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# Effects of lactic acid bacteria and fermentation conditions on physiochemical properties of fermented giant snakehead (*Channa micropeltes*) fish protein

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## Abstract

The objective of this study was to investigate physiochemical properties of fermented giant snakehead (*Channa micropeltes*) gel during fermentation. Lactic acid bacteria including *Lactococcus lactis* and *Lactobacillus plantarum* were added (5 log CFU/100g) into the fish mince prior to being kept at 10 °C and 25°C for 0, 6, 12, 24, 48 h. The properties of fermented gel including gel strength, pH, titratable acidity, whiteness, total volatile bases-nitrogen (TVB-N) and tricholroacetic acid (TCA) soluble proteins were examined. During 48 h of fermentation at 25 °C and 10 °C, giant snake gel inoculated with *L. lactis* and *L. plantarum* resulted in increase in gel strength, titratble acid and TVB-N, whiteness. In contrast, the decrease in expressible moisture, pH and TCA-soluble protein was observed. It could be concluded that lactic acid bacteria could improve textural properties of giant snakehead fish protein during fermentation.

**Keywords:** giant snakehead, fermented gel, Lactic acid bacteria, gel-forming ability

## 1. Introduction

Giant snakehead *(Channa micropeltes*) is carnivorous fish widely found in many tropical countries including Thailand. Generally, this kind of fish is not well known caused by its poor taste and low value for consumption.

Lactic acid bacteria (LAB) are known as the dominant microorganisms in fermented fish products (1, 2) The primary role of LAB is to ferment the available carbohydrates and thereby cause a decrease in pH. The combination of low pH and organic acids (mainly lactic acid) is the main preservation factor in fermented fish products. Generally, pH should be below 5–4.5 in order to inhibit pathogenic and spoilage bacteria (3).

The gel strengthening of fish protein can be achieved by subjecting sols at low temperatures, setting in the range of 0 °C to 40 °C prior to heating (4,5). The gel obtained after setting in this temperature range is generally referred to as 'suwari gel' (6, 7). The polymerization of the myosin heavy chain (MHC) catalyzed by an endogenous transglutaminase (TGase) occurs during low temperature setting, and imparts the enhancement of gel strength prior to heating at higher temperatures (8).

It can be considered that the gel formation of fish protein can be probably enhanced by the polymerization

of protein by TGase activity. However, the textural properties of fermented during fermentation time in the presence of lactic acid bacteria has not been extensively studied. Therefore, the objective of this study is to investigate the effects of lactic acid bacteria and fermentation conditions on physiochemical properties of fermented giant snakehead fish protein.

## 2. Materials and Methods

### 2.1. Preparation of starter culture

Two strains of LAB including *L. lactis* and *L. plantarum* were isolated from traditional fermented meat products produced in the Northeastern of Thailand that kindly supported from Department of Biotechnology Faculty of Technology Mahasarakham University. LAB was subculture in MRS broth at 30 °C for 24 h. Cells were harvested by centrifugation at 10,000g for 15 min at 4 °C, and washed twice with saline water (0.85% NaCl); then the cell pellets were resuspended in the same saline water. After adjusting the level of cells to 5 log CFU/ml, the resulting cell suspension was stored at 4 °C for the inoculation of fish mince.

### 2.2. Surimi preparation

Giant snakehead was bought in a local market. The fish was deheaded, gutted, and scaled. Prepared fish was then manually filleted. Fillet was minced to uniformity using a mincer with a hole diameter of 5 mm. Fish mince was washed with cold 0.1% NaCl (4 °C) solution at a water/mince ratio of 3:1 (v/w). The mixture was stirred for 3 min, and then the wash was followed by manually dewatering with cheesecloth. Surimi was mixed with 4 % sucrose as cryoprotectant. Surimi was packed into polyethylene bags (200 g), frozen at -30 °C in a air blast freezer (Irinox, Cobanese, Italy) and stored at -20 °C until used.

### 2.3 Gel preparation

Surimi was thawed at room temperature. The thawed surimi was chopped for 1 min using chopper (MK-K48 Matsushita Co Ltd, Japan). The chopped surimi was mixed with the mixture of chilled water (to adjust 82% in moisture), NaCl 2.5%, sucrose 4%, 1% garlic and 1 ml of starter containing *L. lactis* or *L. plantarum* (5 log CFU/100g surimi), then chopped for further 3 min. The resulting pastes were stuffed into stainless steel cylinder cases (2.5 cm diameter and 2.5 cm height), and wrapped by polyvinylidene chloride film. These prepared pastes were kept at 10 °C and 25 °C for 0, 12, 24 and 48 h prior to heating at 80 °C for 20 min and subsequently cooling immediately in ice water for 5 min. The resulting gels were kept overnight at 5 °C prior to analyzing gel properties.

### 2.4 Determination of Gel strength

Gel strength regarding to multiplication of breaking force (g) and deformation (cm) was measured by using the texture analyser TA-XT2i (Stable Micro Systems, Godalming, UK) equipped with a spherical plunger (diameter 5 mm; depression speed 60 mm/min).

### 2.5 Determination of pH and Titratable acidity (TA)

pH measurements were done according to the procedure of Wang, 2000 (9). To the sample (5 g), 45 ml of boiling distilled water was added and the mixture was homogenized at 11,000 rpm for 1 min, using an IKA homogenizer (model T25, Selangor, Malaysia). The pH was measured with a digital pH meter (Mettler Toledo 320-s, Shanghai, China). TA expressed as percentage of lactic acid were determined according to the method described by Ikenebomeh, 1989 (10).

### 2.6 Determination of TCA-soluble protein

Three grams of the 2-step heating gel were homogenized with 15 ml of 5% trichloroacetic acid (TCA) solution at 10,000 rpm for 5 min using an Ace Homogenizer (Nihon Seiki Kaisha Ltd., Tokyo, Japan). The filtrate of the resulting homogenate was used according to the Lowry method (11). Tyrosine was used as a standard. The amount of TCA-soluble amino acids and peptides was expressed as μg Tyr-equivalent/g sample.

**2.7 Determination of Total volatile bases-nitrogen (TVB-N)**

TVB-N content was determined using the Conway microdiffusion assay (12). Sample (2 g) was extracted with 8 ml of 4% trichloroacetic acid (TCA). The mixture was filtered using Whatman No. 41 then the filtrate was used for analysis. TVB-N was released after addition of saturated $K_2CO_3$ and diffused into the boric acid solution. The titration of solution was performed and the amount of TVB-N was calculated as mg nitrogen/100 g sample.

**2.8 Determination of whiteness**

The color of fermented gel was determined using a colorimeter (Minolta CR-300, Osaka, Japan). $L^*$, $a^*$, and $b^*$ values were measured and whiteness was calculated as described by Park, 1994 (13), as follows:

$$\text{Whiteness}=100-[(100-L^*)^2+a^{*2}+b^{*2}]^{1/2}$$

**2.9 Statistical analysis**

Analysis of variance (ANOVA) was performed and the mean values were compared based on Duncan's multiple range tests (14).

## 3. Results and Discussion

**3.1 Effect on Gel strength**

Gel strength of giant snakehead inoculated with LAB increased with higher fermentation temperature ($p<0.05$) (Figure 1). In the case of fermentation at 10 °C, gel strength reached the maximum values of 86.4% and 135.4% at 48 h for the both gels inoculated with *L. plantarum* and *L. lactis,* respectively, while the maximum values were 368.9% and 204.4% for the gel inoculated with *L. plantarum* and *L. lactis* in the case of gels setting at 25 °C for 48 h, comparing to the controls. It is generally accepted that setting condition plays a most important role in strengthening surimi gels because of the polymerization of myosin heavy chain through ε-(γ-glutamyl) lysine cross-linking associated with endogenous TGase, resulting in the enhancement of surimi gel quality (15). Setting surimi sol at 25 °C, for an appropriate time, can improve gelling properties of surimi produced from tropical fish (16).

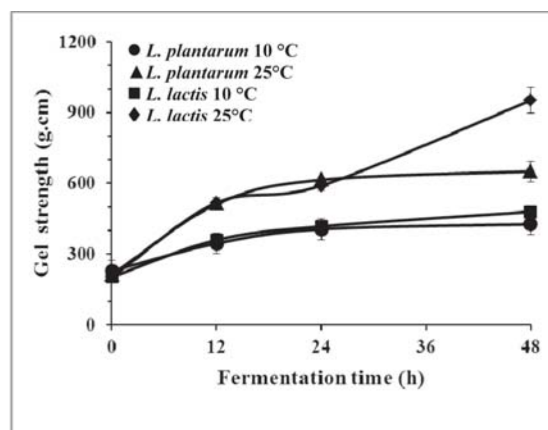


**Figure. 1** Gel strength of giant snakehead fermented gel inoculated with *L. lactis* and *L. plantarum* (5 log CFU/100g) kept at 10 °C and 25°C for 0 h, 12, 24 and 48 h prior to heating at 80 °C for 20 min.

**3.2 Effect on pH**

The initial pH value of giant snakehead gel (about 6.9) decreased sharply to 4.2 within 48 h of fermentation time at 25°C, while slight decrease was observed at 10°C (Figure 2). This can be explained by acid bacteria resulting in rapid production of organic acids at 25°C, especially lactic acid. This results were in agreement with those described by (17, 18) stating that the rapid growth of lactic was observed when fermented at 25°C at which the inhibition of undesirable microorganisms was found. Therefore, the gel fermented at low temperature (10°C) must be cooked before consuming.

**3.3 Effect on Titratable acidity (TA)**

Figure 3 shows that TA was increased with increasing temperature ($p<0.05$). During fermentation at low temperature (10 °C), the slight increase in TA for both gels inoculated with LAB for 48 h was observed. However, the greater increment in TA was found when fermented at 25 °C (0.1 to 1.2 mg/g) for 48 h.

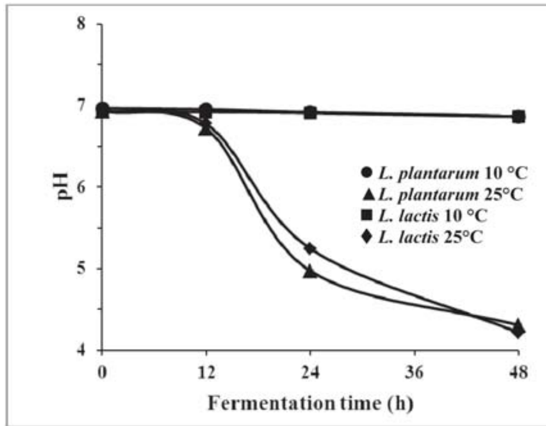


**Figure. 2** pH and of giant snakehead fermented gel inoculated with *L. lactis* and *L. plantarum* (5 log CFU/100g) kept at 10 °C and 25 °C for 0 h, 12, 24 and 48 h prior to heating at 80 °C for 20 min

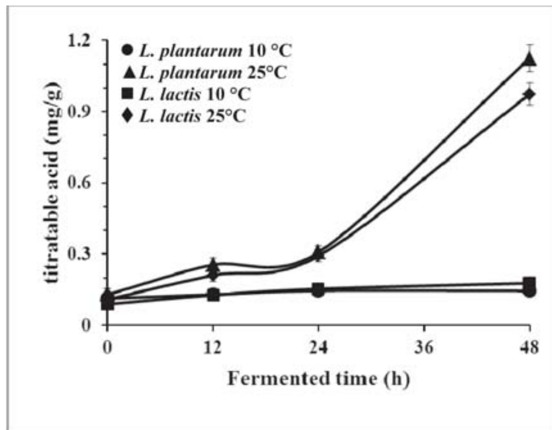


**Figure. 3** Titratable acidity of giant snakehead fermented gel inoculated with *L. lactis* and *L. plantarum* (5 log CFU/100g) kept at 10 °C and 25 °C for 0 h, 12, 24 and 48 h prior to heating at 80 °C for 20 min

**3.4 Effect on TCA-soluble proteins**

Change in TCA-soluble proteins of giant snakehead fermented gel is shown in figure 4. Values of TCA soluble protein changed significantly ($p < 0.05$) depending on fermentation temperature. Fermentation the gel at 25 °C showed higher TCA-soluble proteins than that at 10 °C. After 12 h of fermentation the decrease of TCA-soluble proteins contents was observed, then slightly increased. This suggested that intense proteolysis occurred at higher temperature. This was probably attributed to the enhanced activity of both endogenous and microbial proteolytic enzymes at the higher temperature, especially at low pH, which could stimulate acidic proteinase activity (19).

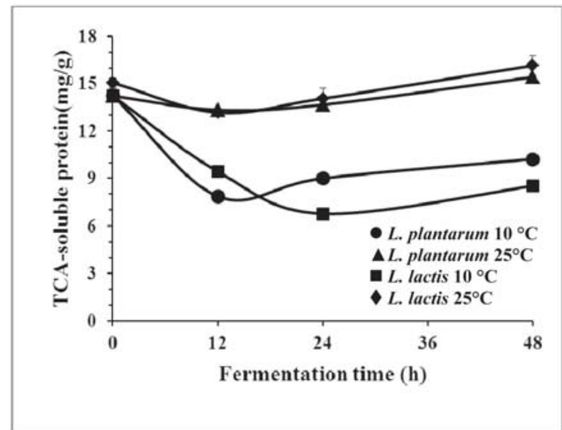


**Figure. 4** TCA-soluble proteins of giant snakehead fermented gel inoculated with *L. lactis* and *L. plantarum* (5 log CFU/100g) kept at 10 °C and 25°C for 0 h, 12, 24 and 48 h prior to heating at 80 °C for 20 min.

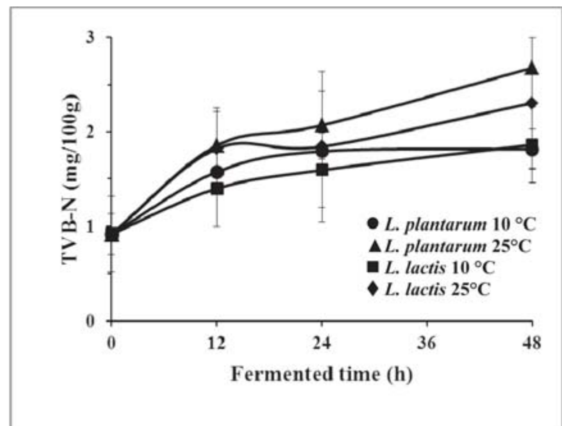


**Figure. 5** Total volatile bases-nitrogen(TVB-N) of giant snakehead fermented gel inoculated with *L. lactis* and *L. plantarum* (5 log CFU/100g) kept at 10 °C and 25°C for 0 h, 12, 24 and 48 h prior to heating at 80 °C for 20 min

**3.5 Effect on Total volatile bases-nitrogen (TVB-N)**

The changes in TVB-N content in the fermented giant snakehead gel are shown in Figure 5. The initial TVB-N value was 0.92 mg/100 g sample. During fermentation at 25°C for 48h, the TVB-N of fermented gel inoculated with *L. lactis* and *L. plantarum* reached maximum values of 2.30 and 2.67 mg/100 g, respectively. TVB-N of the gel fermented at 10 °C increased slowly during the 48 h of the fermentation, reaching the value of 1.86 and 1.82 mg/100 g, respectively. However, only slight increase in the TVB-N values was observed in the gel inoculated with LAB cultures. LAB in meat fermentation could inhibit the accumulation of TVB-N by producing lactic acid and bacteriocins, which could neutralize the TVB-N or inhibit the growth of spoilage bacteria and pathogens (18).

**3.6 Effect on Whiteness**

Whiteness of directly heated gel was showed in the range of 75.4 to 78.9 (Figure 6). After fermentation for 12 h the increase in whiteness of those gels were observed. However, the gel inoculated with LAB that fermented at 25°C was whiter than that at 10 °C. The increase in whiteness might be due to the hydrolysis of water-soluble and salt-soluble proteins (18) and pigment proteins, which consequently make the fermented products more transparent and much whiter (20).

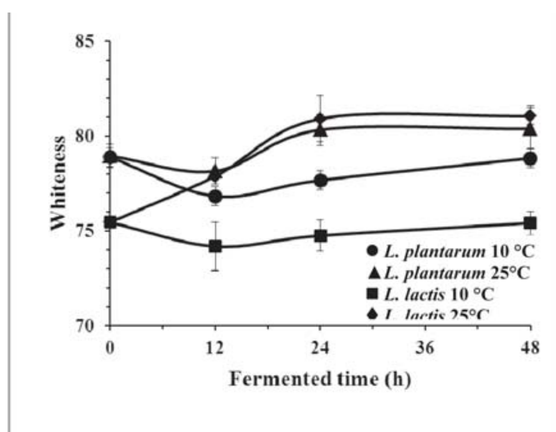


**Figure. 6** Whiteness of giant snakehead fermented gel inoculated with *L. lactis* and *L. plantarum* (5 log CFU/100g) kept at 10 °C and 25°C for 0 h, 12, 24 and 48 h prior to heating at 80 °C for 20 min

# 4. Conclusion

This study suggests that high potential setting conditions of fish protein for an appropriate temperature and time, associated with *L. lactis* and *L. plantarum* in the fermentation process. Therefore, this combination can improve textural and physiochemical properties of fermented fish products from fish protein.

# 5. Acknowledgement

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