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Influence of Substrate and Enzyme Concentration Towards Degree of Hydrolysis for Gelatine

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Abstract: The aim of this study was to investigate the combined effects of substrate concentration and enzyme concentration towards degree of hydrolysis for production of gelatine hydrolysate through enzymatic hydrolysis. Hydrolysis was performed using two types of commercial gelatine sources as substrates; bovine gelatine and fish gelatine with gel strengths of 261 and 257 g, respectively. Alcalase 2.4 L, a microbial protease was used in this study as it was reported to give highest percentage of DH in previous study. During hydrolysis, DH was monitored continuously according to pH-stat method. Results showed that the hydrolysate obtained from a 5% substrate concentration at 2% enzyme concentration gave the highest DH for both type of gelatine; 9.70% when using bovine gelatine and 8.13% when using fish gelatine. This study confirmed that there were significant effects between substrate and enzyme concentration towards the degree of breakdown of peptide chain in gelatine molecule.

Key words: Gelatine, gelatine hydrolysate, enzymatic hydrolysis, alcalase

INTRODUCTION

Fish gelatine used in this study is a type a gelatine while bovine gelatine is a type B gelatine. In order to improve the functional and nutritional properties of food protein, enzymatic hydrolysis was employed (Zhu *et al*., 2006). For the suitability of the enzymes, Alcalase is most preferred as it is widely used for hydrolysis of protein. Research done by Ng and Mohd Khan (2012) reported that Alcalase was the most efficient enzyme among the proteolytic enzymes studied in order to hydrolyze the protein and was able to produce the highest DH of hydrolysate.

A number of studies had shown the differences between these two types of gelatine in several aspects. Research done by Haug *et al*. (2004) studied the comparison between physical and rheological properties for fish and cattle hide gelatine. Ninan *et al*. (2012) made a comparison between three type of fish skin gelatine and bovine gelatine in term of physical, chemical and functional properties. However, comparison on hydrolysate of both type of gelatine is very limited. Thus this study was carried out in order to investigate the combined effect of substrate and enzyme concentration towards the degree of hydrolysis for production of gelatine hydrolysate from tilapia fish and bovine gelatine.

MATERIALS AND METHODS

Chemicals and raw materials: Commercial food grade tilapia and cattle bone gelatine purchased from Halagel Sdn. Bhd. with gel strength of 257 and 261 g, respectively were used as substrates. Alcalase 2.4 L (declared activity of 2.4 au kg^{-1}, density of 1.18 g mL^{-1}), an endoproteinase from *Bacillus licheniformis*, was purchased from Novozymes. All reagents used were of analytical grade.

Proximate analysis: The proximate analysis was carried out on the raw material (gelatine). Moisture and ash contents of the two type of gelatine were determined according to GME Monograph; Standardised Methods for the Testing of Edible Gelatin Version 1. The moisture content was determined according to oven method while ash content was determined by charring the predried sample in crucible at 600°C until a white ash was formed. Protein content of the gelatine was calculated by the differences in total percentage value of the components present in pure gelatine.

Enzymatic hydrolysis: Gelatine solution was prepared by dissolving some amount of substrate which was pure gelatine into required amount of distilled water at temperature of 60°C. Once it had dissolved completely,

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the pH of the gelatine solution was adjusted to pH 8 using NaOH 2N or HCL 2N. Then, Alcalase 2.4 L was added into the homogenized gelatine solution at desired concentrations. The hydrolysis process was conducted for 50 min before been heated at temperature 90°C for 10 min for deactivation of the enzyme. After heat inactivation, the solution was left to cool in room temperature before labeled and kept in refrigerator with temperature about 4°C. The reaction mixture was stirred by a four-blade impeller at speed of 300 rpm. Three sets of hydrolysis experiments were performed in this study where the concentration of the substrate was varied (5, 10 and 25% w/w). In each set, experiments were done with various enzyme concentrations (0.25, 0.5 and 2.0% v/w).

Degree of hydrolysis: Degree of Hydrolysis (DH) was calculated using pH-stat method according to Adler-Nissen (1977). DH was monitored by maintaining the pH value which was at pH 8 during the enzymatic hydrolysis process by addition of NaOH 2N. The base consumption during the hydrolysis process gives DH directly by following this equation by Adler-Nissen (1986):

$$DH = \frac{B \times N_B}{M_P} \times \frac{1}{a} \times \frac{1}{h_{tot}} \times 100\%$$

where, DH is the degree of hydrolysis, B is base consumption (mL), N_B is normality of the base (N), α is average degree of dissociation of the α-NH groups, M_P is mass of protein (g) and h_{tot} is total number of peptide bonds in the protein substrate (11.1 meqv g^{-1} protein for gelatine). According to Kristinsson and Rasco (2000), the degree of dissociation, α was found in the following way:

$$a = \frac{10^{pH - pK}}{1 + 10^{pH - pK}}$$

where, the pK value at different temperatures was calculated based on the following equation by Steinhardt and Beychok (1964):

$$pK = 7.8 + \frac{298 - T}{298T} \times 2400$$

RESULTS AND DISCUSSION

Table 1 below shows the composition for the proximate analysis carried out on the two type of gelatine. The ash and moisture content were calculated according to wet weight basis calculation. From the results, both types of gelatine were mainly composed of protein but slightly higher in fish gelatine. The low percentage of ash content for both gelatine shows that there were only small amount of inorganic minerals present within the gelatine. Total number of experiments designed and the parameters involved for both type of gelatine together with the DH achieved are summarized in Table 2.

Effect of substrate concentration on DH: Effect of substrate concentration towards DH was studied from the experiments done with constant hydrolysis time (50 min) and hydrolysis temperature (60°C). Figure 1a-c and 2a-c shows the trends of DH achieved using various enzyme concentrations (0.25, 0.5 and 2.0%) over various substrate concentrations (5, 10 and 25%) for both type of gelatine. From the Fig. 1a-c and 2a-c, it was noted clearly that the rate of DH were increasing for all time as it is directly calculated from the cumulative base consumption which will always increase in time. However, when comparing the rate of hydrolysis for different substrate concentration (5, 10 and 25%) with constant enzyme concentration, the trends of the DH show a decreasing manner.

Figure 1a-c and 2a-c show that at 5% substrate concentration with any enzyme concentrations used, the rate of DH increased very fast followed by the 10% substrate concentration and leaving the 25% substrate concentration behind. From the results, it is clear that the higher the substrate concentration used, the lower the DH acquired. According to Weber and Nielsen (1991), higher percentage of substrate concentration gives lower value of DH most probably because of the higher substrate

Table 1: Proximate composition of gelatine

Analysis	Fish gelatine	Bovine gelatine
Moisture (%)	12.3000	12.52
Ash (%)	0.0766	0.67
Protein (%)	87.6200	86.81

Table 2: List of experiments and DH achieved using fish and bovine gelatine

Substrate concentration, (%) (Fish gelatine)	Enzyme concentration (%)	Notation	DH(%)
(a)			
5	0.25	FG-01	5.16
5	0.50	FG-02	5.59
5	2.00	FG-03	8.13
10	0.25	FG-04	4.05
10	0.50	FG-05	5.50
10	2.00	FG-06	7.92
25	0.25	FG-07	4.01
25	0.50	FG-08	4.50
25	2.00	FG-09	7.24
(b)			
5	0.25	BG-01	5.75
5	0.50	BG-02	6.53
5	2.00	BG-03	9.70
10	0.25	BG-04	5.99
10	0.50	BG-05	6.21
10	2.00	BG-06	8.84
25	0.25	BG-07	4.50
25	0.50	BG-08	5.49
25	2.00	BG-09	8.94

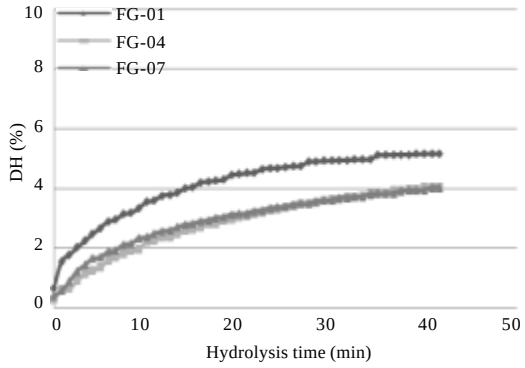

Fig. 1: (a) Hydrolysis using fish gelatine for 0.25% enzyme concentration with various substrate concentrations; FG-01 used 5%, FG-04 used 10% and FG-07 used 25%

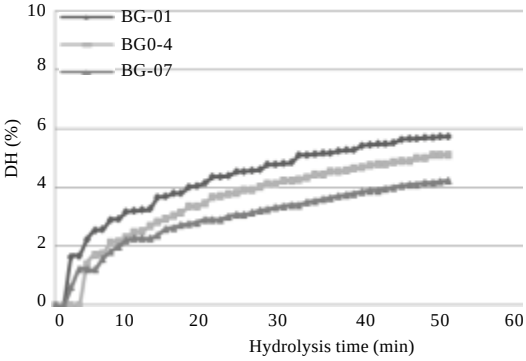

Fig. 2: (a) Hydrolysis using bovine gelatine for 0.25% enzyme concentration with various substrate concentrations; BG-01 used 5%, BG-04 used 10% and BG-07 used 25%

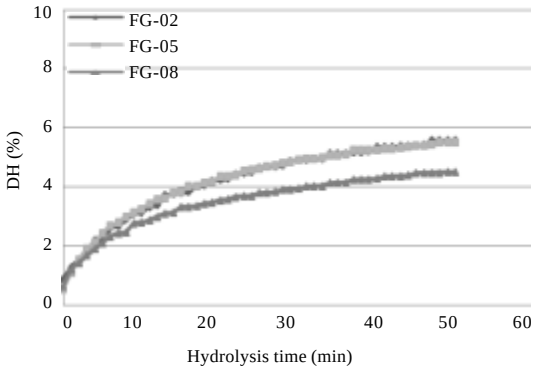

Fig. 1: (b) Hydrolysis using fish gelatine for 0.5% enzyme concentration with various substrate concentrations; FG-02 used 5%, FG-05 used 10% and FG-08 used 25%

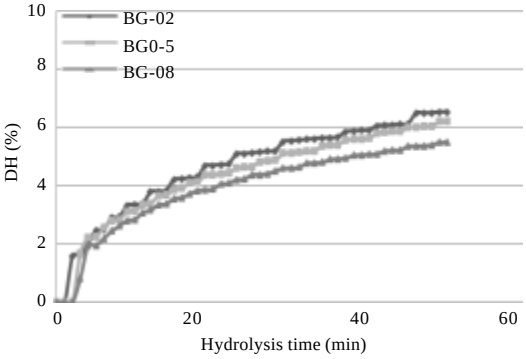

Fig. 2: (b) Hydrolysis using bovine gelatine for 0.5% enzyme concentration with various substrate concentrations; BG-02 used 5%, BG-05 used 10% and BG-08 used 25%

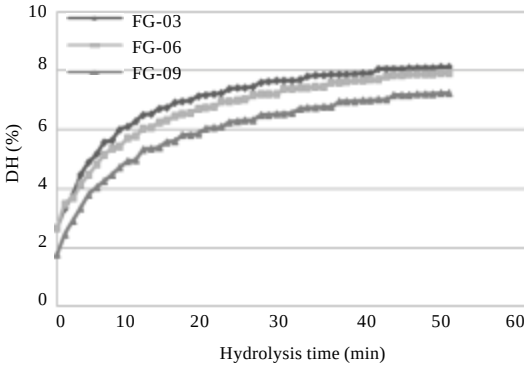

Fig. 1: (c) Hydrolysis using fish gelatine for 2.0% enzyme concentration with various substrate concentrations; FG-03 used 5%, FG-06 used 10% and FG-09 used 25%

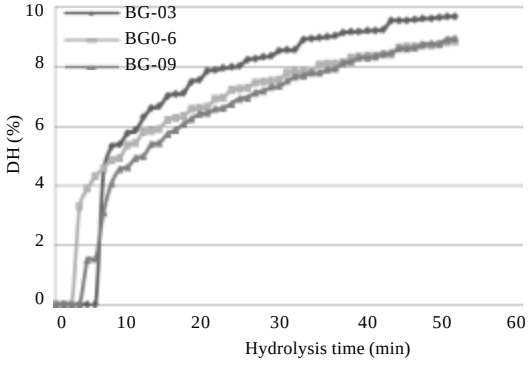

Fig. 2: (c) Hydrolysis using bovine gelatine for 2.0% enzyme concentration with various substrate concentrations; BG-03 used 5%, BG-06 used 10% and BG-09 used 25%

concentration itself or cause by the presence of irreversible endoprotease inhibitor in the substrate. Based on Guadix *et al*. (2000), active enzyme fraction in the solution will be bonded irreversibly with the inhibitor

which presence in the substrate. Hence, this will leads to lower DH as the enzyme are not fully utilize to breakdown the protein structure. Same result was reported by Ordonez *et al*. (2008) using sunflower wholemeal protein concentrate where 5% of substrate concentration gave the highest value of DH (37.8%).

Effect of enzyme concentration on DH: The effect of enzyme concentration towards the DH was studied from the results obtained in the same experiment done. As can be seen from the Fig. 1a-c and 2a-c, at various enzyme concentration (0.25, 0.5 and 2.0%) and constant substrate concentration, the DH values show an increasing trends when the enzyme concentration was increased for both type of gelatine used. However, when enzyme concentration of 2.0% was applied, the rate of reaction increase rapidly compared to other concentration of enzyme used. At 2.0% enzyme concentration together with 5% substrate concentration, DH of 9.70% for bovine gelatine and 8.13% for fish gelatine which were the highest DH for both type of gelatine were obtained.

This result indicates that the presence of higher amount of enzyme will eventually cleaved more peptide bonds in the substrate. Same results were reported in research done by Ng and Mohd Khan (2012) using palm kernel expeller where higher concentration of Alcalase 2.4 L gave a higher value of DH. This result was also supported by research done by Ordonez *et al*. (2008). However, the concentration of the enzyme must be based on the substrate concentration used and the degree of protein breakdown desired as excessive use of enzyme will cost a lot in industry.

CONCLUSION

From this study, it can be concluded that substrate and enzyme concentration show a significant effect towards the enzymatic hydrolysis process of protein. The highest DH achieved when 5% substrate concentration and 2% enzyme concentration were used to produce DH of 9.70 and 8.13% using bovine and fish gelatine, respectively.

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