FUNCTIONAL PROPERTIES OF FRACTIONATED MAIZE PROTEIN HYDROLYSATE OBTAINED BY ULTRAFILTRATION

Orrapun Selamassakul, Natta Laohakunjit and Orapin Kerdchoechuen

School of Bioresources and Technology,

King Mongkut's University of Technology Thonburi

Email address: som_beam@hotmail.com

ABSTRACT The objectives of this study were to improve the functional properties of maize protein by enzymatic hydrolysis for functional and nutritional food ingredients. Maize (Suwan 5) was hydrolyzed by 10% bromelain for 3 h to produce the maize protein hydrolysate (eb-MPH) with the highest foaming capacity and emulsifying capacity. Peptide size control is important for obtaining desirable functional properties, so that the eb-MPH was fractionated by a series of ultrafiltration membrane with selected molecular weight cut-offs (MWCO) of 10, 3 and 1 kDa. Fraction1 (>10 kDa), Fraction2 (3-10 kDa), Fraction3 (1-3 kDa) and Fraction4 (<1 kDa) were analyzed for surface hydrophobicity and functional properties, including foaming capacity, foaming stability and emulsifying capacity. F4 (>10 kDa) had the highest surface hydrophobicity (S_0), followed by F2, F3, and F4, respectively. Result of foaming capacity was positively correlated with the surface hydrophobicity. F1 (>10 kDa) exhibited the greatest foaming capacity of 121.66 ± 2.18%, while F4 (<1 kDa) exhibited least foaming capacity of 38.50 ± 2.18%. For foaming stability, only F1 had the foaming capabilities after 30 min (60.0 6± 2.18%), whereas other fractions do not have the ability to stabilize the air cells of the foam. The correlation between surface hydrophobicity, molecular size of peptide and emulsifying capacity of fractionated MPH was similar to foaming capacity. F1 had the highest emulsifying capacity (4.77 ± 0.22%). Large molecular weight peptides and intact hydrophobic areas of the fractionated MPH were presumed to be associated with the improvement of foaming and emulsifying capacity.

1. INTRODUCTION

Maize or corn (*Zea mays* L.) is known as one of the most nutritional and healthy plant protein sources for human and animal diet. Maize proteins contain a high proportion of hydrophobic amino acids, such as leucine, alanine, proline and isoleucine which can inhibit the propagation of free radical reactions, and they are rich in flavor amino acids like glutamic acid and aspartic acid which give umami taste (Serna-Saldivar, 2010). Therefore, maize protein may become a good source in the preparation of functional foods with a good taste. However, maize protein is mostly soluble in dilute acid or alkali solutions due to its high proportions of hydrophobic amino acids which limited their solubility in aqueous solution under the conditions of pH occurring in most food products, and hence maize protein has rarely been applied as nutritious and functional food (Zheng et al., 2015). Functional properties of maize protein can be improved by enzymatic hydrolysis (Mannheim and Cheryan, 1992). Partial proteolysis of proteins by enzymatic hydrolysis could produce peptides which have smaller molecular size and less secondary structure than native proteins, increasing in the solubility, foaming, gelling, and emulsifying properties when compared with those of the native proteins (Tsumura et al., 2005; Wu et al., 1998). Various studies have reported that a number of proteases such as Alcalase, Papain, and Pepsin have been used to improve the functionalities of plant proteins (Tsumura et al., 2005; Guo et al., 2013). Among these enzymes, stem bromelain, which had an optimal activity over a wide pH range (pH 4.0–8.0), is an interesting protease because bromelain hydrolyzed protein into peptide which consisted of hydrophobic and non-polar amino acid residues at the C-terminal of the peptide chain, increasing in functional properties of protein (Arshad et al., 2014). Several studies have been reported that the presence of proper amino acids, their correct positioning in peptide sequence, hydrophobicity as well as size of peptide are significantly correlated to functional properties of peptides (Tsumura et al., 2005; Klompong et al., 2007). To obtain desirable functional properties, hydrolysis must be carried out under strictly

controlled conditions (Cao et al., 2009). Therefore, the objective of the present study is to examine the functional properties of the maize protein hydrolysate hydrolyzed by bromelain (eb-MPH) with different molecular size of peptides using ultrafiltration which was an effective method to fractionate these proteins into peptides with controlled molecular size.

2. EXPERIMENT

2.1 Materials and chemicals

Maize (Suwan 5) was supplied by Suwanwajokkasikit Field Crops Research. Stem bromelain (E.C. 3.4.22.32; 97,044 U/g) was purchased from K-Much Industry Co., Ltd., Bangkok, Thailand. One unit was defined as the amount of enzyme that liberated 1 mg of tyrosine from a casein substrate in 10 min at a pH of 6.0 and a temperature of 50 °C. All reagents were purchased from Sigma-Aldrich (Milan, Italy) and were of analytical grade.

2.2 Preparation of maize protein hydrolysates

Maize was grinded and sieved through an 80-mesh screen. The powder contained 13.15% protein, 2.51% fat, 1.40% ash, 1.91% fiber, and 81.03% carbohydrate (dry weight). Ten grams of maize powder were dispersed in 100 mL of sterile distilled water, and then pre-incubated at 50ºC for 10 min in order to exert activity of enzyme. The dispersions were incubated at 50ºC for 3 h with 10% bromelain. After hydrolysis, the reaction was stopped by heating at 75ºC for 15 min to inactivate the enzyme. The dispersions were centrifuged at 5,000×g for 15 min, obtaining a supernatant and filtered through filter paper (Whatman No. 1). The eb-MPH solutions were kept in brown bottle glass at -20ºC prior to analysis.

2.2 Fractionation of MPH by ultrafiltration

The MPH solution was fractionated through a series of centrifugal ultrafiltration membrane (Amicon® Ultra, Millipore Corporation, USA) with selected molecular weight cut-offs of 10 and 3 kDa by centrifugation (5000g for 30 min at 25 °C). Ultrafiltration was performed sequentially: the hydrolysate was firstly passed through a 10 kDa membrane; and permeates was further fractionated through 3 kDa. Finally, permeate from 3 kDa was ultrafiltered by stirred cell and disc membrane system with MWCO of 1 kDa. Four fractions were obtained: fraction 1 (>10 kDa), fraction 2 (10–3 kDa), fraction 3 (3–1 kDa), and fraction 4 (<1 kDa), respectively. To facilitate comparison, the eb-MPH and all fractions were adjusted to 20 mg/mL by deionized water for analysis. All fractions were analyzed as follows;

2.2.1 Surface hydrophobicity (S_O). The S_O of all MPH fractions was determined according to the method of Wu et al. (1998), with minor modifications. The samples were serially diluted by 0.01 M phosphate buffer (pH 7.0) to obtain protein concentrations ranging from 0.00125 to 0.03%. Twenty microliters of ANS (8 × 103 M ANS in 0.01 M phosphate buffer, pH 7.0) were added to 4 mL of the diluted protein solutions. Fluorescence intensity (FI) was measured at 390 (excitation) and 470 nm (emission) at 20 ± 0.5 °C, with a constant excitation and emission slit of 5 nm. The FI for each sample with a probe was then computed by subtracting the FI attributed to protein in the buffer. The initial slope of the FI versus protein concentration plot was calculated by linear regression analysis and used as the S_O.

2.2.2 Foaming capacity. The fractionated maize protein hydrolysates (5.0 mL) were mixed with distilled water (5.0 mL). The mixtures were then whipped by homogenizer (Ultra-turrax T25 basic, Germany) at speed of 10,000 rpm to incorporate the air for 1 min at room temperature and the volumes before and after whipping were then recorded. Foaming capacity was expressed as foam expansion at 1 min, while foam stability was expressed as foam expansion during 30 min. The foam capacity and foam stability was calculated according the following equation:

$$\text{Foaming capacity (\%)} = \frac{\text{total volume of foam}}{\text{total volume of solution}} \times 100 \qquad (1)$$

$$\text{Foaming stability (\%)} = \frac{\text{A}-\text{B}}{\text{B}} \times 100 \qquad (2)$$

2.3 Statistical analysis

All the tests were performed in triplicate. Significant differences between each sample were obtained by analysis of variance and Duncan's Multiple Range Test (DMRT) using the Version 9 of the SAS program (SAS Institute Inc., Cary, NC, USA).

3. ANALYSIS

3.1 Functional properties of the eb-MPH

In our previous studies, maize was hydrolyzed by various concentration of bromelain and hydrolysis time to find the optimal condition for producing the eb-MPH which exhibited good functional properties. It was found that the eb-MPH produced by 10% bromelain for 3 h had the highest foaming and emulsifying capacity, whereas the native maize protein did not have these functionalities. (data not shown). Hydrolysis of bromelain significantly improved the foaming capacity of maize protein by decreasing the peptide chain length which lead to a better hydrophobic-hydrophilic balance, low surface tension and a strong tendency to form flexible films around the air bubbles (Paraman, 2007; Cao et al., 2009). The increase in the emulsion capacity of eb-MPH over than that of the native proteins was due to the exposure of the buried hydrophobic groups during proteolysis, increasing in the hydrophobic-hydrophilic balance and the peptides' ability to interact with both water and oil in food systems (Paraman et al., 2007; Cao et al., 2009). From the above results, the eb-MPH which possessed the strongest functional properties was selected for further fractionation using ultrafiltration membranes, in order to obtain more potential functional peptides with the desired foaming capacity and emulsifying capacity.

3.2 Surface hydrophobicity of the ultrafiltrated MPH

The values of surface hydrophobicity (S_O), indicating the number of hydrophobic groups on the surface of peptide were measured by 1,8-anilino naphthalene sulphonate (ANS) as fluorescence probe. Result showed that F1 (>10 kDa) had the highest surface hydrophobicity, followed by F2, F3, and F4, respectively (Table 1). Basically, hydrolysis of protein into smaller peptides might be caused an increase or decrease of surface hydrophobicity, depending mainly on the exposure of hydrophobic groups which are buried inside the core of intact native protein (Wu et al., 1998). In present study, the exposed hydrophobic surfaces of the fractionated MPH declined as the molecular size of peptide decreased. The difference in the surfaces hydrophobicity of fractionated peptides could be due to the presence of small peptides which could have lower hydrophobic binding sites than large peptides, resulting in decreased surface hydrophobicity (Paraman et al., 2007). This result indicated that small peptides have fewer hydrophobic binding sites than larger peptides.

Table 1 Surface hyhydrophobicity of the eb-MPH and its fractions.

Fraction	Surface hydrophobicity
F4 (<1K)	34.25 ± 1.89^{e}
F3 (1-3K)	45.44 ± 3.17^{d}
F2 (3-10K)	105.26 ± 2.72^{b}
F1 (>10K)	190.59 ± 1.32^{a}
eb-MPH	73.70 ± 1.83^{c}
F-test	**
C.V.(%)	2.23
LSD	4.16

[a, b, c] Mean in the same column with different letters are significantly different ($p \leq 0.05$)

3.3 Foaming capacity of the ultrafiltrated MPH

The foaming capacity of the eb-MPH and its fractions to form foams with protein films was determined by measuring their whippability and is shown in Figure 1. After fractionation, all fractions also showed foaming capacity. The fraction corresponding to MW > 10 kDa (F1) had the highest foaming capacity, with a capacity of 121.66 ± 2.18%, as compared to other fractions. The decrease in the foaming capacity with decreasing molecular size of peptide could be due to the presence of short-chain peptides which could have lower hydrophobic binding sites than larger peptides, resulting in a poor integrity to form flexible films (Wu et al., 1998; Paraman, 2007).

For foaming stability, only F1 had the foaming capabilities after 30 min (60.06±2.18), whereas other fractions do not have the ability to stabilize the air cells of the foam because the small peptides do not have the strength needed to maintain a stable foam (Cao et al., 2009). This is in line with other published studies on protein hydrolysates which reported that foaming capacity of hydrolysats could be due to the size and hydrophobicity of peptide (Tsumura et al., 2005; Klompong et al., 2007).

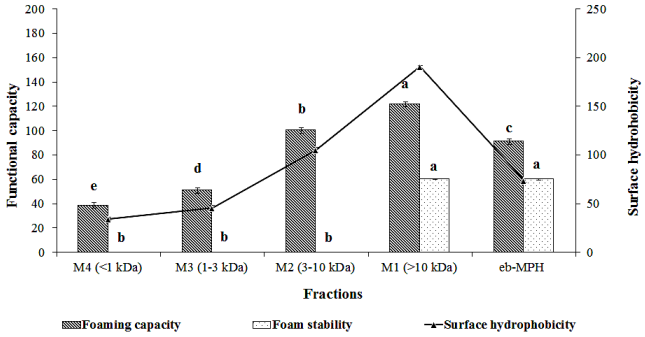


Figure 1 Correlation between surface hydrophobicity, foaming capacity and foaming stability of eb-MPH and its fractions. [a, b, c] The values for the same parameter with different letters are significantly different ($p < 0.05$).

3.4 Emulsifying a capacity of the ultrafiltrated MPH

The emulsifying activity of MPHs under different molecular weight of peptides was determined by the turbidimetric method. The correlation between size of peptides, surface hydrophobicity and emulsifying capacity of the fractionated maize peptides are similar to foaming capacity. Figure 2 showed that F1 (>10 k) showed the highest emulsifying capacity, followed by F2, F3, and F4, respectively. The low molecular weight peptides are less efficient in reducing the interfacial tension and stabilizing the emulsions because they cannot unfold and re-orient at the interface compared with large peptides. Accordingly, the peptides with low molecular weight which had lower surface hydrophobicity may not be amphiphilic enough to exhibit good emulsifying properties (Gbogouri et al., 2004). Accordingly, F1, which contains larger molecular weight peptides, higher amount of hydrophobic amino acids in the peptides and higher surface hydrophobicity than F4, had the highest emulsifying capacity. These results revealed that the effects of surface hydrophobicity on emulsifying capacity were minor compared to those of molecular size.

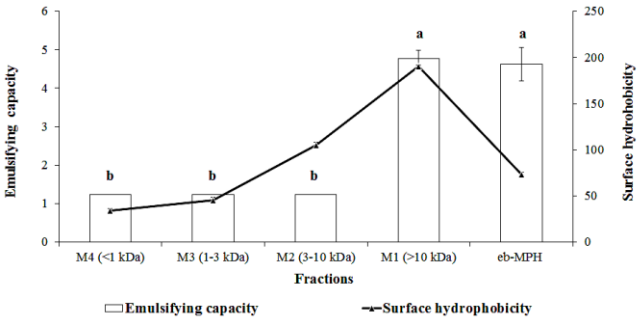


Figure 2 Emulsifying capacity and surface hydrophobicity of eb-MPH and its fractions. Means with different letters on the top of the bars were significantly different ($p < 0.05$).

CONCLUSION

In the present study, all MPH fractions were found to possess foaming capacity and emulsifying capacity. Among four fractions, Fraction1 (molecular weight more than 10 kDa) exhibited the greatest antioxidant activity; whereas Fraction4 (molecular weight less than 1 kDa) had poor functional properties. These results suggested that functional properties of protein were related to the molecular size of peptide and surface hydrophobicity. The large-MW maize peptides (F1) prepared by bromelain modification, followed by ultrafiltration had high foaming and emulsifying capacities and could be used for nutritional supplements and functional enhancement in the cosmetic and health food industries.

REFERENCES

Arshad, Z., Amid, A., Yusof, F., Jaswir, I., Ahmad, K., and Loke, S., Bromelain: an overview of industrial application and purification strategies, *Appl. Microbiol. Biotechnol.*, vol. 98, pp. 7283-7297, 2014.

Cao, X., Wen, H., Li, C., and Gu, Z., Differences in functional properties and biochemical characteristics of congenetic rice proteins, *J. Cereal Sci.*, vol. 50, pp. 184-189, 2009.

Gbogouri, G.A., Linder, M., Fanni, J., and Parmentier, M., , Influence of hydrolysis degree on the functional properties of salmon byproducts hydrolysates, *J. Food Sci.*, vol. 68, pp. 615–622, 2004.

Guo, X., Zhang, J., Ma, Y., and Tian, S., Optimization of limited hydrolysis of proteins in rice residue and characterization of the functional properties of the products, *J. Food Process Preserv.*, vol. 37, pp. 245-253, 2013.

Klompong, V., Benjakul, S., Kantachote, D., and Shahidi, F., Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type, *Food Chem.*, vol. 102, pp. 1317-1327, 2007.

Mannheim, A., and Cheryan, M., Enzyme-modified proteins from corn gluten meal: Preparation and functional properties, *J. Am. Oil Chem. Soc.*, vol. 69, no. 12, pp. 1163-1169, 1992.

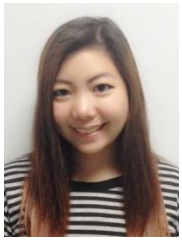
Paraman, I., Hettiarachchy, N. S., Schaefer, C., and Beck, M. I., Hydrophobicity, solubility, and emulsifying properties of enzyme-modified rice endosperm protein, *Cereal Chem.*, vol. 84, pp. 343-349, 2007.

Serna-Saldivar, S. O., *Cereal Grains: Properties, processing, and nutritional attributes*, Taylor & Francis Group, USA, 2010.

Tsumura, K., Saito, T., Tsuge, K., Ashida, H., Kugimiya, W., and Inouye, K., Functional properties of soy protein hydrolysates obtained by selective proteolysis, *LWT-Food Sci. Technol.*, vol. 38, pp. 255-261, 2005.

Wu, W. U., Hettiarachchy, N. S., and Qi, M., Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration, *J. Am. Oil Chem. Soc.*, vol. 75, pp. 845-850, 1998.

Zheng, X. Q., Wang, J. T., Liu, X. L., Sun, Y., Zheng, Y. J., Wang, X. J., and Liu, Y., Effect of hydrolysis time on the physicochemical and functional properties of corn glutelin by Protamex hydrolysis, *Food Chem.*, vol. 172, pp. 407-415, 2015.



Orrapun Selamassakul received the B.Sc. (2008), and M.Sc. (2011) degrees in biochemical technology from King Mongkut's University of Technology Thonburi. She is a PhD candidate from School of Bioresource, King Mongkut's University of Technology Thonburi.

Natta Laohakunjit (Assoc. Prof.) received the B.Sc. (1988), M.Sc. (1996), and D.Sc. (2003) degrees in Postharvest and food process engineering from Asian Institute of Technology. She is a Professor, School of Bioresource, King Mongkut's University of Technology Thonburi.

Orapin Kerdchoechuen (Assoc. Prof.) received the B.Sc. (1976), M.Sc. (1990), and D.Sc. (1996) degrees in Horticulture from Mississippi State University. She is a Professor, School of Bioresource, King Mongkut's University of Technology Thonburi.

Khanok Ratanakhanokchai (Assoc. Prof.) received the B.Sc. (1982), M.Sc. (1985), and D.Sc. (1995) degrees in Applied Chemistry and Biochemistry from Tokyo University of Agricultural and Technology. He is a Professor, School of Bioresource, King Mongkut's University of Technology Thonburi.

Benjawan Thumthanaruk (Assist. Prof.) received the B.Sc. in Microbiology form Chulalongkorn University, M.Sc. in Biotechnology from Mahidol University, and D.Sc. degrees in Food Science & Nutrition from The Ohio State University. She is a Professor, Division of Agro-Industrial Technology, King Mongkut's University of Technology North Bangkok.