Iranian Food Science and Technology Research Journal
Vol. 13, No. 1, Apr. May. 2017, p. 14- 26

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۳، شماره ۱، فروردین-اردیبهشت ۱۳۹۶ ص.۲۶-۱۴

The optimization of the production of anti-oxidative peptides from enzymatic hydrolysis of Pumpkin seed protein

E. Nourmohammadi[1], A.R. Sadeghimahoonak*[2], M. Ghorbani[2], M. Alami[2] and M. Sadeghi[3]

Received: 2015.03.17
Accepted: 2015.10.31

Introduction: Proteins are vital substances for health since they provide nitrogen, amino acids and the energy required for normal body performance. However, the applications of proteins are limited due to their certain properties, such as their low solubility. The enzymatic hydrolysis of proteins is an extensively used approach to produce bioactive peptides and promote the chemical, functional and nutritional properties of proteins. These compounds have interesting biological properties such as anti-oxidative, anti-hypertensive, anti-bacterial, anti-cancer and anti-thrombotic activities. Lipid peroxidation is one of the main reasons behind the deterioration of foodstuffs during processing and storage. In this case, the addition of anti-oxidative compounds is considered as an effective way to improve the shelf-life of lipid containing foods. Due to carcinogenic effect of synthetic anti-oxidative compounds, extensive efforts have been done to find natural anti-oxidative compounds with plant origin during recent years. Pumpkin (Cucurbitapepo) seeds are rich of proteins, unsaturated fatty acids, phytosterols and essential minerals like Zn, K, Ca, Mg, Fe, Cu and P. Oil content of pumpkin seeds is about 40-60%, and mostly consisted of oleic, palmitic and stearic acids. On the other hand, its protein content is about 45-46%, and this amount will reach to 55-56% after defatting. To date, pumpkin seeds have been mainly used for pumpkin oil production. After the oil extraction, a protein-rich by-product (pumpkin oil cake) remains, which is often used for animal feeding. In this study, the enzymatic hydrolysis of pumpkin oil cake protein isolate by a food-grade protease named trypsin was attempted and the optimum treatment was selected based on the DPPH radical scavenging and ferrous ion chelating activities

Materials and Methods: In this study, the optimization of the hydrolysis of pumpkin (*Cucurbitapepo)* oil cake protein was investigated using response surface methodology (RSM) and central composite design (CCD) in order to achieve the maximum DPPH radicals scavenging and metal ion chelating activities. For this purpose, trypsin concentrations of 1-2% and hydrolysis temperatures and times of 35-45 ċ and 2-5 hours were examinedas independent variables.

Preparations of pumpkin oil cake protein isolate (POCPI)

Defatted pumpkin oil cake was dispersed in distilled water (1:10 w/v). The pH was adjusted to 10 with 1N NaOH, mixed for 1 hour at room temperature and centrifuged at 5000g for 20 minutes (Combi514R, South Korea). The supernatant was collected, pH was adjusted to 5 with 1N HCl and centrifugation was performed at the same condition. Supernatant was discarded and precipitate was collected as pumpkin oil cake protein isolate.

Enzymatic hydrolysis

Pumpkin oil cake protein isolate was dispersed in tris-HCl at pH= 8 for trypsin enzymatic treatment (5% w/v). After that, trypsin was added at 1%, 1.5% and 2% and hydrolysis was carried out for 2, 3.5 and 5h at 200 rpm in shaker incubator (8480-VS, South Korea). Hydrolysis temperatures were 35, 40 and 45˚C. At the end of hydrolysis, the enzyme was inactivated for 15 minutes at 85˚C; dispersion was centrifuged at 4000g for 30 minutes, the supernatant was collected and freeze dried.

DPPH radical scavenging activity

An aliquot of 1000 microliterpumpkin oil cake proteinhydrolysate was mixed with 1000 microliter of 0.1mM DPPH solution prepared in 96% ethanol. The mixture was allowed to stand for 60 minutes in the dark and the

1. PhD graduated of Food Science, Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources.
2. Associate Professors, Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources.
3. Faculty Member, Isfahan Cardiovascular Research Center.
(*Corresponding Author Email: sadeghiaz@yahoo.com)

absorbance was read at 517 nm. The blank was prepared with the same manner except that 1000 microliter water was used instead of 1000 microliter pumpkin oil cake proteinhydrolysate.

Ferrous ion chelating activity

Pumpkin oil cake protein hydrolysate(4.7 ml) was mixed with 0.1 ml 2mM $FeCl_2$ and 0.2 ml 5 mM ferrozine and was kept at room temperature for 20 min. The absorbance was read at 562 nm and the blank sample was prepared with the same manner except that 4.7 ml distilled water was used instead of sample.

Results & Discussions: The results of this study, showed that the optimum conditions to reach the maximum DPPH radicals scavenging and metal ion chelating activities were 35 ċ, 5h, 1.1% enzyme concentration and 45 ċ, 2.05h and 2% enzyme concentration that showed DPPH radicals scavenging and metal ion chelating activities of 76.28% and 49.61% respectively. These results were to large extent similar to those suggested by Design Expert software (75.89% and 50.84%). The R^2 was 0.9184% and 0.9761% for DPPH radicals scavenging and metal ion chelating activities respectively. Moreover the adjusted R^2 was estimated to be 0.1333 and 0.1827 for DPPH radicals scavenging and metal ion chelating activities respectively, which suggested the suitability and fitness of proposed model for the considered responses.

Conclusions: Based on the results, pumpkin oil cake protein hydrolysate demonstrated appropriate anti-oxidative and metal ion chelating abilities. The results of this study indicated that pumpkin oil cake protein hydrolysate had the ability to be used as an effective and natural anti-oxidative compound in lipid containing foods.

Keywords: Pumpkin seed, enzymatic hydrolysis, trypsin, Response Surface Method