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CONTENTS

THE FUNCTIONAL PROPERTIES OF ALCALASE PRODUCED THREADFIN BREAM (*NEMIPTERUS JAPONICUS*) PROTEIN HYDROLYSATE

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ABSTRACT

This study was carried out to determine the functional properties of threadfin bream (Nemipterus japonicus) hydrolysate. The hydrolysate exhibited more than 95% solubility over a wide pH range from 2.5 to 11 with low emulsifying property. Foam ability was improved in the hydrolysate as compared to the unhydrolysed threadfin bream muscle. However, the foam was unstable over time.

Keywords: hydrolysate, threadfin bream, hydrolysis

1. INTRODUCTION

Hydrolysis of food protein either by chemical or biological methods results in the modification of the protein in terms of their functional properties. In fish protein hydrolysate, functional properties in fish protein hydrolysate are very important particularly if it is produced for use as food ingredients. By controlling the hydrolysis conditions and proper selection of enzyme, products with the desired functional properties can be obtained. Hydrolysate which is water soluble and has good ability to form stable foam may have various applications in products such as souffles, meringues, sponge cake and ice cream[1]. Functional properties of fish protein hydrolysate such as solubility, emulsifying properties, foam ability and foam stability are largely dependent on the molecular size of the peptides and degree of hydrolysis[2,3]. These factors are influenced by the specificity of enzyme and hydrolysis parameters including the extent of hydrolysis,

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concentration of enzyme and the type of substrate[2,3,4]. Improper control of hydrolysis conditions may produce product which is highly soluble, good in nutritional qualities but lack in other functional properties[5,6]. To date, the functional properties of Alcalase produced threadfin bream hydrolysate has not been reported. This paper reports the study of these properties.

2. MATERIALS AND METHODS

2.1 Materials

Enzyme – Alcalase, a food grade enzyme having a declared activity of 2.4 Aug^{-1} and a density of 1.18 g/ml was obtained from Novo Nordisk Industries (AS, Bagsvaerd, Denmark).
Substrate – Threadfin bream (Nemipterus japonicus) was purchased from Sri Serdang wet market.

2.2 Preparation of hydrolysate

Hydrolysate was prepared according to the pH-stat technique[7]. Hydrolysis was carried out at pH 8.5, 60 °C and enzyme to substrate ratio of 2% for the duration of 2 h. Sodium hydroxide was continuously added to maintain a consistent pH throughout the hydrolysis period.

2.3 Protein solubility (%)

Solubility of the hydrolysate was determined as reported earlier[8] but with slight modification. The protein content of the filtrate was determined by micro-Kjeldahl method[9] and solubility was expressed as percent solubility at a given pH.

2.4 Moisture and fat adsorption

2.4.1 Moisture adsorption[10]

About one gram of hydrolysate was spread in an aluminium tray at 24 °C, relative humidity (RH) 70% for 24 h. Moisture adsorption was calculated as the percent maximum weight gained.

$$\text{Protein solubility (\%)} = \frac{\text{protein content in supernatant}}{\text{protein content in dispersion before centrifuge}} \times 100$$

2.4.2 Fat adsorption[10]

Two grams of hydrolysate was placed in 50 ml centrifuge tube. Then 12 ml of corn oil was added. The content was thoroughly mixed using a glass rod and left for 30 min at room temperature while mixing for 30 sec every 5 min. The content was then centrifuged for 30 min at 3500 rpm using a bench-top centrifuge. Free oil was decanted and fat adsorption was calculated as:

$$\frac{\text{(weight of hydrolysate + oil mixture after centrifugation) – weight of hydrolysate (2 g)} \times 100}{\text{weight of hydrolysate (2 g)}}$$

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2.5 Emulsifying properties

2.5.1 Emulsifying capacity[11]

Three and a half grams hydrolysate or muscle was mixed with 50 ml distilled water and 50 ml corn oil. The mixture was homogenized for 30 sec in a homogenizer at the rate of 10 000 rpm and then centrifuged for 5 min at 4000 rpm. Emulsifying capacity was calculated as the percentage of emulsion to total volume of emulsion and non emulsion.

2.5.2 Emulsifying stability[11]

The samples were prepared similarly as in part 2.5.1 but the mixed samples were initially heated for 30 min at 80 °C prior to homogenization and centrifugation. Emulsion stability was calculated as the ratio of emulsion versus total volume of emulsion and non emulsion. Emulsion stability was expressed as percent emulsifying capacity after heating.

2.6 Foam ability and foam stability[11]

Three grams hydrolysate or muscle was dispersed in 100 ml distilled water. The mixture was homogenized for 1 min using a homogenizer at 10 000 rpm. The mixtuere was poured into 250 ml graduated cylinder and the total volume was recorded. Foam ability was calculated as the volume of foam increased upon whipping whereas foaming stability was expressed as percentage of foam remained after 0.5, 5, 40, 60, 80 and 120 min quiescent period.

3. RESULTS AND DISCUSSION

3.1 Protein solubility

Solubility over a wide pH range is one of the most important functional properties of protein hydrolysate[4]. The hydrolysate produced in this study was more than 95% soluble at a wide pH ranging from 2.5 to 11 (Table 1). A similar finding was reported previously in sardine hydrolysate in which solubility increased with the increased in pH, enzyme-substrate ratio and degree of hydrolysis[12]. However, a minimum solubility between pH 2 to 4 was observed. The increased in hydrolysate solubility is attributed to the formation of smaller peptides and the exposure of the ionizable amino and carboxyl group and thus increasing its hydrophilicity[13,14].

3.2 Moisture and fat adsorption

Table 2 showed that the hydrolysate produced from threadfin bream adsorbed to 20.56% moisture which is considered as highly hygroscopic while having an excellent fat adsorption capacity. This percent moisture adsorption was slightly above that reported in hydrolysate from unwashed meat of shark which was 10.4%[11]. The hydrolysate obtained in this study became sticky over time after exposure to the atmosphere.

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Table 1. Protein solubility of threadfin bream (*Nemipterus japonicus*) hydrolysate at pH 2.5 to 11.

pH	% protein solubility	pH	% protein solubility
2.5	96.19[a]	7	97.02[a]
3	97.14[a]	8	97.19[a]
4	97.19[a]	9	97.17[a]
5	97.11[a]	10	97.17[a]
6	95.35[a]	11	95.13[a]

Notes: Values represent means of three replicates.
Means within a column with different letters are significantly different ($p < 0.05$).

Table 2. Functional properties of threadfin bream muscle and hydrolysate.

Functionality	Muscle	Hydrolysate
Moisture adsorption (70% RH)	ND	20.56
Fat adsorption	ND	152.13
Emulsifying capacity	34.12[a]	3.80[b]
Emulsifying stability	9.45[a]	2.85[b]
Foam ability	23.73[b]	73.33[a]
Foam stability at		
0.5min	35.00[b1]	121.33[a1]
5.0 min	32.53[b1]	69.33[a2]
40 min	25.33[a2]	29.00[a3]
60 min	24.33[a23]	8.33[b4]
80 min	21.33[a3]	7.00[b4]
120 min	17.33[a4]	4.67[b4]

Notes: Values represent means of three replicates.
Means within a row with different letters are significantly different ($p < 0.05$).
Means within a column with different letters and numbers are significantly different ($p < 0.05$).
*ND: Not detected

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3.3 Emulsifying capacity and emulsifying stability

A comparison of emulsifying properties of intact muscle of threadfin bream and its hydrolysate is shown in Table 2. The produced hydrolysate had an emulsifying properties of less than 10%. There were significant differences ($p<0.05$) between emulsifying capacity and stability of muscle and hydrolysate. Emulsifying capacity and stability of hydrolysate were nine and three times lower than that of muscle, respectively. This indicates that emulsifying properties decreased with hydrolysis. High protein constituent in hydrolysate is important to produce a stable emulsion[3]. It was suggested that increasing the degree of hydrolysis cause the decrease in surface hydrophobicity and an increase in the number of low molecular weight peptides which adversely affect the hydrolysate capacity to form a stable emulsion.

3.4 Foam ability and foam stability

Foam ability of the hydrolysate was three times higher than that of the muscle (Table 2). However, foam stability data indicated that the foam produced by the hydrolysate was significantly unstable ($p<0.05$) especially during the first 60 min. The volume was reduced to about four fold within 40 min. In contrary, the foam formed by the muscle was more stable and showed very slight reduction within 2 hr. An improvement in foam ability is attributed to the production of various ranges of amphiphilic peptides having a different hydrophobicity, charge balance and conformation from the intact protein[2].

4. CONCLUSION

Alcalase produced threadfin bream hydrolysate is highly soluble over a wide pH range but exhibited low emulsifying property. Foam ability was improved in the hydrolysate as compared to the unhydrolysed threadfin bream muscle but it was unstable over time.

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