JRIFST

www.journals.rifst.ac.ir
Journal of Research and Innovation
in Food Science and Technology

Research Institute of Food Science and Technology

Volume 7, Issue 1, Spring 2018, Pages 49-64
Document Type: Extended Abstract
DOI: 10.22101/JRIFST.2018.05.19.714

Extended Abstract

Optimization of Enzymatic Hydrolysis of Bee Pollen Protein by Pepsin Based on Antioxidant and ACE Inhibitory Activity and Comparison with Those of Royal Jelly

Atefe Maqsoudlou[1], Alireza Sadeghi Mahoonak[2*], Mohamad Ghorbani[2], Fidel Toldra[3]

1- PhD Student, Department of Food Science & Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
2- Associate, Department of Food Science & Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
* Corresponding author (sadedeghiaz@gau.ac.ir)
3- Professor -Instituto de Agroquímica y Tecnología de Alimentos (IATA), Valencia, Spain

Received: 2016.11.21; **Accepted:** 2017.07.05

Abstract

In this study optimization of the enzymatic hydrolysis condition of bee pollen protein by pepsin and the effect of enzymatic hydrolysis on its antioxidant properties was investigated. Also, the results were compared with antioxidant properties of royal jelly. For this purpose, phenolic compounds, DPPH free radical scavenging activity and ferric ion reducing power of bee pollen and royal jelly were measured. The values of these factors for bee pollen and royal jelly (1000 ppm), were 174 and 1031.71 (mg Gallic acid/ g sample), 67.33% and 95.27% and absorbance of 0.77 and 0.8 at 700 nm, respectively. The highest reducing power of 0.81 was measured in samples which were hydrolyzed by 2% pepsin for 4 h. Also, the highest DPPH radicals scavenging power was 100% which was achieved in hydrolyzed samples by 2% pepsin for 2.5 h. The highest ACE inhibitory activity was 91.49% in hydrolyzed samples by 2% pepsine for 4 h. The results showed that the DPPH radical scavenging power of bee pollen increased from 67.33% to 100% and ACE inhibitory activity increased from 15.54% to 91.49% after enzymatic hydrolysis. The DPPH radical scavenging power and ACE inhibitory activity of bee pollen hydrolysats were comparable to those of royal jelly.

Keywords: ACE, Antioxidant Activity, Bee Pollen, Enzymatic hydrolysis, Royal Jelly

Introduction

Bee pollen, commonly referred as the ‘‘life-giving dust’’, results from the agglutination of flower pollens with nectar and salivary substances of the honeybees (Almeida *et al.*, 2016). Pollen contains 10 to 40% protein, 1 to 13% lipid, 13 to 55% carbohydrates and 2 to 6% minerals. Royal Jelly is produced by enzymatic digesting of bee pollen by proteases and other natural enzymes.It contains 27-41% protein, 30% carbohydrates , 8-19% lipids, minerals, trace elements and some vitamins based on dry weight (Morais *et al.*, 2011;

Bogdanow, 2014). The antioxidant properties of royal jelly and bee pollen, are related to main proteins and phenolic compounds and flavonoids (Nagai & Inue, 2004). The antioxidant activity of peptides includes DPPH, ABTS and hydroxyl radical scavenging activity, Ferric reducing, Ferrous chelating activity (Khantaphant *et al*., 2011). Antioxidant activity of pollen and royal jelly were investigated by different researchers (Bogdanov, 2014; Morais *et al.*, 2011; Nagai & Inoue, 2004). Also antioxidant activity and ACE inhibitory of peptides from anzymatic hydrolysates were reported by researchers (Salampessy *et al*., 2015; Marinova & Tchorbanov, 2010; Liu *et al*., 2008; Nagai *et al*; 2005; Wiriyaphan *et al.*, 2012; Khantaphant & Benjakul, 2008). The objective of present research is Optimization of enzymatic hydrolysis of bee pollen protein by pepsin based on antioxidant and ACE inhibitory activity and comparison with those of royal jelly.

Material and methods

Preparation of extracts

The preparation of the bee pollen extract was performed by mixing the bee pollen using water (1:10) (w/v), on the magnetic stirrer at room temperature for 24 h. The macerates were filtered through filter paper (Whatman) in a Buchner funnel. The obtained extract was centrifuged at 12000 g, then, the obtained supernatant was dried using freeze dryer. The dried bee pollen extract was kept in the dark at room temperature until further analysis (Morais *et al*., 2011). The royal jelly extract were prepared using method as described by (Liu *et al*., 2008).

Determination of total phenolic contents

The total phenolic content of the extracts was recorded using the Folin–Ciocalteu method as described by (Deshpande *et al*., 1987).

DPPH radical-scavenging

DPPH radical-scavenging activity was determined as described by Hmited *et al*. (2011).

Reducing power

The ability of the hydrolysate to reduce iron (III) was determined according to the method of Bougatef *et al*. (2009).

Enzymatic hydrolysis of pollen

Bee pollen was added and homogenized with 5 volumes of distilled water. pH and temperature of the solution were adjusted to pH=3 and 37°C. Enzymes in the concentration range of 1 to 2% w/w were added to the pollen protein solution. Enzymatic hydrolysis lasted 2 to 5 h. Hydrolysis was stopped by boiling for10min.The hydrolysates were centrifuged at 4000x g for 30 min to remove the residue. The supernatants were pooled and then lyophilized (Villanueva *et al*., 1999; Matsuoka *et al*., 2012; Guo *et al*., 2005).

Antioxidant activity and ACE inhibitory of hydrolysates

DPPH radical scavenging ability and reducing power of pollen hydrolysates of pollen hydrolysates were measured. Also ACE-inhibitory activity of pollen hydrolysates was measured that was assayed as reported by Nasri *et al*. (2004).

Statical analysis:

Statical analysis of results before hydrolysis was done by SPSS. Optimization of enzymatic hydrolysis was done by Response Surface Methodology (RSM) in Design Expert software.

Results and discussion

Phenolic compounds

Total phenol value recorded for pollen ranged from 48.15 to 174 mg Gallic acid/g for royal jelly ranged from 9.24 to 87.261 mg Gallic acid/g. Considering that royal jelly is obtained by direct digestion of pollen, the amounts of their phenolic compounds were comparable (Bogdanov, 2014). Phenolic compounds increased by increasing concentration of extract of royal jelly and pollen. Increasing in royal jelly was more than pollen in concentrations of 300 to 1000 mg/L (P<0.05).

Determination of ferric reducing/antioxidant power

Different concentrations of pollen and royal jelly extracts showed various reducing power respectively between 0.29 to 0.8 and 0.74 and 0.77.

DPPH radical scavenging

The lowest and highest DPPH radical scavenging were 1.05 and 67.33 in pollen and 3.75 and 95.27 in royal jelly. DPPH radical scavenging of pollen and royal jelly increased significantly by increasing concentration of extracts (P<0.05). There was significant difference in DPPH radical scavenging of extracts with the highest concentrations (P<0.05). DPPH radical scavenging of phenolic extracts was increased by increasing of their concentration. Royal jelly demonstrated more DPPH radical scavenging activity than pollen that was attributed to its proteins and peptides and their side chains.

Optimization of enzymatic hydrolysis of pollen and anti-oxidant test

The results of the antioxidant activity tests of hydrolysed pollen in the points determined with the central composite design in the RSM are given in Table (1).

Table 1. Results of the antioxidant activity tests of hydrolysed pollen in the points determined with the central composite design in the RSM

Treatment	Enzyme concentration (%)	Time (hr)	DPPH radical scavengering (%)	Reducing power (Absorbance at 700 nm)	ACE inhibitory activity (%)
1	2	1	34.09	0.68	34.09
2	1.5	1	96.51	0.65	35.58
3	1	1	88.58	0.75	67.74
4	2	2.5	100	0.68	44.3
5	1.5	2.5	91.71	0.61	27.93
6	1.5	2.5	93.64	0.57	28.93
7	1.5	2.5	92.71	0.61	27.93
8	1.5	2.5	94.64	0.57	27.93
9	1.5	2.5	95.71	0.61	27.93
10	1	2.5	91.02	0.57	44.22
11	2	4	71.88	0.81	91.49
12	1.5	4	66.69	0.74	58.24
13	1	4	67.24	0.73	57.67

When increasing enzyme concentration and hydrolysis time, the reducing power of hydrolysate was fixed then it was increased, the DPPH radical scavengering of hydrolysate was increased then it was decreased, the ACE inhibitory activity of hydrolysate was decreased then it was increased. Hydrolysate using 2% enzyme concentration in the time of 4 h gave the highest reducing power of absorbance (0.81) and the highest DPPH radical scavengering

(91.49%). Hydrolysate using 2% enzyme concentration in the time of 2.5 h gave the highest DPPH radical scavengering (100%).

Optimization of hydrolysis condition based on antioxidant activity and ACE inhibitory of hydrolysates by RSM

Predicted amounts by RSM and measured amounts are summarized in Table (2).

Table 2. Predicted amounts by RSM and measured amounts

Measured Factores	Predicted Amounts	Measured Amounts	P value
DPPH radical scavengering (%)	71.51	73.71	0.178
Reducing power (Absorbance)	0.8	0.71	0.362
ACE inhibitory activity (%)	91.316	92.793	0.188

Conclusion

In generally it was clear that antioxidant activity and ACE inhibitory of pollen increased by hydrolysis. After hydrolysis DPPH radical scavenging power and ACE inhibitory activity of bee pollen hydrolysates were comparable to those of royal jelly.

References

Almeida, J.F., Reis, A.S., Heldt, L.F.S., Pereira, D., Bianchin, M., Moura, C., Plata-Oviedo, M.V., Haminiuk, C W.I., Ribeiro, I.S., Luz, C.F.P., & Carpes, S.T. (2016). Lyophilized bee pollen extract: A natural antioxidant source to prevent lipid oxidation in refrigerated sausages. *LWT - Food Science and Technology*, *76*(B), 299-305. doi: https://doi.org/10.1016/j.lwt.2016.06.017

Bogdanov, S. (2014). Royal jelly, bee brood: composition, health, medicine: a review. *Bee Product Science*, *28*(3), 118-153.

Bougatef, A., Hajji, M., Balti, R., Lassouedl, I., Triki-Ellouz, Y., & Nasri, M. (2009). Antioxidant and free radical-scavenging activities of smoth hound muscle protein hydrolysates obtained by gastro intestinal proteases. *Food Chemistry*, *114*(4), 1198-1255. doi: https://doi.org/10.1016/j.foodchem.2008.10.075

Deshpande, S., Chryan, M., & Salunkhe, D. (1987). Tanin analysis of food products. Critical Review in *Food Nutrition, 24*, 41-49. doi: https://doi.org/10.1080/10408398609527441

Guo, H., Kozuma, Y., & Yonekura, M. (2005). Isolation and properties of antioxidative peptides from water-soluble royal jelly protein hydrolysate. *Food Science and Technology Research*, *11*(2), 222-230. doi: https://doi.org/10.3136/fstr.11.222

Hmidet, N., Balti, R., Nasri, R., Sila, A., Bougatef, A., & Nasri, M. (2011). Improvement of functional properties and antioxidant activities of cuttlefish (*sepia officinalis*) muscle proteins hydrolyzed by bacillus mojavensis A21 proteases. *Food Research International*, *44*(9), 2703-2711. doi: https://doi.org/10.1016/j.foodres.2011.05.023

Khantaphant, S., & Benjakul, S. (2008). Comparative study on the proteases from fish pyloric caeca and the use for production of gelatin hydrolysate with antioxidative activity. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *151*(4), 410-419. doi: https://doi.org/10.1016/j.cbpb.2008.08.011

Khantaphant, S., Benjakul, S., & Kishimura, H. (2011). Antioxidative and ACE inhibitory activities of protein hydrolysates from the muscle of brownstripe red snapper prepared using pyloric caeca and commercial proteases. *Process Biochemistry*, *46*(1), 318-327. doi: https://doi.org/10.1016/j.procbio.2010.09.005

Liu, J.R., Yang, Y.C., Shi, L.S., & Peng, C.C. (2008). Antioxidant properties of royal jelly associated with larval age and time of harvest. *Journal of Agriculture and Food Chemistry*, *56*(23), 11447-11452. doi: https://doi.org/10.1021/jf802494e

Marinova, M.D., & Tchorbanov, B.P. (2010). Preparation of antioxidant enzymatic hydrolysates from honeybee-collected pollen using plant enzymes. *Enzyme Research*, *41*, 5949-50. doi: http://dx.doi.org/10.4061/2010/415949

Extended Abstract

Matsuoka, T., Kawashima, T., Nakamura, T., Kanamaru, Y., & Yabe, T. (2012). Isolation and characterization of proteases that hydrolyze royal jelly proteins from queen bee larvae of the honeybee, *Apis mellifera*. *Apidologie, 43*(6), 685-697. doi: https://doi.org/10.1007/s13592-012-0143-z

Morais, M., Moreira, L., Feás, X., & Estevinho, L.M. (2011). Honeybee-collected pollen from five portuguese natural parks: Palynological origin phenolic content antioxidant properties and antimicrobial activity. *Food and Chemical Toxicology*, *49*(4),1096-1101. doi: https://doi.org/10.1016/j.fct.2011.01.020

Nagai, T., & Inoue, R. (2004). Preparation and the functional properties of water extract and alkaline extract from royal jelly. *Food Chemistry, 84*(2), 181-186. doi: https://doi.org/10.1016/S0308-8146(03)00198-5

Nagai, T., Inoue, R., Suzuki, N., Myoda, T., & Nagashima, T. (2005). Antioxidative ability in a linoleic acid oxidation system and scavenging abilities against active oxygen species of enzymatic hydrolysates from pollen Cistus ladaniferus. *International Journal of Molecular Medicine, 15*(2), 259-263. doi: https://doi.org/10.3892/ijmm.15.2.259

Salampessy, J., Reddy, N., Kailasapathy, K., & Phillips, M. (2015). Functional and potential therapeutic ACE-inhibitory peptides derived from bromelain hydrolysis of trevally proteins. *Journal of Functional Foods, 14*, 716-725. doi: https://doi.org/10.1016/j.jff.2015.02.037

Villanueva, A., Vioque, J., Sánchez-Vioque, R., Clemente, A., Pedroche, J., Bautista, J., & Millán, F. (1999). Peptide characteristics of sunflower protein hydrolysates. *Journal of the American Oil Chemists Society, 76*(12), 1455-1460. doi: https://doi.org/10.1007/s11746-999-0184-2

Wiriyaphan, C., Chitsomboon, B., & Yongsawadigul, J. (2012). Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. *Food Chemistry, 132*(1):104-111. doi: https://doi.org/10.1016/j.foodchem.2011.10.040

Extended Abstract