DONG THAP UNIVERSITY JOURNAL OF SCIENCE
Tạp chí Khoa học Đại học Đồng Tháp
Natural Sciences Issue
ISSN 0866-7675 | e-ISSN 2815-567X

DOI: https://doi.org/10.52714/dthu.ns.2438.1835

THE EFFECT OF TEMPERATURE AND SALINITY ON PROTEIN RATE OF PURPLE-SPOTTED BIGEYE DURING PRESERVATED BY NITROGEN- SLURRY ICE

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Article history

Received: 05/6/2025; Received in revised form: 26/6/2025; Accepted: 28/6/2025

Abstract

The paper shows experiments of 13 samples with three repeat times, indicating the effect of temperature and salinity in a Nitrogen-slurry ice solution on Purple-Spotted Bigeye (Priacanthus tayenus) protein rate. The result shows that the protein Quadratic model is significant with a p-value of less than 0.05. The temperature affects strongly the protein rate of the samples during preservation by the Nitrogen-slurry ice solution. The equation of protein related to temperature and salinity was Protein rate equals to $19{,}99 - 0{,}91A + 0{,}15B - 1{,}66A^2 - 0{,}56B^2$*, where A is Temperature, B is Salinity. The protein rate of the fish was kept at 19.9% during 07 days in storage solution with temperature of -1.5 °C and salinity of 25‰ (the fish was stored in normal ice 4 days onboard before keeping in the experiment solution). The experiment suggested that, after landing from fishing boats, the Purple-Spotted Bigeye (Priacanthus tayenus) can preserve in the Nitrogen-slurry ice of temperature range from -1.4 °C to -1.9 °C and salinity from 22‰ to 28‰ from in least 07 days to remain the high rate of protein over 19%.*

Keywords: *Nitrogen-slurry ice, Protein rate, Purple-spotted bigeye, Salinity, Temperature.*

Cite: Dinh, X. H. (2026). The effect of temperature and salinity on protein rate of purple-spotted bigeye during preservated by nitrogen- slurry ice. *Dong Thap University Journal of Science, Online First*, 1-11. https://doi.org/10.52714/dthu.ns.2438.1835

SỰ ẢNH HƯỞNG CỦA NHIỆT ĐỘ VÀ HÀM LƯỢNG MUỐI ĐỐI VỚI TỶ LỆ PROTEIN CỦA CÁ SƠN THÓC TRONG QUÁ TRÌNH BẢO QUẢN BẰNG ĐÁ SỆT – NITƠ

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Lịch sử bài báo

Ngày nhận: 05/6/2025; Ngày nhận chỉnh sửa: 26/6/2025; Ngày duyệt đăng: 28/6/2025

Tóm tắt

Bài báo thể hiện kết quả của 13 thí nghiệm với 3 lần lặp, chỉ ra sự ảnh hưởng của nhiệt độ và độ mặn đối với tỷ lệ protein của cá Sơn thóc (Priacanthus tayenus) trong dung dịch đá sệt ni tơ. Kết quả chỉ ra rằng, mô hình bậc hai protein là phù hợp với giá trị tin vậy trên 95%. Nhiệt độ ảnh hưởng đáng kể đến tỷ lệ protein của các mẫu thử nghiệm trong quá trình bảo quản bằng đá sệt Ni tơ. Phương trình protein liên quan đến nhiệt độ và độ mặn là tỷ lệ Protein $= 19{,}99 - 0{,}91A + 0{,}15B - 1{,}66A^2 - 0{,}56B^2$*, trong đó A là nhiệt độ, B là độ mặn. Tỷ lệ protein của cá này giữ ở mức 19.9% sau 07 ngày bảo quản bằng đá sệt Ni tơ với độ mặn là 25‰ và nhiệt độ ở -1.5^{o}C (Cá này đã được bảo quản bằng đá xay truyền thống 07 ngày trên tàu trước khi được lưu giữ trong môi trường đá sệt ni tơ). Thí nghiệm đề xuất rằng, sau khi cập bến, cá Sơn thóc có thể bảo quản trong đá sệt ni tơ ở dải nhiệt độ từ -1.4^{o}C đến -1.9^{o}C và độ mặn từ 22‰ đến 28‰ để duy trì ít nhất 07 ngày nữa với tỷ lệ protein cao hơn 19%.*

Từ khóa: *Cá sơn thóc, đá sệt ni tơ, độ mặn, nhiệt độ, tỷ lệ protein.*

1. Introduction

Purple-Spotted Bigeye (*Priacanthus tayenus*) appears commonly in bottom trawl fishery in Vietnam but its value has been wasted before selling to factories because of stored by the traditional ice during fishing to transporting activities. According to Nguyen et al. (2016) indicated that, the protein rate of some bottom fish from trawl fishing is under 18,5% during preserving by tradiontal ice over 07 days.

To study on the influence of temperature and salinity to balance between percentage of frozen water and keeping extension of storage time is nessessary. It means that maintaining higher 18,5 % protein rate of purple-spotted bigeye during long time preservation over 07 days after fishing port landing is the goal of this article.

The data used is collected from results of project of completing slurry ice combined with Nitrogen to preserve port-harvest products from off-shore marine fisheries in Ben Tre province.

2. Theoretical overview and research history

Slurry ice (*iceflow, nanoice*) is a homogeneous mixture of micrometer ice crysticals and liquid. The solution for making slurry ice has freezing point that is lower than which of fresh water. The solutions of Natri clorua, Ethanol, Ethylene glycol, and Propylene glycol are used normally for slurry ice-making in the industry field. In the field of seafood preservation, natural seawater solution is normally used to create slurry ice(Kauffeld et al., 2005). There are some advantages of slurry ice as liquid form, fast and uniform cooling, temperature from -1 ^{o}C to -2 ^{o}C, non-crystallization in hard mass, and non-refreezing (Kauffeld et al., 2010). The ability of adjusting the ice concentration up to 60% and the salt content in the range of 20÷30‰ in the ice slurry ensures maximum preservation results without damage to delicate fish and avoids excessive salt uptake by the fish (Kauffeld et al., 2010). Additionally, complete coverage of the fish surface by the slurry ice mixture also protects sufficiently fish tissues from the action of oxygen and therefore from lipid oxidation and dehydration (Huidobro et al., 2002).

The Nitrogen Ultra Fine Bubble technology is a technique of creating nitrogen air bubbles with micrometer size. These bubbles are invisible to the naked eye, it does not rise to the surface of the water and can remain in the water for a long time. The microscopic air bubbles carry a negative charge, so they cannot combine with each other to form larger air bubbles for rising to water's surface like regular air bubbles. They can attract other organic substances with a positive charge, which means, they can purify water well. With the absorption effect of the Nano nitrogen bubbles, it will discharge dissolved oxygen from the water and, as a result, inhibit the growth and activity of aerobic bacteria. In addition, the Nano Nitrogen bubbles are effective in preventing the oxidation of fat from the outer surface to the inside of the fish's body. Thus, it effectively prevents rancidity, degeneration, and commercial value reduction of fish meat (Research Institute for Marine Fisheries, 2016). It is important to note that oxygen is almost totally expelled from ice crystals as they are formed (Porstal et all., 1981).

Nitrogen - Slurry ice technology creates a preservation environment at the minus temperature from -1^{o}C to -2^{o}C and dissolved oxygen concentration less than 2 mg/liter. According to Hung etc (2023), the mixture is suitable for requirement of fish preservation in terms of days extending onboard over 20 days (Hung & Cuong, 2023). The initial salt content of 20% to 30% is optimal and it can help determine the potential for long preservation of catch on board and during transport, improving seafood quality (Melinder et al., 2015). Combined refrigeration technology allows the creation of a mixture of water and ice at a near-freezing

temperature of -1.5^{o}C to preserve seafood in order to form a crystallization mechanism and maintain the original characteristics of the food. (Ando, et al., 2004).

Fish storage at temperatures between 0°C and -4°C is called super chilling or partial freezing. The shelf life of various fish and shellfish can be extended by storage at subzero temperatures. Super chilling extends the shelf life of fish products. The technique can be used, for example where productive fishing grounds are so far from ports and consumers that normal icing is insufficient for good quality products to be landed and sold (Ikuko et al., 1984). The shelf life was predicted by the square root model at -1°C, -2°C for a product that keeps in the ice is 17, 22 days, respectively, which days match with time cruise on sea. The percentage of frozen water in super-chilled fish is highly temperature-dependent (-1°C = 19%; -2°C = 55%) (Ronsivalli et all., 1981). It has been suggested that negative effects of super chilling on drip loss, appearance, and texture of cod and haddock are due to formation of large ice crystals, protein denaturation and increased enzymatic activity in the partially frozen fish (Love & Elerian, 1964). In Japanese studies with seabass, carp, rainbow trout and mackerel, it has been shown that the drip loss as well as several biochemical and chemical deteriorative reactions were reduced in super-chilled fish, compared to traditonal ice storage (Uchiyama et al., 1974); (Kato et al., 1974); (Uchiyama et al., 1978); (Uchiyama et al., 1978). As opposed to cod and several other fish species, the prime quality of super-chilled shrimp from Pakistan was increased from 8 days in ice to 16 days in NaCl-ice at -3°C (Fatima et al., 1988).

Structural proteins (actin, myosin, tropormyosin and actomyosin), which constitute 70% to 80 % of the total protein rate in fish muscle tissue. These proteins are soluble in neutral salt solutions of fairly high ionic strength. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (<0.15 M). This fraction constitutes 25-30 % of the protein. The cold brining of herring should be done with a mixture of salt and brine (FAO, 1995). The amount of salt is 10% to the weight of raw fish and the ratio of fish to brine is 1: 0.2 (Won, 2008). The high concentration of salt has been shown to prevent microbial spoilage in similar products (Andersen et al., 2007). Pacific Whiting may be stored at 0 ºC ÷ 2 °C, but at (0 ÷ 1) g/100 ml salt concentration (Choi et al., 2008). The protein of purple-spotted bigeye (*Priacanthus tayenus*) sample ranged from 12.46÷31.14% (Abdul, 2022), but the fish was preservated 07 days by trational ice method on trawl fishery only kept under 18,5% protein rate after landing in Viet Nam fishing port (Nguyen et al., 2016). So, developing a new method to preserve fish onboard or at fishing port is an important mision to treat with the status of port-harvest lost (protein rate lost) in Vietnam currently.

3. Materials and research methods

3.1. Experimental place

The study was researched at the laboratory of The South Research Sub-Institute for Marine Fisheries and sampling at middleman store in Ben Tre province. The samples were analyzed in the Eurofins Scientific Centre.

3.2. Main experimental equipment

The devices were included in this study:

- A slurry ice machine, ice capacity of 03 tons per 24 hours, power consumption of 10 kW, 03 phases, 380 ÷ 400 V, 50 ÷ 60 Hz.

- An insulated tank: volume 300 liters.

- A Nitrogen Nano-machine: capacity 1 m^3/h, DO < 2 mg/l.

- Temperature measure: HANNA - HI93501 (Range -10 to + 80).

- DO measure: HANNA HI 98193.

- Salinity measure: Hydrometer.

- Analysis software: Design Expert 2.

3.3. Experimental period

The study was researched from July to September 2024.

3.4. Experimental design

There are 13 experiments with at temperatures of -1^{o}C, -1.5^{o}C and -2^{o}C, salinity of 20‰, 25‰ and 30‰ at preserved rate of fish/ice with 1/1. The salinity controlling was set up by mixing seawater with fresh water. The samples were preserved during 07 days after the fish (the fish was preserved by traditional ice onboard 07 days) landed from fishing boat and kept fixation during moving to laboratory. The number of experiments was set up by Design Expert model. The subzero temperature and salinity ranges are determined by many research results previously at -1^{o}C to -2^{o}C and 20‰ to 30‰ is suitable for fresh fish storage. This article will test which temperature and salinity value matching with the highest protein rate possible.

The Central Composite Designs of experiments provide modeling of the response surface.

Table 1. The experiment design

Code	A:Temperature (^{o}C)	B:Salinity (‰)
CS11	-1	30
CS12	-1.5	25
CS13	-2	30
CS21	-1.5	30
CS22	-1.5	25
CS23	-2	20
CS31	-1.5	25
CS32	-2	25
CS33	-1.5	20
CS41	-1.5	25
CS42	-1	20
CS43	-1.5	25
CS44	-1	25

The other outside effective factors were controlled to remain.

3.5. Data analysis

The data were analyzed by Excel software and Design expert software.

Central Composite Design model is used to design sample number with a three-level fractional factorial.

4. Results and discussion

Protein rate in of all samples were recorded higher than 16,8% during the preservation period of 07 days. This is the high protein rate although the fish was preserved over 07 days onboard by traditional ice.

Table 2. The results of optimal experiments

No.	Code	Temperature (^oC)	Salinity (‰)	Protein (%)
1	CS11	-1	30	17
2	CS12	-1,5	25	19,8
3	CS13	-2	30	18,9
4	CS21	-1.5	30	19,5
5	CS22	-1.5	25	20,1
6	CS23	-2	20	18,7
7	CS31	-1.5	25	20,5
8	CS32	-2	25	19
9	CS33	-1.5	20	19
10	CS41	-1.5	25	20,1
11	CS42	-1	20	16,8
12	CS43	-1.5	25	20,3
13	CS44	-1	25	17,3

The Design Expert show the final equation in terms of coded factors.

$P = 19{,}99 - 0{,}91A + 0.15B - 1{,}66A^2 - 0.56B^2$.

Where: P: Protein; A: Temperature; B: Salinity.

Response 1: Protein. The ANOVA analysis for Quadratic model is shown below.

Table 3. The analysis results of ANOVA for quadratic model of protein effect by temperature and salinity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	**17.46**	**5**	**3.49**	**32.38**	**0.0001**	**significant**
A-Temperature	5.04	1	5.04	46.76	0.0002	
B-Salinity	0.13	1	0.13	1.25	0.3001	
AB	3.55	1	3.55	3.29	1.0000	
A^2	7.65	1	7.65	70.91	< 0.0001	
B^2	0.87	1	0.87	8.14	0.0246	
Lack of Fit	0.22	3	0.07	0.56	0.67	**not significant**
R^2						0.95
Adjusted R^2						0.92
C.V%						1.73

Where: df: degree of freedom; R^2: Reliability. C.V%: Coefficient of Variation.

The **Model F-value** of 32.38 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. **P-values** less than 0.05 indicate model terms are significant. In this case A, A^2, B^2 are significant model terms. The **Lack of Fit F-value** of 0.56 implies the Lack of Fit is not significant relative to the pure error. There is a 67.02% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good, the model to fit. Although the independent Salinity (B) value was not less than 0.05, B^2 is significant, and the model is significant too. Also, comparing with the previous studies, salinity content in solution of fish preservation impacted maintenance quality of the fish, B value is kept for this suitable model.

Table 4. Coefficients im terms of coded factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	19.99	1	0.136	19.67	20.31	
A-Temperature	-0.91	1	0.134	-1.23	-0.59	1.00
B-Salinity	0.15	1	0.134	-0.16	0.46	1.00

AB	0.00	1	0.16	-0.38	0.38	1.00
A^2	-1.66	1	0.19	-2.13	-1.20	1.17
B^2	-0.56	1	0.19	-1.03	-0.09	1.17

The factors are orthogonal, then the VIFs are 1. As a rough rule, VIFs less than 10 are tolerable.

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor.

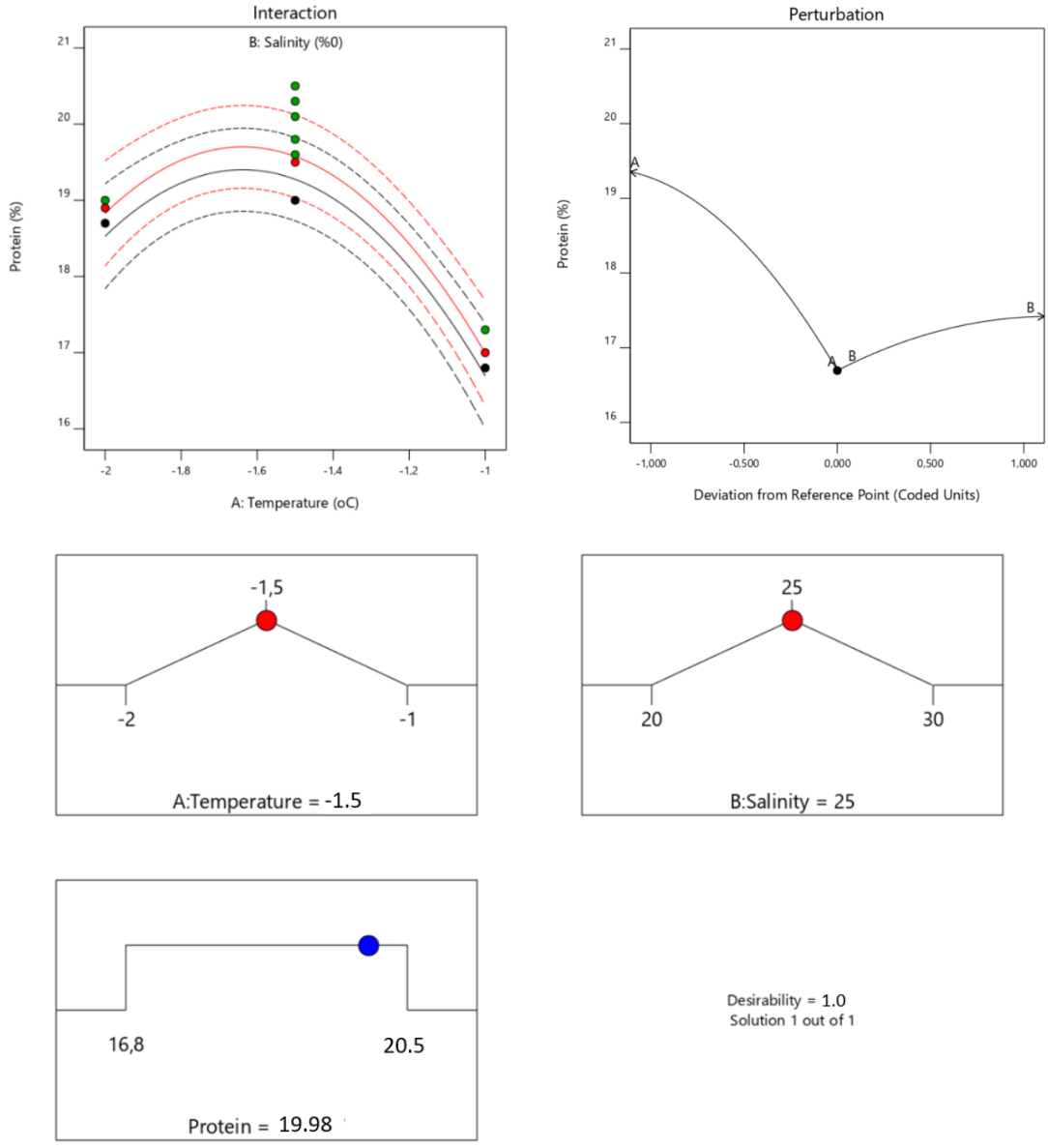

Figure 1. The relationship between temperature, salinity and protein rate change

The protein of the fish can be kept at 19.98% during storage for the next 7 days in Nitrogen - Slurry-ice at temperatures of -1.5ºC and salinity of 25‰ after being kept 4 days onboard in traditional ice. According to Hung and Thi (2019), the preservation temperature for yellow fin tuna is also recommended at -1.5 ºC for solution of slurry ice. Merritt (1965) found that cod stored at -2°C for 10 days had an appearance and texture inferior to fish stored at 0°C in ice. Although, low temperature is getting low spoilage of fish, not at all species (Merritt, 1965). As FAO (1995) showed that each species match with each temperature level for storage to extend period of preservation. The good protein rate in this suggestion reached 19.98% for the fish was stored in a Nitrogen-Slurry ice environment, this value is higher than the fish's protein rate from trational ice storage method. In comparison to the protein rate at the initial phase (the protein rate of the fish after 07 days of storage by traditional ice onboard is 20.1%), the decline of protein rate after 7 days of preservation in the Nitrogen-slurry ice mixture was acceptable. The salinity of the solution is at 25‰, it is not RSW system, is mud or spongy appearance, so, fish muscle is not penetrated by chilling salt.

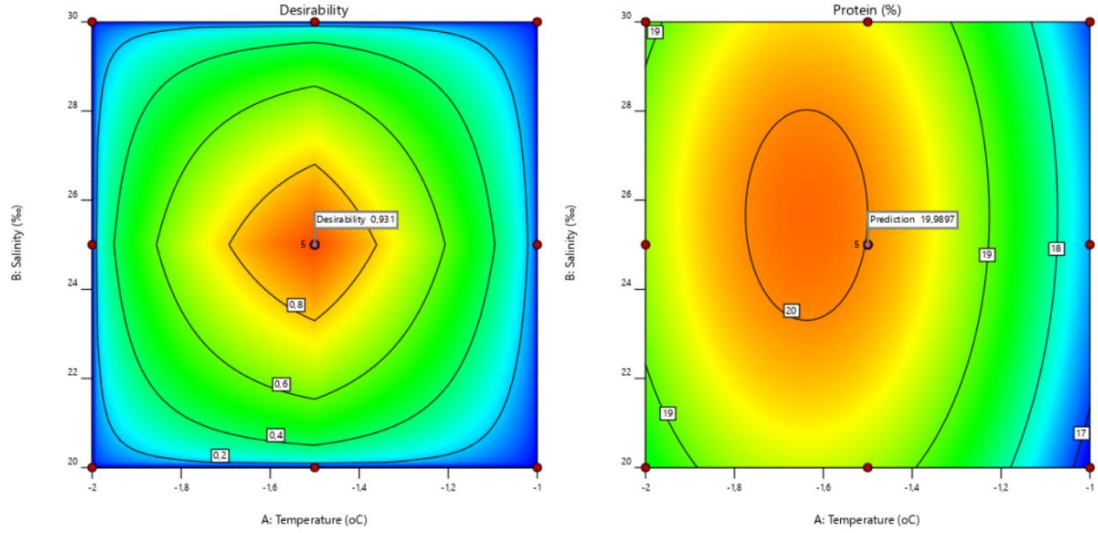

Figure 2. The prediction of the best range of temperature and salinity for keeping high protein rate in the Purple-Spotted Bigeye stored by Nitrogen-slurry ice solution

A prediction, after landing from fishing boats, the Purple-Spotted Bigeye (*Priacanthus tayenus*) can preserve in the Nitrogen-slurry ice of temperature range from -1.4 ºC to -1.9 ºC and salinity from 22‰ to 28‰ during at least 07 days to remain high rate of protein. For the shelf-life and quality of anchovies, the best quality was observed with the samples that were brined at 22‰ and 26‰ salt concentrations and stored at 4±1°C (Karaçam et al., 2002). The protein presented the loose structure with a relative low stability when it was immersed in a monovalent solution with a salt concentration of 0.8 mol/L (Dongqing et al., 2022). Acetic acid levels increased with temperature and decreased with salt concentration (Lisbeth et all., 1995).

This research only focuses on optimized value of temperature and salnity for the same sample collection at fishing port, but not making test directly on fishing boat after deck-up net. It means, the initial protein rate of the fish is higher than its at fishing port. Otherwise, the impact of age, sex, developmental stage of the fish sample was not been concerned yet in this article.

5. Conclusion

The protein Quadratic model is significant with a p-value of less than 0.05. The temperature affects strongly the protein rate during preservation by the Nitrogen-slurry ice

solution. Temperature and salinity of Nitrogen-Slurry ice solution remain from -1.4^{o}C to -1.9^{o}C and 22‰ to 28‰ respectively can maintain high protein rate of the Purple-Spotted Bigeye (*Priacanthus tayenus*) during storage more 07 days after landing from fishing boats.

The Nitrogen – slurry ice technology is suitable for fresh fish storage onboard or at fishing port landing to solve port-harvest lost issue of Vietnam marine fisheries. However, need some more researches on impact of age, sex and developmental stage on protein rate during storage by the ice.

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