Effect of Heating on Antioxidant Activity on Edible Bird Nest

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ABSTRACT

Edible bird nest (EBN) is a traditional Chinese food that is nutritious and therapeutic since ancient times. This study found that raw EBN has crude protein content of 58.28%. The aim of this research is to study the effect of heat treatment with different time (30 min, 60 min, 120 min and 180 min) and temperature (60°C, 80°C and 100°C) on the protein solubility, degree of hydrolysis (DH), peptide content and also the antioxidant activity of EBN analyzed through DPPH, ABTS and FRAP assays. According to the results obtained, protein solubility (3,302.56 μg/ml), DH (0.95%), peptide content (820.33 μg/ml), DPPH (46.15%), ABTS (94.21%) and FRAP (3.581 mgTEAC/g) activities of EBN hydrolysates showed the highest values at temperature 100°C for 180 min. This study showed that the positive results increased when the heating time and temperature applied were increased until the optimum level had reached at 100°C with duration time of 120 min- 180 min.In conclusion, EBN has the potential to be used in the production of innovative product from the point of its fucntional antioxidant properties that can benefit the consumer's health.

Keyword: Edible bird's nest, heat treatment, antioxidant

INTRODUCTION

Edible bird's nest is known as "yan wo (燕窝)" in Chinese, "edible bird's nest (EBN)" in English or "ensou" in Japanese. Edible bird nest is made by swallows, especially species of the genus *Collocalia*. Edible bird nest is often used in traditional Chinese medicine and Chinese cuisine. The most commonly eaten in the form of bird's nest soup and also been added into variety of dishes. EBN somewhat scarce, making them expensive and vulnerable to fraud. Edible bird nest plays an important role in traditional Chinese medicine which is to improve health, such as improving the quality of the skin, reducing symtomps of asthma, and strengthens the immune system (Cranbrook 2002). More than half of the composition of the bird's nest compose of protein (Marcone 2005), the composition of the EBN are: 0.14 -1.28% lipid, ash 2.1%, 25.62-27.76% carbohydrates and 62-63% protein. The main bioactive

component in EBN is the glycoproteins. Traditionally, EBN is cooked by soaking in boiling water and cooked with rock sugar and red dates according to one's preference. EBN was cooked with a double boiling method with various time and temperature depending on the types of EBN. Prolong boiling may effect EBN in terms of its functionality and bioactivities but there are still less research on the effect of time and temperature during boiling of EBN to its antioxidant activity. This study was focussed on the study of the effect of heat treatment at different time and temperatures on antioxidant activity of EBN.

METHODOLOGY

EBN was obtained from bird's house around Pahang, Malaysia and was supplied clean. The bird nest obtained was stored in air tight containers and kept at ambient temperature until further analysis. For the first part, EBN samples was prepared by direct boiling and double boiling at 3 different temperature (60°C, 80°C and 100°C) in five duration time (30 minutes, 60 minutes, 120 minutes, 180 minutes and 240 minutes). Next is the analysis such as protein content, protein solubility, degree of hydrolysis, measurement of peptides content and antioxidant activity were carried out on the EBN samples.

Protein Content. Determination of protein content was measured using Kjeldahl method (AOAC 1990).

Protien Solubility. Soluble protein content was determined using Bradford assay proposed by Bradford (1976) with a slight modification on the ratio (1:4). Standard curve was prepared using Bovine Serum Albumin (BSA) as a standard.

Degree of Hydrolysis and Peptide Content. Degree of hydrolysis and peptide content of EBN was determined using o-phthaldialdehyde (OPA) by Church et al. (1983) with a slight modification.

Antioxidant Activity. Antioxidant activity was determined using three different methods; 1,1- diphenyl-2-picrylhydrazyl (DPPH) as proposed by Brand-Williams *et al.* (1995), ferric reducing antioxidant power (FRAP) reported by Benzie dan Strain (1996) and 2,2'-Azinobis-(3-Etilbenzthiazolin-6-Acid Sulphonic) as proposed by Re *et al*. (1999).

Statical Analysis. Data obtained were analysed statistically with analysis of variance and Duncan test using SPSS Version 20 (SPSS 2011) to identify the significance difference among the samples (*$p<0.05$).

RESULTS AND DISCUSSION

On this study, the protein content of the edible bird nest was at 58.28% and this result is comparable to previous studies as shown in Table 1.1. Based on the studies, it can be concluded that the protein content is the range of 58 – 65%. The protein content in EBN samples may differ due to the harvesting season, habitat and the diet of swiflets. Based on the study conducted by Norhayati *et al*. (2010), EBN harvested in peninsular Malaysia during rainy season contains more protein compared to other seasons. Besides that, according to Burhanuddin (2004), EBN harvested in industrial areas contains less protein compared to non-industrial areas. This may be because industrial areas may contributed to less insect population which is the main protein diet to the swiflets.

Table 1.1 Protein content of EBN from various studies

Sample	Protein (%)
Edible Bird Nest (EBN)	58.28
Marcone (2005)	62.0 to 63.0
Zainab et al. (2013)	59.8 to 65.8
Utomo et al. (2014)	57.49
Halimi et al. (2014)	58.5

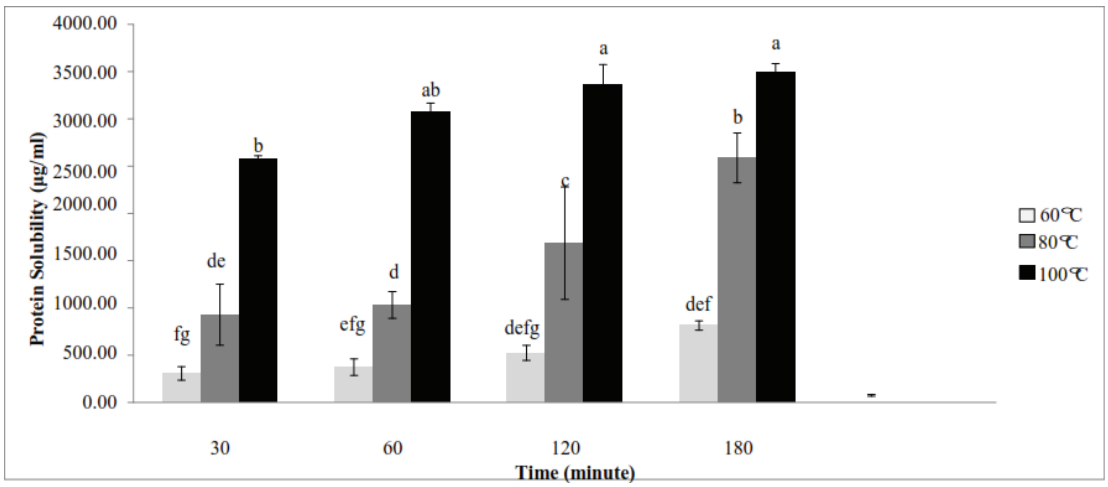

Figure 1.1 Protein solubility of EBN treated with different time and temperature

Based on Figure 1.1, as the hydrolysis time and temperature increases, the solubility of protein also increases. Regardless all three heat treatment temperatures, 180 minute of boiling time recorded the highest protein solubility followed by 120 minutes. Meanwhile, the temperature of 100℃ recorded the highest solubility throughout all duration from 30 – 180minute. According to Csapo *et al*. (2008), increase in hydrolysis time on protein causes

the release higher amount of amino acids and short chain peptides that improves the solubility of the hydrolysate. Besides that, prolong hydrolysis time and high temperature causes protein degradation and produces more soluble by-products (Chobert et al., 1988; Linder et al., 1996; Gbogouri et al., 2004;).

The highest DH was recorded at 100°C, 180 minutes with a value of 0.95%. The higher the hydrolysis time and temperature, more peptides bonds will be broken and producing smaller sized molecules. According to Mukhin and Novikov (2001), hydrolysis rate increase with the increase of temperatures and at low temperature, the hydrolysis rate decreases and reducing the protein solubility. By observing the effect of hydrolysis time on DH, hydrolysis time at 180 minutes was seen to record the highest DH and hydrolysis time at 30 min recorded the lowest DH at all temperature. This may be due to the limited response time during heat treatment where the boiling at 30 min causes inefficient and incomplete cleavage of peptide bond.

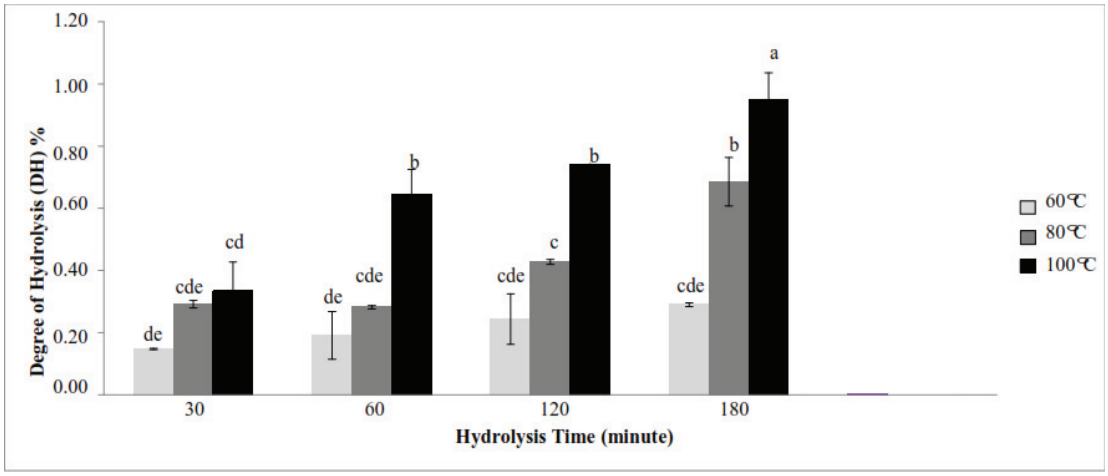

Figure 1.2 Degree of hydrolysis (DH) of EBN treated with different time and temperature

Table 1.2 Peptide content of EBN treated with different time and temperature

Hydrolysis Time (min)	Temperature (°C)		
	60	80	100
30	5.33±2.89^{e}	30.33±7.64^{e}	147.00±16.39^{d}
60	10.33±8.78^{e}	56.17±12.58^{e}	250.33±32.60^{c}
120	16.17±3.82^{e}	193.67±58.60^{cd}	805.33±31.30^{a}
180	26.17±23.80^{e}	384.50±37.50^{b}	820.33±66.90^{a}

Peptide content analysis have been conducted to quantify peptides in samples which are responsible for the antioxidant effect. When the hydrolysis time and temperature increases, the peptide content also increases. Peptide content had the highest amount at

100°C, 180 minute of boiling time. According to Morais *et al*. (2013), in his study, the increase in hydrolysis time can produce higher amount of short chained peptides and free amino acids. He also reported that there was significant correlation between DH and peptide content in the protein samples. Based on this study, EBN samples that subjected to highest temperature and longest boiling, had the highest DH and produces higher peptide content which also correlates to better protein solubility.

The DPPH, ABTS and FRAP antioxidant activity of EBN at 100 ° C, 120 min showed significantly the highest activity, which was at 43.90%, 93.68% and 3.440 mgTEAC / g respectively and there is no significant difference ($p>0.05$) observed between antioxidant activity at 100 ° C, 180min. Therefore, this study also proved that with proper treatment, EBN able to provide a good source of protein, peptides with then translated to high antioxidant activity.

Table 1.3 Antioxidant activity of EBN treated with different time and temperature

Hydrolysis Time (min)	Temperature (°C)								
	DPPH %			ABTS %			FRAP mg TEAC/g		
	60	80	100	60	80	100	60	80	100
30	5.07± 0.58^{de}	4.92± 0.55^{de}	8.80± 0.24^{cd}	76.30± 0.45^{f}	85.13± 2.41^{de}	89.15± 0.59^{c}	0.027± 0.01^{g}	0.914± 0.05^{e}	1.454± 0.02^{d}
60	6.37± 0.26^{de}	7.04± 0.48^{de}	17.57± 4.72^{b}	82.62± 1.16^{e}	88.71± 0.61^{c}	91.18± 0.69^{bc}	0.062± 0.01^{g}	1.869± 0.14^{c}	1.896± 0.12^{c}
120	9.85± 0.88^{cd}	14.24± 1.22^{bc}	43.90± 7.05^{a}	85.82± 0.18^{d}	91.18± 0.18^{bc}	93.68± 0.52^{ab}	0.319± 0.01^{f}	2.688± 0.01^{b}	3.440± 0.07^{a}
180	11.82± 0.40^{bcd}	17.78± 0.44^{b}	46.15± 4.01^{a}	90.13± 0.45^{c}	93.77± 0.62^{ab}	94.21± 0.30^{a}	0.397± 0.03^{f}	3.574± 0.04^{a}	3.581± 0.02^{a}

CONCLUSION

This study showed that EBN treated at high temperature and extended hydrolysis time produces better quality EBN hydrolysate. Therefore, further studies have to be conducted with much higher temperature and longer hydrolysis time to estimate the time at temperature of functional degradation of EBN

ACKNOWLEDGEMENTS

The authors would like to thank to Universiti Kebangsaan Malaysia (UKM) towards the research and findings of this paper and Innovation Center for Confectionary Technology (MANIS) for laboratory facilities. The financial support by Dana Inovasi ST-2016-013 is gratefully acknowledged

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