruits and consequently led to stable FN levels after month 2. Addition of the fruits tended to increase the Maillard reaction, by which L^* was decreased and a^* and b^* were increased. Conclusively, papaya and pineapple can increase FN. Pineapple caused quality failure due to mold proliferation. Supplementation with papaya in RFF is therefore better than with pineapple in term of YM suppression and is recommended.

Keywords: rotten fermented fish, unripe papaya, ripe pineapple, by-product fish frame, tilapia fillet processing, Plara Hnong

Introduction

Fermented fish is a home-based fish product consumed in Southeast Asian countries including Thailand, *Lao People's Democratic Republic (Lao PDR)*, Cambodia, and Vietnam. The product is known as Pladak, Prahok, and Mum in Lao PDR, Cambodia, and Vietnam, respectively. In Thailand, the product has different local names. In the central region of the country, it is called Plara. In the northeast, it is called Pladak (National Research Council of Thailand, 1982). Thai fermented fish is a mixture of fish, salt, and rice bran or roasted rice, allowed to ferment at ambient temperature for at least 6 months (Krusong, 2004). Hemwihok and Hongsaeng (2013) divide fermented fish into two groups according to the type of rice added to

the product. The first group, relying on roasted rice for its production, is known locally as Plara Kaw Kou. The second group, relying on rice bran for its production, is known as Plara Rum. The first group is found in central Thailand, while the latter group is common in the northeast of the country. Both groups can be prepared by mixing fish: salt: roasted rice/rice bran at a ratio by volume of 3: 2: 1 (Chuaphohak, 2006; Hemwihok and Hongsaeng, 2013).

Plara Rum (a fermented fish mixed with rice bran) can be divided into three types according to the size of the fish and the ratio of salt. Rotten fermented fish is one of these three types. It is known locally as Plara Hnong. Small natural fish species and a low salt content are used for rotten fermented fish production. The low salt content causes a stronger *pungent* odor compared to

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regular fermented fish (Hemwihok and Hongsaeng, 2013). Sudapan (2011) reported that fish: salt: rice bran at a ratio by volume of 4: 1: 1 is used for the production of rotten fermented fish. Reducing the salt ratio from 1 to 0.5 can produce a more pungent odor. Sauce from rotten fermented fish is added in small amounts to the sauce from regular fermented fish to enhance the flavor. Netphakdee (2013) found that a ratio of rotten fermented fish sauce: regular fermented fish sauce of 1: 9 (v/v) yielded the most desirable flavor of a dressing sauce used for northeastern-style papaya salad.

Using small natural fish species for the production of rotten fermented fish is limited to the rainy season when natural fish is abundant. Using tilapia frame, an underutilized by-product from tilapia fillet processing, as an alternative material may enable year-round production of rotten fermented fish. Using tilapia frames for the production of rotten fermented fish has not been recorded to date. However, using the frames for the production of regular fermented fish was carried out by Chomnawang et al. (2013) and Gawborisut et al. (2013). Using the frames for production of rotten fermented fish, which can be boiled and further used for fermented-fish sauce production, may efficiently use the by-product.

Addition of unripe papaya and ripe pineapple, containing protease enzymes, to this rotten fermented fish product may benefit hydrolysis of the frame meat which harbors a high amount of connective tissue (Bechtel, 2003). Papaya and pineapple contain natural proteolytic enzymes called papain and bromelain, respectively. Addition of commercial papain and bromelain enzymes in fish sauce production to accelerate protein hydrolysis has been extensively reviewed by Beddows (1998) and Batista (2007). However, the enzymes may not be accessible by small-scale fermented fish producers. Using unripe papaya and ripe

pineapple locally available for the production should be explored. Experiments using pineapple stems and unripe papaya for fish sauce production were recorded by Diaz-Lopez and Garcia-Carreno (2000). Only pineapple is commercially used in fish sauce production. Using pineapple cores to accelerate the production of fish sauce from surimi waste was studied by Sangjindavong et al. (2009). To our knowledge, using unripe papaya and ripe pineapple for accelerating the production of rotten fermented fish has not been recorded. The objective of this experiment was to investigate the effect of unripe papaya and ripe pineapple supplementation on the quality of rotten fermented fish produced from tilapia frames.

Materials and Methods

Material preparation

Experimental rotten fermented fish was composed of minced tilapia frame, salt, rice bran, and minced fruits (unripe papaya and ripe pineapple). Frozen tilapia frames (10 kg/block) were purchased from Grobest Frozen Co. Ltd (Nakhon Phanom, Thailand) and kept in a freezer until used. The frames were thawed overnight in a refrigerator ($4\pm 2^{\circ}\text{C}$), washed twice using potable water, and chopped into small pieces. They were then minced twice into paste using a commercial meat mincer equipped with grinding plates of 3- and 7-mm pore size (Thai Amnoury, Khon Kaen, Thailand). Grinding plates with a pore size of 7mm were used for the first mince, and a pore size of 3 mm was used for the second mince. The minced frame was sampled and analyzed for its moisture content using the AOAC method (1990). It was then packed in polyethylene bags, iced, and further used for preparing rotten fermented fish.

Salt was obtained from a supermarket (*Tesco Lotus, Khon Kaen, Thailand*). Rice bran was purchased from a local rice supplier (Kaw

Thong, Khon Kaen, Thailand), roasted on the stovetop until golden brown, cooled to room temperature, and used immediately. Whole unripe papaya (Kak Dum variety) and ripe pineapples (Pattavia variety) were purchased from a local market (Sri Mueang Wholesale Market, Khon Kaen, Thailand). The top end of the papaya was cut off, then it was sliced in half lengthwise, seeded, washed twice, and chopped into small pieces. The top and bottom ends of the pineapples were cut off. The unpeeled pineapples were washed twice and chopped into small pieces. Papaya and pineapple were minced separately using a mincer equipped with a grinding plate with a pore size of 7 mm. The minced fruits were analyzed for their moisture content using the AOAC method (1990). Analysis showed that the unripe papaya and ripe pineapple had a moisture content of 91.87% and 82.57%, respectively.

Rotten fermented fish preparation

Papaya and pineapple rich in proteases were supplemented in rotten fermented fish and compared with control, supplemented with distilled water. Three treatments of rotten fermented fish, supplemented with distilled water (control), unripe papaya, or ripe pineapple, were prepared. Five kilograms of minced frame was mixed with distilled water, papaya, or pineapple until the moisture content in the mixture reached 70%. Minced tilapia frame with a moisture content of 70% was proved to lower mold proliferation in rotten fermented fish (unpublished preliminary experiment). Experimental rotten fermented fish was prepared by combining four parts of minced tilapia frame with one part of salt and one part of roasted rice bran by volume, according to Sudapan (2011). Rotten fermented fish was divided into four portions. Each portion was tightly packed a glass jar. The surface of the rotten fermented fish in the jar was covered with a polyethylene plastic sheet. Plastic meshes were

place onto the plastic sheet to press down the mixture, making it submerge fully under the brine. The jars were then loosely capped and kept in a room at ambient temperature (28–32 °C). Rotten fermented fish was sampled in months 0, 2, 4, and 6 and analyzed for its quality parameters.

Analysis of quality parameters

Yeast and mold count (YM) was enumerated according to Mislivec and Stack (1989). Proper serial dilutions were spread onto acidified potato dextrose agar and incubated at 23 ± 0.1 °C for 5 days. The YM count was in the range of 15–150 CFU. The level of YM was expressed as log CFU/g.

Formol nitrogen was determined according to Beddows et al. (1976). One gram of fermented fish was mixed thoroughly with 40 ml of deionized water, kept in a refrigerator overnight, stirred at room temperature for 1 h, and filtrated through a Whatman No. 4 filter paper. The liquid was titrated to pH 7.0 with 0.1 N NaOH. Then 10 ml of formalin solution (38% v/v) was added to the neutralized liquid. The neutralized liquid was titrated to pH 8.5 with 0.1 N NaOH. Formol nitrogen (mg nitrogen/g fermented fish) was calculated using equation (1).

$$\text{Formol nitrogen} = \frac{\text{ml NaOH (pH 7.0–8.5)} \times \text{Normality of NaOH} \times 14}{\text{Weight of sample}} \quad (1)$$

CIE color values (L^* , a^* , and b^*) were analyzed using a Konica Minolta CM-2600dspectrophotometer (Konica Minolta, Inc., Japan). The L^* value indicates lightness; a^* and b^* are the red/green and yellow/blue coordinates, respectively.

Statistical analysis and experimental design

The experimental design was a 3×5 split-plot arrangement in a randomized complete block design (RCBD). The main plot was treatment (distilled water, papaya, and

pineapple), and the sub-plot was fermentation period (0, 2, 4, and 6 months). Statistical analysis was conducted using the IBM SPSS Statistics 21 program (IBM, Armonk, New York) at a 95% confidence level. The experiment was repeated three times using tilapia frames from three different production lots.

Results and Discussion

YM counts are illustrated in Table 1. The counts were not significantly different among the treatments in month 0 ($P>0.05$). After that, YM in the control decreased as fermentation time increased, and it was not detected in month 6. Unfavorable conditions for mold growth during fermentation such as an anaerobic/micro aerobic environment and lack of suitable nutrients, especially sugar, may have caused the reduction of YM in the control. In the sample containing papaya, reduction of YM was obvious. YM was not detected after month 0. The antifungal property of papaya may have contributed to the reduction in YM. The inhibitory effect of papaya or papaya extract on YM including *Rhizopus stolonifera* (Romasi et al., 2011), *Aspergillus*

niger, *Penicillium notatum*, *Fusarium msolani*, and *Candida albicans* (Tewari et al., 2014) has been documented. Ripe pineapple yielded YM counts in months 0–6 of 3.10–5.56 log CFU/g. Jay et al. (2005) stated that a variety of yeast genera can usually be found on fruits, and many yeasts are capable of using sugar. Pineapple may contain some YM species, especially halophilic ones, capable of survival in a saline environment. Sugar in ripe pineapple may support their proliferation in rotten fermented fish after month 2. The Thai Industrial Standards Institute (2014) requires fermented fish to contain $YM < 3$ log CFU/g. The results showed that the control sample, containing YM of 1.73 log CFU/g, passed the requirement in month 4. YM in the sample containing unripe papaya reached an undetectable level in month 2. Supplementation of unripe papaya enabled the product to meet the YM requirement 2 months earlier than the control. The sample containing pineapple failed to meet the YM requirement. The addition of unripe papaya, therefore, is advantageous for lowering YM, thus helping the product to meet the requirement before other treatments.

Table 1 Changes in yeast and mold, total plate count, and lactic acid bacteria in rotten fermented fish supplemented with unripe papaya and ripe pineapple during a fermentation period of 6 months

Treatments	Fermentation period (months)			
	0	2	4	6
Yeast and mold (log CFU/g)				
Distilled water (control)	5.06±0.34 ^{ab}	3.22±2.80 ^{aAB}	1.73±3.00 ^{aA}	ND
Unripe papaya	5.12±0.14 ^a	ND	ND	ND
Ripe pineapple	5.16±0.14 ^{ab}	3.10±2.70 ^{aA}	4.70±0.38 ^{bAB}	5.56±0.19 ^B
Formol nitrogen (mg nitrogen/g fermented fish)				
Distilled water (control)	0.01±0.00 ^{aA}	0.02±0.00 ^{bB}	0.02±0.00 ^{bB}	0.02±0.00 ^{aB}
Unripe papaya	0.01±0.00 ^{aA}	0.03±0.00 ^{aBC}	0.03±0.00 ^{aC}	0.02±0.00 ^{aB}
Ripe pineapple	0.01±0.00 ^{aA}	0.03±0.00 ^{abB}	0.03±0.00 ^{aB}	0.02±0.00 ^{aB}

Mean values in the same column for each quality parameter followed by different lowercase letters are significantly different at a 95% confidence level.

Mean values in the same row for each quality parameter followed by different uppercase letters are significantly different at a 95% confidence level.

ND= below detection limit (<15 CFU/g)

Formol nitrogen, reflecting the degree of protein hydrolysis (Zhong et al., 2015), is illustrated in **Table 1**. The results showed that the levels of formol nitrogen were not significantly different among treatments in month 0 ($P>0.05$). In months 2 and 4, formol nitrogen in samples containing unripe papaya and ripe pineapple increased significantly ($P<0.05$). However, in month 6, supplementation with the fruits produced no effect on formol nitrogen. Proteolytic enzymes in papaya and pineapple may benefit the hydrolysis of rotten fermented fish, thus increasing formol nitrogen in months 2 and 4. It was noticeable that the degree of hydrolysis in the samples containing papaya and pineapple dropped to 0.01 mg/g in month 6. Unfavorable high salt content may have decreased the activity of protease enzymes from papaya and pineapple, by which formol nitrogen was lowered. Ooshiro et al. (1981) concluded that the effects of adding papain and bromelain to assist fish sauce production are seen sharply only for a few days early in the fermentation period, after which the enzyme activity becomes weak due to the high salt concentration.

The CIE color values obtained are illustrated in **Figure 1**. In month 0, L^* values were not significantly different among the treatments ($P>0.05$). Values for the samples containing

distilled water, unripe papaya, and ripe pineapple ranged from 46.74 to 48.35. After that, L^* in the control (distilled water) increased rapidly during fermentation. The L^* value of rotten fermented fish supplemented with papaya was almost constant in months 0–4. However, the value increased to 48.83 in month 6. In the samples containing pineapple, L^* values mostly remained constant throughout the fermentation period. Low L^* values in samples containing papaya and pineapple may have been caused by the Maillard reaction. An increase in amino acids, hydrolyzed from fish meat during the extended fermentation period, may have caused them to interact more with reducing sugar broken down from starch in rice bran or sugar in the fruits, causing a decrease in L^* values. Additionally, skin of unpeeled papaya and pineapple in the product may have also contributed to the lower L values. a^* values of the samples containing ripe pineapple were significantly higher than those for the control in month 6. b^* values of the samples containing pineapple were also significantly higher than those for the control in months 2 and 6. It was noticeable that the a^* and b^* values of all samples showed increasing trends during fermentation, which may have been caused by the Maillard reaction.

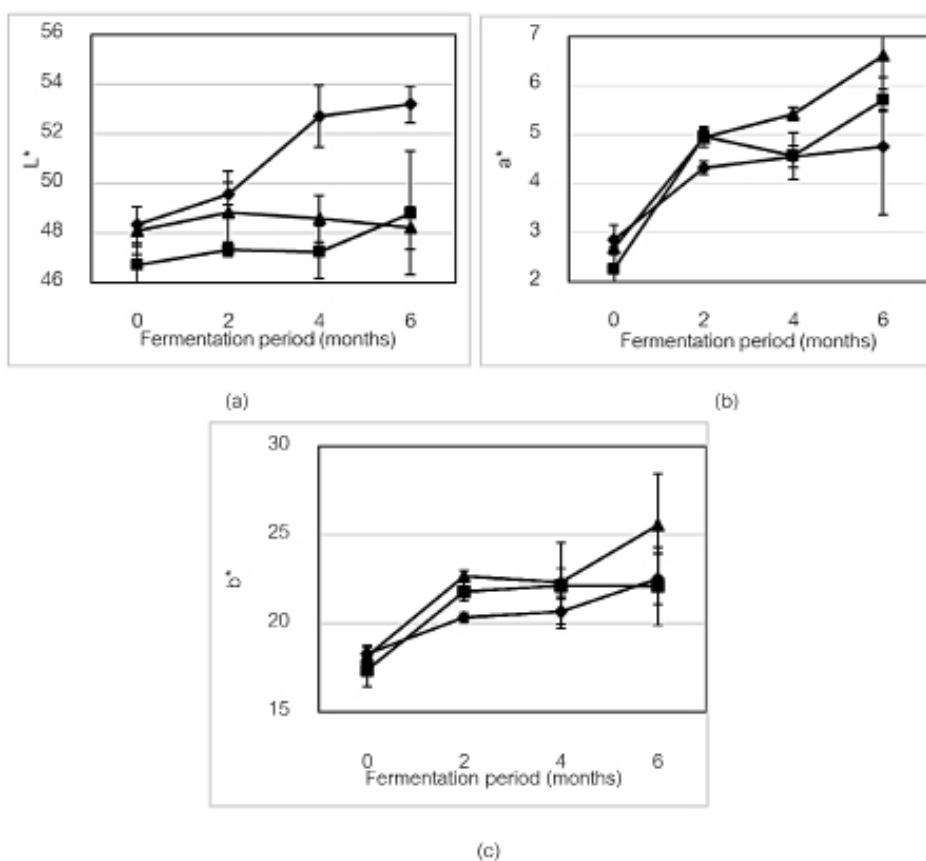


Figure 1 CIE L^* (a), a^* (b), and b^* (c) color values of rotten fermented fish supplemented with unripe papaya and ripe pineapple during fermentation. —◆— Distilled water (control), —■— unripe papaya, —▲—ripe pineapple

Conclusions

Supplementation with unripe papaya and ripe pineapple increased the formol nitrogen content compared to the control. However, values in the samples containing both fruits were not improved during fermentation. A high salt content or reduction in pH may stop the activity of bromelain and papain in the fruits during fermentation. Supplementation with ripe pineapple caused YM proliferation of $> 3 \log$ CFU/g, which failed the product specification

standards. Unripe papaya caused a reduction in YM to an undetectable level in month 2, thus passing the microbial quality standard. Supplementation with unripe papaya was therefore advantageous for suppressing yeast and mold, enabling the product to meet the YM quality standard faster than other treatments. It also improved formol nitrogen of rotten fermented fish. Fruit supplement tended to increase the Maillard reaction, by which L^* was decreased and a^* and b^* were increased. Supplement of papaya and pineapple tended to increase

Maillard reaction, thus decreasing L* value and increasing a* and b* values for rotten fermented fish. In conclusion, pineapple caused quality failure due to mold proliferation. Supplementation with papaya in RFF is therefore better than with pineapple in term of YM suppression and is recommended.

References

- AOAC. 1990. Official Method of Analysis. 17th Edition. Association of Official Analytical Chemists, Washington, D.C.
- Batista, I. 2007. By-catch, underutilized species and underutilized fish parts as food ingredients. P.171-191. In: F. Shahidi. Maximizing the Values of Marine By-Products. CRC Press, New York.
- Bechtel, P.J. 2003. Properties of different fish processing by-products from pollock, cod, and salmon. J. Food Process. Preserv. 27:101-116.
- Beddows, C. A., M. Ismail, and K. H. Steinkraus. 1976. The use of bromelain in the hydrolysis of mackerel and the investigation of fermented fish aroma. J. Food Technol. 2:379-388.
- Beddows, C. G. 1998. Fermented fish and fish products. P.416-434. In: B. J. Wood. Microbiology of Fermented Food, Volume 1. 2nd Edition. Blackie Academic & Professional, New York.
- Chomnawang, C., K. Huaisan, and J. Huaisan. 2013. Development of Fermented Fish Paste from Tilapia Frame. Full Report. Faculty of Agro Industry, Kalasin Technology, Kalasin (translated from Thai).
- Chuaphohak, P. 2006. Local Products from Aquatic Animals. Kasetsart University Press, Bangkok (translated from Thai).
- Diaz-Lopez, M. and F. L. Garcia-Carreno. 2000. Applications of fish and shellfish enzymes in food and feed products. P.571-618. In: N.F. Hard and B.K. Simson. Seafood Enzymes. Marcel Dekker, Inc., New York.
- Gawborisut, S., K. Huaisan, and P. Srisakultiew. 2013. Technologies for producing value-added food and leather commodities from tilapia processing industry by-products (Plara sauce from tilapia frame). National Research Council of Thailand, Bangkok (translated from Thai).
- Hemwihok, K. and S. Hongsaeng. 2013. North-Eastern Food. 1st Edition. Sangdad Publishing Co. Ltd, Bangkok (translated from Thai).
- Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern Food Microbiology. 7th Edition. Springer, New York.
- Krusong, W. 2004. Production of Thai fermented fish: Plara, Pla-som, Som-fak. P.707-718. In: K. H. Steinkraus. Fermentations in World Food Processing. 2nd Edition. Marcel Dekker, New York.
- Mislivec, P. B. and M. E. Stack. 1989. Mycotoxin prevention and control in food grains. <https://bit.ly/2Mnasil>. Accessed 18 Nov. 2013.
- Morton, R. D. 2001. Aerobic plate count. P.63-67. In: F.D. Downes and K. Ito. Compendium of Methods for the Microbiological Examination of Food. 4th Edition. American Public Health Association (APHA), Washington, D.C.

- National Research Council of Thailand. 1982. Report on Thai Traditional Fermented Food Research Project. National Research Council of Thailand, Bangkok.
- Netphakdee, A. 2013. Ratio of fermented-fish sauce, ground fermented fish flesh, and rotten fermented fish suitable for production of concentrate fermented-fish sauce. Senior Project. Department of Fisheries, Khon Kaen University, Khon Kaen(translated from Thai).
- Ooshiro, Z., T. Ok, H. Une, S. Hayashi, and T. Itakura. 1981. Study of use of commercial proteolytic enzymes in production of fish sauce. Mem. Fac. Fish. Kagoshima Univ. 30:383-394.
- Puladan-Muller, C., M. Madsen, P. Sophanodora, L. Gram, and P. Lange-Moller. 2002. Fermentation and microflora of Pla-som, a Thai fish product prepared with different salt concentration. Int. J. Food Microbiol. 73:61-70.
- Romasi, E. F., J. Karina, and A. J. N. Parhusip. 2011. Antibacterial activity of papaya leaf extracts against pathogenic bacteria. Makara J. Technol. 15:173-177.
- Sangjindavong, M., J. Mookdasanit, P. Wilaipun, P. Chuapoehuk and C. Akkanvanitch. 2009. Using pineapple to produce fish sauce from surimi waste. Kasetsart J. (Nat. Sci.) 43:791-795.
- Sim, K.Y., F.Y. Chye, and A. Anton. 2015. Chemical composition and microbial dynamics of Budu fermentation, a traditional Malaysian fish sauce. Acta Aliment. 44:185-194.
- Sudapan, P. 2011. Plara (fermented fish). Matichon Technol. Chawbaan 23:60-67 (translated from Thai).
- Tewari, B. B., G. Subramanian, and R. Gomathinayagam. 2014. Antimicrobial properties of *Carica papaya* (papaya) different leaf extract against *E. coli*, *S. aureus* and *C. albicans*. Amer. J. Pharma. Pharmacotherapeutics 1:25-39.
- Thai Industrial Standards Institute. 2003. Thai Community Product Standard No. 37/2546 (Plara: Fermented Fish). Thai Industrial Standards Institute, Ministry of Industry, Bangkok.
- Thai Industrial Standards Institute. 2014. Thai Community Product Standard No 37/2557 (Plara: Fermented Fish). Thai Industrial Standards Institute, Ministry of Industry, Bangkok.
- Uchida, M., J. Ou, B. Chen, C. Yuan, X. Zhang, S. Chen, Y. Funatsu, K. Kawasaki, M. Satomi, and Y. Fukuda. 2005. Effects of soy sauce koji and lactic acid bacteria on the fermentation of fish sauce from freshwater silver carp *Hypophthalmichthys molitrix*. Fish. Sci. 71:422-430.
- Zhong, C., M. Nakanishi, J. Geng, E. Okazaki, M. Cao, W. Weng, and K. Osako. 2015. Comparison of non-volatile taste-active components in fish sauce produced from lizardfish *Saurida wanieso* viscera under different conditions. Fish. Sci. 81:581-590.