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Some functional properties of oat bran protein concentrate modified by trypsin

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Abstract

Oat bran protein concentrate (OBPC) was prepared from oat bran, and hydrolyzed using trypsin. Protein hydrolysates of three different degrees of hydrolysis (4.1%, 6.4% and 8.3% respectively) were obtained. SDS-PAGE analysis demonstrated that oat globulin was the major protein component in OBPC. After trypsin treatment, acidic polypeptides were partly degraded into large peptides ($M_r = 29{,}000–33{,}000$) and small peptides ($M_r < 20{,}000$); however, basic polypeptides were almost intact. The functional properties of the resulting products were compared with those of control OBPC. Marked changes in the protein functionality were caused by proteolysis. The solubility, water-holding capacity, emulsifying activity and foaming ability of the hydrolysates gradually increased with the increase in DH. However, the oil-holding capacity, emulsifying stability and foaming stability of the hydrolysates reduced to a certain extent.
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Keywords: Oat bran protein concentrate; Enzymatic hydrolysis; SDS-PAGE; Degree of hydrolysis; Functional properties

1. Introduction

Oat protein preparations are high quality cereal protein preparations. The good nutritional quality of oat proteins has been well established by animal feeding studies and amino acid analysis (Hischke, Potter, & Graham, 1968; Robbins, Pomeranz, & Briggle, 1971; Wu, Sexson, Cavins, & Inglett, 1972). However, under slightly acidic and neutral pH levels, which are usually suitable for food systems, oat proteins have poor functional properties (Ma, 1983; Ma, 1985; Ma & Khanzada, 1987). This constitutes a major limitation to the extensive utilization of oat proteins for human consumption. In order to make better use of oat proteins as food ingredients, it would be necessary to further improve their functional properties.

Modification of a protein is usually realized by physical, chemical, or enzymatic treatments, which change its structure and consequently its physicochemical and functional properties (Chobert, Sitohy, & Whitaker, 1988). Chemical modification has been used to improve functional properties of oat proteins derived from oat flours (Ma, 1985; Ma & Khanzada, 1987; Ma & Wood, 1987; Ponnampalam, Goulet, Amiot, & Brisson, 1987). Compared with chemical treatments, enzymatic modification may be preferable because of milder process conditions required, easier control of the reaction and minimal formation of by-products (Mannheim & Cheryan, 1992). Enzymatic hydrolysis has been widely used to improve the functional properties of proteins, such as solubility, emulsification, gelation, water- and fat-holding capacities, and foaming ability, and to tailor the functionality of certain proteins to meet specific needs (Arzu, Mayorga, Gonzalez, & Rolz, 1972; Kim, Park, & Rhee, 1990; Kuehler & Stine, 1974; Ma, 1985; Panyam & Kilara, 1996; Ponnampalam et al., 1987; Puski, 1975).

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It has been reported that the extent of proteolytic degradation of food proteins affects greatly the functional properties of the hydrolysates (Adler-Nissen, 1977; Diniz & Martin, 1997; McNairney, 1984; Puski, 1975; Quaglia & Orban, 1987). Limited proteolysis can improve the functional properties of proteins by changing the molecular size, conformation, and strength of the inter- and intramolecular bonds of the protein molecules (Ryan, 1977). However, protein functionality can be impaired if excessive enzymatic modification takes place (McNairney, 1984). Therefore, the proteolysis reaction must be carefully monitored and controlled in order to manufacture products with desired functionality.

Oat bran is a by-product during oat grain processing and contains high protein content. It can be used as a good resource not only of water-soluble dietary fiber, but also of proteins. However, the distribution of Osborne fractions in oat bran is different from that of oat flour (Nnanna & Gupta, 1996). This probably causes oat bran protein concentrate (OBPC) to have some different functional properties from those reported of oat flour proteins. In addition, it is by far not clear how the extent of proteolysis influences the functional properties of OBPC. The objective of the present investigation was to evaluate the functional properties of OBPC as influenced by the extent of enzymatic hydrolysis.

2. Materials and methods

2.1. Materials

Oat bran was obtained from Rongkang Cereal and Oil Co. Ltd. (Shanxi, China). Trypsin PTN6.0S (E.C.3.4.21.4), a food-grade serine protease with a proteolytic activity of 1250–1450 usp-u/mg, was purchased from Novo Industry (Copenhagen, Denmark). All the other reagents were of analytical grade.

2.2. Preparation of protein hydrolysates

OBPC was prepared from defatted oat bran by the method of Ma (1983). The protein product obtained had a protein content of 73.4% ($N \times 6.25$). One liter of OBPC dispersion (7 g protein/100 ml) was adjusted to pH 8.0 with 2 M NaOH and heated to 45 °C. Trypsin dissolved in water (1.05 g/10 ml) was added to the reaction mixture in order to obtain a final E/S ratio of 1.5%. The pH–stat technique was used, and the pH was maintained at 8.0 by the addition of 0.5 M NaOH. The reaction was allowed to proceed under constant stirring, temperature and pH, and then stopped at the desired end-point by heating at 100 °C for 10 min. The reaction mixture was then spray-dried and stored at 4 °C until its use in the functional property tests. In addition, two controls, including untreated control (UC) and heat-treated control (TC), were also prepared. UC was the OBPC without any heat treatment, and TC was prepared exactly the same way as the enzyme-treated products, but without the addition of enzyme.

2.3. Control and estimation of degree of hydrolysis (DH)

Protein hydrolysates of three different DH were obtained by varying the time of hydrolysis. The amount of alkali consumed was measured and used to calculate the DH, defined as the ratio between the total number of peptide bonds cleaved and the total number of peptide bonds in the protein substrate (Adler-Nissen, 1986). The percent DH was calculated according to the following equation:

$$\mathrm{DH}(\%) = \frac{B \cdot N}{\alpha \cdot M_{\mathrm{p}} \cdot h_{\mathrm{tot}}} \times 100$$

where B and N refer to the volume (ml) of NaOH consumed during the proteolysis of the substrate and its normality respectively; α represents the average degree of dissociation of the α-NH_2 groups in the protein substrate; M_{p} is the mass (g) of the protein ($N \times 6.25$); and h_{tot} is the total number of peptide bonds in the protein (mmol/g $N \times 6.25$), which was determined to be 7.31 according to the composition of amino acid in the substrate.

2.4. Determination of TCA soluble nitrogen (NTCA)

Samples were drawn from the enzymatic reaction mixture at intervals to determine the TCA-soluble nitrogen by the method described by Adler-Nissen (1986). Five milliliters of TCA (200 g/l) solution was added to an equal amount of sample solution. After 1 h at room temperature, the dispersions were centrifuged at 10,000*g* for 10 min. The supernatants were collected and analysed for nitrogen contents by the Kjeldahl procedure. The percent NTCA was expressed as:

$$\mathrm{NTCA}(\%) = \frac{\text{TCA soluble N in supernatant}}{\text{Total N in sample}} \times 100$$

2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Laemmli (1970). The gel system consisted of a 12.75% polyacrylamide-resolving gel (pH 8.9) and a 4% stacking gel (pH 6.7). The lengths of the resolving and stacking gels were 12 and 3 cm, respectively, with a gel thickness of 1.5 mm. Two milligrammes of samples were dispersed in 1 ml buffer (0.01 M Tris–HCl, pH 8.0, containing 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue), heated in a boiling H_2O bath for 3 min, and then centrifuged (10,000*g*, 10 min) prior to application to the gel slab. Fifteen microliters of supernatants were loaded in each lane. Electrophoresis was carried out at a constant current of 15 mA. Protein bands were stained by immersion of the gels in a 0.05% (w/v) Coomassie Brilliant Blue R-250 solution in 45% (v/v) methanol and 9% (v/v) acetic acid. The gels were destained with 7.5% (v/v) acetic acid.

2.6. Functional properties

Functional properties, except for emulsifying properties, were determined on samples of product on an as is basis. Emulsifying properties were measured using sample dispersions of concentrations adjusted to an equal protein basis.

2.6.1. Solubility

The solubility of samples was evaluated by the nitrogen solubility index (NSI) according to Ponnampalam et al. (1987) with minor modification. Hundred microgrammes samples were suspended in 15 ml distilled water and the pH of the system was adjusted to the desired values using either 0.1 M HCl or 0.1 M NaOH. The suspensions were stirred magnetically for 30 min at room temperature and centrifuged at 4000*g* for 30 min. The supernatants were analysed for nitrogen contents and the percentage of soluble nitrogen was calculated at each pH value.

2.6.2. Water-holding capacity (WHC)

Five hundred milligrammes of samples were dispersed in 20 ml distilled water with a vortex mixer for 30 s in graduated centrifuge tubes. After standing for 6 h at room temperature, the dispersions were centrifuged at 2000*g* for 30 min. The volume of the supernatants was determined, and WHC was expressed as ml of water absorbed per g of protein sample.

2.6.3. Oil-holding capacity (OHC)

Five hundred milligrammes of samples were added to 5 ml corn oil in graduated centrifuge tubes, and mixed for 30 s with a vortex mixer. The oil dispersions were centrifuged at 2000*g* for 30 min. The volume of oil separated was measured and OHC was expressed as the ml of oil absorbed per gram of protein sample.

2.6.4. Emulsifying properties

Emulsifying activity index (EAI) and emulsifying stability index (ESI) were measured according to the method described by Pearce and Kinsella (1978). Portions of 24 ml sample solutions (1 mg protein/ml) of varying pH (3, 5, 7, and 9) were homogenized with 8 ml soy oil for 1 min at a speed of 10,000 rpm. Afterwards 50 μl emulsions were drawn from the bottom of the vessel, and diluted with 10 ml 0.1% SDS solution. The absorbance was measured at 500 nm at 0 min (A_0) and 10 min (A_{10}) after emulsion formation. EAI and ESI were calculated as follows:

$$\mathrm{EAI}\ (\mathrm{m}^2/\mathrm{g}) = \frac{2 \times 2.303 \times A_0 \times N}{c \times \varphi \times 10{,}000}$$

$$\mathrm{ESI}\ (\mathrm{min}) = \frac{A_0 \times 10}{A_0 - A_{10}}$$

where N represents a dilution factor; c is the weight of protein per unit volume (g/ml); and φ is the oil volumetric fraction (0.25).

2.6.5. Foaming properties

Foaming properties were determined by the method of Fernandez and Macarulla (1997) with minor modifications. Portions of 40 ml sample solutions (20 mg/ml) of varying pH (3, 5, 7, and 9) were mixed thoroughly using a homogenizer at 10,000 rpm for 3 min. Foaming ability (FA) was calculated as the percent increase in volume of the protein dispersion upon mixing. Foam stability (FS) was estimated as the percentage of foam remaining after 30 min and 60 min.

2.7. Statistical analysis

Three repetitions of each test were carried out to characterize the functional properties. The data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS 10.0.

3. Results and discussion

In this research, heat treatment was applied to inactivate the enzyme. To differentiate between the effects of enzyme and heat treatments, two controls, UC and TC, were used. The difference between the two controls indicates the effect of the heat treatment on the functional properties.

3.1. Enzymatic hydrolysis of OBPC

The hydrolysis of OBPC with trypsin was monitored for 4 h by the degree of hydrolysis. Fig. 1 shows a typical hydrolysis curve obtained under the conditions used. In the course of enzymatic treatment, the DH increased sharply in the first 2 h, and then smoothly in the following

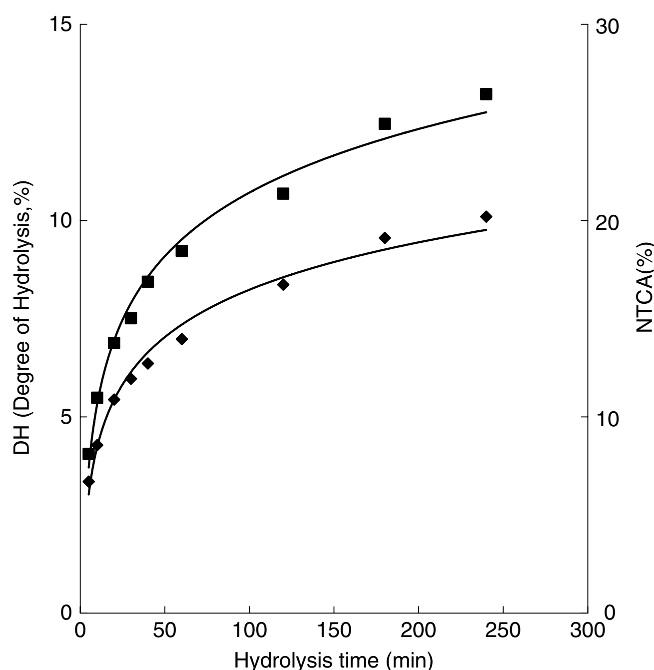

Fig. 1. Time course of OBPC hydrolysis. DH (%) (♦); NTCA (%) (■).

period. The DH (%) reached 4.1, 6.4 and 8.3 at time-points of 10 min, 40 min and 2 h respectively after initiation of hydrolysis, but at 4 h it only increased to 10.1 (Fig. 1). The reasons for the decrease of the rate of hydrolysis with the progressing of the hydrolysis mainly include: (1) decrease in specific peptide bonds available for enzyme action; (2) enzyme inactivation; and (3) competition between the native protein and the peptides being constantly formed during hydrolysis. TCA-soluble nitrogen contents as a function of hydrolysis time is also shown in Fig. 1. TCA-soluble nitrogen formed during hydrolysis, as a measure of proteolysis, is usually used although it is not a precise measure of the peptide bonds cleaved. In this study, it was found that the NTCA (%) correlated well with the DH (%), suggesting that the peptides formed during the initial stages of hydrolysis served as the substrate for the formation of smaller peptides soluble in TCA.

3.2. SDS-PAGE

Fig. 2 shows the SDS-PAGE patterns of the control proteins and the hydrolysates. Protein markers and their molecular weights are shown in Fig. 2Ab and 2Ba. Fig. 2Aa and 2Ac show the patterns of UC and TC, respectively. It was observed that heating did not markedly change the SDS-PAGE pattern of OBPC. Moreover, the patterns are similar to that of the oat globulin, suggesting globulin is the major protein component in OBPC. This is in agreement with previous reports (Brinegar & Peterson, 1982; Peterson, 1978). Electrophoresis of OBPC in the buffer with reducing agent exhibited two major bands ($M_r = 38{,}000$ and 22,000) corresponding to acid polypeptide (AP) and basic polypeptide (BP) of oat globulin, respectively (Peterson, 1978). The SDS-PAGE patterns of protein hydrolysates of three different DH (4.1%, 6.4% and 8.3%) are shown in Fig. 2Bc, Bd, and Be, respectively. It was observed that the bands corresponding to AP almost disappeared, while several new bands ($M_r = 29{,}000$–33,000) appeared, suggesting a partial hydrolysis of AP. However, the $M_r = 22{,}000$ bands showed no obvious changes. This indicated that BP was intact and resistant to trypsinolysis. According to the structure model proposed by Plietz, Zirwer, Schlesier, Gast, and Damaschun (1984), AP is mainly located at the surface of the protein molecules, while BP is usually buried inside. Therefore, AP can more easily access the catalyzation active point of enzyme than BP. Some researchers (Dudek, Horstmann, & Schwenke, 1996; Kamata & Shibasaki, 1978; Plumb & Lambert, 1990; Schwenke, 2001) also obtained similar results in studying other plant proteins, and indicated that limited tryptic hydrolysis of 11 S globulins resulted in the splitting of the surface-exposed regions of α-chains, while caused no observable changes of β-chains. In addition, the SDS-PAGE patterns of enzymatic hydrolysates were alike despite the difference in DH. This indicated that the large peptides observed were somewhat resistant to further proteolysis.

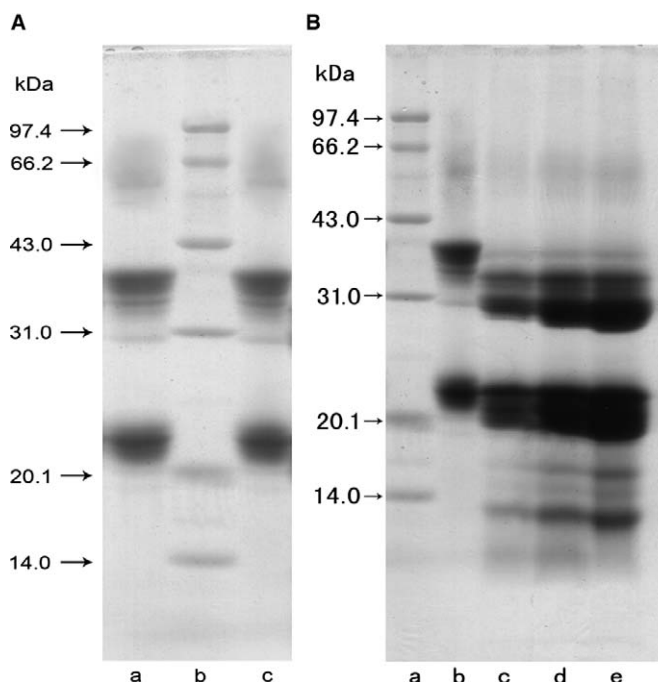

Fig. 2. SDS-PAGE patterns of the controls and the hydrolysates. (A) a, UC; b, molecular mass markers; c, TC and (B) a, molecular mass markers; b, TC; c, DH 4.1%; d, DH 6.4%; e, DH 8.3%.

Some peptides of M_r ranging from 20,000 to $<14{,}400$, were also produced during the process of enzymatic hydrolysis (Fig. 2B). Moreover, the amount of the very small peptides, which are soluble in TCA, increased with the development of hydrolysis (indicated in NTCA result). Next to these peptides, other peptides ($M_r < 10{,}000$) might also exist; however, they are too small to be detected under the analytical electrophoresis conditions.

3.3. Functional properties

3.3.1. Solubility

Solubility is one of the most important characteristics of proteins because it is not only important by itself, but also influences other functional properties. Good solubility of proteins is required in many functional applications, especially for emulsions, foams and gels, because soluble proteins provide a homogeneous dispersibility of the molecules in colloidal systems and enhance the interfacial properties (Zayas, 1997).

The pH-solubility profiles of the two control proteins and the hydrolysates are shown in Fig. 3. Both UC and TC presented typical bell-shaped solubility curves with minimum solubility (4.7% and 7.3%, respectively) at pH 5, which is the isoelectric point of oat bran protein, and high solubility at acidic and alkaline pH. At pH 9.0 the solubility of UC and TC reached 83.2% and 89.3%, respectively. It is interesting to note that heat treatment of OBPC resulted in a slight increase in solubility. In general, for most food proteins, heat treatment (100 °C for 10 min) may cause their extensive or complete denaturation, and

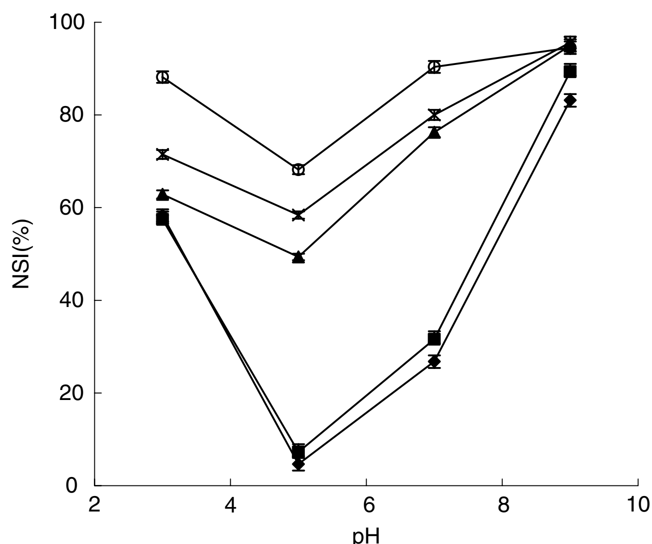

Fig. 3. Effect of pH on nitrogen solubility of the controls and the hydrolysates. Means ± standard deviation. UC (◆); TC (■); DH 4.1% (▲); DH 6.4% (×); DH 8.3% (○).

subsequently loss of solubility to a certain degree. However, oat globulin has fairly high heat stability with a denaturation temperature around 110 °C (Harwalkar & Ma, 1987; Ma & Harwalkar, 1987). The extent of denaturation of the control OBPC due to heat treatment in this study was probably not high enough to impair its solubility. On the contrary, a slight increase in solubility due to heating was observed, which could be attributed to slight hydrolysis taking place during heat treatment. Enzymatic hydrolysis increased markedly the solubility of OBPC at all pH values, especially around the isoelectric point. Compared with that of TC, the solubility of the DH 8.3% hydrolysate increased to 68.2% from 7.3% at pH 5. The enhanced solubility of the hydrolysates could be attributed to: (1) demolishment of the tight structure of the proteins and large peptides by enzymatic hydrolysis and heat treatment; (2) decrease in the molecule size of polypeptide chains; and (3) exposure of more charged and polar groups to the surrounding water. In addition, all hydrolysates presented similar patterns of NSI, which could be due to the fact that the specificity of the enzyme used was the same in all these cases.

It has been also reported that trypsin-hydrolyzed oat protein concentrate exhibits a broad solubility curve with greatly improved solubility though the relevant DH is not considered (Ma, 1985). The results in this study, therefore, are consistent with this report.

3.3.2. Water- and oil-holding capacity

Heat treatment improved the WHC of the control protein (Table 1). Ponnampalam et al. (1987) also made a similar observation, and indicated that the improvement of WHC might be due to the effect of heat treatment on the solubility of TC. However, protein was not the only factor that influenced WHC during heating. Carbohydrates, including starches, fibres and so on, also played a significant role. Particularly, starch granules can swell extensively in presence of excess water during heating causing gelatinization. Therefore, the increase in WHC could be partly attributed to starch swelling caused by heat treatment. WHC of enzymatic hydrolysates increased in comparison with that of TC. Moreover, WHC of the hydrolysates increased with increase in DH. Although the DH 8.3% sample had lower WHC value than the DH 6.4% sample, this was not statistically significant. Because the part of the protein that is soluble does not contribute to the WHC (inherent to the technique used), the observed increase in WHC with increasing DH (and solubility) could be due to the increase in accessibility of non-protein components in the samples to the surrounding water (e.g. fibres, starches, etc.) by removal of protein.

Table 1
Water- and oil-holding capacities of protein samples[a]

Samples	Bulk density (g/ml)	WHC[b] (ml/g)	OHC[c] (ml/g)
UC	0.39 ± 0.07	1.94 ± 0.15	2.50 ± 0.19
TC	0.35 ± 0.05	2.04 ± 0.30	2.96 ± 0.37
DH:4.1%	0.44 ± 0.02	2.13 ± 0.09	1.99 ± 0.07
DH:6.4%	0.47 ± 0.10	2.27 ± 0.21	1.78 ± 0.11
DH:8.3%	0.51 ± 0.06	2.25 ± 0.13	1.51 ± 0.07

[a] All data were expressed by mean values of triplicates ± standard deviation.
[b] Water-holding capacity.
[c] Oil-holding capacity.

The OHC of TC was higher than that of UC (Table 1). Enzymatic modification decreased the OHC of OBPC compared to TC. Ma (1985) has also reported similar results. The mechanism of fat absorption has been attributed mostly to physical entrapment of oil (Dench, Rivas, & Caygill, 1981; Kinsella, 1976; Wang & Kinsella, 1976). Compared with the bulk density of TC (0.35 g/ml), those of enzymatic hydrolysates were much higher (Table 1). The difference in the surface area of the protein particles in those protein samples may therefore affect the amount of oil absorbed.

3.3.3. Emulsifying properties

The EAI and ESI of the controls and hydrolysates at four different pH values are shown in Table 2. For UC and TC, the emulsifying activities were both the lowest at the isoelectric point (pH 5.0), and increased with pH changes from pH 5.0. At pH 5.0, the minimum emulsifying activities of UC and TC are respectively 7.3 and 8.1 m^2/g, which are presumably because of their flocculation caused by minimum protein charge. Heat treatment of OBPC did not show much influence on emulsification behaviour as indicated by the similarities between the two controls.

After trypsin treatment, the tendency of the emulsifying activities of all hydrolysates with pH is similar to that of TC. Meanwhile, improvements of emulsifying activity of the hydrolysates were observed compared with that of TC,

Table 2
Effect of pH on emulsifying properties of protein samples[a]

Samples	pH	EAI[b] (m^2/g)	ESI[c] (min)
UC	3.0	27.6 ± 0.3	110.0 ± 1.2
	5.0	7.3 ± 0.2	115.9 ± 2.5
	7.0	20.4 ± 0.4	46.2 ± 0.2
	9.0	45.3 ± 2.1	76.9 ± 0.8
TC	3.0	25.8 ± 1.1	104.7 ± 2.0
	5.0	8.1 ± 0.7	118.4 ± 1.3
	7.0	18.9 ± 1.4	52.6 ± 0.7
	9.0	48.1 ± 3.1	83.6 ± 0.4
DH:4.1%	3.0	32.6 ± 0.1	39.3 ± 0.2
	5.0	31.0 ± 0.5	50.9 ± 1.6
	7.0	88.1 ± 0.4	55.6 ± 0.3
	9.0	151.3 ± 1.3	87.3 ± 0.5
DH:6.4%	3.0	48.6 ± 0.3	62.9 ± 0.3
	5.0	36.9 ± 0.4	75.0 ± 0.6
	7.0	85.1 ± 0.5	55.0 ± 0.1
	9.0	185.1 ± 2.5	55.1 ± 0.4
DH:8.3%	3.0	42.7 ± 0.6	125.0 ± 3.5
	5.0	39.5 ± 1.1	210.9 ± 4.3
	7.0	89.2 ± 0.8	89.6 ± 0.3
	9.0	194.3 ± 2.4	89.1 ± 0.8

[a] All the data are expressed by mean values of triplicates ± standard deviation.
[b] Emulsifying activity index.
[c] Emulsifying stability index.

especially at pHs above 5.0, which could be attributed to the increase in solubility and hydrophobicity of enzymatic hydrolysates. On the one hand, the solubility of protein was an important prerequisite for film formation because rapid migration to and adsorption at the oil–water interface was critical. A positive correlation between solubility and emulsifying capacity of proteins has been reported (Felix, Hill, & Diarra, 1990; Volkert & Klein, 1979). On the other hand, trypsin treatment and subsequent heat treatment might also uncover buried hydrophobic groups, which could improve the hydrophilic–lipophilic balance for better emulsification.

Enzymatic hydrolysates had lower emulsifying stability compared with TC. This may be caused by the increase of the quantity of small peptides after enzymatic digestion of proteins. Since these peptides are shorter and less globular, they will form less stable protein layers around the oil droplets that offer less resistance to coalescence or Ostwald ripening (Puski, 1975; Singh & Dalgleish, 1998).

The improvement in emulsifying properties of trypsin-treated oat protein concentrate has been reported (Ma, 1985). However, the effect of pH on emulsifying stability was not considered in his study. In addition, the denaturation degrees of enzymatic hydrolysates due to heat treatment, which are critical to emulsifying stability, were different in both studies. In the present study, heat treatment used might have denatured the larger protein components so that they had less emulsion stability. These can offer an explanation to the difference of emulsion stability in both studies.

3.3.4. Foaming properties

Proteins in dispersions cause lowering of surface tension at the water–air interface, thus creating foaming capacity (Surowka & Fik, 1992). The effects of pH on foaming ability and stability of the controls and the hydrolysates are presented in Table 3. For TC, the minimum FA (97.7%) was observed at pH 5.0. FA increased in highly acidic and alkaline regions. Meanwhile, alkaline pH improved FA much more than acidic pH. Compared with that of 97.7% at pH 5.0, a foaming ability of 144.1%, which is equivalent to an increase of 46.4%, was observed at pH 9.0. However, the effect of pH on FS was opposite to that on FA. In addition, all samples presented similar patterns of FA and FS values as affected by pH.

The effect of pH on foaming capacity and stability of cowpea seeds has also been reported by Aluko and Yada (1995). In highly acidic and alkaline regions, the protein molecules become net charged. This development leads to electrostatic repulsion, which weakens the hydrophobic interaction but facilitates the flexibility of the protein molecules. Thereby, the increase in FA of OBPC under highly acidic and alkaline conditions might be attributed to the increase in the flexibility of the protein molecules, which diffuse more rapidly to the air–water interface to encapsulate air particles, leading to enhanced foaming (Chau & Cheung, 1998). Moreover, the increase in protein solubility away from pI could account for higher foaming capacity (Aluko & Yada, 1995).

Earlier researchers (Aluko & Yada, 1995; Buckingham, 1970) reported that protein stabilized foams were more sta-

Table 3
Effect of pH on foaming properties of protein samples[a]

Samples	pH	FA[b] (%)	FS[c] (%)	
			30 min	60 min
UC	3.0	107.5 ± 3.4	79.3 ± 2.4	42.4 ± 0.2
	5.0	95.6 ± 2.3	85.3 ± 1.5	72.5 ± 1.8
	7.0	126.5 ± 1.7	63.0 ± 1.4	47.5 ± 1.5
	9.0	135.5 ± 3.5	59.7 ± 0.1	32.8 ± 2.2
TC	3.0	104.3 ± 1.8	80.0 ± 1.0	46.6 ± 0.9
	5.0	97.7 ± 2.1	82.5 ± 0.9	70.7 ± 1.1
	7.0	132.5 ± 3.2	62.6 ± 2.1	51.3 ± 0.8
	9.0	144.1 ± 1.9	55.3 ± 0.5	43.4 ± 1.3
DH:4.1%	3.0	146.6 ± 3.3	26.5 ± 1.3	14.1 ± 0.2
	5.0	114.8 ± 2.6	75.6 ± 2.3	53.8 ± 1.6
	7.0	163.5 ± 1.7	43.8 ± 1.8	29.8 ± 1.2
	9.0	171.5 ± 2.6	39.3 ± 1.7	23.6 ± 0.6
DH:6.4%	3.0	158.1 ± 2.4	21.7 ± 0.4	7.8 ± 0.1
	5.0	141.9 ± 3.3	56.2 ± 1.9	38.1 ± 1.2
	7.0	168.9 ± 2.9	48.0 ± 2.6	34.0 ± 0.1
	9.0	175.7 ± 1.6	43.5 ± 1.4	23.1 ± 0.7
DH:8.3%	3.0	163.5 ± 2.3	15.3 ± 0.6	5.0 ± 0.1
	5.0	150.7 ± 1.5	33.6 ± 0.5	33.6 ± 0.8
	7.0	173.6 ± 0.4	47.9 ± 1.3	31.9 ± 1.5
	9.0	190.5 ± 3.8	45.0 ± 0.7	26.6 ± 0.4

[a] All data are expressed by mean values of triplicates ± standard deviation.
[b] Foaming ability.
[c] Foaming stability.

ble around pI of the protein than any other pH. Around the isoelectric pH, lack of repulsive interactions of protein molecules enhanced favorable protein–protein interactions and formation of a viscous film at the interface. More protein molecules absorbed at the air–water interface facilitated the formation of stable molecular layers in the interface, which imparted stability to the foam.

All hydrolysates had better FA than the controls. The results could be mainly explained by the favorable effect of an increase in protein solubility on the formation of foams. The improvement in foaming capacity for enzyme-modified food proteins has also been reported (Ma, 1985; Puski, 1975). However, after trypsin treatment, the decrease of FS was pronounced. Previous study (Turner, 1969) indicated that to make a stable protein containing foam, some larger protein components were needed. In this study, the sufficient denaturation of larger protein components due to heat treatment could cause loss of the foam stabilizing capacity.

4. Conclusions

The experiments demonstrated that trypsin treatment could only selectively cleave some peptide bonds in AP, and alter markedly the functional properties of OBPC, such as the solubility, water- and oil-holding capacities, emulsifying properties, and foaming properties. Moreover, the functional properties of the hydrolysates were closely correlated with the extent of enzymatic hydrolysis. In the DH range studied, the solubility, water-holding capacities, emulsifying activity and foaming ability of resulting hydrolysates gradually increased with the increase of DH, but their oil-holding capacities, emulsifying and foaming stability showed more or less decreases. In summary, trypsin-treated OBPC exhibits satisfactory functional properties as required in food processes, and therefore has a bright prospect of applications in the food industry.

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