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Emulsifying Properties of Sesame Seed Protein Hydrolyzates

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Sesame seed protein isolates have been hydrolyzed using enzyme trysin and mixed proteases. The degree of hydrolysis (6, 8 and 10 %) was determined by the pH-stat method. The emulsifying activity, emulsion stability, effect of time and temperature on emulsio stability were determined. The effect of pH and degree of hydrolysis on the emulsification properties were also studied.

Key Words: Sesame seed protein, Emulsification properties.

INTRODUCTION

The use of proteins as food additives has been gaining importance because of several advantages in that they are natural constituents of foods and addition of proteins would enhance the nutritional value of the food. It is necessary that proteins for potential application as food additives should possess desirable functional properties like emulsification, foaming, viscosity, water retention and fat absorption, *etc.*, The property of emulsification is used in sausages, bologna, soups and cakes.

The formation and stabilization of emulsions are favoured by the addition of emulsifying agents, which are surface-active agents. An emulsifying agent works by reducing the surface tension. Some examples of emulsifiers are salts of oleic acid, phospholipids, hydrocolloids, glycerol esters, *etc.*[1].

Many food emulsions are stabilized proteins, which form a protective layer around the fat globules. In aerated emulsions such as ice cream, frozen desserts, whipped cream and toppings, the air cells are stabilized by adsorbed fat globules, fat crystals and proteins.

The demand for proteins in human diet is not met fully necessitating the identification/development of new protein sources. Oilseeds are rich sources of proteins. Seeds of sesame, cotton, castor and peanuts have been primarily harvested for their oil content.

Sesame seed is one of the most valuable oil seeds, called the queen of the oil seed crop because of its high yield of good quality of oil and meal. It contains about 50 % oil and about 25 % protein by weight. The protein contents of the meal varies from 30-60 % by weight and contains relatively higher percentage of methionine.

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Literature sources indicate the determination of emulsification capacity, emulsion stability and emulsion activity with regard to the emulsification properties. Chobert *et al.*[2] have studied the emulsifying capacity, activity and emulsion stability of casein and whey proteins modified enzymatically with trypsin by turbidimetric method. The minimal length of peptides required to produce a stable oil in water emulsion system has been determined by Singh and Dalgleish[3].

The present paper deals with the recovery of proteins and protein hydrolyzates from sesame seed for human consumption by ecofriendly enzymatic method and evaluate their physical and functional properties for potential application as food additives.

EXPERIMENTAL

Preparation of protein isolates: In the present work defatted sesame seeds were taken as the substrates. The proteins were extracted by alkaline extraction followed by isoelectric precipitation. The proteins were hydrolyzed using enzyme trypsin and mixed proteases. The mixed proteases were produced by solid-state fermentation using *Bacillus amyloliquefaciens*, a GRAS certified organism. Protein content was estimated by Kjeldahl method of nitrogen estimation and by the method of Lowry[4].

Preparation of protein hydrolyzates: The sesame seed protein were hydrolyzed enzymatically using trypsin and mixed proteases to produce protein hydrolyzates. In order to produce protein hydrolyzates of varied functional properties, it was necessary to monitor the degree of hydrolysis of proteins. The degree of hydrolysis is defined as the percentage of the cleaved peptide bonds during hydrolysis compared to the total quantity of available peptide bonds. A pH-stat technique was used for monitoring the degree of hydrolysis. In this method the pH of the reaction mixture in unbuffered condition (free water hydrolysis) was monitored and maintained constant at the desired value by titrating with a standard solution of sodium hydroxide. The reaction was terminated at the desired degree of hydrolysis (6 , 8 and 10 %) by decreasing the pH so as to inactivate the enzyme using trichloroacetic acid. After 0.5 h of incubation the contents were centrifuged. The residue was discarded and the centrifugate was utilized for the determination of emulsification properties.

Emulsifying activity and emulsion stability: The emulsification properties were determined by the turbidimetric method as reported by Chobert *et al.*[2]. The hydrolyzate and the groundnut oil were stirred in the ratio of 3:1 in a shaft stirrer at 4000 rpm for 1 min. Aliquots were immediately pipetted out from the emulsion and diluted 1000 fold with sodium chloride containing 0.1 % SDS pH 7.0. The absorbance of the samples was recorded at 500 nm. The readings were taken at regular intervals for 2 min for 30 min. The initial absorbance was taken as the emulsifying activityas reported by Monteiro and Prakash[5].The emulsion stability has been determined graphically from the plot of absorbance as a function of time.

The emulsifying activity is quantitatively expressed as emulsifying activity index (EAI) according to the equation:

$$EAI = 2T/\Phi c$$

where T = turbidity = 2.3A/L, A = absorbance at 500 nm, L = light path in metres, Φ = oil phase volume = 0.25, C = concentration of protein = 0.25 %.

The effects of temperature as well as time on emulsion stability were studied. The emulsion prepared as above was held at room temperature for 24 h. The solution was again stirred for 1 min. Aliquots were pipetted out and diluted 1000 fold with 0.1 M sodium chloride containing 0.1 % SDS pH 7.0. The absorbance of the samples was recorded at 500 nm for 0.5 h at 2 min intervals.

The emulsion was heated at 80 ºC for 0.5 h. The emulsion was cooled, again stirred at 4000 rpm for 1 min. The turbidity was measured as above. The emulsion stability was calculated using the following equation:

$$\Delta EAI\% = \frac{EAI(RT) - EAI(80\ ^{o}C)}{EAI\ (RT)}$$

RESULTS AND DISCUSSION

The effect of trypsin-mediated hydrolysis on the emulsifying activity of sesame seed protein is shown in Fig. 1.

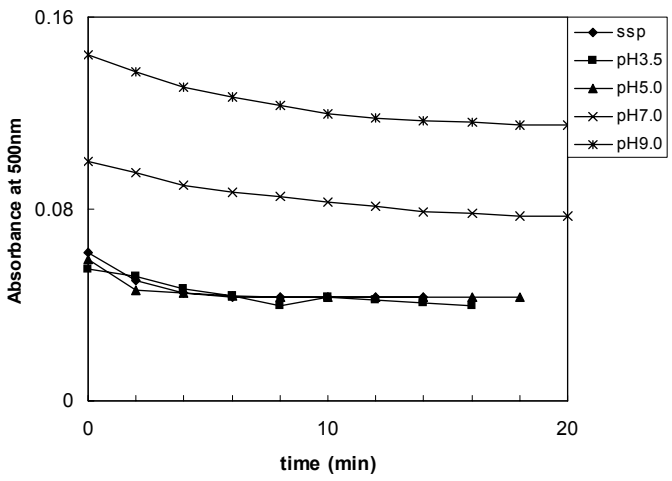

Fig. 1. Variation of emulsifying activity of trypsin mediated sesame seed protein hydrolyzate at different pH values (6 % degree of hydrolysis)

The hydrolyzate has been found to have a higher emulsifying capacity at 6 % degree of hydrolysis at higher pH values compared to protein isolates.

A study on the effect of degree of hydrolysis showed that that the emulsifying activity decreased as the degree of hydrolysis was varied from 6 to 10 %. On the

other hand, the emulsifying activity was found to increase from pH 3.5 to pH 9.0 at a given degree of hydrolysis. From the plots of absorbance *vs.* time, the emulsion stability can be calculated. At 8 % degree of hydrolysis, the emulsion stability has been found to show a maximum of 9.4 (±) 1 min and a minimum of 2 (±) 1 min at 6 % degree of hydrolysis at pH 3.5.

It is evident from Fig. 2 that enzymatic hydrolysis has been effective in improving the emulsifying activity at higher pH values. At a particular degree of hydrolysis, the emulsifying activity shows an increase with an increase in the pH value.

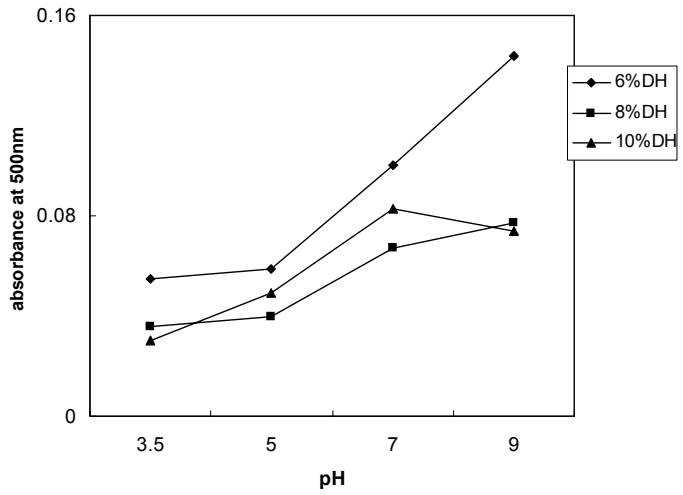

Fig. 2. Variation of emulsifying activity of trypsin mediated sesame seed protein hydrolyzate at different degree of hydrolysis (DH) and pH values

The effect of time and temperature on the emulsion stability of trypsin mediated sesame seed protein hydrolyzate is shown in the Tables 1 and 2. The emulsion stability was higher at higher pH values and decreased with increasing degree of hydrolysis values. The emulsifying activity of the sesame seed protein is not much improved by the mixed protease hydrolysis. This is evident from the Figs. 3 and 4.

The emulsifying activity has been found to be higher at lower degree of hydrolysis and higher pH values. The hydrolysate at 8 % degree of hydrolysis at pH 9.0 showed a maximum emulsion stability of 8 ± 1 min while a minimum of 3.2 ± 1 min is shown by the hydrolysate at 6 % degree of hydrolysis at pH 3.5.

The effect of time and temperature on emulsion stability has also been favoured by increase in pH, but decreased with increasing degree of hydrolysis. The emulsions at 6 and 8 % degree of hydrolysis were found to be stable to heat treatment at higher pH values (Tables 1 and 2). Enzymatic hydrolysis has improved the stability of the hydrolyzates to increasing time and temperature.

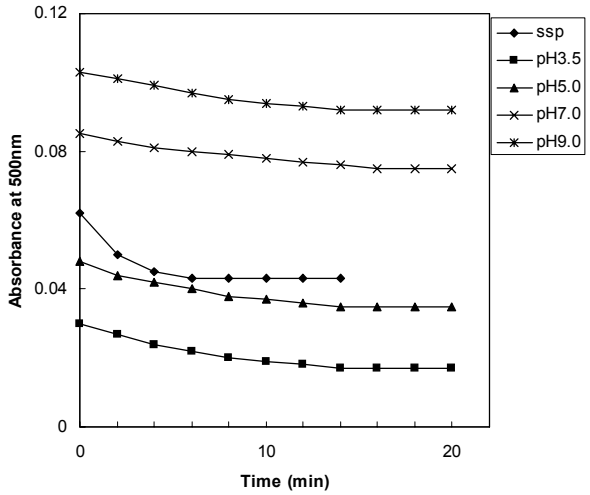

Fig. 3. Variation of emulsifying activity of mixed protease mediated sesame seed protein hydrolyzate at different pH values (6 % degree of hydrolysis)

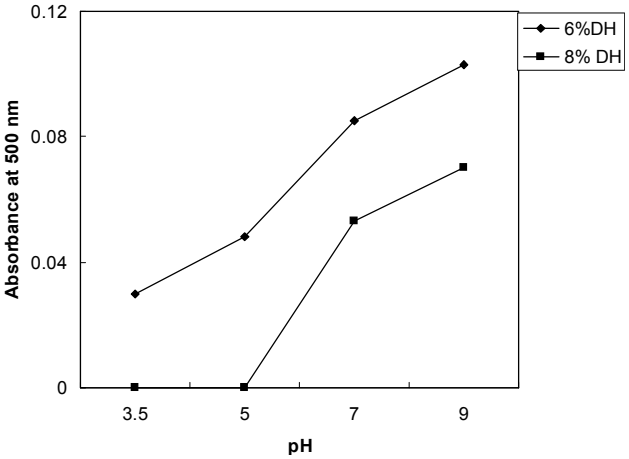

Fig. 4. Variation of emulsifying activity of mixed protease mediated sesame seed protein hydrolyzate at different degree of hydrolysis (DH) and pH values

TABLE-1
EFFECT OF TIME ON THE VARIATION OF EMULSION STABILITY OF SESAME SEED PROTEIN HYDROLYZATES AT DIFFERENT DEGREE OF HYDROLYSIS (DH) AND pH VALUES

Enzyme	DH (%)	Protein isolate pH 7.0	pH 3.5	pH 5.0	pH 7.0	pH 9.0
Trypsin	6	11.2	–	13.3	6.0	4.1
	8	–	–	–	11.9	9.0
	10	–	–	–	19.7	11.6
Mixed protease	6	–	–	–	4.7	4.8
	8	–	–	–	–	14.2

TABLE-2
EFFECT OF TEMPERATURE ON THE VARIATION OF EMULSION STABILITY OF SESAME SEED PROTEIN HYDROLYZATES AT DIFFERENT DEGREE OF HYDROLYSIS (DH) AND pH VALUES

Enzyme	DH (%)	Protein isolate pH 7.0	pH 3.5	pH 5.0	pH 7.0	pH 9.0
Trypsin	6	11.2	–	16.6	11.0	9.7
	8	–	–	–	12.0	11.6
	10	–	–	–	19.7	11.7
Mixed protease	6	–	–	–	10.5	9.7
	8	–	–	–	–	14.2

From the above discussions, it is clear that the pH stat method has been found to be effective for monitoring the enzymatic hydrolysis reaction in the preparation of protein hydrolyzates. The method can be easily scaled-up and offers a viable control to monitor and control the extent of hydrolysis reaction to achieve protein hydrolyzates with better functional properties.

The residue obtained after preparing the protein hydrolyzates by pH-stat method is amenable for use as cattle feed though with a reduced protein content. Hence the enzymatic method of recovering proteins as protein hydrolyzates from non-conventional protein sources is ecofriendly as it does not generate any waste.

Trypsin has been found to be efficient with better rates of hydrolysis compared to bacterial proteases. The mixed protein hydrolyzates produced by trypsin catalyzed hydrolysis of sesame seed protein have been found to contain better emulsification property compared to protein isolates.

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