EFFECT OF TREATMENT WITH THE ENZYME ACTINIDIN ON THE FUNCTIONAL PROPERTIES OF COMMON CARP PROTEINS

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Many commercially enzymes are available for hydrolysis of fish protein to improve the yield and properties of fish protein hydrolysate. The purpose of this study was to use actinidin, the sulfhydryl protease from kiwi fruit (Chinese gooseberry) to solubilize common carp proteins and to investigate the effect of this treatment on the functional properties of the solubilized proteins. Actinidin was purified by salt precipitation followed by DEAE-Sephadex column chromatography. The common carp homogenized muscles were subjected to different concentrations of the enzyme and the mixtures were incubate at 37 °C and pH 7.4. After 1 hr the mixture was centrifuged at 1250g and the nitrogen solubility index (NSI), gel forming property, water holding capacity and emulsion stability and foaming capacity of the hydrolysate significantly improved. SDS-PAGE showed extensive digestion of the muscle proteins by actinidin. These results suggest the actinidin can replaced the commercially available proteases to be applied in fish industry to prepare protein hydrolyate.

Key words: Common carp, actinidin, protein solubility, functional properties, SDS-PAGE

I. INTRODUCTION

Fish muscles compromise 45-50% of the weight of fish and is the main eatable part of the fish, ~20 of which is proteins. The muscle proteins are responsible for many of the functional properties important in fish industry [1]. These properties include gel forming and water holding capacity, emulsion forming capacity and foam formation [2]. Many of these properties can be improved by chemical and enzymatic modifications of proteins. Enzymes are extensively used in meat industry to improve the recovery of proteins from animal sources and to tenderize meats [1,3]. Enzymatic hydrolysis breaks down proteins in to smaller fragments with distinct functional and sensory properties which are different than the original un-hydrolyzed proteins. Many commercial enzymes are available and are extensively used in meat and fish industry, including, alkalase, bacterial proteases, papain from papaya and other plant proteases [4,5]. In this study we investigated the effects of purified actinidin, the proteasae from kiwi fruit on the solubility and other functional properties of common carp proteins.

II. MATRIALS AND METHODS

Actinidin was purified from Hiward variety of Kiwi fruit as described by McDowal, (1970) with some modification [5]. The method included preparation of the kiwi fruit homogenate, precipitation of proteins by adding ammonium sulfate to 65% saturation followed by centrifugation at 15000x g at 4 °C for 10 min.and separation of proteins on a 30X5 cm DEAE-Sephadex column (Pharmacia, Upsala, Sweden). A linear NaCl gradient of 0 to 1.0 mol/L in citrate buffer, pH 5.5 was used to elute absorbed proteins. Fractions corresponding to different peaks were collected and dialyzed against distilled water. Solutions of proteins of each fraction were concentrated on

ethylene glycole and were used for subsequent studies. Proteolytic activity of actinidin was determined using hemoglobin as substrate according the method described by Matilus, et al., (1999)[6]. Effect of actinidin on the nitrogen solubility index (NSI) was studied. The NSI was determined as described by Inklaar and Fortin (1969)[7] with some modifications. Different volume of a 10 mg/ml solution of purified actinidin containing 0.0126 units/ml was added to the fish samples in the presence of 0.05 M. phosphate buffer, pH 7.4 After holding at 37 ºC for 1 h, the samples were homogenized and centrifuged at 1250xg for 15 min. The nitrogen content of the supernatant was determined by the standard micro-Kjeldahl procedure. The NSI was calculated by dividing the nitrogen content of supernatant by the total nitrogen content multiplied by 100.

The supernatants which were used for determination of NSI were also used for SDS-PAGE studies. Slabs for SDS-PAGE were formed according to the discontinuous buffer system of Laemmli (1970)[8]. The method of Hung and Zayas (1992)(9) was used for determination of WHC. A Whatman No.2 filter paper was soaked in saturated KCl and dried under vacuum. The meat (0.3 g) was placed on the paper and two plastic plates measured by 6×6×0.8 inches were placed above and under the paper. A one-kg weight was placed on the top plate. After 20 min, the area of the pressed meat and the total area of the moistened paper was measured using an area measurement system (Delta-T Devices Ltd, London, England). WHC was then calculated as follows:

$$WHC = \left(1 - \frac{B - A}{A}\right) \times 100$$

where B is the area of the moistened filter paper and A is the area of the pressed meat. The method of Hung and Zayas (1992)[9] with some modifications was used for measurement of ES. Samples of uncooked sausage emulsions (10 g) were mixed with 10 mL distilled water at 25 ºC for two min in a laboratory homogenizer and at 1500×g for five min. The emulsion was immediately centrifuged at 1500×g for five min and the amount of separated water was measured and used for the calculation of ES as described below. The volume of separated water was subtracted from the volume of water in the formulation divided by volume of water in the formulation multiplied by 100.

The effect of pH and temperature on the solubility of actinidin-treated fish proteins was studied by adding 1 ml of supernatant to buffers with pH's of 4-8 held at 20-60 ^{o}C and NSI was determined as described above.

III. RESULTS AND DISCUSSION

The effect of different concentrations of actindin on NSI of common carp proteins is shown in Table 1. At all concentrations the solubility of actinidin-treated samples were significantly higher than the un-treated sample. As the concentration of the enzyme increased the solubility increased. However, at higher activities of the enzyme, a sharp decrease in solubility was observed. These results indicate that when the level of the protease activities is increased, the highly fragments peptides probably aggregate and produce insoluble fractions that tend to precipitate. This phenomena was visually obvious when these samples were prepared. SDS-PAGE results further confirmed break down of many of the major fish muscle proteins into smaller polypeptide chains. Table 2 shows the solubility of fish proteins treated with actinidin at different pH's. At all pH's actinidin significantly improved the solubility of proteins. The minimum solubility improvement was at pH 5.0, close to the pI of muscle proteins. Table 3 shows the effect of hydrolysis on the thermal stability of fish proteins. The actinidin-treated fish proteins were more soluble at all temperatures than untreated samples. Actinidin improved emulsion capacity (EC) of fish proteins. The untreated fish proteins showed 0.618 ± 0.01 ml oil emulsified /mg protein while this value was 1.38± 0.038 ml oil emulsified /mg protein. However our data indicated lower emulsion stability (ES) of the hydrolysis products (Table 4) probably because the smaller peptide fragments have lower capacity to orient themselves at the

oil/water interface for longer times. The water holding capacity of the untreated fish proteins was 4.83 ± 0.76 ml water/gram fish protein while this value improved to 5.66 ml water per gram fish protein. Many reports on the application of actinidin on meat tenderization have been published [3,10,11]. Actinidin improves protein solubility and other functional properties of meat and meat praoducts made from beef or meat of other species. Our results further indicate that this enzyme application can be extended to marine meat sources as well.

IV. CONCLUSION

Taken together, the results of NSI analysis and SDS-PAGE suggested that actinidin increases the solubility of fish protein by digesting the proteins into smaller units which give rise to improved functional properties such as WHC and emulsifying properties that are required when fish protein hydrolysates are to be used in different products.

V. ACKNOWLEDGEMENT

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VI. REFERENCES

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Table 1: Effect of different concentrations of actinidin on the solubility of common carp proteins.

Units actinidin/g fish meat	% change in NSI
0	0
0.114	+53.0± 2.5[a]
0.132	+64.0 ± 5.5[a]
0.150	+73.9 ± 3.8[a]
0.176	+1.4 ± 8.0
0.352	+23.4 ± 7.8[a]
0.528	+09.8 ± 3.2[a]

In each column different superscripts letters indicate significant difference at $p<0.05$).

Table 2. Effect of pH on the solubility of common carp proteins treated with 0.150 units actinidin/g fish meat.

pH	%change in NSI as compared with control
3	+62.6 ± 0.5[a]
4	+79.0 ±1.3[b]
5	+17.1 ± 12.[c]
6	+52.5 ± 5.1[d]
7	+66.7 ± 6.6[a,b]
8	+75.4 ± 7.3[b]

The superscripts indicate significant difference at $p<0.05$) with respect to un-treated samples.

Table 3. Effect of temperature on the solubility of common carp proteins treated with 0.150 units actinidin/g fish meat.

Temperature (^{o}C)	%change in NSI as compared with control
20	+83.8 ± 7.7[a]
30	+82.8 ±5.0[b]
40	+85.3 ±5.3[c]
50	+61.7 ± 6.0 [d]
60	+74.7 ± 5.3[a]

In each column different superscripts letters indicate significant difference at $p<0.05$).

Table 4. Effect of actinidin on the emulsion stability (ES) of common carp proteins

Emulsion Stability ((ES)Min)	Untreated fish proteins (ml oil emulsified /mg protein)	Actinidin-treated fish proteins (ml oil emulsified /mg protein)
15	94.6±1.3[a]	89.3± 3.5[b]
30	86.6±1.3[a]	72.9 ±5.5[b]
45	79.5 ± 0.7[a]	60.9±1.5[b]
60	77.3±3.5[a]	53.3±1.3[b]

In each row different superscripts letters indicate significant difference at $p<0.05$).