

# Characterization of gelatin from pink salmon (*Oncorhynchus gorbuscha*) skin

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## INTRODUCTION

In the United States, the production of pink salmon is approximately 200,000 tons in good harvest years, accounting for 60% of the total salmon production. Deep processing products of pink salmon include various peeled canned on frozen fillets and segments.

## AIM

Due to the outbreak of mammalian diseases, as well as some ethnic and religious issues, the utilization of gelatin from land animals is limited. Most wastes from processing factories have not been utilized efficiently but instead are being dumped in water. Utilizing collagen and gelatin from pink salmon skin (PSK) can improve the added value of fish processing and reduce the environmental pollution caused by improper disposal.

## METHOD

Scanning electron microscopy was performed by the method of Jongjareonrak et al. (2006) with slight modifications. The 6.67% (w/v) gelatin gel (frozen at -80 °C and sliced) with a thickness of 2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 1 h. The samples were rinsed with distilled water three times and dehydrated in ethanol with a serial concentration of 30%, 50%, 70%, 80%, 90%, and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with platinum β palladium. The specimens were observed with a SEM at an acceleration voltage of 30 kV.

## RESULTS

### Electrophoresis

Figure 1 shows that the PSK collagen contains at least two different  $\alpha$  chains ( $\alpha_1$  and  $\alpha_2$ ), and the molecular weight of  $\alpha_1$  and  $\alpha_2$  is between 116 and 158 kDa. Basic structures such as  $\beta$ ,  $\alpha_2$ , and trimers were similar to those of cod collagen electrophoresis profile. The collagen of PSK was identified as type I (Wu, Kang, & Xiao, 2007), which is the dominant constituents of fish skin (Mahmoodani, et al., 2014). This result agrees with those for other fish species (Liang et al., 2012). The subunit molecular weight ( $\alpha_1$ ,  $\alpha_2$ , and  $\beta$  chain) from PSK collagen is higher than that from cod skin collagen. No other bands were found in Lane 2, indicating high purity of the extracted collagen.

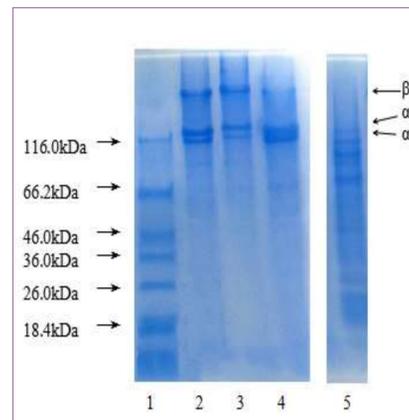


Fig.1. SDS-polyacrylamide gel electrophoresis of pink salmon collagen. Lane1: Marker; Lane2: pink salmon skin collagen; Lane3: catfish skin gelatin; Lane4: cod skin collagen; Lane5: pink salmon skin gelatin

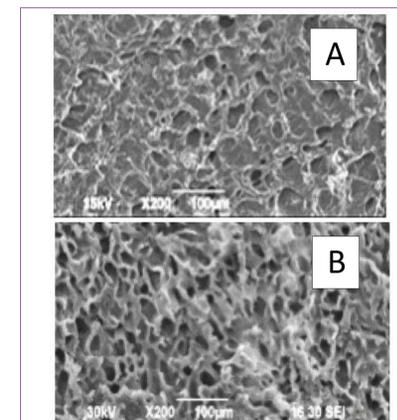


Fig.2. Scanning electron microscopy (SEM) ( $\times 200$ ) of the gelatin gels formed by pink salmon (A) and silver carp (B)

### Scanning electron microscopy (SEM)

The gel microstructures of gelatins from PSK and silver carp skins were observed by SEM. They both showed interconnected pore networks. However, the differences were observed in these networks. The pores of PSK gelatin were larger than those of silver carp gelatin, indicating that the structure is fragile and easy to collapse. This phenomenon occurred because pink salmon is deep-sea fish with low growth temperatures, and silver carp is a freshwater fish with strong adaptability and cold tolerance (Duan, Zhang & Zhao, 2006).

Table 1. Melting point of the gelatin extracted from pink salmon skins

	time consumed (s)	temperature (°C)
initial collapse	273	19.4
Complete collapse	46	21.9

Table 2. Solidification of gelatin gel at different concentrations

Concentration (%)	1.5	2.0	2.2	2.3	2.4	2.5	2.6
Phenomenon	×	×	×	×	o	o	o

o: the gel formed; ×: the gel did not form.

## CONCLUSIONS

The results showed that the PSK collagen was type I, and the denaturation temperature was approximately 16.0 °C. The foaming capability and stability of PSK gelatin were 48.6% and 32.1%, respectively. The melting point was approximately 20.7 °C, and the minimum solidification concentration was 2.4%. The pores of PSK gelatin were larger than freshwater fish gelatin, indicating that the structure is fragile and easy to collapse. It can be concluded that gelatin from pink salmon skin has great potential in food processing applications.

## ACKNOWLEDGEMENTS

This work was supported by the "Blue Project" young academic leaders in the universities of Jiangsu province (KQN1408).

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# Effect of microwave irradiation nonuniformity on the digestion and allergenicity of the glycated ovalbumin

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## INTRODUCTION

- Ovalbumin has antigenicity.
- Glycation can be used as a natural modification method to change the functional properties of proteins.
- The properties of glycated ovalbumin after digestion are rarely studied.

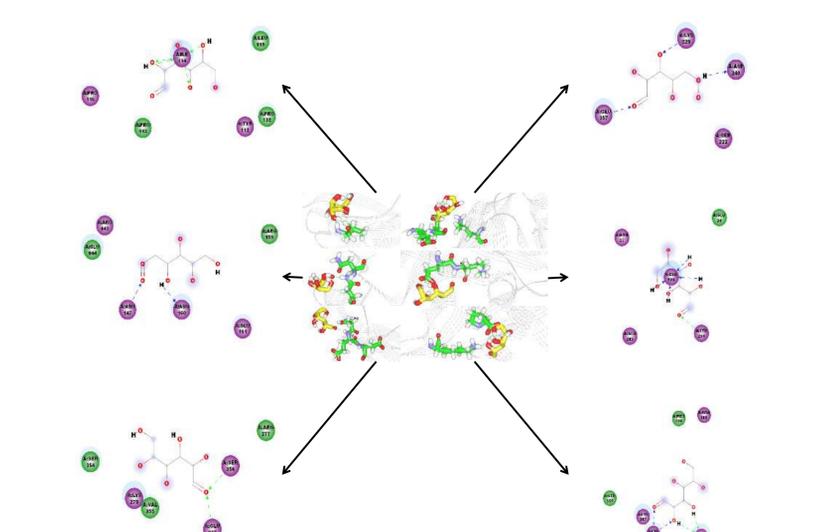
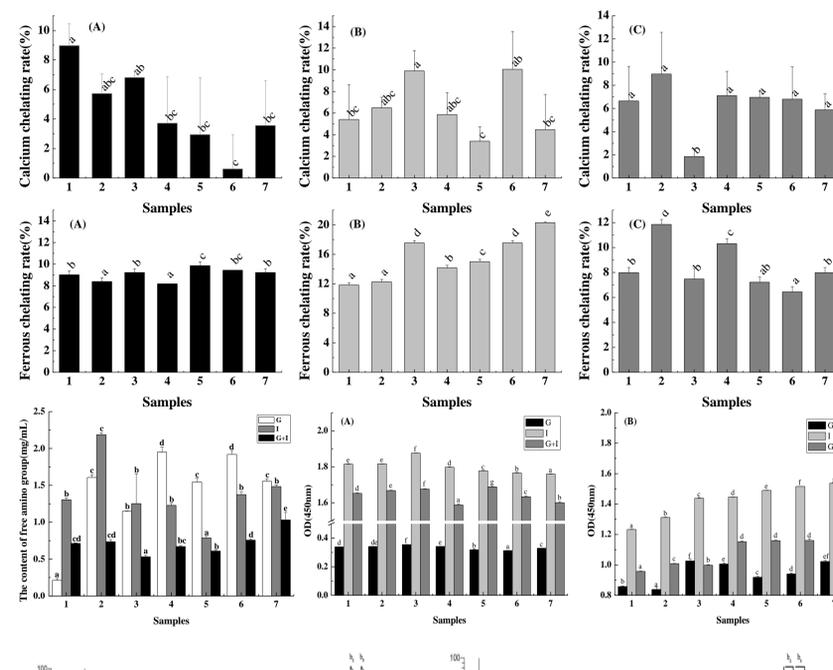
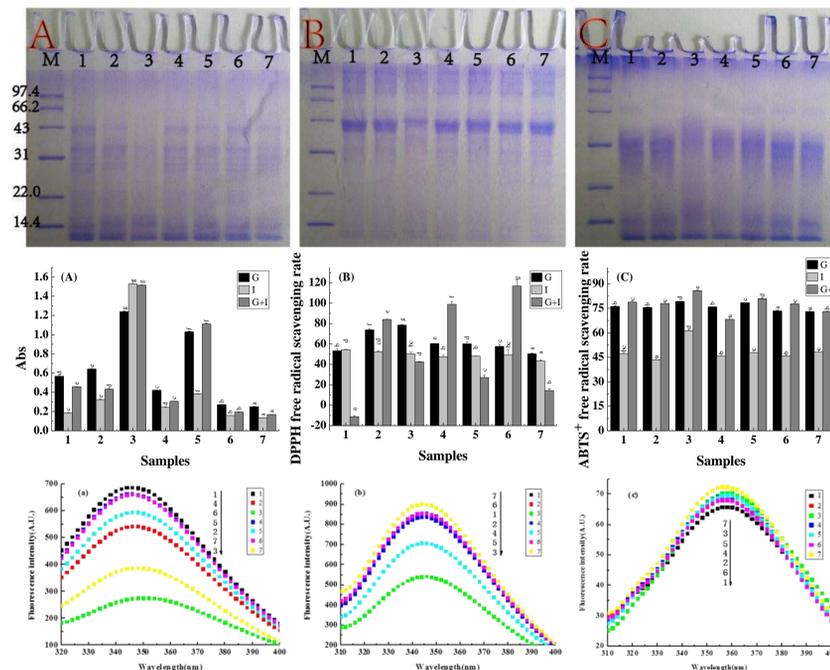
## AIM

- To analyze the characteristics of glycated ovalbumin after digestion
- To analyze the allergenicity of glycated ovalbumin after digestion

## METHOD

- Ovalbumin was glycated by microwave irradiation and then digested by different methods.
- SDS-PAGE, Free Amino groups, Ions chelating capacity, Antioxidant activity, Allergenicity were detected to evaluate the digestibility of ovalbumin.
- Molecular docking and mass spectrometry were used to elucidate the mechanism of microwave glycation and allergy.

## RESULTS



## CONCLUSIONS

- The results showed that microwave can unfold the structure of OVA and promote glycation.
- The glycated OVA was digested more easily in gastric fluid than intestinal fluid.
- More calcium and ferrous ions attached to the glycated samples and the glycated samples had better antioxidant abilities.
- As for the allergenicity, glycation could reduce the IgG binding while increasing the IgE binding.

## ACKNOWLEDGEMENTS

NSFC(31560458) NSFC(21706111) 2016BCB23017

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## INTRODUCTION

Previous studies mainly focused on the how ultrasonic effect the structure and functional properties of  $\beta$ -Lg. However, there are few studies on the changes of the allergenicity potential and antioxidant activity of ultrasonicated  $\beta$ -Lg during digestion in vitro.

## AIM

The overall goal of this research was to study ultrasonic pretreatment and gastric and gastroduodenal digestion affect the structure,  $\beta$ -Lg's digestibility, antioxidant and allergenicity potential of  $\beta$ -Lg.

## METHOD

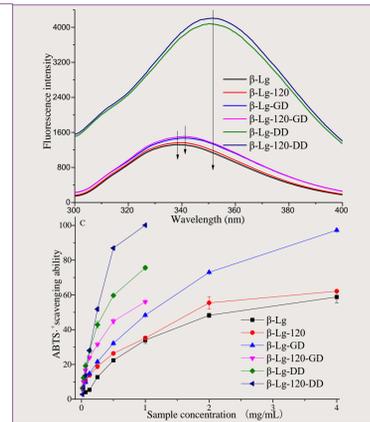
- Ultrasonic treatment
- In vitro gastric and duodenal digestion
- Tricine-SDS-PAGE
- Molecular weight distribution
- Intrinsic fluorescence emission spectroscopy
- IgG/IgE binding ability
- ABTS<sup>•+</sup> scavenging activity assay
- Cellular antioxidant activity (CAA) assay

## RESULTS

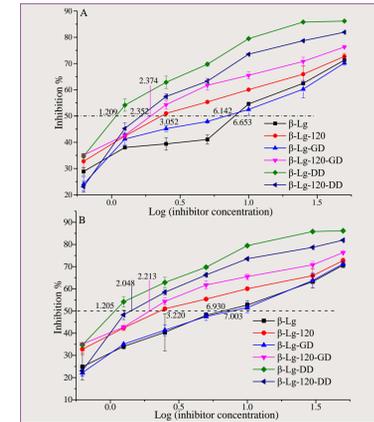
After  $\beta$ -Lg was treated by ultrasonic prior to digestion in vitro, the treated  $\beta$ -Lg showed high intrinsic fluorescence emission and a red shift of  $\lambda_{max}$ , this may be accounted for that the ultrasonic promotes the exposure of enzymatic cleavage site around Trp, enhances enzymatic hydrolysis, leading to the destruction of the structure around Trp, meanwhile prompting the Trp buried inside the conformation to be exposed.

The treated  $\beta$ -Lg showed more of the hydrolytic products and high antioxidant activity, the reason may be that ultrasonic pretreatment can change the structure of  $\beta$ -Lg and expose more digestion sites, resulting in the formation of peptides with smaller molecular weight and free amino acids, thus, the hydrolytic degree of  $\beta$ -Lg was enhanced by pepsin and trypsin. It indicated that ultrasonic pretreatment could effectively improve the antioxidant activity of  $\beta$ -Lg during in vitro digestion, attributed to the improvement the susceptibility of  $\beta$ -Lg during in vitro digestion, producing many small-molecule antioxidant peptides.

Native  $\beta$ -Lg was resistant to gastric digestion and retained its allergenicity. However, the allergenicity of ultrasonicated  $\beta$ -Lg after gastric digestion was increased due to ultrasonic promotes the production of peptides with intact structure and immunogenicity. Ultrasonic causes the unfolding of protein molecules, thereby increasing the accessibility of exposed hydrophobic amino acids to pepsin. Furthermore, these fragments have intact structure and immunogenicity, which exposes more allergenicity loci and antigen epitope. After gastrointestinal digestion, the IgG/IgE binding ability of  $\beta$ -Lg was increased, this may be due to the fact that more allergenic sites and conformational epitopes was exposed through the gastrointestinal tract, resulting in a significant increase in protein allergenicity. But, ultrasonicated  $\beta$ -Lg has a diametrically opposite results because the increase of small peptides with the decreasing of immunogenicity. Therefore, the structural changes of  $\beta$ -Lg by ultrasonic and gastrointestinal digestion were responsible for improving the antioxidant activity and reducing the IgG/IgE binding activity.



Effect of ultrasonic on the Intrinsic fluorescence emission spectra and ABTS<sup>•+</sup> scavenging activity of  $\beta$ -Lg during digestion in vitro.



Changes of ultrasonic pretreatment on the IgG (A), IgE (B) binding ability of  $\beta$ -Lg during digestion in vitro.

samples	Fraction relative area		
	F1/Tot	F2/Tot	F3/Tot
$\beta$ -Lg	1.00	—	—
$\beta$ -Lg-120	1.00	—	—
$\beta$ -Lg-GD	0.81	0.03	0.16
$\beta$ -Lg-120-GD	0.74	0.05	0.21
$\beta$ -Lg-DD	0.18	0.23	0.59
$\beta$ -Lg-120-DD	0.15	0.22	0.62

The areas of fractions correspond to F1 (10 to 13 min), F2 (13 to 15.5 min) and F3 (15.5 to 35 min), Tot (F1+F2+F3).  
F1 (18.3 KDa-5 kDa); F2 (5 kDa-1 KDa); F3 (<1 KDa)

## CONCLUSIONS

Ultrasonic pretreatment can improve the susceptibility of  $\beta$ -Lg during in vitro digestion, produce many small-molecule antioxidant peptides, thus upgrade the antioxidant activity compared with those of untreated sample. And, the IgG/IgE binding ability of ultrasonicated  $\beta$ -Lg after gastric digestion was increased. But, ultrasonicated  $\beta$ -Lg digested by gastrointestinal tract presents a completely opposite result. Ultrasonic increased the hydrolysis of  $\beta$ -Lg by trypsin and produced more small peptides that causes the decrease of IgG/IgE binding ability. Thus, ultrasonic pretreatment-assisted with digestion in vitro had a significantly positive effect on the antioxidant and IgG/IgE binding activity of  $\beta$ -Lg by altering the structure, which will be more likely to provide the theoretical basis for preparing the low sensitivity of dairy products in future.

## ACKNOWLEDGEMENTS

This work was supported by the Chinese National Natural Science Foundation (No. 21878135) and earmarked fund for China Agriculture Research System (CARS-45).

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FSMILE 2020

November 24-25, 2020

# Effect of extraction temperature on the gelling properties and identification of porcine gelatin



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## INTRODUCTION

Gelatin is a product obtained by the partial hydrolysis of collagen. Porcine gelatin is the most widely used on the market, and its safety has also attracted public attention, due to the outbreak of foot and mouth disease (FMD). Religious believers such as Muslims and increasing rates of vegetarianism have further enhanced the importance of porcine gelatin traceability.

## AIM

The main objective of this study was to obtain characteristic tryptic peptides of porcine gelatin that did not change at different extraction temperatures. Our findings should provide some useful information for porcine gelatin traceability.

## METHOD

- 55 °C, 65 °C, and 75 °C were used to extract gelatin from porcine skin.
- Shear stress rheological detection techniques and a texture analyzer were applied to evaluate the gelling properties of porcine gelatin such as the gel strength, gelation point and melting point to confirm the effect of the extraction temperature on the functional properties.
- HPLC and linear-ion trap (LTQ)/Orbitrap high-resolution mass spectrometry were combined to investigate the differences in porcine gelatins extracted at various temperatures.

## RESULTS

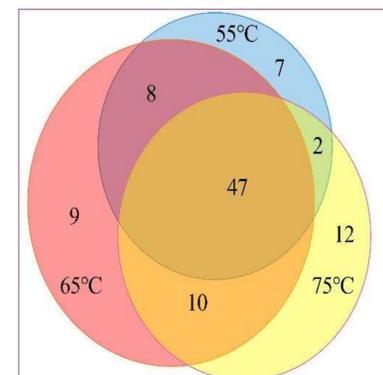
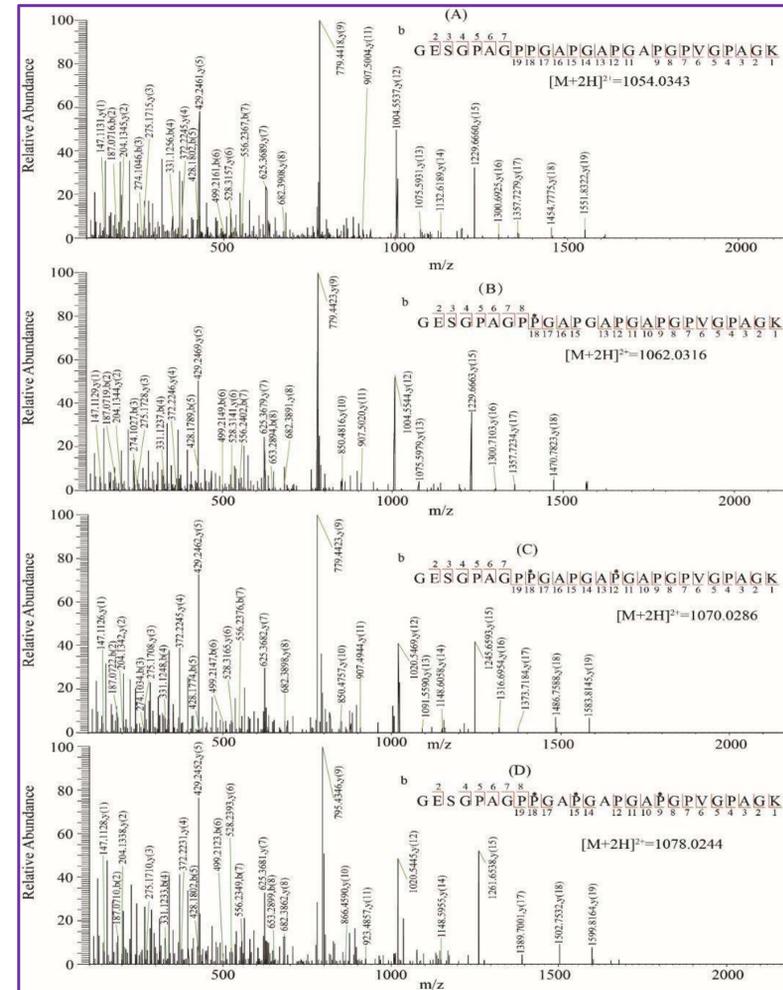
### Gelling properties

The extraction temperature significantly affected the gelling properties of porcine gelatin. When the extraction temperature increased from 55 °C to 75 °C, the gel strength of the porcine gelatin decreased from 717.36 g to 183.55 g. The gelation point declined from 21.30 °C to 9.08 °C, and the melting point decreased from 30.23 °C to 21.46 °C.

Samples	55 °C	65 °C	75 °C
Gel strength (g)	717.36±0.71a	638.10±9.44b	183.55±3.26c
Gelation point (°C)	21.30±0.50a	17.31±0.50b	9.08±0.35c
Melting point (°C)	30.23±0.49a	26.73±0.51b	21.46±0.35c

### Identification

Different extraction temperatures resulted in the gelatins with various performances, which would further affect the traceability of porcine gelatin. Firstly, according to the multiple sequence alignment software, 69 and 70 of the theoretical sequence fragments were found for porcine gelatin and bovine gelatin, respectively. Based on accurate mass and tandem mass spectrometry, 64, 74, and 71 characteristic tryptic peptides were identified from porcine gelatins extracted at 55 °C, 65 °C, and 75 °C, respectively. **A total of 47 common characteristic peptides were detected in tryptic hydrolysates of porcine gelatins.** In general, the extraction temperature showed a certain degree of impact on the traceability identification of porcine gelatin. Therefore, the identification of the porcine gelatin should refer to those common tryptic peptides that were not affected by the extraction temperature.



Different modification level identification by tandem mass spectrometry for tryptic peptides.

Venn analysis of porcine gelatin tryptic peptides extracted at 55 °C, 65 °C, and 75 °C.

## CONCLUSIONS

When the extraction temperature increased from 55 °C to 75 °C, the gel strength, the gelation point and the melting point of the porcine gelatin decreased. Compared with the established theoretical sequence fragment database of porcine gelatin and bovine gelatin, 64, 74, and 71 tryptic porcine peptides in gelatins were extracted at 55 °C, 65 °C, and 75 °C, respectively. Notably, regardless of the extraction temperature, 47 common peptides were detected in the tryptic hydrolysates of porcine gelatins. Using these common tryptic peptides can effectively improve the accuracy of the porcine gelatin identification. The influence of different factors in the extraction process on the traceability of gelatin needs to be further researched to improve the credibility of gelatin identification.

## ACKNOWLEDGEMENTS

This study was supported by the National Key R&D Program of China (No. 2018YFD0901101), the National Natural Science Foundation of China (No. 31660487, 31860428), and the Earmarked Fund for China Agriculture Research System (CARS-45).

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# Effects of chitosan coating on quality and protein characteristics of large yellow croaker (*Pseudosciaena crocea*) during ice storage

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FSMILE 2020  
November 24-25, 2020

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## INTRODUCTION

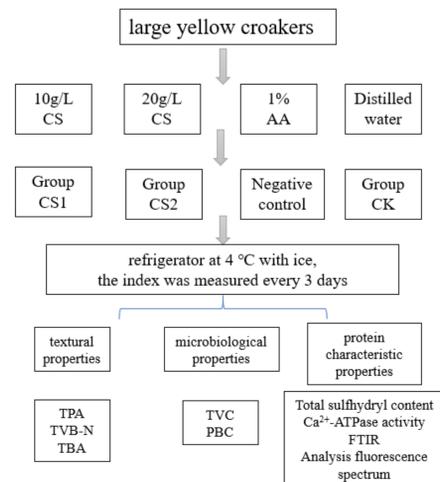
- Large yellow croaker (*Pseudosciaena crocea*), as a traditional species of marine fish, has been favored by many consumers for its good taste and high nutritional values. Due to its high nutritive content, large yellow croaker is highly perishable like the other fishery products. It will deteriorate rapidly without preservation, which always cause great economic losses.
- Chitosan (CS) is a natural polysaccharide with semi-permeable membrane, can be used to coat on fish, fruit, vegetables, meat and so on as a protective film to improve quality and extend shelf life.

## AIM

To investigate the effects of chitosan (CS) coating on quality and protein characteristics of large yellow croaker (*Pseudosciaena crocea*) during ice storage.

## METHOD

Ice-fresh large yellow croakers with an average weight of 500±50 g and length of 340±20 mm.



## RESULTS

Tab.1 Effects of chitosan coating on the changes of Hardness, Springiness and Chewiness in *Pseudosciaena crocea* during ice storage

Index	group	0	3	6	9	12	15
Hardness/g	CK	3291.60±242.68 <sup>AA</sup>	2335.33±821.42 <sup>BB</sup>	2089.84±415.39 <sup>BB</sup>	1832.98±302.42 <sup>CC</sup>	1666.95±324.13 <sup>CC</sup>	1280.71±118.37 <sup>BB</sup>
	AA	3316.13±396.05 <sup>AA</sup>	2319.25±656.66 <sup>BB</sup>	2413.53±338.50 <sup>AA</sup>	1674.67±182.53 <sup>CC</sup>	2049.87±141.71 <sup>BB</sup>	1522.61±328.15 <sup>BB</sup>
	CS1	3260.99±586.94 <sup>AA</sup>	2462.59±697.80 <sup>BB</sup>	2308.60±107.72 <sup>AA</sup>	2111.47±83.80 <sup>BB</sup>	2332.81±113.62 <sup>AA</sup>	2045.54±319.09 <sup>AA</sup>
	CS2	3188.12±423.46 <sup>AA</sup>	2735.61±282.68 <sup>AA</sup>	2476.23±292.07 <sup>AA</sup>	2741.13±50.20 <sup>AA</sup>	2462.30±102.73 <sup>AA</sup>	2269.19±290.17 <sup>AA</sup>
Springiness/mm	CK	0.51±0.15 <sup>AA</sup>	0.43±0.03 <sup>AA</sup>	0.50±0.06 <sup>AA</sup>	0.45±0.05 <sup>AA</sup>	0.42±0.03 <sup>AA</sup>	0.44±0.04 <sup>AA</sup>
	AA	0.37±0.22 <sup>AA</sup>	0.50±0.11 <sup>AA</sup>	0.49±0.03 <sup>AA</sup>	0.55±0.02 <sup>AA</sup>	0.49±0.02 <sup>AA</sup>	0.47±0.07 <sup>AA</sup>
	CS1	0.49±0.03 <sup>AA</sup>	0.43±0.01 <sup>AA</sup>	0.51±0.06 <sup>AA</sup>	0.49±0.10 <sup>AA</sup>	0.48±0.03 <sup>AA</sup>	0.38±0.03 <sup>BB</sup>
	CS2	0.40±0.01 <sup>AA</sup>	0.42±0.03 <sup>AA</sup>	0.45±0.04 <sup>AA</sup>	0.52±0.02 <sup>AA</sup>	0.45±0.10 <sup>AA</sup>	0.56±0.09 <sup>AA</sup>
Chewiness/N	CK	597.85±300.34 <sup>BB</sup>	298.80±117.97 <sup>BB</sup>	395.59±79.37 <sup>BB</sup>	329.76±103.74 <sup>BB</sup>	259.92±59.08 <sup>BB</sup>	222.71±46.95 <sup>BB</sup>
	AA	848.17±686.10 <sup>AA</sup>	445.30±174.80 <sup>AA</sup>	386.68±41.35 <sup>BB</sup>	348.65±80.41 <sup>BB</sup>	423.41±84.28 <sup>AA</sup>	261.02±114.24 <sup>BB</sup>
	CS1	572.16±127.31 <sup>BB</sup>	272.06±78.69 <sup>BB</sup>	441.11±136.82 <sup>AA</sup>	362.01±111.40 <sup>BB</sup>	465.37±91.40 <sup>AA</sup>	297.56±79.97 <sup>BB</sup>
	CS2	381.67±116.50 <sup>CC</sup>	329.13±33.10 <sup>BB</sup>	356.43±128.03 <sup>BB</sup>	467.79±70.51 <sup>AA</sup>	435.37±214.18 <sup>AA</sup>	492.54±139.47 <sup>AA</sup>

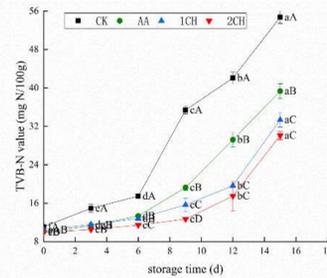


Fig.1 Effects of chitosan coating on TVB-N value of *Pseudosciaena crocea* during ice storage

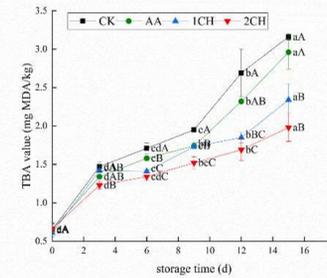


Fig.2 Effects of chitosan coating on TBA value of *Pseudosciaena crocea* during ice storage

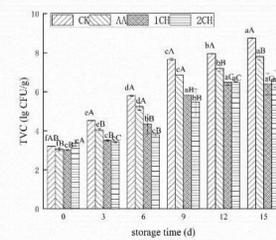


Fig.3 Effects of chitosan coating on TVC(a) and PBC(b) in *Pseudosciaena crocea* during ice storage

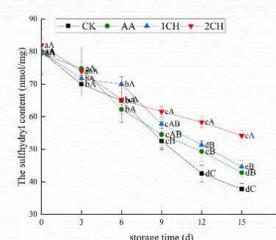
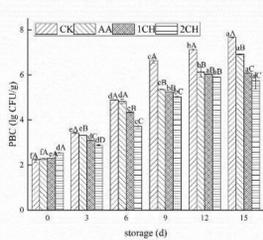


Fig.4 Effects of chitosan coating on sulfhydryl content of *Pseudosciaena crocea* during ice storage

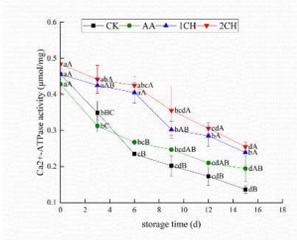


Fig.5 Effects of chitosan coating on Ca2+ -ATPase of *Pseudosciaena crocea* during ice storage

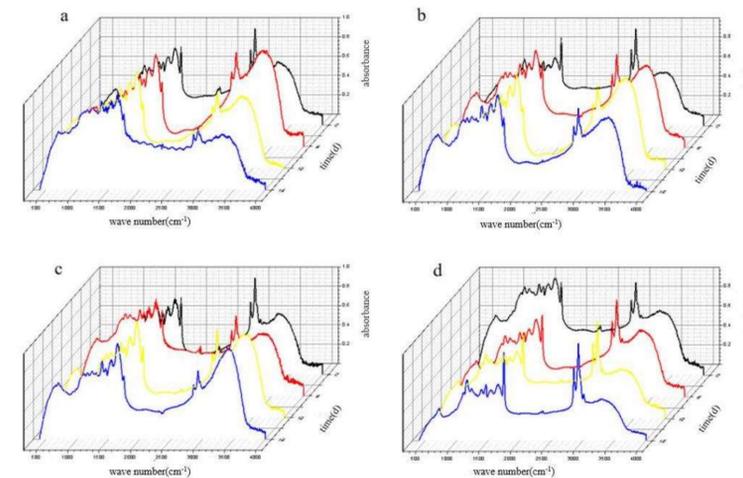


Fig.6 Effects of chitosan coating on FT-MIR spectra of myofibrillar proteins in *Pseudosciaena crocea* during ice storage

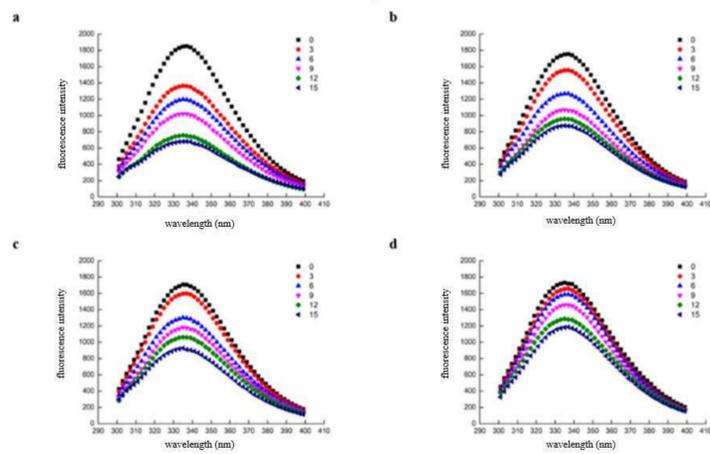


Fig. 7 Effects of chitosan coating on the intrinsic fluorescence intensity (IFI) of myofibrillar protein in *Pseudosciaena crocea* during ice storage

## CONCLUSIONS

- Chitosan treatment could effectively inhibit the activity of endogenous enzymes and microbial growth.
- Chitosan treatment also protect the invariability and structural changes of proteins in large yellow croaker (*Pseudosciaena crocea*) during ice storage.

## ACKNOWLEDGEMENTS

The study was financially supported by National Key R&D Program of China (2019YFD0901602), China Agriculture Research System (CARS-47-G26), Ability promotion project of Shanghai Municipal Science and Technology Commission Engineering Center (19DZ2284000)

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FSMILE 2020
November 24-25, 2020

Effects of different drying methods on the quality and nonvolatile taste compounds of Black Carp



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INTRODUCTION

As an effective method of food processing and preserving, drying not only improve the quality of the product, but also extend shelf life. The reasonable drying will give food seductive color and unique flavor.

AIM

The purpose of this study was to investigate the effects of hot air drying and vacuum drying on quality and nonvolatile taste compounds of black carp. Such as texture, color, amino nitrogen content, ATP-related compounds, free amino acid and Equivalent Umami Concentration.

METHOD

Sample preparation: Taking dorsal meat out and cut it into 3cm x 2cm x 1.5cm fish pieces. The sample was spread in in hot air drying oven and vacuum drying oven and dried in atmospheric pressure at 50 °C for 12h. Samples were taken out every two hours.

Moisture content: The determination of moisture content referred to the GB 5009.3-2016.

Hardness and Color: A TA-XT Plus texture analyzer , a P/2 (diameter 2mm) flat-head stainless steel cylindrical probe and a portable colorimeter CR-20 were used to measure the hardness and color of the fish pieces.

SEM observation: The microstructure of the sample was observed by a scanning electron microscope

Amino nitrogen: Amino nitrogen (ANN) content was measured using a potentiometric titrator.

ATP-related compounds: Use Waters 2695 high performance liquid chromatography equipped with COSMOSIL 5C18-PAQ liquid chromatography column and SPD-10A (V) detector.

Free amino acids: Use automatic amino acid analyzer (L-8800, Hitachi, Japan) to determine and analyze free amino acids.

RESULTS

The moisture content of the sample was 78.55% for fresh, 45.86% for HD 12 hours and 56.75% for VD 12 hours. When HD was dried for 8 hours, and VD was dried for 12 hours, the moisture content of both two drying methods was close to 55%. The hardness of black carp after drying was significantly increased compared to fresh, which is related to its contention of moisture, protein, fat and muscle tissue state. When HD reached 8 hours, and VD reached 12 hours, the hardness of HD was higher than that of VD.

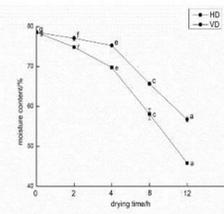


Fig 1. Changes in moisture content of black carp in two drying methods. Different lowercase letters show that the mean values are significantly different (p < 0.05)

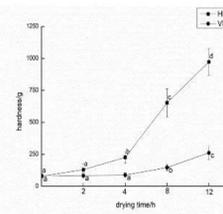


Fig 2. Changes in the hardness of black carp in two drying methods. Different lowercase letters show that the mean values are significantly different (p < 0.05)

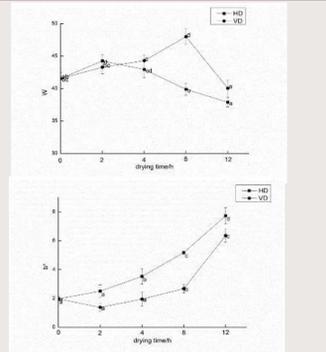


Fig 3. Changes in color of black carp in two drying methods. Different lowercase letters show that the mean values are significantly different (p < 0.05)

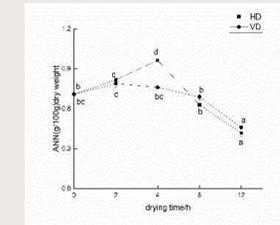


Fig 4. Changes in ANN content of black carp in different drying methods (dry weight). Different lowercase letters show that the mean values are significantly different (p < 0.05)

As the drying time increase, W of HD and VD had the same trend, first increased and then decreased. They reached the highest value in 2 hours and 8 hours respectively. The b\* of HD and VD was continuously increased during drying time, and it related to fat oxidation.

The ANN content in HD and VD is shown in Figure 4. During the drying process, ANN content of HD and VD both showed a first increased and then decreased trend. Hot air drying changes more significantly than vacuum drying.

Table 1: Changes in ATP-related compound of black carp in different drying methods (mg/100g dry weight). Columns include drying method, time (h), IMP, ATP, ADP, HXR, AMP, Hx, IMP, AMP.

Note: Different lowercase letters in the same column and part show a significant difference (p < 0.05)

Table 2: Changes in free amino acid of black carp in HD and VD (mg/100g dry weight). Columns include amino acid species, time (h), and content (mg/100g dry weight).

Note: Different lowercase letters in the same column and part show a significant difference (p < 0.05)

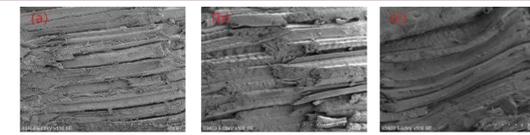


Fig 5. Changes in microstructure of black carp in two drying methods (x100). (a) Fresh, (b) HD for 8 hours, (c) VD for 12 hours

Fresh black carp muscle fiber bundles were arranged neatly and uniform thickness. After the HD, the black carp muscle fibers were partially broken, the muscle fiber structure of black carp after VD was still complete

From Table 1, the contents of AMP and IMP of HD were higher than those of VD, indicating that the black carp was more delicious after being dried by HD.

From Table 2 and Table3, When the drying time reached 12 hours, the TFAAs content of HD was 2135.93 mg/100g dry weight, while the TFAAs content of VD was 2163.95 mg/100g dry weight. The TFAAs content of two drying method did not have much difference, suggesting that the prolonged drying time make the protein hydrolysis completely, and the change was not significant.

CONCLUSIONS

This paper takes black carp as the research object and compares the quality changes of hot air drying and vacuum drying during the drying process. Studies have shown that during the drying process of black carp, the b\* and hardness increased, the whiteness, amino nitrogen, ATP-related compounds, free amino acids first increased and then decreased. IMP, Glu, Pro, Ala and Gly showed a great contribution to umami and sweetness.

ACKNOWLEDGEMENTS

This study was funded by a Key R&D Program (No. 2018YFD0901003).

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# Comparison of the flavor substances and protein degradation of black carp (*Mylopharyngodon piceus*) pickled products during steaming

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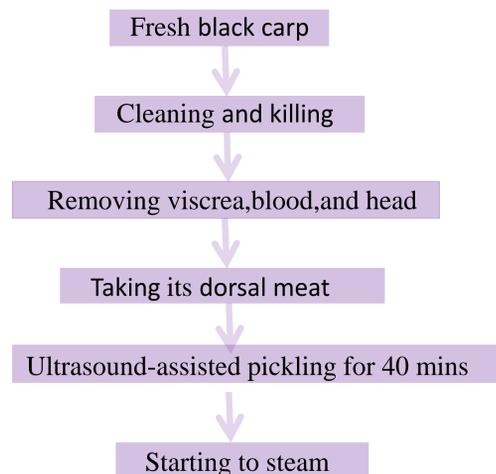
## INTRODUCTION

- Black carp (*Mylopharyngodon piceus*), a kind of carnivorous fish, is one of the four traditional freshwater farmed fish species that distribute in the south of the Yangtze River, China. In 2019, its national production amounted to 679.6 thousand tons.
- Appropriate thermal processing conditions not only gives the products their distinctive color, flavor, and texture, but also kills microorganisms to enhance the product's quality and safety. Besides, it can increase the digestibility of protein through denaturation, which facilitates nutrients' absorption for human.

## AIM

In order to investigate the influence of different steaming times on the flavor changes of black carp pickled products, this study mainly used high-performance liquid chromatography (HPLC) to analyze the nucleotides and free amino acids during steaming. The protein degradation was also discussed. The results of this paper provide suitable conditions for steaming black carp and provide theoretical and technical support for further processing of black carp.

## METHOD



## RESULTS

As shown in Tab. 1, during the steaming of pickled black carp, the Thr, Gly, and Ala showed higher contents in umami and sweet amino acids. The content of Thr was highest (23.98 mg/100 g) at 8 min, while that of Gly reduced significantly to the minimum (23.86 mg/100 g) at this point. The content of Ala increased then decreased. The reduction was most evident at 8 min, and the value at 12 min was the lowest (8.86 mg/100 g). However, their contents were far below thresholds (260 mg/100 g, 130 mg/100 g, and 60 mg/100 g, respectively), indicating that the three free amino acids had little effect on the flavor during steaming. Bitter amino acids presented higher contents of Lys and His. Lys content, which was almost constant during steaming, reached the maximum value of 120.36 mg/100 g at 14 min. Besides, the content of His increased significantly to its maximum of 270.95 mg/100 g at 8 min. Both of the values exceeded their thresholds (50 mg/100 g and 20 mg/100 g, respectively), which was not conducive to the flavor formation. There has been research pointed out that Lys and His are major components of free amino acids in freshwater fish, which is consistent with the result in this paper.

Tab. 1 Changes of main free amino acids content of black carp meat during steaming

Types	Threshold	Contents (mg/100 g)					
		4 min	6 min	8 min	10 min	12 min	14 min
Thr <sup>*</sup>	2600	21.23±1.25 <sup>a</sup>	23.77±1.71 <sup>a</sup>	23.98±0.29 <sup>a</sup>	17.63±0.23 <sup>b</sup>	18.90±0.40 <sup>b</sup>	15.30±0.53 <sup>b</sup>
Gly <sup>*</sup>	1300	34.13±1.43 <sup>a</sup>	32.39±2.20 <sup>a</sup>	23.86±0.02 <sup>b</sup>	27.34±1.88 <sup>b</sup>	24.86±0.50 <sup>b</sup>	28.61±0.79 <sup>b</sup>
Ala <sup>*</sup>	600	17.20±0.12 <sup>a</sup>	17.43±1.18 <sup>a</sup>	14.38±0.17 <sup>b</sup>	10.88±0.49 <sup>b</sup>	8.86±0.23 <sup>b</sup>	12.69±0.41 <sup>b</sup>
Lys <sup>▲</sup>	500	117.75±8.38 <sup>a</sup>	119.09±3.64 <sup>a</sup>	118.47±1.2 <sup>a</sup>	118.87±0.80 <sup>a</sup>	119.48±1.85 <sup>a</sup>	120.36±1.78 <sup>a</sup>
His <sup>▲</sup>	200	223.48±14.06 <sup>a</sup>	244.47±15.00 <sup>b</sup>	270.95±7.18 <sup>b</sup>	233.48±11.74 <sup>b</sup>	259.06±5.89 <sup>b</sup>	222.73±7.53 <sup>b</sup>

<sup>\*</sup> represents fresh and sweet amino acids; <sup>▲</sup> represents bitter amino acids; Data in the same row with different letters are significantly different ( $p < 0.05$ ) during steaming

As shown in Tab. 2, the content of nucleotides varied during time. As the time extends, the IMP content predominated because the slow degradation of AMP into HxR during ATP degradation resulted in IMP accumulation. This content increased significantly from 8 min to 10 min, after which no obvious change was observed. It reached the maximum value of 210.85 mg/100 g at 14 min. The threshold of IMP was 25 mg/100 g, and its TAV was greater than one consistently, indicating that IMP contributed to the flavor mostly. The AMP content increased along with the extension of the steaming time and reached the maximum value of 8.56 mg/100 g at 14 min. The threshold of AMP was 50 mg/100 g, and its TAV was less than one consistently, indicating that AMP had an unobvious effect on the taste. Hx, as the final product of ATP degradation, is associated with bitterness. Its content fell then increased. During steaming, the value plummeted at 6 min and reached a minimum of 0.65 mg/100 g at 10 min. Then, it increased significantly to 0.79 mg/100 g at 12 min.

Tab. 2 Changes of nucleotide compounds content of black carp meat during steaming

Steaming time/min	Contents (mg/100 g)								TAV
	IMP	ATP	ADP	AMP	Hx	HxR	IMP	AMP	
4	204.97±12.01 <sup>a</sup>	11.73±1.22 <sup>a</sup>	9.36±0.19 <sup>a</sup>	5.87±0.57 <sup>a</sup>	1.08±0.10 <sup>a</sup>	24.38±1.66 <sup>a</sup>	8.20±0.48 <sup>a</sup>	0.12±0.01 <sup>a</sup>	
6	191.28±8.21 <sup>a</sup>	11.50±0.90 <sup>a</sup>	8.70±0.49 <sup>a</sup>	6.12±0.36 <sup>a</sup>	0.70±0.06 <sup>a</sup>	19.47±0.59 <sup>a</sup>	7.65±0.32 <sup>a</sup>	0.12±0.01 <sup>a</sup>	
8	186.00±4.54 <sup>a</sup>	9.31±0.93 <sup>a</sup>	9.16±0.13 <sup>a</sup>	6.40±0.11 <sup>a</sup>	0.90±0.03 <sup>a</sup>	24.37±1.44 <sup>a</sup>	7.44±0.18 <sup>a</sup>	0.13±0.01 <sup>a</sup>	
10	209.08±3.83 <sup>a</sup>	15.64±0.38 <sup>a</sup>	9.70±0.63 <sup>a</sup>	7.37±0.29 <sup>a</sup>	0.65±0.02 <sup>a</sup>	21.34±0.68 <sup>a</sup>	8.36±0.15 <sup>a</sup>	0.15±0.01 <sup>a</sup>	
12	201.32±2.62 <sup>a</sup>	16.53±0.65 <sup>a</sup>	9.34±0.89 <sup>a</sup>	7.49±0.23 <sup>a</sup>	0.79±0.02 <sup>a</sup>	22.74±0.37 <sup>a</sup>	8.05±0.11 <sup>a</sup>	0.15±0.01 <sup>a</sup>	
14	210.85±6.70 <sup>a</sup>	26.34±1.35 <sup>a</sup>	9.67±0.40 <sup>a</sup>	8.56±0.46 <sup>a</sup>	0.69±0.03 <sup>a</sup>	21.25±1.23 <sup>a</sup>	8.44±0.27 <sup>a</sup>	0.17±0.01 <sup>a</sup>	

Data in the column with different letters are significantly different ( $p < 0.05$ ) during steaming.

Fig. 1 shows the variation trend of sensory assessment during the steaming. During heating, the muscle texture was affected by reduced water holding capacity, protein denaturation, connective tissue, and contractile actomyosin, etc. Meanwhile, the color, especially the gloss, changed obviously. The sensory assessment first rose and then fell during the steaming. The score increased significantly to 82.33 at 6 min

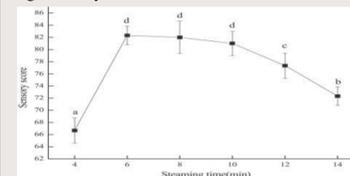


Fig. 1 Changes of sensory score of black carp meat during steaming. Data with different letters are significantly different ( $p < 0.05$ )

Fig. 2 displays the PCA of black carp by the electronic tongue during steaming, with the result shown in the form of a 2D scatter plot composed of two coordinate axes (PC1 and PC2). The closer the two samples are, the more similar the taste characteristics will be. As shown in Fig. 2, the sum of accumulated variance contribution rates of PC1 and PC2 was 95.37%, which was greater than 85%, indicating that the two principal components contained sufficient information to reflect the total variance of the entire data set. Besides, the DI value reached 87, showing a higher discriminability.

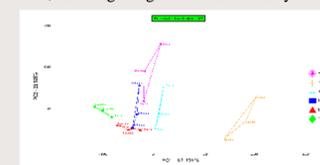


Fig. 2 The two-dimensional PCA plot of electronic tongue results recorded of black carp meat during steaming

Figure 3 shows the EUC value calculated throughout steaming, which decreased initially and then increased. At 8 min, the value plummeted to the lowest 1.42 g MSG/100 g. In other words, the umami intensity of each gram of carp was equivalent to 1.42 g of monosodium glutamate (MSG). The change was not significant after 10 minutes. The EUC value reached its maximum at 4 min, which was 1.94 g MSG/100 g. The value at 6 min did not change significantly compared to that at 4 min, and the EUC value was 1.81 gMSG/100 g. The MSG threshold is 0.03 g/100 mL. During the steaming process, all TAV of EUC values were greater than one, indicating that the taste of black carp is primarily contributed by the synergistic effect between nucleotides and amino acids.

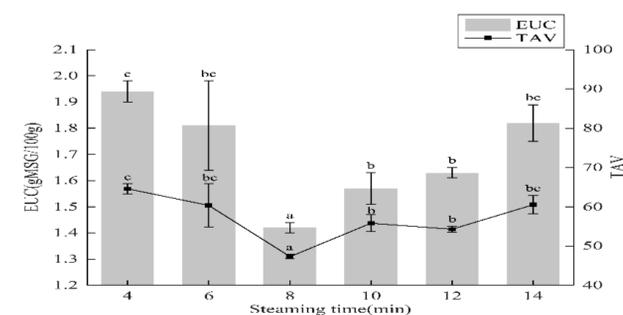


Fig. 3 Changes of EUC value and its TAV of black carp meat during steaming. Data with different letters are significantly different ( $p < 0.05$ )

## CONCLUSIONS

This paper studied the flavor changes of pickled black carps during steaming (4-14 min) and found that the former can be significantly affected by heating conditions. Throughout the process, the meat presented the most satisfying gloss, tenderness, and chewiness at 6-8 minutes, which greatly improved its sensory score. As steaming time extended, proteins gradually degraded, producing more flavor precursor substances. When being steamed for 6 minutes, its umami and sweet amino acid content were the highest, with the TAV of IMP being 7.65, which proves that IMP was the most significant contributor to the taste. Besides, the electronic tongue principal component analysis was adopted to distinguish the flavor profile during steaming. According to the study, 6-8 min is an ideal steaming time for black carps, which provides a theoretical basis for quality control during the heating process.

## ACKNOWLEDGEMENTS

This study was supported by the National Key R&D Program of China (Grant No. 2018YFD0901003)

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# Pectin combined with plant essential oils inhibit water migration, myofibril proteins degradation and muscle tissue enzyme activity of vacuum packaged large yellow croaker (*Pseudosciaena crocea*) during ice storage

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## INTRODUCTION

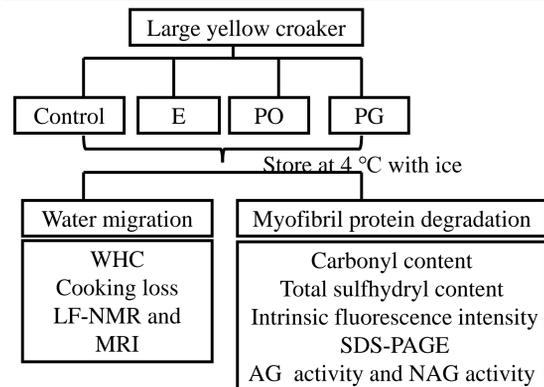
- Large yellow croaker (*Pseudosciaena crocea*), an economical marine-cultured fish species, is widely welcome for its excellent nutritional composition and delicious taste.
- Plant essential oils obtained from natural plant and have been recognized as safe substances by the US Food and Drug Administration.
- Pectin has attracted much attention owing to its non-toxic, odorless, renewable, biodegradable and low permeability of pectin coating, it is a good barrier to cut off oxygen.

## AIM

The effects of pectin combined with plant essential oils on water migration, myofibrillar proteins (MPs) and muscle tissue enzyme activity of vacuum packaged large yellow croaker (*Pseudosciaena crocea*) during ice storage at 4±1°C were investigated.

## METHOD

Group	Treating methods
Control	samples treated with distilled water
E	samples treated with 2.5% Ethanol
PO	samples coated with 2.5% Pectin and 0.4% oregano essential oil
PG	samples coated with 2.5% Pectin and 0.4% ginger essential oil



## RESULTS

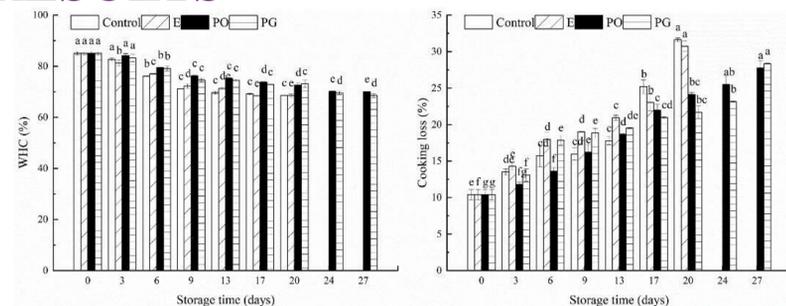


Fig. 1 Changes in water holding capacity (A) and cooking loss (B) of large yellow croaker stored in ice at 4 ° C. a-g: Different superscript values indicate significant differences in the same treatment group in different days ( $P < 0.05$ ).

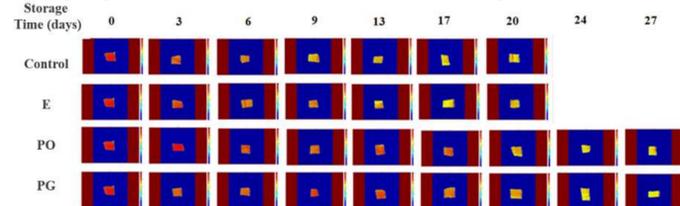


Fig. 2 Changes in MRI of large yellow croaker stored in ice at 4 ° C.

Tab.1 Changes in the percentage of  $T_{21}$  of large yellow croaker stored in ice at 4 ° C

Storage time (days)	0	3	6	9	13	17	20	24	27
$P_{T21}$	Control	2.56	2.03	1.70	1.64	1.40	1.26	0.94	
	E	2.56	2.07	1.85	1.55	1.46	1.17	1.15	
	PO	2.56	2.41	2.07	1.89	1.63	1.57	1.47	1.33
	PG	2.56	2.32	1.82	1.77	1.84	1.38	1.35	1.27
$P_{T22}$	Control	97.18	95.38	94.85	94.62	94.40	93.07	93.31	
	E	97.18	95.19	95.00	94.70	94.24	94.19	93.51	
	PO	97.18	96.21	95.71	95.82	95.38	95.31	94.21	94.83
	PG	97.18	96.18	95.91	95.46	95.30	94.68	95.00	94.76
$P_{T23}$	Control	0.26	2.59	3.45	3.74	4.20	5.67	5.75	
	E	0.26	2.74	3.15	3.75	4.30	4.64	5.34	
	PO	0.26	1.38	2.22	2.29	2.99	3.12	4.33	3.84
	PG	0.26	1.50	2.27	2.77	2.86	3.94	3.65	4.12

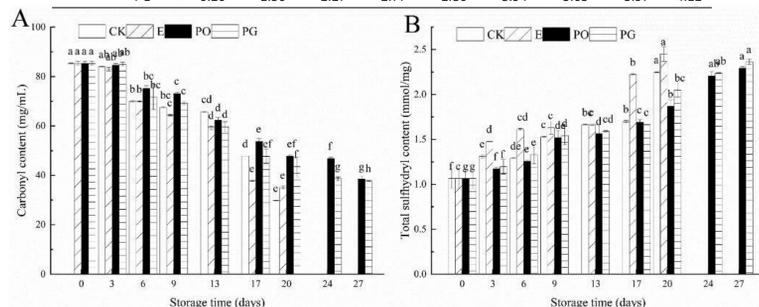


Fig. 3 Changes in carbonyl content (A) and total sulfhydryl content (B) of large yellow croaker stored in ice at 4 ° C. a-g: Different superscript values indicate significant differences in the same treatment group in different days ( $P < 0.05$ ).

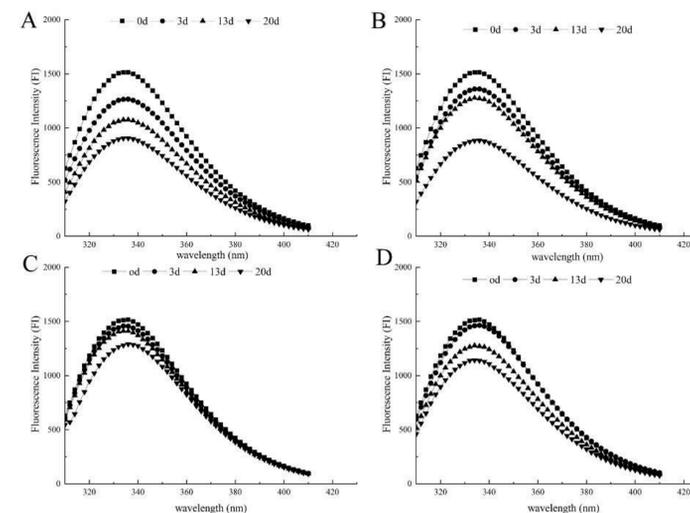


Fig. 4 Changes in intrinsic fluorescence intensity (IFI) of large yellow croaker stored in ice at 4 ° C.

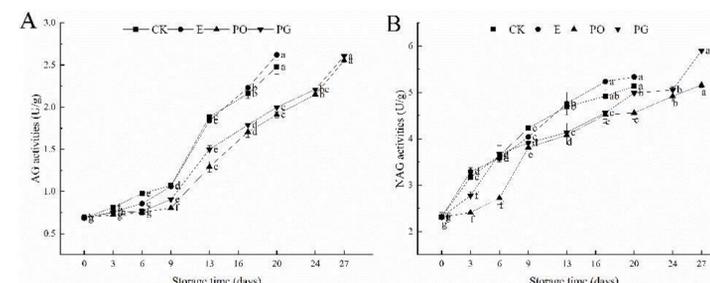


Fig. 5 Changes in the activities of AG (A) and NAG (B) of large yellow croaker stored in ice at 4 ° C. a-g: Different superscript values indicate significant differences in the same treatment group in different days ( $P < 0.05$ ).

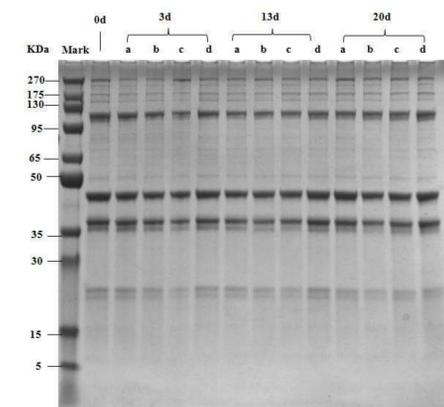


Fig.6 Changes in SDS-PAGE of large yellow croaker stored in ice at 4 ° C.

## CONCLUSIONS

- Compared with the control group, PO and PG group prevent adverse texture changes by reducing cooking loss, retarding the decrease of WHC and inhibiting the content of free water.
- PO and PG also have significant protective effects on protein oxidation, including preventing carbonyl and IFI, inhibition of endogenous enzyme activity, and a decrease in the total sulfhydryl content, inhibiting endogenous enzyme activity, and reducing the total amount of sulfhydryl groups.
- pectin combined with essential oil could effectively slow down the decrease of MPs during refrigeration.
- Overall, pectin combined with plant essential oils had the best effect on maintaining the freshness of quality preservation of large yellow croaker.
- These results showed that adding EOs to pectin coating could extend the shelf life of yellow croaker for another 7 days at least.

## ACKNOWLEDGEMENTS

The study was financially supported by National Key R&D Program of China (2019YFD0901602), China Agriculture Research System (CARS-47-G26), Ability promotion project of Shanghai Municipal Science and Technology Commission Engineering Center (19DZ2284000)

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# Effect of different types of collagen peptides derived from shortbill spearfish (*Tetrapturus angustirostris*) on hyaluronidase inhibition activity

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**Keywords:** Collagen peptides, Hyaluronidase inhibition activity, type I & V

## INTRODUCTION

Hyaluronidase (HAase) has been confirmed as an inflammation trigger (Fig. 1). Although the HAase inhibition activity of several plants has been reported, the relative research of peptides is quite limited and there is no evidence on the type V collagen peptides isolated from fish.

Given this background, we investigated the HAase inhibition activity (Fig. 6) of collagen peptides from fish skin and muscle.

## MATERIALS & METHODS

Type I and V collagen peptides were obtained from the skin and muscle of shortbill spearfish (*Tetrapturus angustirostris*) (Fig. 2) according to the scheme shown in Fig. 3. The hyaluronidase inhibition activity was measured based on the method of Meyer [1]. The degree of hydrolysate (DH%) (Fig. 4) and SDS-PAGE (Fig. 5) were also investigated.

On the other hand, the ultrafiltrates of crude peptides (MWCO: 30000) and fractions eluted by RP-HPLC were subjected to measurement of hyaluronidase inhibitory activity.



Fig. 2 The head, skin and muscle of Shortbill spearfish (*Tetrapturus angustirostris*)

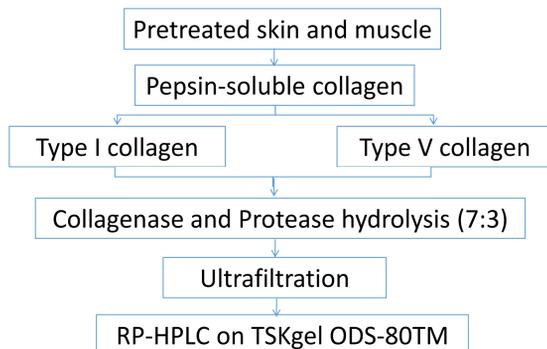


Fig. 3 Isolation scheme of type I and V collagen peptides

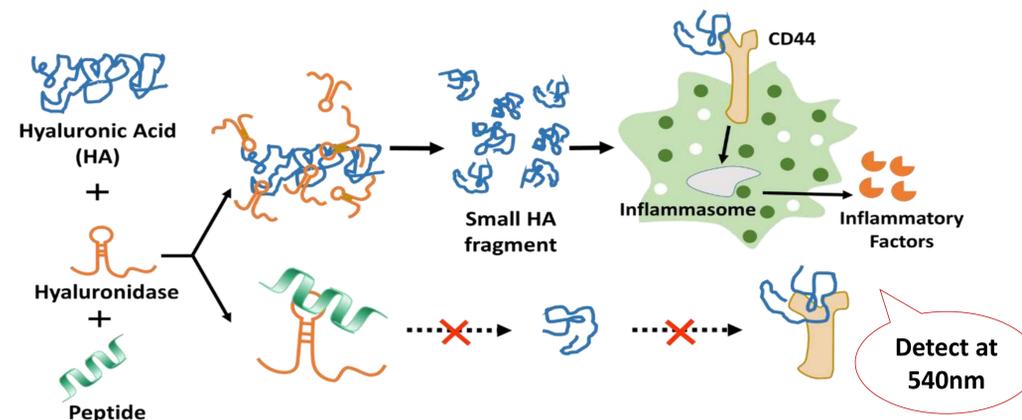


Fig. 1 Reaction cascade for hyaluronidase inhibition activity of peptide

## RESULTS

The peptide distribution of collagen peptides hydrolyzed from type I and V collagen of skin (TAS-I and TAS-V) and muscle (TAM-I and TAM-V) of shortbill spearfish was investigated by SDS-PAGE and DH% assay (Fig. 4, Fig. 5). The results showed that the molecular weight of type I collagen peptides were less than 30KDa. TAS-I with higher DH% (24.8%) and lower molecular distribution showed higher HAase inhibition activity (39.6%) compared with TAS-V and TAM-V, indicating that the smaller peptide attributed to higher HAase inhibition activity (Fig. 4).

Regarding further study on HAase inhibition activity of smaller peptides by RP-HPLC, it was 7.4 times higher in purified fractions of TAS-V and the inhibition rate increased by 8.3% in purified fractions of TAS-I, compared with crude peptides respectively (Fig. 7). Compared with plant extracts, the F-3 fraction of TAS-V showed a less HAase inhibition rate (Table 1). On the other hand, the higher HAase inhibition activity of type I collagen peptides may attribute to the more hydrophobic fractions existed.

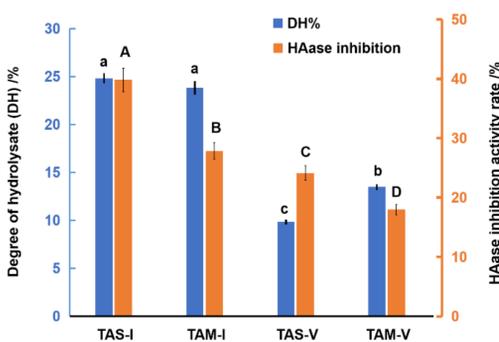


Fig. 4 DH% and HAase inhibition rate of crude type I and V collagen peptides of skin and muscle derived from shortbill spearfish

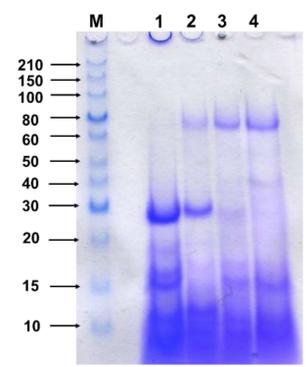


Fig. 5 Molecular distribution of crude type I and V collagen peptides of skin and muscle derived from shortbill spearfish

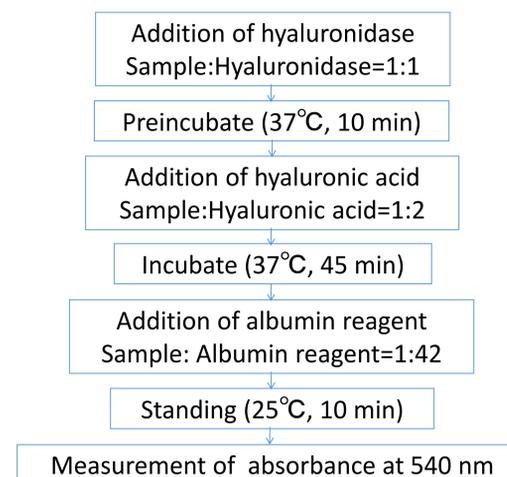


Fig. 6 The flow chart of hyaluronidase inhibition assay

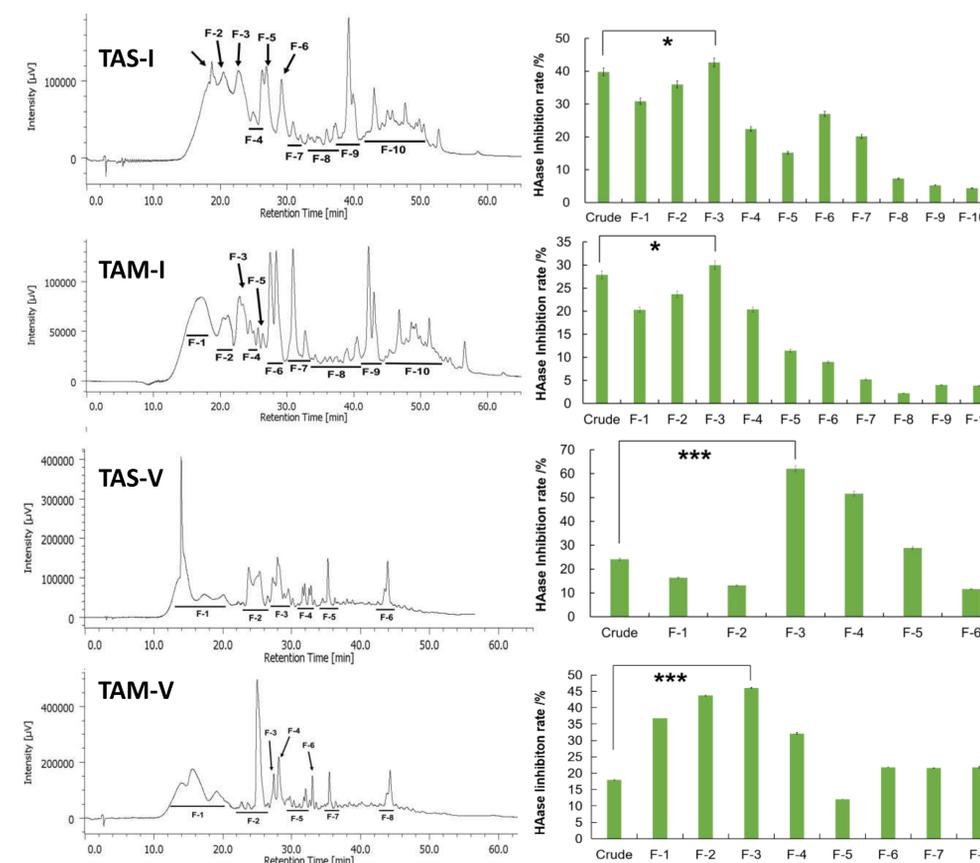


Fig. 7 Peptides mapping by RP-HPLC and the hyaluronidase inhibition activity of eluted fractions of type I and V collagen peptides from of skin and muscle derived from shortbill spearfish

## CONCLUSIONS

It was concluded that the type I collagen peptides isolated from the skin of shortbill spearfish possessed higher HAase inhibition activity. On the other hand, since any peptides fractionated from type V collagen of the skin showed higher HAase inhibition rate compared with crude peptides, it seemed that it is necessary to study further. To the best of our knowledge, this is the first report about the bioactivity of type V collagen peptides, also the information about HAase inhibition activity of collagen peptides are very limited. So the results of our study provide a meaningful research idea.

Table 1 comparison of HAase inhibition rate of various samples

Sample	Inhibiton rate	Conc.
The leaf of <i>Azadirachta indica</i>	67% [2]	0.50 mg/mL
Squid skin collagen hydrolysate	> 50% [3]	1.71 mg/ml
Glutathion	> 50% [4]	1.20 mg/ml
F-3 fraction of TAS-I	42%	1.00 mg/ml
F-3 fraction of TAS-V	60%	1.00 mg/ml

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## INTRODUCTION

**Supply chain**

**Round with ice**

**Fillet with ice**

**Consumer**

**Benefits of farmed cherry salmon**

- Post-catch processing is possible
- Can maintain freshness
- Production adjustment is possible
- There is no Anisakis

**Objective: What is better way for distribution??**

**AIM: To investigate the effect of post-catch handling (Killing and transportation) on the freshness of farmed salmon. Clarify.**

## METHOD

**Preparation**

ATP and pH measured at 0, 4 or 5, 24, 48 and 72h  
Store in a refrigerator at 4 °C

Index freshness

$$K \text{ value (\%)} = \frac{HxR+Hx}{ATP+ADP+AMP+IMP+HxR+Hx} \times 100$$

## RESULTS

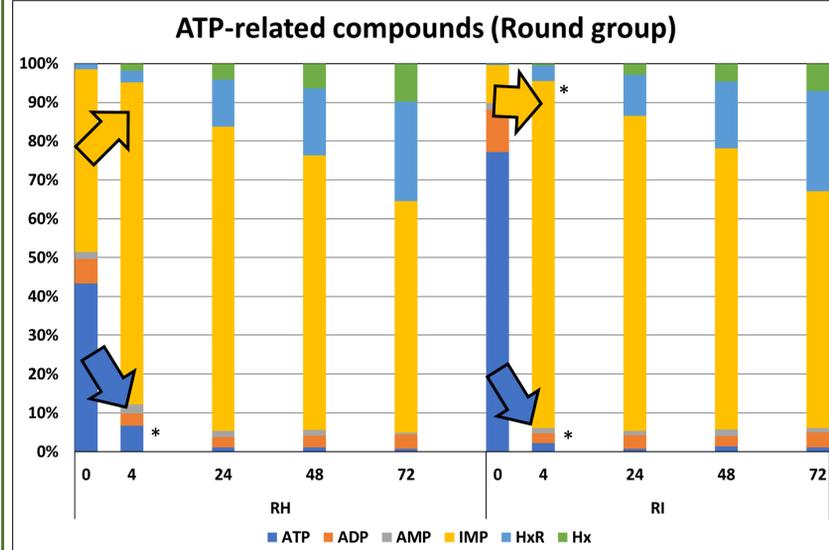


Fig. 1 Change in ATP related compounds of two round groups (\*p<0.05)

ATP decreased and IMP increased significantly from 0 to 4 hours.

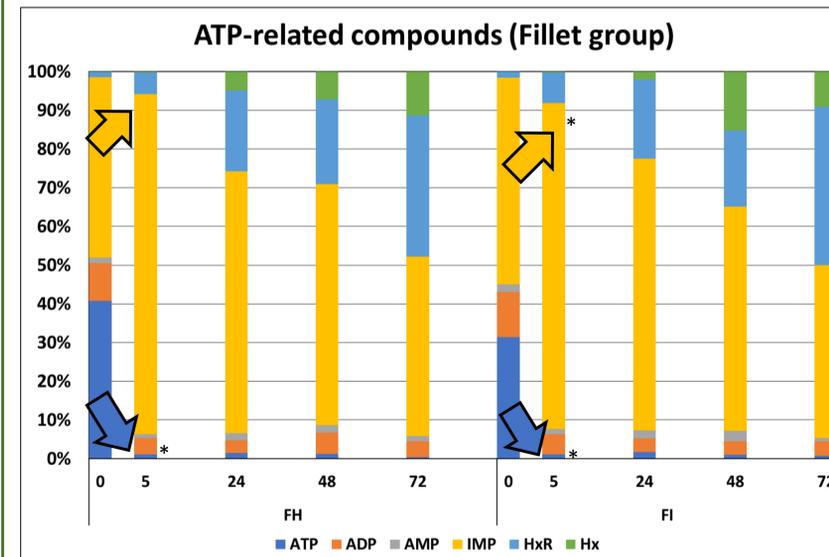


Fig. 2 Change in ATP related compound in the two fillet groups. (\*p<0.05)

IMP gradually decreased after 4 hours.

**Result 1: In farmed cherry salmon, ATP decreased and IMP increased quickly after killing and then decreased.**

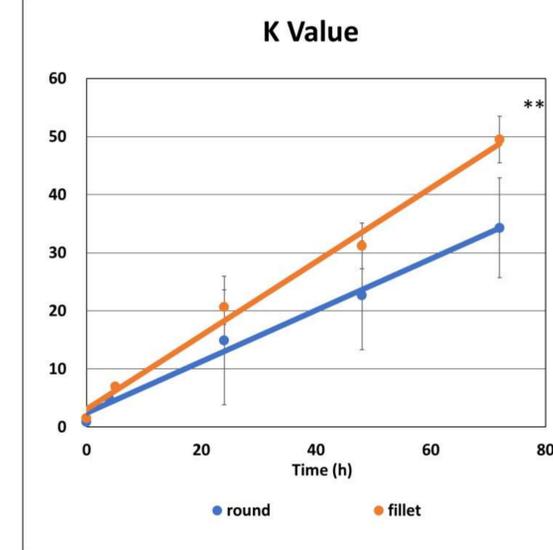


Fig. 3 Changes in K value in the round and fillet sample during strage. (\*\*p<0.01)

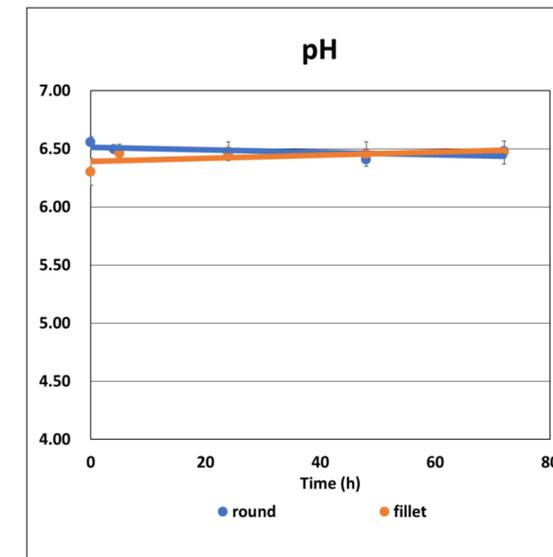


Fig. 4 Changes in pH of round and fillet samples during storage.

**Result 2: Round transport suppressed the increase in K value compared to that of fillet transport.**

## CONCLUSIONS

- It was clarified that the ATP in post-mortem cherry salmon decreased rapidly and IMP accumulated in 4-5 h.
- The increase of K-value for the round transportation group showed less than that of fillet one.
- Considering the supply chain from fish catch to consumption, it suggested that the round transportation might be better than fillet one.
- Moreover, the effect of transportation temperature should be further studied in detail.

**Keep round for as long as possible**

**Consumer**

## ACKNOWLEDGEMENTS

The project was funded by JSPS 19H05611.

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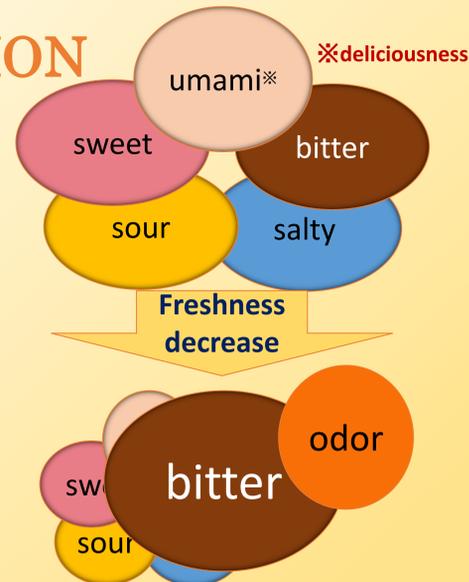
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## INTRODUCTION



Ascidian  
(*Halocynthia roretzi*)

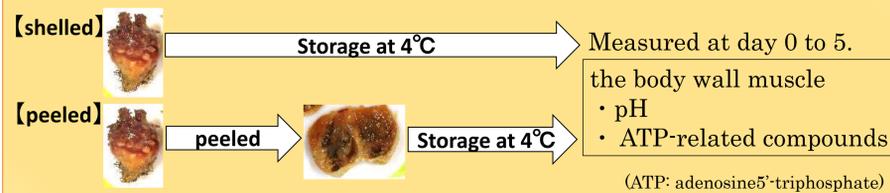


- Problems:
1. Fisheries adjustment
  2. Lack of freshness keeping technology
  3. Short distribution channel

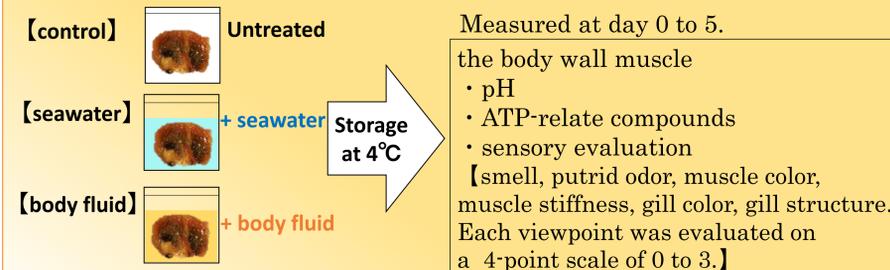
**Aim:** we tried to collect knowledge about changes in the freshness of them and examine effective methods for maintaining freshness.

## METHODS

### (1) Comparison of changes in freshness between shelled and peeled ascidians



### (2) Comparison of three storage methods for peeled ascidians



## RESULTS

### (1) Comparison of changes in freshness between shelled and peeled ascidians

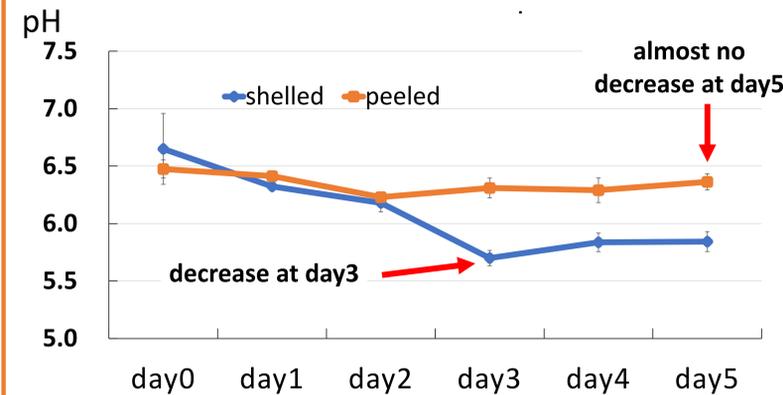


Fig.1 Changes in pH between shelled and peeled ascidians at 4°C.

The pH of shelled ascidian decreased to pH 5.7 on day3, but the peeled ascidian kept around pH 6.3 at day 5.

Most of the individuals with shell became dead on day 3, which gave significant effects of digestive juices and feces immediately thereafter.

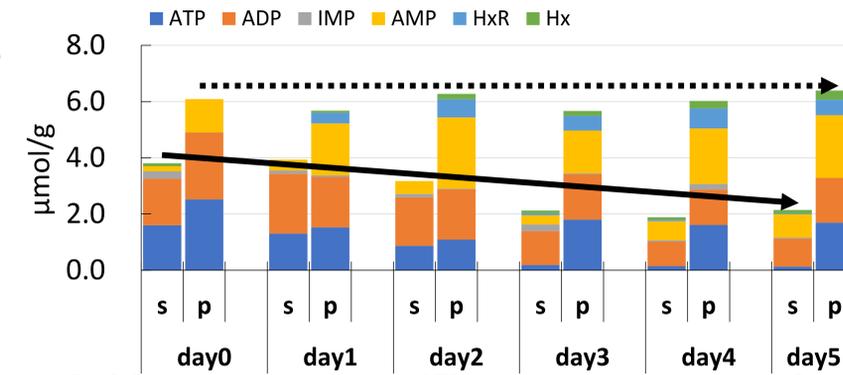


Fig.2 Changes in the amount of ATP-related compounds from day0 to day5. (s:shelled, p:peeled)

The ATP-related compounds of shelled ascidian decreased from day 3, but the peeled ascidian kept unchanged at day 5.

### (2) Comparison of three storage methods for peeled ascidians

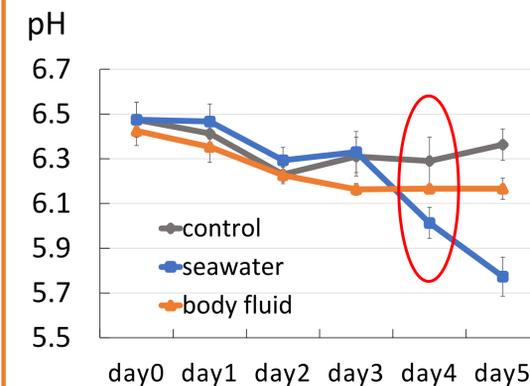


Fig.3 Changes in the pH of control, storage with seawater and with body fluid at 4°C.

The pH of three storage conditions decreased to 6.3 (control), 6.0 (with body fluid) and 6.1 with seawater, respectively on day 4

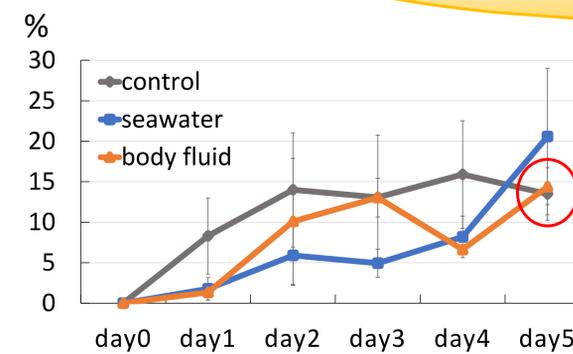


Fig.4 Changes in the K value of control, storage with seawater and with body fluid at 4°C.

The K values of the control and the sample with body fluid were lower than 20% till day 5.

The control pH and K value were the highest of all, but sensory evaluation score was the lowest. However body fluid storage obtained the best score on sensory evaluation.

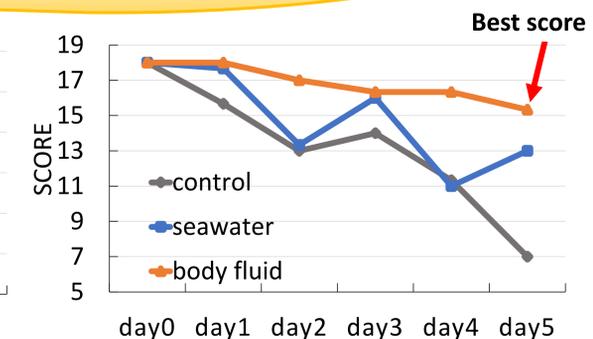


Fig.5 Changes in score of sensory evaluation. (Total score in each 0~3points of smell, putrid odor, muscle color, muscle stiffness, gill color and gill structure)

The sensory evaluation score decreased to 7 points for the control group on day 5. The body fluid storage had the highest score of 15 points due to less odor and blackening.

## CONCLUSIONS

1. Maintain freshness in the long term  
Shelled < Peeled

2. [body fluid] Possible for long-distance transportation. Can be eaten raw in good condition at least 4 days.

3. [body fluid] K value > [control] Odor > Appearance

It is necessary to evaluate the freshness of ascidians comprehensively.



Vinegared Ascidian



Hoyaboy

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