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The Optimization of Hydrolyzed Protein Production with High Anti-Oxidation Ability from Sesame Meal by Response Surface Methodology

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Abstract

The purpose of this study was to produce a hydrolyzed protein from sesame seed meal using response surface methodology (RSM).The sesame meal protein is a low-value product, often used for feeding animals, but it can be hydrolyzed to protein hydrolysate with high nutritional value using alkalase enzyme. The factors that were considered in this study to achieve the highest level of antioxidant activity were temperature (40-55 °C), time (30-180 min) and ratio of enzyme to substrate (1-3%) whose effects, as 3 independent variables, were evaluated on DPPH free radical scavenging activity and this effect fitted by quadratic equation. The results showed that optimal conditions for reaching the highest antioxidant activity were temperature 52.07 °C, time 125.49 min and ratio of enzyme to substrate was 3% and under this condition DPPH free radical scavenging activity was 66.5%. Also, the results showed that the production of hydrolyzed protein was highly affected by the hydrolysis conditions, so different hydrolysis conditions including time, temperature and enzyme to substrate ratio showed significant effect on the properties of final hydrolyzed product. The results showed that hydrolyzed sesame protein can be used in food formulation as a natural antioxidant.

Keywords: Enzyme Hydrolysis, Hydrolyzed Protein, Optimization, Response Surface Method, Sesame Meal

Introduction

Sesame (*Sesamum indicum*) belongs to pedaliaceae family which is an important oilseed. It also contain high amount of protein (Rastegar, 2005) which can be used for production of protein hydrolysate. Bioactive peptide are product of protein hydrolysis which usually contain 2-20 amino acid sequence with molecular weight less than 6000 Da (Alaiz, Beppu, Ohishi, & Kikugawa, 1994). Bioactive peptide show different characteristics like antioxidant, antihypertensive, antimicrobial, chelating activity, anticancer, but some are multifunctional

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and show more than one characteristics (Sarmadi & Ismail, 2010). Bioactive peptides are inactive in protein structure, but after release using enzymes or microorganisms, they exert different biological activity like antioxidant properties. These properties can be measured with different test like 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, chelating activity, hydrogen donating ability, singlet oxygen quenching activity (Li, Jiang, Zhang, Mu, & Liu, 2008). Bioactive peptide can be prepared using different protein sources like soybean (Chen, Muramoto, & Yamauchi, 1995), groundnut (Tang *et al*., 2012), African yam bean seed (Ajibola, Fashakin, Fagbemi, & Aluko, 2011), gingerbread plum (Amza, Balla, Tounkara, Man, & Zhou, 2013). Sesame seed meal is a byproduct produced after oil extraction which can be used as a good source for production of hydrolysed protein. In the present study sesame seed meal were used for production of protein concentrate and then the protein hydrolysis condition was optimized to achieve maximum antioxidant activity using alkalase enzyme. To do this, different conditions of enzymatic hydrolysis including hydrolysis time, temperature and enzyme to substrate ratio were optimized using response surface methodology to achieve the maximum DPPH radical scavenging activity in hydrolyzed product.

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Material and methods

Sesame seed was purchased from Vejen factory, Gorgan and then its oil was extracted by solvent extraction using hexane. In the next step, de-oiled sesame seed meal was mixed with water in ratio of 1:10 and its protein was extracted using temperature 50-55 °C, at pH 9.5 (using 0.1 M NaoH) and then it was centrifuged at 4000×g for 10 min. In the next step, the pH of supernatant was adjusted on 4.9 and centrifuged at 8000× g for 20 min. The extracted protein was subjected to enzymatic hydrolysis using alkalase enzyme. In this step the extracted protein was mixed with water in 1:10 ratio and pH was adjusted to 8 using Tris-Hcl buffer system. The enzymatic hydrolysis was done using different enzyme ratio (1-3%), temperature 40-50 °C and different hydrolysis time of 30-180 min. At the end of enzymatic hydrolysis reaction was stopped by placing reaction flask in water bath at temperature 85 °C during 20 min. Finally the hydrolysed solution was centrifuged at 7600×g and the supernatant was freeze-dried (Meshgin Far, 2012). The factors that were considered in this study to achieve the highest level of antioxidant activity were temperature, time and ratio of enzyme to substrate whose effects as 3 independent variables in hydrolysis were evaluated on DPPH free radical scavenging activity and this effect fitted by quadratic equation. Chemical composition of sesame seed meal and protein hydrolysate was measured according to AOAC (2008) Antioxidant activity of hydrolysed protein was measured using DPPH radical scavenging method according to (Bougatef *et al*., 2009).

Results and discussion

As shown in Fig. (1a), with increasing hydrolysis time and enzyme to substrate ratio (E/S ratio), the anti-oxidant activity increases slightly. This figure shows that increased enzyme activity in a constant time also has an effect on changes in anti-oxidant activity. The justification for the anti-oxidant activity changing over time is related to changes in the peptide chain length by increasing the amount of hydrolysis. Also, many studies have shown that peptides with a lower molecular weight exhibit higher anti-oxidant activity (Rajapakse, Mendis, Byun, & Kim, 2005). Fig. (1b) shows variations in the percentage of DPPH free radical scavenging activity at different temperatures and hydrolysis time. Based on the results, the temperature above 49 °C showed the highest effect on DPPH free radical scavenging activity, while the increase in time after 120 min showed a greater effect. Changes in the size, amount, and structure of amino acids and peptides, as a result of the hydrolysis time, affect the anti-oxidant activity (Wu, Chen, & Shiau, 2003). Fig. (1c), indicates changes in the

percentage of DPPH free radicals scavenging activity in different enzyme to substrate ratio and temperatures. The results showed that the increase in temperature from the mid to upper amount showed higher effect on the DPPH free radical scavenging activity and also the enzyme to substrate ratio from the middle to upper amount increases the DPPH free radical scavenging activity.

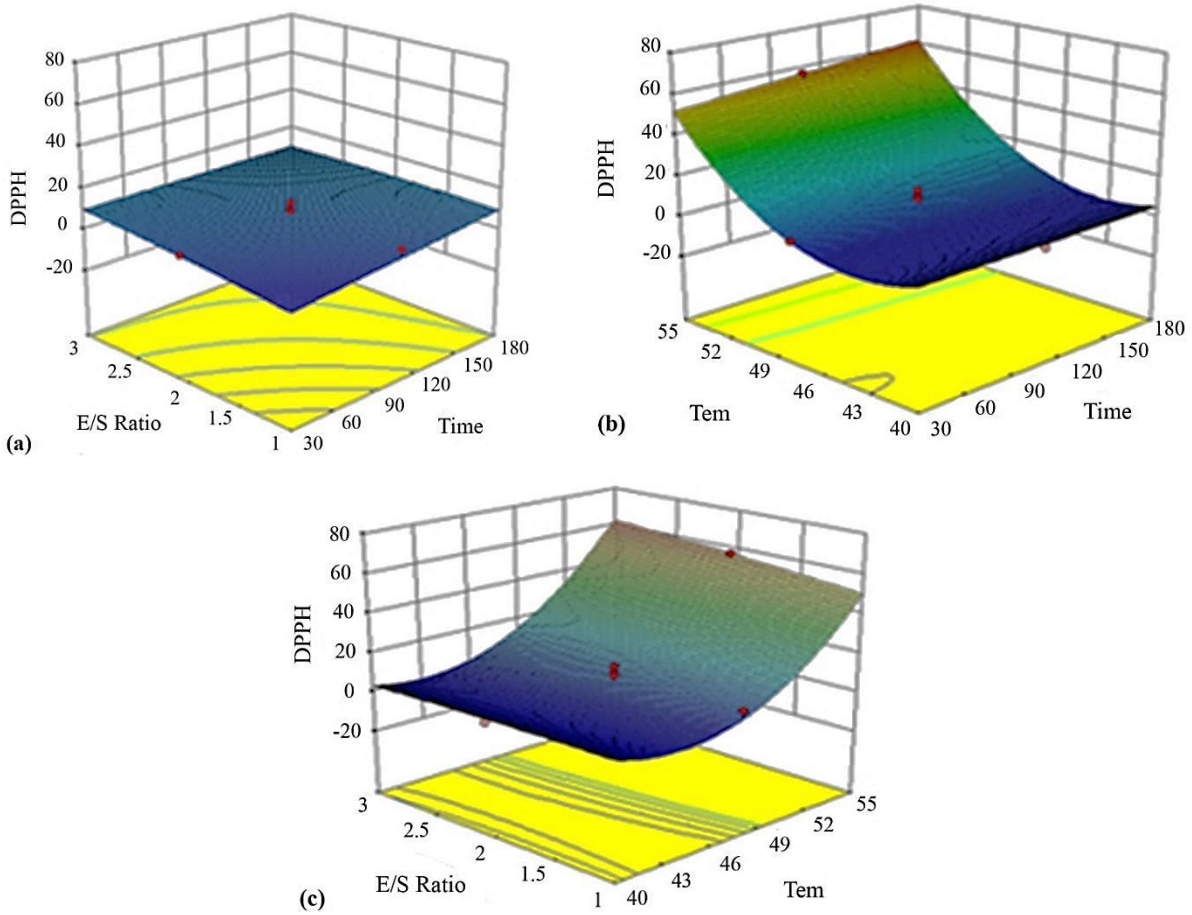

Fig. 1. Response surface plot for change in DPPH radical scavenging activity as affected by two different hydrolysis parameter when the third parameter is in optimal condition (a) E/S ratio versus hydrolysis time (b) hydrolysis time versus temperature (c) E/S ratio versus hydrolysis temperature

Conclusion

The results showed that optimal conditions for reaching the highest antioxidant activity were temperature 52.07 °C, time 125.49 min and ratio of enzyme to substrate ratio of 3 which under this condition DPPH free radical scavenging activity was 66.5%. The results showed that hydrolyzed sesame protein meal can be used in food formulation as a natural antioxidant.

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