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Effect of eel head protein hydrolysates on the denaturation of grass carp surimi during frozen storage

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Abstract

Protein hydrolysates (SH, TH) were prepared from eel head by enzymatic treatment using protease. The eel head hydrolysates were used as a natural food preservative by adding to grass carp surimi at the 10\% ranging. We compared the effect of protein hydrolysates of eel head with traditional antifreeze on the denaturation, gel strength and contents of the salt soluble protein in grass carp surimi during frozen storage at -20\( ^\circ \text{C} \) for 80 days. The addition of eel head hydrolysate markedly decreased the activation rate of the Ca-ATPase. The contents of the salt soluble protein in the grass carp surimi containing eel head hydrolysates were higher than those without eel head hydrolysates (control). Therefore, the gel strength of grass carp surimi media with eel head hydrolysate lower than the others. The result suggests that eel head hydrolysate could suppress the denaturation of grass carp surimi during frozen storage.

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Keyword: Eel waste; Protein hydrolysate; Ca-ATPase; Denaturation; Frozen storage;

1. Introduction

Utilization of waste from food industry has gained an increased interest in these years. Such as the aquatic product processing, the ranging of fishery processing industry waste was about 40\%~50\% [1]. At present, most of these waste was processed into fishmeal and silage [2]. Processing techniques for aquatic product waste are needed to convert the underutilized wastes into more marketable, valuable and
acceptable products [3]. Enzymatic hydrolysis of protein is a viable method for it could effective utilization of nutrients, and it avoids the extremes of physical and chemical treatments [4].

Compared to other food stuffs, the denaturation of aquatic products after catch occurs very fast, and it is very difficult to store over a long time even in ice [5]. Frozen storage is widely used as a long time preservation method, which retards the spoilage mechanisms of fish protein [6]. However, biochemical changes during freezing are inevitable associated with some of fish protein, as the alteration of protein solubility [7], the decline of gel strength [8,9], and Ca-ATPase inactivation [10]. Adding antifreeze has been used to prevent such unwanted changes. The protective effects of sugars, amino acids, organic acids and phosphates were examined [11]. Sugar mixed with compound phosphate and sorbitol, which has a good preventive effect against freeze-denaturation, was applied to process frozen surimi [12].

Several investigators have devoted considerable effect to develop protein hydrolysate from the waste of aquatic product processing by enzymatic hydrolysis [3]. However, there were few of reports about the effect of eel head protein hydrolysate on the denaturation of grass carp surimi during frozen storage.

2. Methods

2.1. Materials

Heads of eel were obtained from Jiangxi Dong Hai Food Co., Ltd. and used as raw materials for preparation of protein hydrolysate by enzymatic hydrolysis. Fresh grass carp were purchased from Happy Buy Supermarket (NanChang, China) and used for preparation of surimi. The papain was purchased from GuangXi PangBo Biotech Ltd. Alcalase 2.4L was purchased from Novozymes enzyme preparation Co.

2.2. Preparation of eel head protein hydrolysates

2.2.1. Hydrolysate of hydrolyzed by single enzyme (SH)

The eel heads were steamed by high temperature and high pressure for 40 min to inactivate endogenous hydrolyzing enzymes. Thereafter, the eel heads was added with a 4-fold volume of distilled water, and then crushed by the grinder. The mixture was adjusted to pH 9.0 with 2N NaOH at 60°C for an optimal enzyme activity. The Alcalase enzyme was added at 48 AU/kg and continuously hydrolyzed with stirring for 2h. In the hydrolytic process, we added 2N NaOH to maintain the mixture at pH9.0. After filtering the hydrolysate, the mixture was heated to 100°C for 10 min and the lipid layer was removed from the surface. After cooling, the hydrolysate was filtrate by 8 layer gauze. After adjusting the pH to 7.0 with 8N HCl, the hydrolysate was subjected to low temperature cryodesiccation (FreeZone2.5, Labconco Inc.) to obtain a dried product.

2.2.2 Hydrolysate of hydrolyzed by two kinds of enzyme (TH)

The first enzyme step was according to the single enzyme hydrolyzed method. After hydrolyzed 2h, the mixture was adjusted to pH 6.5 with 8N HCl at 65°C. The papain was added at 2%(w/w) and continuously hydrolyzed with stirring for 4h. After filtering the hydrolysate, the mixture was heated to 100°C for 10 min and the lipid layer was removed from the surface. After cooling, the hydrolysate was filtrate by 8 layer gauze. After adjusting the pH to 7.0 with 8N HCl, the hydrolysate was subjected to low temperature cryodesiccation (FreeZone 2.5, Labconco Inc.) to obtain a dried product.
2.3. Preparation and frozen storage of grass carp surimi

The surimi was prepared according to the method of Kazufumi, Mohammed and Koichi [13] with slight alteration. The grass carp surimi were beheaded, gutted and washed. Skin and bones were removed using knives. The remaining meat was cut into mince and washed twice with 5 volumes of distilled water at a cool temperature. After washing, the excessive water in the meat was removed using a centrifuge machine (1000×g for 10 min; Evolution RC, Shanghai Zhongkewubaihao Biotech Ltd.). The remaining was the grass carp surimi.

SH and TH (10g) were added to 100 g of the surimi. After mixing, about 300g of each sample was sealed into a valve bag, and stored at -25°C. 4% sugar, 4% sorbitol and 0.3% compound phosphate were added to 100 g of the surimi in another group. As a control, surmi without added hydrolysate were stored in the same manner.

2.4. Measurement of surimi Ca-ATPase activity and salt extractable protein

The surimi samples were thawed in a cold room at about 4°C after various periods of storage at -20°C. After addition of five volumes of 0.05 M KCl-20mM Tris-maleate buffer (pH7.0), the specimens were homogenized in an agitator (JJ-1 Aitator, JinTan Chengdong Instrument factory) at 670 rpm for 20 min and the homogenate was centrifuged at 9000×g for 20 min. And then removed the filter liquor by filtrated with 8 layer gauze. After addition of five volumes of 0.6 M KCl-20mM Tris-maleate buffer (pH7.0), the remaining insoluble were homogenized stored in a cold room at 4°C for 1h. Obtaining the filtrate after centrifuged at 9000×g for 20 min.

The myofibrillar Ca-ATPase activity was then measured by the following method [14]. The myofibril samples of 0.2-0.4mg were incubated at 25°C in the presence of 100 mM ATP and the reaction was terminated by adding 15% trichloroacetic acid. The free inorganic phosphate was measured by colorimetry.

The contents of salt soluble protein in grass carp surimi was measured by biuret method [15] using bovine serum albumin as the standard. The content of bovine serum albumin was corrected by the Kjeldahl method.

The Ca-ATPase (KD) of surimi was calculated using the following formula [16] KD= (InCo - InCt) / t; where Co and Ct denote the relative activity of surimi Ca-ATPase before and after t days of frozen storage, respectively.

2.5 Gel Preparation

The gel preparation was prepared according to the method of Krisana [17] with a slight alteration. The frozen surimi was thawed at 4°C, and then mixed with 2.5% salt by using a homogenizer (Modle JJ-2, Jintan Ronghua Instrument Manufacture Co. Ltd). The sol obtained was stuffed into plastics cylinders of 3.0cm inner diameter and 10cm length. Surimi gels were prepared by heat setting at 40°C for 40 min in a water bath ( Modle HH-4, Jintan Ronghua Instrument Manufacture Co. Ltd). Then, the gels were cooked at 90°C for 30 min, and cooled at room temperature.

2.6 Gel Strength Analysis

The gel strengths of surimi gels were tested by using a TA-XT2 Texture Analyser ( Satable Micro Systems Ltd, Surrey, UK.). All the cooked gels were compressed at the speed of 1.0 mm/second using a cylindrical shaped probe of 1.0 cm in diameter. Then, the changes in applied force were recorded.
3. Results

3.1. Surimi Ca-ATPase activity

The surimi Ca-ATPase inactivation with SH, TH or tradition antifreeze was markedly retarded on the day following freezing, and then gradually decreased thereafter up to the 80 days (Fig.1). In contrast, the Ca-ATPase activity in the control dropped quickly to approximately 33.66% of the initial value on the day following freezing, and further decreased gradually until 80 days (remaining activity, 3.32%), showing a biphasic denaturation pattern. Although the extent of denaturation of surimi with SH and TH was markedly retarded. The group, added TH, could remain in 98.51% after 5 days. The other group which added SH remained in 77.24%. These two groups showed a higher Ca-ATPase activity than the control group and the traditional antifreeze group. Thus the hydrolysates suppressed freeze-induced denaturation of surimi, and TH had a higher suppressive effect than SH at all concentration levels.

![Fig.1. effect of SH, TH and traditional antifreeze on the changes in Ca-ATPase activity of surimi protein during freezing at -20°C. Surimi without additions was the control. Traditional antifreeze: (△), Control: (◇), SH: (×), TH: (○).](image)

3.2. The contents of salt soluble protein in surimi

The contents of salt soluble protein in surimi increased markedly after added hydrolysates (Fig.2.). In contrast, the amount of salt soluble protein in the control decreased to 26.71% of the initial value within 20 days of freezing, which was more or less unchanged up to 80 days. These findings showed that protein hydrolysates can inhibit the decrease of the salt soluble protein. And the TH is higher than that surimi with SH.

![Fig.2. The contents of salt soluble protein in surimi](image)
Fig. 2. Changes in the contents of salt soluble protein in surimi during freezing at -20°C. Surimi without additions was the control. Traditional antifreeze: (△), Control: (○), SH: (×), TH: (□).

3.3 The gel strength of grass carp surimi

The gel strength of grass carp surimi declined visibly after added protein hydrolysates (Tab.1.). Especially, the SH group declined to 4215.24, and the TH group reduced to 4336.77. These findings showed that both TH and SH would decrease the gel strength of grass carp surimi.

Tab. 1 Change of surimi gel strength during freezing at -20°C (n=6)

<table>
<thead>
<tr>
<th></th>
<th>Gel strength of fresh surimi (g • cm)</th>
<th>Gel strength of storage 20 days (g • cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>106303.68</td>
<td>9531.74</td>
</tr>
<tr>
<td>Traditional group</td>
<td>106303.68</td>
<td>39934.08</td>
</tr>
<tr>
<td>SH group</td>
<td>106303.68</td>
<td>4215.24</td>
</tr>
<tr>
<td>TH group</td>
<td>106303.68</td>
<td>4336.77</td>
</tr>
</tbody>
</table>

4. Discussion

In this study, we attempted to elucidate the effect of SH and TH, as a natural suppressor, of the freeze-induced denaturation and on the contents of salt soluble protein of grass carp surimi. As shown in Fig1, the Ca-ATPase activity of the control declined signally on the day following freezing and then slowly declined afterwards up to 80 days, which was similar to the freeze-denaturation pattern of carp [16]. Though, the group with SH and TH also showed the freeze-denaturation, it proceeded more slowly, suggested the suppressive effect of SH and TH against freeze-induced denaturation of surimi. This result was similar to the reports of Yasumitsu et al. [18] and Hossain et al. [6] who postulated that protein hydrolysates and peptides might have the effect of prevent freeze-induced denaturation of fish muscle protein.

The amount of salt soluble protein increased in the surimi, after added SH and TH. The peptides and protein hydrolysates could steady the water around protein, and restrain the protein denaturation [19].

Gel-forming is a complex process. It contains peptide chains dissociation and agglutination [20]. The peptides and protein hydrolysates might prevent the dissociation or the agglutination, and all that will decrease the gel strength.

5. Conclusions

The results indicated that SH and TH from eel head could be used as food additives to improve the functional properties of foods. Our results showing that the addition of hydrolysate to grass carp surimi increased the amount of salt soluble protein, but they declined the gel strength.

Acknowledgments

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