# Effect of Cooking and Preservation Time on Fish Balls Quality Produced from *Pangasius hypophthalmus* Meat by-Product

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## Abstract

The demand for Tra fish (*Pangasius hypophthalmus*) fillets is increasing every year which also increases the number of fish meat byproducts. Approximately 10% of *P. hypophthalmus* meat is discarded after the production of fish fillets. This study aimed to develop fish balls from the fishmeat byproduct of *P. hypophthalmus* fillet then investigated the effect of cooking methods and preservation time on the alteration of its texture, brightness, and total bacterial count (TBC). The raw material, minced byproduct and fish balls were analyzed for pH, protein, fat, and moisture contents. The protein content in Tra fish reached 7.35% and increased to 37.14% after the completion of the processing stages for the finished product. Blanching for 4 minutes at 90°C and steaming for 4 minutes at 100°C resulted in good texture and brightness of fish balls. However, a more effective reduction in total bacterial count was observed during the blanching process. Fish balls were preserved by freezing at -40 °C for 42 days and still maintained stable brightness. However, TBC increased significantly after 7 days, and conversely for the texture of fish balls. A finding on the stability of texture and TBC of fish balls when continued preservation from 7 to 42 days. The utilization and use of by-products from the fish fillet processing industry contribute to improving the economic value of the aquaculture industry.

Keywords: Blanching, freezing preservation, fish balls, *Pangasius hypophthalmus*, pre-treatment

## Introduction

*Pangasius hypophthalmus*, also known as Tra fish, is a freshwater catfish commonly growing in Southeast Asia, particularly in the Mekong basin (Guimarães et al., 2016; Jeyakumari et al., 2016). Besides the nutritional value, this fish has white meat, lacks a fishy smell, and is relatively low priced, which attracts many consumers (Rao et al., 2013; Usydus et al., 2011). Therefore, this fish has been sold in over 80 countries worldwide between 2009 and 2013. In addition, Vietnam is noted as the top producer of *P. hypophthalmus* among seven other Asian countries, including Laos, Bangladesh, Malaysia, Cambodia, China, and Indonesia (Karl et al., 2009; Phan et al., 2009; Tong Thi et al., 2013).

The demand for boneless and skinless fish products is increasing, especially in developed countries such as the Netherlands, the United States, and Germany (Karl et al., 2009; Phan et al., 2009). At the same time, the amount number of fish meat by-products after filleting is also escalated. Therefore, processing technology is necessary to give added value to these by-products.

On the other hand, in the Vietnamese and international markets, the main product of *P. hypophthalmus* is fish fillets. Currently, product variety from *P. hypophthalmus* in the Vietnamese market is limited, which allows the development of another product by utilizing its meat by-product.

Among other processed food, the fish ball has become one of the most favorable products in the market. Therefore, the utilization of fish meat by-products to develop fish balls is very interesting to be done. It was emphasized that the type of fish, as the main ingredient, influences the cooking method for making fish balls. A previous study produced fish balls from a mixture of *P. hypophthalmus* and *Sarda orientalis* meat using two methods: incubation at 50 °C for 60 minutes followed by heating at 100°C for

30 minutes, and pressure steaming at 120 °C and 15 lbs/inch$^2$ for 20 minutes (Hoque et al., 2021). Another report applied heat treatment to make fish balls from *Hypophthalmichthys molitrix* by soaking the meat in warm water (40 °C) for 30 minutes, then increasing the temperature gradually to 90 °C for 20 minutes (Fan et al., 2022). Previously, Kok et al. (2013) developed fish balls from a meat mixture of *Nemipterus* sp., *Priacanthus* sp., *Johnius* sp., *Saurida* sp., and *Upeneus* sp., by cooking them at 90 °C for 10 minutes. Nevertheless, the effect of increasing time and temperature was clearly shown on the texture of the Bighead Carp muscle (*Aristichthys nobilis*) in a previous study (Jiang et al., 2018). Besides, each type of fish requires different processing conditions to ensure the texture and sensory quality of the product. In addition, the original properties of food, preservation method, conditions, and period are crucial to extending the shelf life of the product. A previous study showed that frozen *Mystus seenghala* muscle at -12 °C slowed down microbial growth, with total plate count reaching 7.77 ± 0.2 log CFU/g after three weeks (Gandotra, 2012). Alkuraieef et al. (2020) reported that frozen preservation (-18 °C) effectively maintained mackerel pellets' quality for six months with a total plate count reaching 2 x $10^2$ log CFU/g. At the same time, it was noted that the sensory value of the meat texture did not change.

The by-product from the *P. hypophthalmus* fillet industry was utilized to develop a fish ball product to add value. Since the utilization of *P. hypophthalmus* fillet industry by-product has never been reported, this study aimed to investigate the effect of two cooking methods on the fish ball's physiochemical quality and the effect of preservation on the alteration of fish ball quality.

## Material and Methods

### Samples

*P. hypophthalmus* was raised and caught in the Mekong Delta, Vietnam. About 20 kg of the leftover parts of *P. hypophthalmus* is collected while shaping fillets at the Hung Phuc Company in Can Tho City, Vietnam. The leftover parts were washed at the factory and transported to the laboratory for 20 minutes under refrigerated conditions, with ice packs maintaining a temperature of 1-2°C. Raw materials are stored at -10 °C for up to 24 hours before processing.

### Equipment and Chemicals

Some devices were used to support analysis, such as Colorimeter, Minolta - Japan, Texture Analyzer TA-XT2i, England, Soxhlet System – Spain, and Kjeldahl System – Germany, pH measuring equipment HI2211 pH/ORP Meter, Hanna Instruments Ltd. US and some chemicals were used, such as $NaHCO_3$ (>99%), diethyl ether (>99%), $H_2SO_4$ (>99%), $CuSO_4.5H_2O$ (>99%), $K_2SO_4$ (>99%), NaOH (>97%), $H_3BO_3$ (>99%) – which was purchased at Merck.

### Processing

Raw materials were washed immediately with 0.3% $NaHCO_3$ and 0.3% salt after thawing to about 50%, then were sliced with an average size of 0.2 cm. The seasoning mixture, including sugar, salt, pepper, starch, dried garlic, monosodium glutamate, polyphosphate, and sorbitol, was added to the ingredients. The mixture was ground (*) and created spheres with an average weight of 10 g. The pellets were blanched at 40 °C for 10 minutes to increase the cohesion of the meat tissues (Kok et al., 2013). Two cooking methods were applied for 3–6 minutes, inclosing (1) steaming at a temperature of 100 °C (2) blanching at a temperature of 80–100 °C (Kok et al., 2013). After heating, the sample was drained in a cold room (20–25 °C). After the samples had been drained and cooled, the samples were stored in a refrigerator at a temperature of (4–5 °C) before being stored at -40 °C. This process aimed to prevent evaporation and water condensation on the surface of the products, resulting in minimizing the possibility of mold growth (Kok et al., 2013). The product was frozen at a temperature of -40 °C for six weeks. A previous report has shown that preserving seafood at -40 °C is the best way to retain some biological activities and reduce fat oxidation in seafood (Secci & Parisi, 2016). Inactivation of harmful microorganisms and reduced fat oxidation have been shown during the preservation of meat and fish in deep freezing conditions between -30 °C and -80 °C (Ojagh et al., 2014), and the formation of peroxide and malondialdehyde was gradually inhibited when the temperature was reduced from -25 to -45 °C (Indergård et al., 2014; Karlsdottir et al., 2014).

### Texture Determination

The Texture Analyzer TA-XT2i was used to analyze the texture of Tra fish balls. HDP/BSK probes were used during compression at a speed of 2 mm/s (Soliman et al., 2022).

### Determination of Brightness (L*)

The Colorimeter device was used to determine the brightness of the product, which used CIELab color space to determine the L* value. The L* value ranges from 0 to 100, corresponding to the color range from black to white.

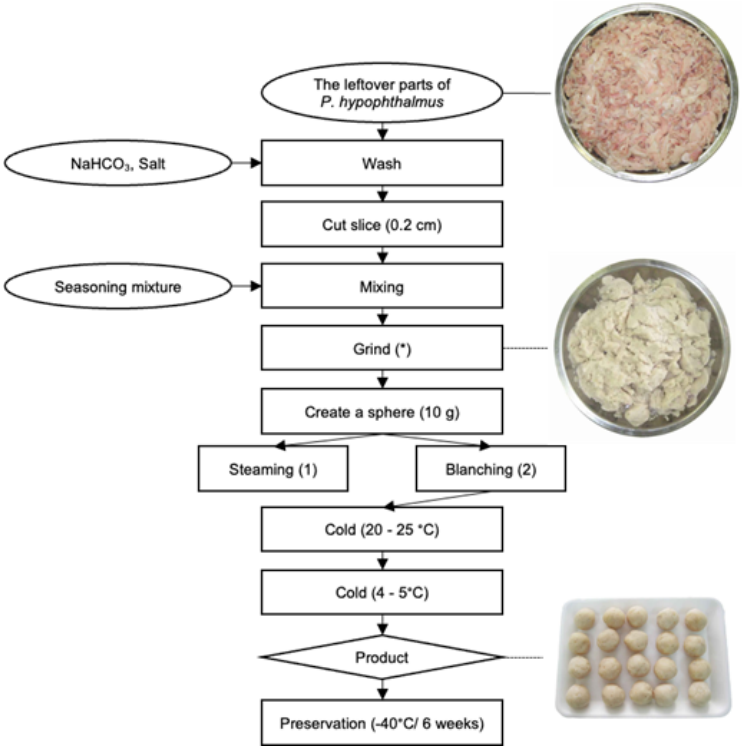


Figure 1. Processing and preservation process of fish balls from *P. hypophthalmus.*

## Determination of Total Bacterial Count (TBC)

Determination of TBC was carried out as described in a previous study. Bacterial cultures were diluted two-folded and spread onto the 10 cm diameter petri dishes, followed by incubation at 37 °C for 18-24 hours. The total number of bacteria in the sample was determined in units of CFU/g (Khanom et al., 2017).

## Determination of Lipid Content

Fat content was determined by the Soxhlet method, as described in a previous study. Firstly, 10 g of the sample was crushed, then wrapped with filter paper and placed in the extraction tube. Approximately 350 mL of diethyl ether was used as the extraction solvent for 8-12 hours. On average, there are 7-8 spills of solvent per hour. After extraction, the sample bag was dried at 50 °C three times, and measured the weight difference after extraction (Mohammadpour et al., 2019).

## Determination of Protein Content

The determination of protein content was described in a previous study. The sample was digested in $H_2SO_4$, while a mixture of $CuSO_4.5H_2O$ and $K_2SO_4$ was used as a catalyst for releasing nitrogen in the protein, and the nitrogen as a retained $NH_4^+$. NaOH 30% was used to liberate $NH_3$. The distillation process draws $NH_4^+$ into an absorption flask containing $H_3BO_3$, which is then titrated (Chang & Zhang, 2017).

## Determination of Moisture Content (MC)

The moisture content was determined based on the description in the previous study. The moisture content was determined as the weight loss before and after drying (Vu et al., 2022).

## Determination of pH

The pH value is determined with the HI2211 pH/ ORP Meter manufactured by Hanna Instruments Ltd. A 20g sample is in a glass beaker, and the pH probe is directly inserted into the mixture. The pH is determined under conditions of 30°C with three repetitions.

## Analysis of Statistical

Microsoft Excel software (Redmond, WA, USA) and Statgraphics Centurion XV version 15.1.02 was used for data analysis (Vu et al., 2022).

# Result and Discussion

## Composition of Input *P. hypophthalmus*, Grind (*), and Product

The chemical composition analysis of *P. hypophthalmus* at the grinding stage showed protein,

lipid, and pH changes. However, there was no change in MC (moisture content) after the grinding process compared to the raw material. The moisture content of *P. hypophthalmus* reached 77.09 ± 1.89%. This result is similar to the pool barb (*Puntius sophore*) and about 8% higher than *Garra abhoyai* (68.25 ± 0.39%) (Sarjubala et al., 2018). After pre-treatment (wash), the presence of $NaHCO_3$ and NaCl had an effect on water preservation in fish muscle ($p<0.05$). The formation of aggregated β-structures at the expense of α-helices has increased water retention in fish (Åsli et al., 2016). On the other hand, polyphosphate was added during the mixing process, which helped enhance the binding capacity between water and fish meat (Carneiro et al., 2013). The close connection between muscle fibers and animal tissue and enhanced water retention. Therefore, in the grind stage, P. hypophthalmus did not change moisture content significantly ($p>0.05$).

Table 1. Raw material composition, grind stage (*), and product

| Components | Raw materials | Grind (*) | Fish balls |
|---|---|---|---|
| MC (%) | 77.09 ± 1.89[a] | 76.23 ± 0.86[a] | 45.22 ± 0.18[b] |
| Protein (%) | 7.35 ± 0.05[b] | 14.41 ± 0.08[a] | 37.14 ± 0.18[a] |
| Lipid (%) | 10.92 ± 0.16[b] | 4.78 ± 0.09[c] | 13.37 ± 0.41[a] |
| pH | 6.4 ± 0.03[b] | 7.42 ± 0.17[a] | 7.36 ± 0.03[a] |

Note: Different letters in the row indicate a statistically significant difference ($p<0.05$) and vice versa

The results are similar to the study of the effects of $NaHCO_3$ and NaCl on the water content in *Pollachius virens* L. (Åsli et al., 2016). The protein content was evaluated to reach 7.35 ± 0.05 %. This result is within the average protein value (6 - 10%) of other fish species (Anchovy, Sardine, Capelin, and Herring). The protein value reached 14.41 ± 0.08 % after preliminary processing with $NaHCO_3$ and NaCl, two times higher than the control. A previous report showed that increasing the pH value of chicken meat from 5.5 to 7.0 also promoted protein content (Zhu et al., 2018). The increase in protein content was proportional to the rise in pH. This result is explained by the dependence of pH on the isoelectric point of the muscle material when the pH is further away from the isoelectric point of the muscle material. The meat of the material increases the solubility of the protein. In this case, the higher the pH value, the further away from the isoelectric point of the fish (pH 5.5), corresponding to an increase in solubility. However, approximately 60% of the fat content was reduced compared to the raw material after grinding process. This has resulted in an increase in the percentage of protein in the same unit mass of the sample. The results resemble a previous report on washing surimi-like materials from duck meat with NaCl and $NaHCO_3$ (Ramadhan et al., 2014). Another report mentioned that the grinding process lost fat in the material (Carpenter, 2010). Fat loss during grinding is explained by severing the bonds between the meat tissues, and the fat molecules are cut into small pieces to easily adhere to the equipment wall and the processed tools contact.

The pH value increased from 6.4 ± 0.03 to 7.42 ± 0.17. The results were consistent with the previous report in which the pH value was significantly increased when $NaHCO_3$ was added during the soaking of fish muscle (Åsli et al., 2016). Another report indicated that the pH value of surimi-like material from duck meat increased from 6.5 to 7.3 when three washes were conducted using NaCl. Furthermore, pH increased to 8.4 when the washing process involved $NaHCO_3$ (Ramadhan et al., 2014). A similar report of $NaHCO_3$ increasing pork pH by 0.46 units was previously reported (Zhu et al., 2018). Besides, the moisture content (MC) decreased significantly after processing, with MC remaining at 45.22 ± 0.18%. This can be explained by the fact that the high temperature during the cooking process of fish balls causes the water in them to evaporate, resulting in a decrease in MC. The reduction in MC leads to increased protein and fat content per gram of dry weight.

## The Investigation of Steaming Time

Steaming is cooking food products using steam as the heat transfer medium (Huang et al., 2013). The steaming process has a statistically significant effect on the brightness (L*) of the product ($p<0.05$). The L* value after immersion in water at 40 °C reached 34.14 ± 0.5. The L* value tends to increase as the steaming time is increased to 4 min. However, the L* value decreases after the 4$^{th}$ minute of steaming and remains at 50.55 ± 0.42. Besides, the investigation of steaming time from 5 to 6 minutes did not find a statistically significant difference ($p>0.05$). The color change of the product during autoclaving is explained by the conversion of iron(II) oxide, which is dark red in myoglobin, to iron(III), which is light brown upon heating (Wójciak et al., 2014). Applying too-high heat will cause the cell tissue to shrink, and the density of browning components will shrink. The color of the product becomes dark brown. Besides the significant influence of the steaming time on the change in brightness of the product, the product texture also changes due to the shrinkage and dehydration of the raw materials (Sehrawat et al., 2016). The force required to break the product increased with increasing

the steaming time to 4 min (450.72 g/cm$^2$). The increase in the steaming time causes the temperature to affect all parts of the product. After being heated and cooled, the protein in the product will appear precipitated, and the meat tissue shrinks and becomes stiffer (Brewer, 2012). The results are similar to a report on fish texture, as increasing the blanching time (40 °C) requires more force to break the fish texture (Liu et al., 2011). However, at high temperatures for a long time, the proteins are easily decomposed, and the bonds between the tissues are broken and decomposed, leading to the phenomenon of soft texture (Bax et al., 2012).

Table 2. Effect of steaming time on the change in brightness (L*), TBC, and Breaking force (BF) of the product

| Time (min) | BF (g/cm$^2$) | TBC ($10^4$ CFU/g) | Brightness (L*) |
|---|---|---|---|
| 0 | 286.45$^e$ | 560 ± 23.09$^a$ | 34.14 ± 0.5$^d$ |
| 3 | 401.68$^b$ | 4.2 ± 0.25$^b$ | 47.66 ± 0.51$^c$ |
| 4 | 450.72$^a$ | 2.8 ± 0.10$^c$ | 54.33 ± 0.42$^a$ |
| 5 | 378.97$^c$ | 2.5 ± 0.15$^d$ | 50.55 ± 0.42$^b$ |
| 6 | 361.69$^d$ | 2.4 ± 0.21$^d$ | 51.34 ± 0.69$^b$ |

Note: Different letters in the same column indicate a statistically significant difference ($p<0.05$) and vice versa

The temperature of the cooking process not only affects the color and texture of the product but also separates a part of the water in the product and destroys microorganisms that cannot withstand high temperatures present on the product. The remaining TBC on the product is inversely proportional to the heating time of the autoclave. The TBC of the original sample was 5.60 ± 0.23 × $10^6$ CFU/g. After autoclaving at 100 °C for 3 minutes, TBC decreased significantly and remained at 4.20 ± 0.25 ×$10^4$ CFU/g. The trend of microorganism survival decreased as the steaming time continued to increase to 6 minutes, with the TBC value reaching 2.4 ± 0.21 × $10^4$ CFU/g. The results are consistent with a previous report on applying 121 °C temperature in the pasteurization of food products (Li & Farid, 2016). A similar effect of using heat was shown to reduce the number of microorganisms (Sagar & Suresh Kumar, 2010).

## Effect of Blanching Time and Temperature on Brightness (L*)

Blanching is one of the methods used to cook food products by utilizing water as a heat transfer medium at high temperatures. The heat transfer occurs directly in the boiling water, which has a much higher heat transfer coefficient than steam at the same temperature.

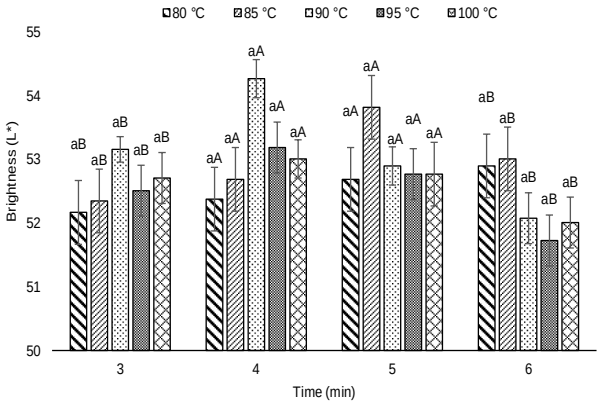


Figure 2. Effect of blanching time/temperature on brightness change of products (L*). Different lowercase and uppercase letters represent statistically significant differences in temperature and time, respectively ($p<0.05$).

Temperature and time significantly affected the quality of products cooked by blanching. The investigated blanching time of 3 - 5 min tended to increase brightness. At the temperature of 80 °C, the brightness L* tends to increase continuously, reaching the highest value at 6 minutes (52.90 ± 0.52). During the blanching process at 85 °C, the L* value increased until the 5$^{th}$ minute, reaching the highest value (53.82 ± 0.55), and the L* value tended to decrease when blanching continued until the 6$^{th}$ minute. Temperature 90 – 100 °C, the L* values both tended to increase to the highest when blanching for 4 min and decreased as the blanching time continued to increase to 6 min. Transferring heat from the environment into the product at lower temperatures takes longer. The slower color change rate because converting iron (II) into iron (III) requires the influence of temperature. At high temperatures, the shorter the heat transfer rate from the aqueous medium to the center of the material, the faster the color transition of the pigment molecule. The rapid darkening at higher temperatures is since the shrinkage of the colored molecules in the fish muscle occurs faster than blanching at low temperatures.

## Effect of Blanching Time and Temperature on BF

Temperature and time are two factors that significantly affect the texture of the product through the connective tissue (Roldán et al., 2013). High temperature/time will cause the bonds between tissues to break, and the texture is easily destroyed. The product cooking rate decreases at too low a temperature (Purslow et al., 2016). At the same time, the process of cooking and hardening the product will decrease when the blanching time is not enough for the heat to affect the entire position of the product.

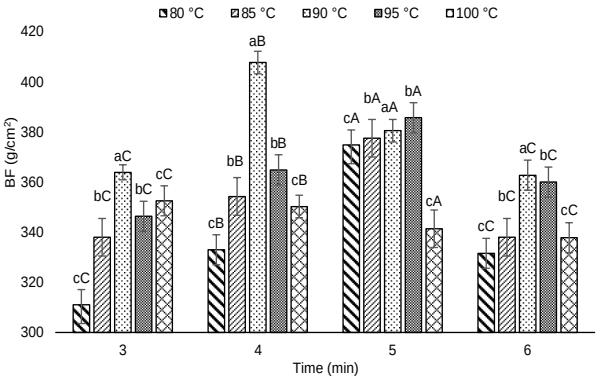


Figure 3. Effect of blanching time and temperature on the BF change of the product. Different lowercase and uppercase letters represent statistically significant differences in temperature and time, respectively ($p<0.05$).

The investigation results showed that there is a statistically significant influence on the breaking force (BF) ($p>0.05$) in the temperature range from 80 to 85 °C. BF improved by increasing the blanching time from 3 to 5 minutes. BF tended to decrease gradually as the blanching time increased to 6 minutes. At 90 °C, BF reached its maximum value at 4 min (407.7 g/cm$^2$). At 100 °C, BF decreases with increasing time from 3 to 6 minutes. The increase in BF due to the precipitation of proteins after the heating process creates product hardness, and the rapid dehydration causes shrinkage of the meat tissue, increasing the adhesion between the meat tissues. A report on fish texture after heat treatment showed that the force required to break the material increased with increasing cooking time (Liu et al., 2011). The breakdown of proteins and the bonds between the tissues, leading to the softened texture, was shown in a previous report (Bax et al., 2012). At the same level of time, an increase in temperature affects the texture of the product ($p<0.05$). The general trend shows that the highest value is always achieved at 90 °C. At 100 °C, the texture of the product is the least stable and tends to decrease with increasing treatment time. The higher the temperature, the more the proteins are denatured, leading to the loss of function of the proteins, which leads to the destruction of the bonds between tissues. At the same time, the product texture becomes soft and brittle.

## Effect of Blanching Time and Temperature on TBC

Temperature and blanching time are two critical factors that affect the quantity of bacteria in the environment. When temperature and blanching time increase, the number of bacteria usually decreases as their growth, regeneration, and distribution processes are affected. However, the impact of temperature and holding time on the total bacterial count depends on many factors, such as the type of bacteria. Therefore, adjusting temperature and holding time is crucial for controlling the number of bacteria in the environment.

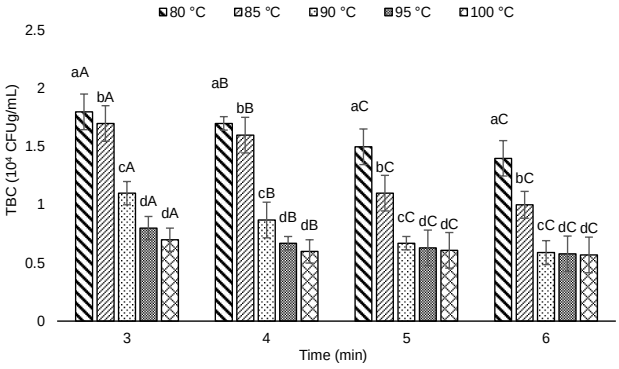


Figure 4. Effect of blanching time and temperature on the change of TBC in the product. Different lowercase and uppercase letters represent statistically significant differences in temperature and time, respectively ($p<0.05$).

Each microorganism has an optimal operating temperature range. Most microorganisms survive and thrive at room temperature (30 – 35 °C). At low temperatures < 20 °C and medium temperatures between 40 – 70 °C, the growth of microorganisms is inhibited. However, some microorganisms less resistant to heat are destroyed at higher temperatures. Some previous reports have applied high temperatures to minimize the microbial content in raw materials (Li & Farid, 2016). The blanching process at 80 – 100 °C temperature significantly decreased TBC ($p<0.05$). At 3 and 4 min, TBC decreased continuously with increasing temperature, and the lowest TBC value was at 100 °C ($0.7 \times 10^4$ CFU/g), respectively. TBC rapidly reduced at 5 and 6 min, increasing temperature from 80 to 85 °C. However, no change in TBC was observed with increasing temperature from 90 – 100 °C, and it was stabilized at $5.7 \times 10^3$ CFU/g. The thermal instability of some microorganisms explains this decline, and the increase in temperature and time causes the microorganisms to be destroyed. The implementation is for a long time (5 - 6 minutes), and at high temperatures, the less resistant microorganisms are destroyed, and the heat-stable microorganisms remain. On the other hand, microbial washout during blanching occurs. This reduces the amount of heat-stable microorganisms in the product.

## Effect of Frozen Preservation Time on the Brightness and Break Force (BF)

Food color is easily changed under the influence of enzymes and microbial content. The preservation

methods that help prevent this change are based on inhibiting the destructive activity of microorganisms on muscles and cells and inhibiting the activity of certain enzymes. The frozen preservation process investigated during 42 days showed good color retention of the product by the cryogenic method ($p>0.05$). The brightness value (L*) of the product is stable at 52.67 ± 0.70. The L* value does not have this change because it limits the growth and cell destruction of food spoilage microorganisms and prevents the oxidation of the pigment of the product.

Table 3. Effect of frozen preservation time on the change in brightness (L*) of the product

| Time (days) | Brightness (L*) |
|---|---|
| 0 | 52.67 ± 0.70[a] |
| 3 | 52.44 ± 0.61[a] |
| 7 | 52.09 ± 0.73[a] |
| 14 | 53.75 ± 0.52[a] |
| 28 | 53.46 ± 0.63[a] |
| 42 | 53.25 ± 0.51[a] |

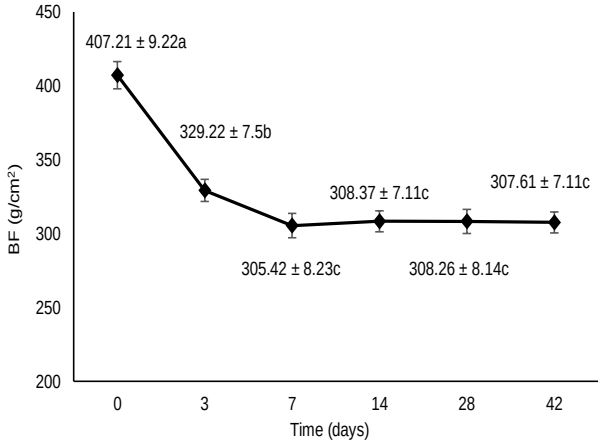


Figure 5. Effect of frozen preservation time on the change of BF in the product. Different letters represent statistically significant differences ($p<0.05$).

The freezing process directly impacts the texture change of food with statistical significance ($p<0.05$). The texture of the product after freezing and evaluated after thawing showed deterioration after 14 days of preservation (308.37 g/cm$^2$). During the first 14 days of preservation, new ice crystals were formed, the connective tissues between cells were broken, the free water content in the material was frozen, and the space between cells was increased during transfer turning solid water into liquid form in the raw material causing compression of the bonds, resulting in the breakdown of bonds between cells (Bilbao-Sainz et al., 2020; Sun et al., 2019). After 14 days of freezing, the texture of the product became stable by rearrangement of the product texture. There was no significant increase in ice crystals and rupture of muscle fibers and tissues of the products ($p>0.05$). Similar results were obtained on frozen preservation Cephalopod Muscles with texture deterioration during the first day and stabilized for 30 days of preservation (Gokoglu et al., 2018).

## Effect of Frozen Preservation Time on the Various TBC

Freezing plays an important role in inhibiting the growth of microorganisms, except spores resistant to low temperatures (Dai et al., 2021). Some microorganisms that can grow in cryogenic environments increase with increasing food preservation time (Magan, 2007).

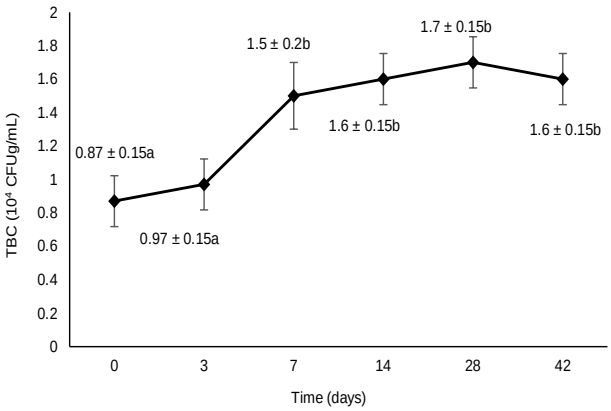


Figure 6. Effect of frozen preservation time on the change of TBC in the product. Different letters represent statistically significant differences ($p<0.05$).

The investigation showed that TBC initially reached $0.87 \times 10^4$ CFU/g and tended to grow strongly in the first seven days of preservation ($1.5 \times 10^4$ CFU/g). After 28 days of preservation, TBC tends to increase slowly. On day 28$^{th}$, TBC reached $1.5 \times 10^4$ CFU/g. Continuing to increase the preservation time to 42 days, TBC tends to decrease insignificantly. The increase in TBC is due to the growth of microorganisms capable of surviving in a frozen environment. The freezing process partially inhibits microbial growth but does not completely inhibit microorganisms.

On the other hand, the process of formation and growth of ice crystals has broken the cell wall, providing a source of nutrients for the growth and development of microorganisms (Sasidharan et al., 2011). The size of the ice crystals could influence the slow proliferation of TBC. This did not increase

significantly with prolonged preservation, not increasing the number of disrupted cells. Microorganisms do not have enough nutrients for growth and begin the death process when prolonged for more than 28 days. A lack of oxygen degrades some aerobic microorganisms. At the same time, microorganisms present in frozen products are inhibited due to the harsh growth environment. After 42 days of preservation, TBC has not exceeded the allowable limit of the Ministry of Health, Vietnam, on TBC on fish products. Results were similar to a previous report of increased microbial content in Nile Tilapia (*Sarotherudun galiaenus*) after 60 days of cryopreservation (Arannilewa et al., 2006).

## Conclusion

This study succeeded in evaluating changes in texture, brightness, and total bacterial count in the blanching and steaming of the fish meatball production process from the by-product of Pangasius hypophthalmus processing. The nutritional and physical criteria of input materials have changed through the stages of fish balls processing. The protein content tended to increase through stages and the highest protein in fish balls. Two optimal steaming conditions (4 min/100 °C steam) and blanching (4 min / 90 °C water) produce good texture and color. However, the steaming process is less effective in reducing the total bacterial count (TBC) than the blanching process. The frozen preservation process at -40 °C effectively maintains brightness stability. However, the texture of fish balls tends to decrease during the first 15 days and then remain stable until the 42$^{nd}$ day, which is inversely proportional to the TBC. The research results have found suitable conditions and methods for making fish balls from *P. hypophthalmus*. This promotes the solution and maximum utilization of leftover meat from the fish fillet process. At the same time, diversifying products from *P. hypophthalmus* helps to enhance the economic efficiency of the seafood processing industry.

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## Supplementary Materials

Supplementary materials is not available for this article.

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