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# Anchovy paste production from lactic acid fermentation of Anchovy fish found in the Gulf of Thailand

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## Abstract

Anchovies are small fishes of the Engraulidae familynative to the Mediterraneanand southern European coastlines. It is widely used as seasoning ingredient in European cuisine. In Thailand, anchovies were used to make dried fish and fish sauce. Anchovy paste is made from fermented anchovy fillets being ground into paste. Producing anchovy paste from anchovies found in the Gulf of Thailand will be an alternative way of producing high value anchovies-based product. *Lactobacillus pantarum* isolated from ka-pi (fermented krill) and *Tetragenococcus halophilus* isolated from imported anchovy fillets were used as lactic acid bacterial starter for the experiments. This research compared two fermentation processes of making anchovy paste. Process 1: anchovies were fermented in brine for 4 months with and without lactic acid bacterial starter. The cured fish fillets were then ground into paste and blended with olive oil. Process 2: anchovies were fermented in brine with and without lactic acid bacterial starter overnight, then left to air dry in order to reduce the amount of moisture for one day. Next, the fishes were pounded and anaerobic fermented in closed containers for four months before being turned into paste by blending with olive oil.The changes in physical, chemical and microbiological properties of the fish during fermentation were investigated. The bacterial inoculated products of Process 1 had the highest lactic acid concentration and the lowest pH value. The sensory evaluation indicated that theinoculated product of Process 1 had higher over all acceptable score than the un-inoculated product. When the experimented and imported anchovy paste products were used in making Caesar salad dressing, the differences went undetected by trained panelists. Therefore, the anchovy paste made of anchovy fishes from the Gulf of Thailand can be sold commercially and help increase the income for Thai fishermen. This new product can also be exported and replace the need for imported anchovy paste in Thailand.

**Keywords**: Anchovy paste, Anchovy fish, Fermented fish, Lactic acid bacteria

# 1. Introduction

Anchovies are small silvery fishes in the Engraulidae family, related to herring. They are found in scattered areas throughout the world's oceans.There are about 140 species in 16 genera, found in the Atlantic, Indian, and Pacific Oceans. Anchovies are abundant in the Mediterranean, and are regularly caught on the coasts of Italy, France, Turkey and Spain.Anchovies are used extensively in cuisines throughout Southern Europe. Anchovy paste is a paste made from ground anchovy fillets. The paste is available in many grocery and specialty stores, usually in the form of small tubes. It has a very distinctive salty, fishy flavor and is popularly taken not only as side dishes, but also as an ingredient in many types of salad dressings.It is also used as ingredients in variety of sauces, including Worcestershire sauce(1). When anchovy paste is manufactured, the filleted and cured fish are ground into an evenly textured paste. The curing process is what creates the distinctive intense anchovy flavor, and it ensures that the anchovy paste will have long shelf life. In the Gulf of Thailand, 14 species in 2 genera are found. Thai anchovies (*Stolephorus dubiosus*) are normally used as raw materials for fish sauce manufacturing and dried fish products. Due to high cost of anchovy products that are results of European manufacturing, producing anchovy paste from anchovies from the Gulf of Thailand will be an alternative way of producing high value anchovies-based product.

Curing of anchovies is a conventional process to obtain a product with a specific aroma and taste as a result of biological activities on the flesh. The term "preserved fish" refers to fish prepared by enzymatic curing, or maturation, in which salt is added and acts on muscles, viscera, microorganisms and enzymes, developing microorganism which produces lactic acid, lowering the pH and making the product resistant to the development of putrefying bacteria (2). Anchovies are salted and fermented at low temperature (10–20 °C) for 2–4 months to produce flavor. Since the fermentation processes involve the increase of organic acids into high quality of fermented product through the action of microbes, it contains relatively large amounts of lactic acid bacteria (LAB)(3). LAB has been reported to be the predominant microorganisms in most of fermented foods. They play important roles in fish preservation and fermentation processes and are considered technologically fundamental. LAB are able to decrease pH by lactic acid production, produce bacteriocins to prevent the growth of pathogenic and spoilage microorganisms, provide diversity by the modification of raw material to obtain new sensory properties, and improve stability of the shelf life of fish products. LAB also contributes to the flavor, color and texture development(4).Therefore, lactic acid bacteria are generally recognized as safe (GRAS), specification of origin, non-pathogenic and antibiotic resistance characteristics should also be used as starter culture in fermentation(5).

Saithong et al.(6) used two strains (high acid producers) of lactic acid bacteria (LAB): *Lactobacillus plantarum* IFRPD P15 and *Lactobacillus reuteri* IFRPD P17 as starter for producingpla-som, one kind of Thai fermented fish products. *Lactobacillus plantarum* and *Lactobacillus reuteri* were dominant as acid producers (decreased pH in pla-som). *Tetragenococcus* is the only genus of lactic acid bacteria (LAB) that can thrive in a high salt environment (18% NaCl). *Tetragenococcus halophilus* was reported to be isolated from soy sauce (7). It has been speculated that they could playa significant role in the flavor and aroma development of the fish sauce.Udomsil et al.,(8) reported that *T. halophilus* appeared to play an important role in volatile compound formation during fish sauce fermentation.

The objective of this research was to investigate a suitable process for producing anchovy paste from anchovy fish founded in the Gulf of Thailand while maintaining similar quality of the European made products. The traditional method of making fermented anchovy in Europe and the method of making kapi (fermented small shrimp and fish) in Thailand were applied with and without the lactic acid bacterial starter. The finished products were evaluated for consumer acceptability when compared with the imported products from Europe.

## 2. Materials and methods

**2.1 Isolation of Lactic acid bacteria from imported anchovy, anchovy paste, and shrimp paste**

5 samples of imported anchovy fillets and 2 samples of anchovy paste were obtained from the supermarkets in Bangkok. 10 samples of traditional kapi made from krill were purchased from local market in Thailand (Krabi, Samut Songkhram provinces). All samples were enriched in de Man Rogosa and Sharpe broth supplemented with 3% NaCl (MRS, Merck Darmstadt, Germany) and anaerobically incubated at 37°C for 14 day. 0.1ml from each enriched samples were poured plated in MRS agar with 3% NaCl and 1% $CaCO_3$ and anaerobicallyincubated using the Gas Pack system at 37°C for 3-5 days. The typical colonies that had clear zone surrounding were randomly picked and purified.The cultures were identified according to their morphological, physiological, and biochemical characteristics following Bergey's manual of systematic bacteriology. To increase shelf life, the purified isolates were stored at -80°C. The pure cultures were kept in MRS broth supplemented by 20% glycerol (w/w).

**2.2 Anchovy fermentationand Anchovy paste preparation**

Anchovies (*Stolephorus* sp.) and marine salt,supplied by Pichai fish sauce industry from Chonburi province, were used as the raw materials for anchovy fermentation. Two processes of the anchovy paste production were designed as shown in Fig 1. Process 1: mixture of anchovy fish and marine salt at the ratio of 3:1was fermented with and without lactic acid bacterial starter (1%w/w) in closed containers at 10-15°C for 4 months, and then the cured fish fillets were ground into paste and blended with olive oil. Process 2: mixture of anchovy fish and marine salt at the ratio of 10:1 was fermentedwith and without lactic acid bacterial starter (1%w/w)at 25-30°C for 1 day, then left to air dried before grinding and anaerobic fermenting in closed containers for 4 monthsat room temperature (25-30°C). Anchovy paste was made by blending the completely fermented mixturewith olive oil.The lactic acid bacterial starter was used at the rate of 1% w/w.

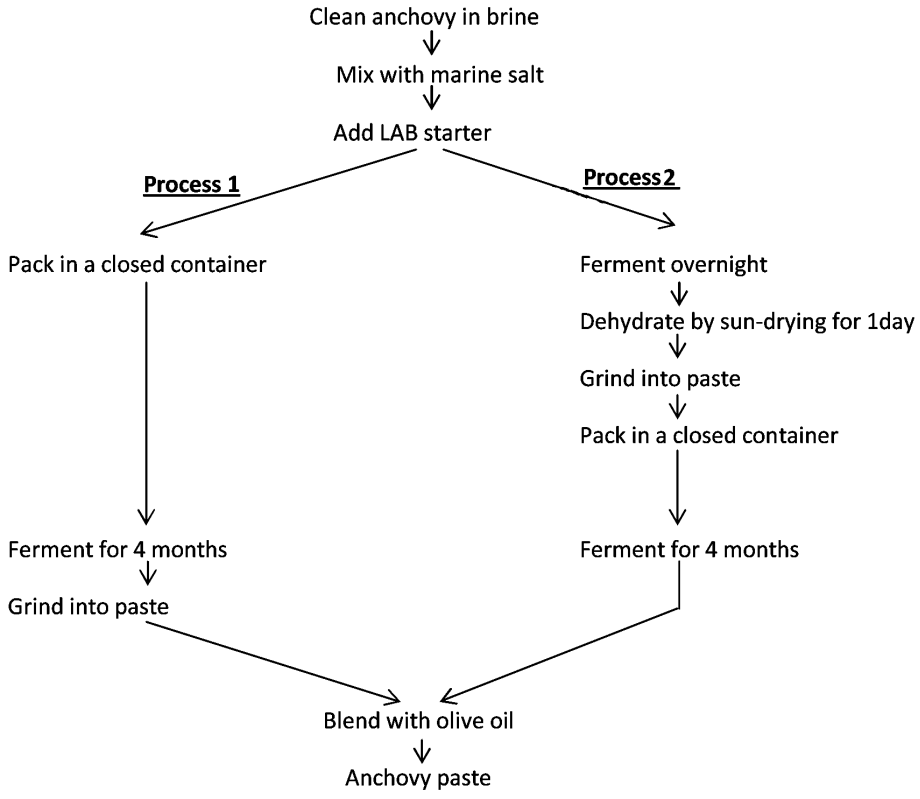


**Figure.1** Flow chart of experimental anchovy paste process 1 and process 2 (left and right)

**2.3 Physical and chemical analysis**

The pH and acidity were determined according to the method of AOAC (1995). The salt content was analyzed following Mohr's method (9). Moisture, ash, fat, protein and carbohydrate contents of anchovy paste products were determined according to AOAC (10). Amount of lactic acid produced in GYP medium by the bacteria were analyzed by UV method (11). The total volatile acid produced by the bacteria cultured in fish hydrolysate for 14 days was determined by adjusting the pH of the broth to 2.5 prior to steam distillate and titrate the condensate with 0.1 N NaOH. The amount of total volatile acid in mg was $CaCO_3$/l.

**2.4 Sensory evaluation of the experimental products**

The sensory evaluation was performed to determine the preference of the consumer between the experimental and traditional products. The test was revealed by 30 panels selected from members of the Microbiology Department of King Mongkut's University of Technology Thonburi. Water and unsalted biscuit were provided to cleanse the palate between samples. A preference test was carried out using hedonic scales consisting of 5 levels with 1representing extreme dislike and 5representing high satisfaction. The tested attributes were color, texture, odor, taste, and overall acceptability. The experimental and imported anchovy paste products were used in making Caesar Salad dressing and was put through thesame comparative test(12).

**2.5 Statistical analysis**

All analyses were carried out in triplicate, and results were expressed as mean values with standard deviations. The analysis of variances and means comparison by LSD post-hoc test were used with level of significant at 0.05 for analysis the data.

## 3. Results and discussion

**3.1 Isolation and identification of lactic acid bacteria**

6 isolates of lactic acid bacteria were isolated from 5 samples of canned imported anchovy fillets. The bacteria were identified according to Bergey's manual of systematic bacteriology (13) to be *Pediococcus acidilactici*2 isolates (A22and A68);*Pediococcus pentosaceus*1 isolate (A12); *Tetragenococcus halophilus*1 isolates (A66); and *Lactobacillus plantarum*2 isolates (A31 and A43). 10 isolates of lactic acid bacteria were isolated from 14 samples of Kapi. The bacteria were identified according to Bergey's manual of systematic bacteriology (13) to be *Pediococcus acidilactici*2 isolates (K73 and K91); *Tetragenococcus halophilus*2 isolates (K53and K103); and *Lactobacillus plantarum*3 isolates (K72, K93, and K124); and *Lactobacillus delbrueckii*3 isolates(K102, K172, and K215).

**3.2 D+L lactic acid and total volatile acid production of the isolates**

The efficiency of acid production of each bacterial isolate was demonstrated in Table 1. The *Lactobacillus plantarum*K93 isolated from Kapi sample gave the highest amount of lactic acid produced in GYP medium at 5.26 g/l. The *Tetragenococcus halophilus* A66 isolated from imported anchovy fillets generatedthe highest amount of total volatile acid at 9.09 mg $CaCO_3$/l when cultured in the fish hydrolysate. Therefore, these 2 isolates were used as bacterial starter in the experimental fermentation by individual culture and mixed culture.

**Table 1.** Lactic acid and total volatile acid production of the bacteria isolated from imported anchovy and Kapi samples

| Bacteria | Lactic acid (g/l) | Total volatile acid (mg $CaCO_3$/l) |
|---|---|---|
| *P. acidilactici*A22 | 6.28 | 8.23 |
| *P. acidilactici*A68 | 6.06 | 7.77 |
| *P. acidilactici*K73 | 7.45 | 8.08 |
| *P. acidilactici*K91 | 8.24 | 8.00 |
| *P. pentosaceus*A12 | 7.83 | 8.18 |
| *T. halophilus*A66 | 7.00 | 9.09 |
| *T. halophilus* K53 | 7.56 | 8.33 |
| *T. halophilus* K103 | 7.54 | 8.55 |
| *L. plantarum* A31 | 7.39 | 3.25 |
| *L. plantarum* A43 | 7.26 | 2.50 |
| *L. plantarum* K72 | 8.23 | 2.08 |
| *L. plantarum*K93 | 8.26 | 3.64 |
| *L. plantarum* K124 | 7.88 | 2.03 |
| *L. delbrueckii* K102 | 7.41 | 2.53 |
| *L. delbrueckii* K172 | 7.56 | 2.43 |
| *L. delbrueckii* K215 | 7.15 | 2.55 |

**3.3 Changing in pH and acidity of the fermenting material during fermentation**

When anchovy fishes were fermented following process1 and 2 with and without bacterial inoculums, the individual and mixed culture of *Lactobacillus* K93 and *Tetragenococcus* A66 were inoculated at 1%w/w to the fermenting fish. The changing in pH and acidity in term of amount of lactic acid during fermentation were demonstrated in Fig.2. For process1, whole anchovies were fermented for 4 months. The treatment with bacterial mixed culture could decrease the pH of the fermenting fish faster than the other treatments. The pH level of the final products was between 6.18to 6.28. The *Lactobacillus* K93 treatment gave the highest amount of lactic acid in the product at 3.47 g/l. For Process 2, whole fish were fermented for one day,then grounded and anaerobic fermented in the closed container. All the treatments of the process2 started at the pH level between 6.20 to 6.27 which was lower than of those of the process1 (6.59 to 6.45)and final pH level between 5.87 to 6.12. The mixed culture treatment gave the lowest pH and highest amount of lactic acid at 2.0g/l. All the treatments with bacterial inoculation enhanced the lactic acid fermentation than the uninoculated treatments.

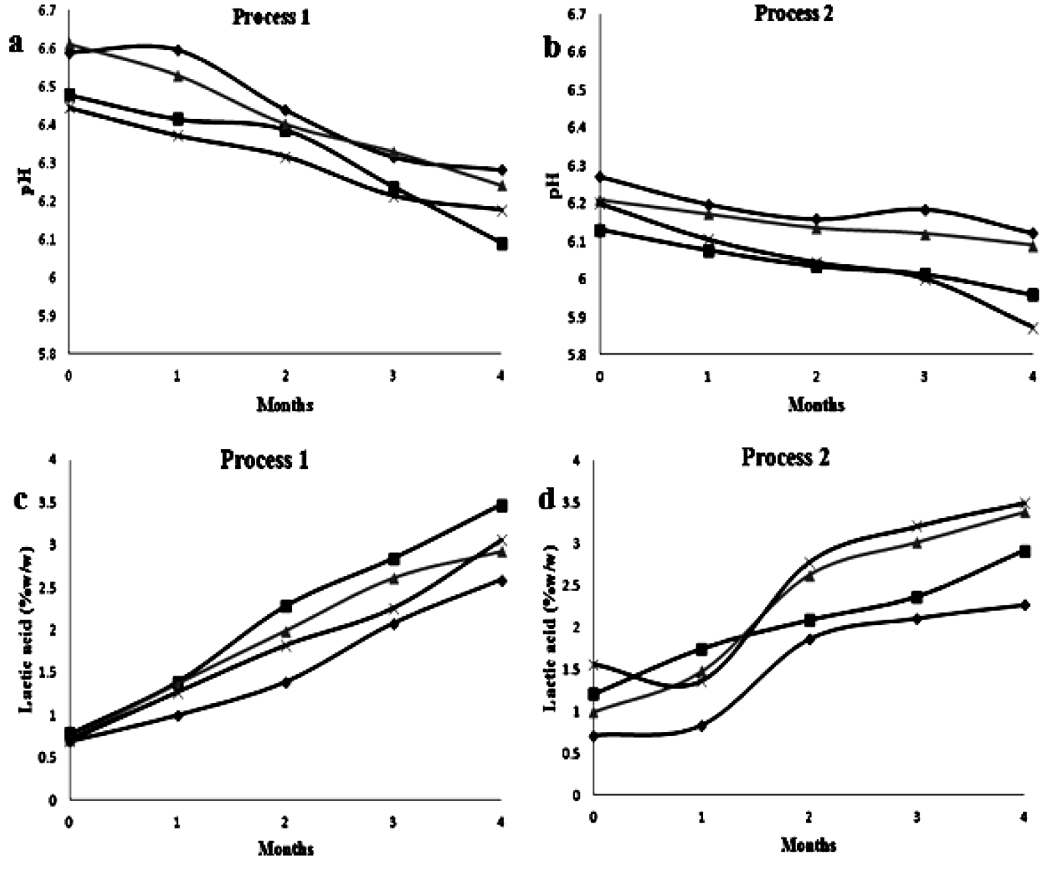


**Figure 2.** Changing in pH and acidity of the fermenting material during fermentation.

**3.4 Composition of the experimental anchovy paste**

The chemical compositions of anchovy paste products were determined as shown in Table2. The anchovy paste products made by process2 had higher protein and ash content than the products of the process1, while the anchovy paste products of the process1 had high moisture content.

**3.5 ensory evaluation**

The results of the sensory evaluation of anchovy paste by the panelists indicated a more positive response to the texture, taste and overall liking of anchovy paste inoculated with starter culture rather than anchovy paste made by natural fermentation (control);however, the experimental anchovy of all treatments were accepted less than the commercial product (Data shown in table 3). While the sensory evaluation of all experimental anchovy paste from ka-pi processing were not significantly different from the commercial product, when the experimental and imported anchovy paste products were used in making Caesar Salad dressing and performing the same-difference test (12), the differences went undetected by trained panelists ($p>0.05$)

**Table 2.** Chemical compositions of the experimental anchovy paste products

| Process | Proximate analysis (%w/w) | | | | |
|---|---|---|---|---|---|
| | **Moisture** | **Protein** | **Fat** | **Carbohydrate** | **Ash** |
| Process1 | | | | | |
| Control | 56.1 | 14.2 | 10.1 | 18.3 | 1.3 |
| *Lactobacillus* K93 | 53.4 | 14.8 | 8.7 | 21.4 | 1.7 |
| *Tetragenococcus* A66 | 53.3 | 14.2 | 8.2 | 22.6 | 1.7 |
| Mixed culture | 54.4 | 14.3 | 7.8 | 22.3 | 1.2 |
| Process2 | | | | | |
| Control | 35.1 | 18.9 | 10.9 | 22.8 | 12.3 |
| *Lactobacillus* K93 | 41.4 | 14.2 | 9.1 | 24.4 | 10.9 |
| *Tetragenococcus* A66 | 44.1 | 14.8 | 7.5 | 22.5 | 11.1 |
| Mixed culture | 38.4 | 15.3 | 8.3 | 25.4 | 12.6 |

**Table 3.** Sensory attributes in experimental anchovy fermentation and commercial anchovy paste products .

| | Sensory evaluation[i] | | | | |
|---|---|---|---|---|---|
| | **Color** | **Texture** | **Odor** | **Taste** | **Overall** |
| Anchovy paste | 3.50a | 3.13a | 3.77a | 3.57a | 3.70a |
| Process1 | | | | | |
| Control | 2.80b | 2.62b | 2.90b | 2.38c | 2.69b |
| *Lactobacillus* K93 | 2.70b | 3.03ab | 3.07a | 2.60bc | 2.77b |
| *Tetragenococcus* A66 | 2.73b | 2.93ab | 2.63b | 2.60bc | 2.73b |
| Mixed culture | 2.93b | 3.13a | 2.67b | 2.90b | 2.93b |
| Process2 | | | | | |
| Control | 3.17a | 3.61a | 3.01a | 3.03a | 3.11a |
| *Lactobacillus* K93 | 3.16a | 3.54a | 3.33a | 3.11a | 3.30a |
| *Tetragenococcus* A66 | 3.08a | 3.51a | 3.00a | 3.14a | 3.27a |
| Mixed culture | 3.19a | 3.72a | 3.08a | 3.19a | 3.22a |

(i)0 = dislike extremely; 5 = like extremely.

(ii) Means with the same letter within the column are not significantly different ($P < 0.05$).

## 4. Conclusion

This research has successfully produced anchovy paste using local raw material, the anchovy fishesfrom in the Gulf of Thailand. The comparison between the original processing (Europe standard) and the ka-pi processing (Thailand), and using the LAB starter culture for rapid fermentation and high quality, finally, we can substitute the ka-pi processing for anchovy paste production. The experimental anchovy paste is similar quality with commercial anchovy paste. This new product can increase values to the raw material found in Thailand.

## 5. Acknowledgement

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