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Effectiveness of cryoprotectants in fish cake production from three-spot gourami (*Trichopodus trichopterus*)

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Three-spot gourami (*Trichopodus trichopterus*) has good nutritious quality, easy to make, but having stiff bones so many people are afraid, especially for children. In order to improve the added value of this fish, we have attempted to utilize the three-spot gourami converting into fish cake with different cryoprotectants as gelling agent. Different cryoprotectant agents such as carboxymethyl cellulose, alginate, gellan gum in various concentrations (0.2%, 0.25%, 0.30%, 0.35%, 0.40%) on the physico-chemical, microbiological, and sensory characteristics of three-spot gourami fish cake was studied. Our results showed that carboxymethyl cellulose 0.35% was the most adequate for fish cake production. This fish could be utilized to process into fish cake to enhance the added value. Three-spot gourami fish cake was rich in nutritious constituents, and can be used as enrichment for humans to alleviate malnutrition.

Keywords: *Trichopodus trichopterus*, fish cake, cryoprotectant, carboxymethyl cellulose, alginate, gellan gum.

INTRODUCTION

Although three-spot gourami (*Trichopodus trichopterus*) is not the main fish species in Mekong delta, the output of this fish is also hundreds of tons each year in the forms of fish sauce and dry-salted product. It's popularly sold as fresh fish in the local markets and used in rural families every day. It's facing over exploitation. It has a very important role in the natural food chain. Because the fish is omnivorous, it is possible to eat scraps, plants, insects, crustaceans in pond to help clean the algae and not give them overgrowth. It is very easy to feed, very healthy and due to the secondary respiratory organs, often to breathe in the water, be able to live in a low-oxygen environment. It is generally absent from fast flowing streams and rivers. It typically occurs in heavily vegetated, shallow or standing lowland waters including ponds, ditches, rice paddies,

canals, swamps, marshes and wetlands (Shima et al. 2017). The behavior of *Trichopodus trichopterus* to perform various tastes of chemical substances is commonly presented among aquatic species (Kasumyan & Mouromtsev 2020). Nest-building of *Trichopodus trichopterus* is influenced by the performance of male (Degani & Ziv 2016).

Frozen storage for longer periods causes rubbery texture in meat and fish products (Maity et al. 2018). Hydrocolloids are used as cryoprotectant in different food applications such as gelling, thickening, stabilizing, emulsifying. Incorporation of cryoprotectants can restrain the protein dehydration so as to prevent protein freeze denaturation of myofibrillar proteins during frozen storage, hence preserving the gel-forming ability of fish cake (Anwar et al. 2013). Food additives often used with different concentrations in the production of fish cake. Concentration of

myofibrillar proteins is one of the important factors for improving gel strength and elasticity of fish cake. A reduction in water soluble protein increases the concentration of myofibrillar proteins, thus enhancing the functional properties of fish cake. The gelling process entails the association of long myofibrillar protein chains which produces a continuous three-dimensional network in which water and other components are trapped. As a result, a visco-elastic gel is obtained (Sánchez-González et al. 2008). Improvement of rheological properties and gel forming capacity depend on kind and concentration of cryoprotectants supplemented (Jo et al. 2001; Benjakul et al. 2003; Lin & Chao, 2001).

Value-added seafood may be minced, breaded, surimi, fish cake products (Kamari and Shabanpour, 2013; Hwang et al. 2013). They can provide essential nutrients as a potential diet for malnourished children. One of the most vital food processing technologies is the production of low-cost and available fish use as value-added fish products (Shaviklo et al. 2013). There were few studies mentioned to the fish cake processing. Boiled fish cake was prepared by using acetic acid-treated cuttlefish bone (Kimura et al. 1991). Enoki mushroom was added to fried fish cake (Koo et al. 2001). The effects of shiitake mushroom on the textural properties of fried fish cake were investigated (Son et al. 2003). A fried fish cake was prepared by using cultured king oyster mushroom (*Pleurotuseryngii*) and silver white croaker (*Pennahiaargentata*) surimi to enhance its physiological effects (Kim et al. 2003). A steamed fish cake with added 5% ground citrus fruits with skin was developed (Yang and Cho 2007). A fried fish cake with added white *P. cocos* powder was prepared (Shin et al. 2009). The effect of king oyster mushroom on the textural and physicochemical properties of steamed cuttlefish (*Sepia esculenta*) fish cake was investigated (Chung et al. 2010). The quality characteristics of fried fish cakes containing rice flour were examined (Kwon and Lee 2013). Fish cake was prepared using five mixed fish species (Begum et al. 2017). Effect of different cryoprotectants such as sucrose, sorbitol, tripolyphosphate, carrageenan, modified starch, xanthan gum on the physico-chemical, microbiological, and sensory characteristics of surimi were investigated (Minh et al. 2019). Our research emphasized on the effectiveness of different cryoprotectant agents such as carboxymethyl cellulose, alginate, gellan gum in various concentrations (0.2%, 0.25%, 0.30%, 0.35%, 0.40%) on the physico-

chemical, microbiological, and sensory characteristics of three-spot gourami fish cake.

MATERIALS AND METHODS

Material

Three-spot gouramifishes were naturally collected in ponds and rivers in Bac Lieu province, Vietnam in January 2020. After harvesting, 25 kg of fishes was kept in flake ice in ice-chest and conveyed to laboratory in one trip as soon as possible for experiments. Apart from three-spot gourami, we also used other ingredients such as carboxymethyl cellulose, alginate, gellan gum, NaCl, sugar, pepper, garlic, monosodium glutamate.

Researching method

25 kg of three-spot gourami fishes was washed thoroughly with potable water several times to remove foreign matter. Scales, fins and intestines were carefully removed and washed with tap water to avoid contamination. Fish was thoroughly crushed by grinder convert into fish paste. Various ingredients of salt (0.4%), sugar (0.1%), pepper (0.02%), garlic (0.02%), monosodium glutamate (0.01%) as additives were added into the minced fish. Minced fish was then mixed with 3 cryoprotectants such as carboxymethyl cellulose, alginate, gellan gum in same concentration 0.2%. After finding the suitable cryoprotectant agent based on optimal values of the moisture content, crude protein, yield, chewiness, water-holding capacity, total plate count and overall acceptance; the effect of 5 different cryoprotectant concentrations (0.2%, 0.25%, 0.30%, 0.35%, and 0.40%) was also verified. The optimal variables were evaluated by moisture content %, crude protein %, yield %, chewiness (kgf), water holding capacity (%), total plate count (cfu/g), and sensory score. Two experiments with total 24 lots of samples were involved. Three replicates were applied in each treatment.

Moisture content (%) was determined by comparing the weights of the sample with the electronic balance. Crude protein (%) was measured by AOAC (2000). The yield of the treatments (%) was calculated by the ratio between the weight of the raw fish used and the weight of the final fish cake. Chewiness (kgf) was determined Texture Analyzer. Water holding capacity (%) was measured by the method of Himonides (1999). The total plate count (cfu/g) was enumerated by Petrifilm - 3M. The

organoleptic score was evaluated by a group of specialists (9 members) using nine point Hedonic scale. The experiments were run in triplicate with three different lots of samples. The data were presented as mean±standard deviation. The significance level was set to $\alpha = 0.05$.

$$\mu = \bar{X} \pm 1.96 \times \frac{s}{\sqrt{N}}$$

Where μ is the mean of the population, X is the mean of the sample, s is the standard deviation of the sample, and N is the sample size. The critical value would be 1.96. Statistical analysis was performed by the Statgraphics Centurion XVI.

RESULTS AND DISCUSSION

From table 1 and 2, the optimal cryoprotectant was shown at carboxymethyl cellulose 0.35% so this variable was appropriated for three-spot gourami fish cake production.

Table 1 revealed that carboxymethyl cellulose was superior to alginate, gellan gum in physico-chemical, microbiological, and organoleptic attributes of fish cake. At incorporation of 0.2%, carboxymethyl cellulose induced the highest the moisture content (74.05±0.01%), crude protein (11.42±0.00%), yield (66.85±0.03%), chewiness (5.81±0.03kgf), water-holding capacity (58.76±0.00%), and overall acceptance (7.13±0.03) but the lowest total plate count (1.51x10²±0.00cfu/g). Meanwhile, table 2 showed that incorporation of carboxymethyl cellulose in different concentrations to the physico-chemical, microbiological, and organoleptic attributes of fish cake. Among various concentrations, 0.35 % of carboxymethyl cellulose supplemented into fish cake resulted to the highest the moisture content (75.68±0.02%), crude protein (11.25±0.00%), yield (67.89±0.01%), chewiness (5.57±0.01kgf), water-holding capacity (59.92±0.02%), and overall

acceptance (8.03±0.03) but the lowest total plate count (1.25x10²±0.01cfu/g). The role of cryoprotectants in the fish cake production is very essential to maintain the gel-forming stability during freezing and thawing (Kuhn & Soares 2002). They avoid the proteins denaturation during freezing by binding water and proteins and support the gel structure after thawing by diminishing the intermolecular aggregation of the proteins. The oscillations in the texture parameters may be due to the breaking of myosin, which leads to an increase in the semi gel fluidity, causing the separation of some protein grids already existent. The air inside the gel interferes on the attainment of the texture since the increase of the pressure accomplished during the test causes the disruption of the structure (Visessanguan et al. 2000). Moreover, moisture can be affected by the presence of salts and metal ions in the mixture because they interfere in the formation of hydrogen bounds between proteins and water. The water binding provided by the cryoprotectants could increase the yield.

Mechanical attributes of fish cake can be improved by the supplementation of various hydrocolloids such as konjac, carrageenan, locust bean, xanthan gum, and microbial transglutaminase (Ramirez et al. 2002; Ramirez et al. 2000; Gomez-Guillen et al. 1997). The addition of alginates has been mentioned to weaken fish paste gels (Lee et al. 1992). Carboxymethyl cellulose enhanced the breaking force of Alaska pollock surimi. The increase in the breaking force induced by carboxymethyl cellulose vanished upon increasing the degree of substitution of hydroxyl groups to carboxymethyl groups. It enhanced the breaking strain and breaking force and reduced the amount of expressible water but were unsuccessful in the case of coarser particles (Niwa et al. 1997).

Table 1: Effect of various cryoprotectants (carboxymethyl cellulose, alginate, gellan gum) at 0.2% to physico-chemical, microbiological, and organoleptic attributes of fish cake

Variables	Cryoprotectant		
	Carboxymethyl cellulose	Alginate	Gellan gum
Moisture (%)	74.05±0.01 ^a	73.69±0.02 ^b	74.25±0.01 ^{ab}
Crude protein (%)	11.42±0.00 ^a	12.01±0.03 ^b	11.83±0.02 ^{ab}
Yield (%)	66.85±0.03 ^a	64.51±0.01 ^b	65.04±0.00 ^{ab}
Chewiness (kgf)	5.57±0.01 ^a	5.31±0.00 ^b	5.42±0.01 ^{ab}
Water holding capacity (%)	58.76±0.00 ^a	50.39±0.02 ^c	54.25±0.02 ^b
Total plate count (cfu/g)	1.51x10 ² ±0.00 ^c	6.45x10 ² ±0.03 ^a	3.19x10 ² ±0.00 ^b
Sensory score	7.13±0.03 ^a	5.79±0.01 ^c	6.42±0.02 ^b

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

Table 2: Effect of various concentrations of carboxymethyl cellulose (0.20%, 0.25%, 0.30%, 0.35%, and 0.40%) to physico-chemical, microbiological, and organoleptic attributes of fish cake

Variables	Carboxymethyl cellulose				
	0.20%	0.25%	0.30%	0.35%	0.40%
Moisture (%)	74.05 ±0.01 ^b	74.82 ±0.03 ^{ab}	75.03 ±0.00 ^{ab}	75.68 ±0.02 ^a	75.71 ±0.01 ^a
Crude protein (%)	11.42 ±0.00 ^a	11.39 ±0.00 ^a	11.31 ±0.01 ^{ab}	11.25 ±0.00 ^{ab}	11.23 ±0.02 ^b
Yield (%)	66.85 ±0.03 ^b	67.13 ±0.02 ^{ab}	67.79 ±0.02 ^{ab}	67.89 ±0.01 ^a	67.92 ±0.00 ^a
Chewiness (kgf)	5.57 ±0.01 ^b	5.65 ±0.00 ^{ab}	5.74 ±0.00 ^{ab}	5.81 ±0.03 ^a	5.83 ±0.03 ^a
Water holding capacity (%)	58.76 ±0.00 ^b	59.02 ±0.03 ^{ab}	59.61 ±0.03 ^{ab}	59.92 ±0.02 ^a	59.95 ±0.02 ^a
Total plate count (cfu/g)	1.51x10 ² ±0.00 ^a	1.49x10 ² ±0.00 ^a	1.36x10 ² ±0.01 ^{ab}	1.25x10 ² ±0.01 ^{ab}	1.04x10 ² ±0.02 ^b
Sensory score	7.13 ±0.03 ^b	7.45 ±0.02 ^{ab}	7.86 ±0.02 ^{ab}	8.03 ±0.03 ^a	8.06 ±0.02 ^a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ($\alpha = 5\%$).

CONCLUSION

Fish cake is processed from three-spot gourami comprising salt-soluble proteins isolated from fish meat. It is a refined fish myofibrillar protein manufactured through numerous step-by-step processes. To inhibit denaturation and to lessen the damage of gel quality during cold storage, cryoprotectants such as carboxymethyl cellulose, alginate, gellan gum are added to fish cake for demonstration. We have successfully manufactured the fish cake from three-spot gourami with carboxymethyl cellulose 0.35% as cryoprotectant. It can improve the rheological and textural characteristics of fish cake by changing the viscosity and water-binding ability. Fish cake is highly nutritious for human and mainly malnourished children

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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