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Characterization of Freeze- and Spray- Dried Edible Bird's Nest Hydrolysates

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Abstract

The purpose of this study was to investigate the physicochemical properties of freeze-dried (FD) and spray-dried (SD) EBN hydrolysates. Raw uncleaned (RUC) EBN and by-products of EBN are enzymatically hydrolyzed with bromelain to obtain the EBN hydrolysates. Scanning electron microscopy (SEM) was used to study the structure while the nitrite content, antioxidant activity, total sialic acid and total polysaccharide were determined to study their chemical properties. SEM images revealed similar microstructures between the two FD hydrolysates produced from different raw material. On the other hand, SEM images showed different microstructures between SD and FD hydrolysates produced from the same raw material (by-product of EBN) but dried with different drying methods.All samples contained less than 30 ppm nitrite. All EBN hydrolysates exhibit antioxidant activity (DPPH free radical scavenging activity: 10.81 -19.48%; ABTS free radical scavenging activity: 96.39 -99.14%) and contain high sialic acid content (17.47 – 19.13%).

Keywords: Edible bird's nest, Enzymatic hydrolysis, Sialic acid, Scanning electron microscope.

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1. Introduction

Edible bird's nests (EBN) are built by swiftlets with the saliva they regurgitate [1]. The nutritional value and pharmacological activities of bird's nest have been proven by scientific research, such as: anti-viral; anti-aging; improving learning and memory; combating oxidative and nitrosative stress in Parkinson's disease; and acting as a chondro protectant [2–8]. Raw uncleaned (RUC) EBNs are EBNs harvested from farms either caves or swiftlet house, are raw material for all EBNs product. Raw cleaned (RC) EBNs are EBNs that have undergone cleaning processes. Basically, RC EBNs refers to product after primary process, the steps are: sort RUC EBN- soaking softening- cleaning impurities and picking of feathers - shaping- drying- sterilizing -grading and packing [1]. The primary process is time consuming, especially the picking step.

Picking step is an important step as it controls the cleanliness and recovery of the RC EBN product. Primary process is a process that is dependent on labor, the experience, and the skill of the staff as these factors directly affect the product cleanliness and recovery rate from RUC EBN to RC EBN. EBN hydrolysates were defined as small biomolecules broken down from EBN by physical, chemical, or enzymatic treatments (biotechnology process) [9–14]. EBN hydrolysates are used in downstream products. The raw material for this biotechnology process is the RC EBN [15–17]. Recently, a study was reported on the use of enzymatic hydrolysis for recovering the glycopeptide from EBN by-product (consists of EBN residues, dirt, feathers, and foreign matters) [18]. The study showed that the EBN hydrolysates have antioxidants and probiotic activity. The sialic acid content and antioxidant activity of EBN have also drawn research attention.

EBN hydrolysate showed higher sialic acid content and antioxidant activity compared to RC EBN (after primary processing) [16,19]. Spray drying and freeze drying are commonly employed techniques in the food industry to transform liquid substances into powdered form [20]. These methods contribute significantly to food preservation, enhance product stability, and simplify transportation and storage without the necessity of refrigeration [21]. Spray drying is the predominant technology for converting liquid feed into powder due to its cost-effectiveness. This process involves the transformation of liquid materials into powders via a spray dryer's atomizer, where they come into contact with hot air, potentially altering their composition. Conversely, freeze drying employs ice sublimation under reduced pressure to rapidly freeze products while maintaining their high food quality. The porous structure of lyophilized materials promotes swift rehydration, preserving the nutritional content and sensory attributes of the product, including flavor compounds.

Despite these advantages, freeze drying is not widely adopted in the drying industry because it is energy-intensive, resulting in higher production costs [20-21]. In this study, we performed enzymatic hydrolysis (as cleaning process) on heavy feather RUC EBN and EBN by-products. We produce EBN hydrolysate from heavy feather RUC EBN without primary processing in order to reduce processing waste and reliance on manpower. On the other hand, EBN hydrolysate produced from by-product EBN is a potential value-added product. The purpose of this study was to understand freeze-dried and spray dried EBN hydrolysates through structural and chemical characterization and to determine the quality of the product/ EBN hydrolysate.

2. Materials and Methods

The raw materials (cup shaped RUC EBN with heavy feather and by- product of EBN) were supplied by Think Birdnest Sdn. Bhd. (Malaysia). The EBN hydrolysate was prepared using a combination of heat treatment and enzymatic hydrolysis with bromelain enzyme (Brand: NOW, USA). The raw materials were ground into powder using mortar then weighed, washed, and soaked with distilled water. It was then double boiled at 100 °C for 30 min. Subsequently, the sample was cooled to 50 °C. Bromelain (2400 GDU/g) was added to the samples after cooling at 0.6% of EBN dry weight. Then, it was enzymatically hydrolyzed at 50 °C for 1 hour. After enzymatic hydrolysis, the sample was filtered, and the filtrate was EBN hydrolysate. The hydrolysate was then double boiled at 80 °C for 20 min to denature the bromelain. The hydrolysates were subsequently freeze-dried (FD) or spray-dried (SD).

Freeze-dried and spray-dried EBN hydrolysates were characterized for structural properties, nitrite content, antioxidant activity, total sialic acid content, and total polysaccharide content. Scanning electron microscopy (SEM) was used to study the structural properties of EBN samples. EBN sample was stuck to the carbon tape and evenly coated with platinum. The sample was studied under a field emission SEM (JEOL, JSM-7600F, Japan). Nitrite content analysis was done as follow: EBN hydrolysate 0.4 g was mixed with 20 ml distilled waster, then incubated at 80 °C for 30 mins. İt was then centrifuged at 8, 000 rpm, 5 mins.

5 ml of supernatant (as sample) was added with 1 level blue microspoon ready-to-use NO_2-1 reagent (Cat. No. 1.14776.0001 Merck Millipore). The mixture was then shaken to dissolve the reagent, and incubated at room temperature for 10 minutes. Absorbance was measured at 543 nm. Nitrite standard solution was used as standard. 2,2-di (4-tertoctylphenyl)-1-picrylhydrazyl (DPPH) and 2,2'-Azinobis-(3-Etilbenzthiazolin-6-Asid Sulphonic) diammonium salt (ABTS) free radical scavenging activity were used to study the antioxidant activity of the EBN hydrolysate. DPPH assay: 1 ml of EBN sample (EBN weight: 11.36 mg/ml) was added to 14 ml of DPPH reagent (0.036 mM). Distilled water was used as blank, and for the control sample, distilled water was used instead of EBN. The mixture was incubated in the dark for 30 min. The sample/control was then filtered with a PTFE syringe filter (0.45 µm) after reaction. DPPH absorbance was read at 517 nm. ABTS assay: 0.2 ml of sample (11.36 mg/ml) was added to 1.8 ml of ABTS reagent (absorbance= 0.7 ± 0.2, at 734 nm).

Distilled water was used as blank, and for the control sample, distilled water was used instead of EBN. The mixture was incubated in the dark for 10 min. The sample/control was then filtered with a PTFE syringe filter (0.45 µm) and measured at 734 nm. The periodate-resorcinol assay [16,22] was used to analyze the total sialic acid content in the sample. 2 mg/ml sample was used in this assay. Absorbance was measured at 580 nm (SHIMADZU UV-VIS Spectrophotometer mini-1240, Japan) and N-Acetylneuraminic acid (analytical standard) was used as standard for this assay. The phenol-sulfuric acid method [16,23] was used to analyze the total polysaccharide content in the sample (2.0 mg/mL). Glucose monohydrate was used as standard. The sample absorbance was read at 490 nm (SHIMADZU UV-VIS Spectrophotometer mini-1240, Japan).

3. Results and Discussions

The EBN hydrolysate produced by (1) RUC EBN was named EBN_{rh}; and (2) by- product of EBN was named EBN_{ch}. Figure 1 shows the SEM micrograph of the EBN hydrolysate. Two freeze-dried hydrolysates (different raw material), FD EBN_{rh} and FD EBN_{ch}, showed similar microstructures. On the other hand, EBN hydrolysates from the same raw material (by-product of EBN) but with different drying techniques were different in microstructure. The SD hydrolysate exhibited round and smaller particles (**Fig. 1e and f**), which may be due to atomization during spray drying, resulting in smaller round atomized droplets. The FD hydrolysate appeared in the form of flakes and larger particles (**Fig. 1a–d**), which may be due to the lack of force in the freeze-drying method to break up the frozen liquid into droplets during evaporation [20]. Similar microstructures were observed for SD and FD egg white powders [20]. Compared with fish skin hydrolysate powder, both FD hydrolysates have similar microstructures, but were different from SD hydrolysate microstructure. SD EBN hydrolysate showed a more rounded morphology [24]. No contaminants such as mites, feathers and pollutants were observed in the hydrolysates.

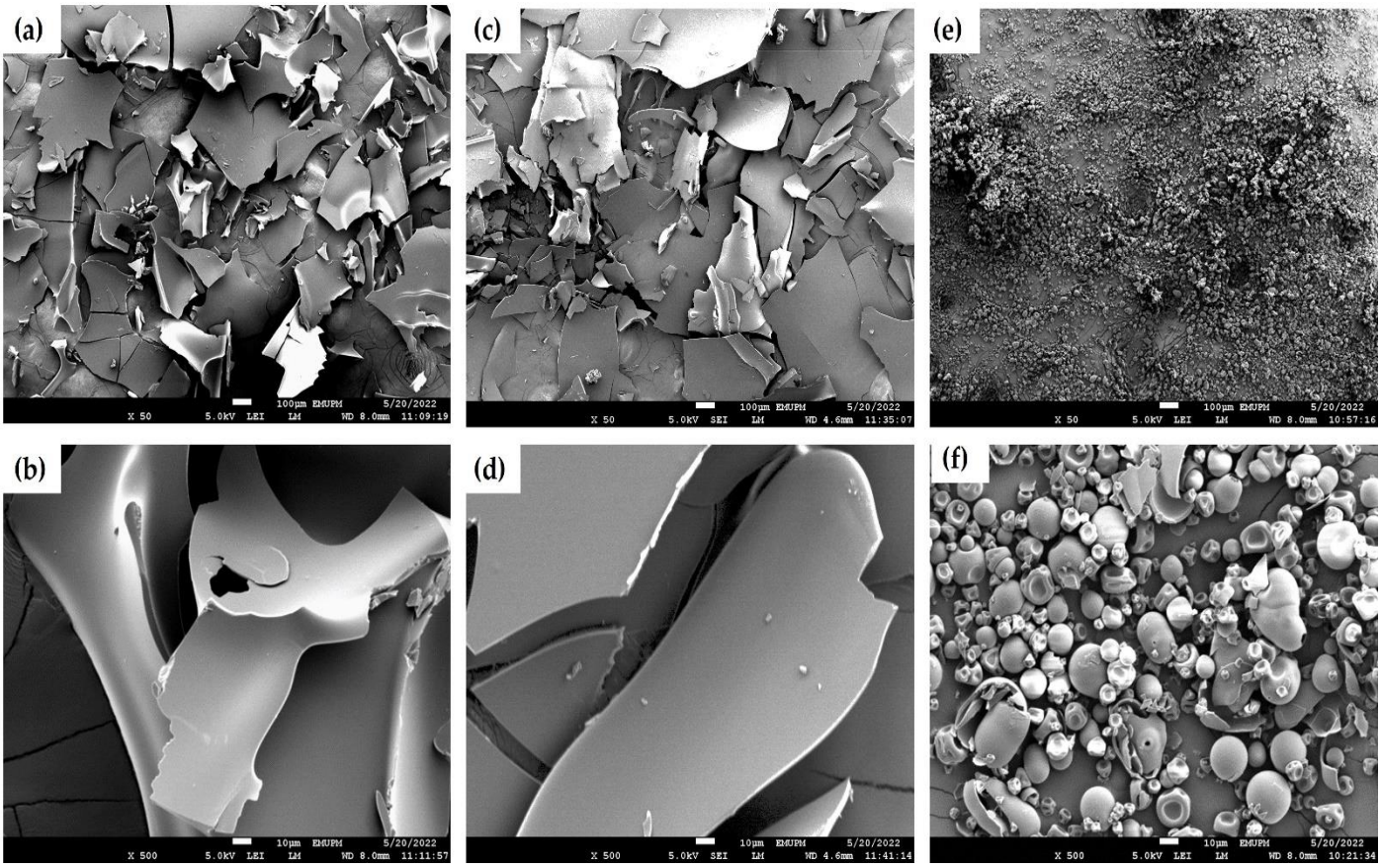

Fig. 1. SEM micrograph of FD EBN_{rh} at magnification of (a) 50x, (b) 500x; FD EBNch at magnification of (c) 50x, (d) 500x; and SD EBN_{ch} at magnification of (e) 50x, (f) 500x.

Table 1. Chemical properties of EBN hydrolysates (Mean ± SE)

Sample	Nitrite (ppm)	DPPH Free Radical Scavenging Activity (%)	ABTS Free Radical Scavenging Activity (%)	Total Sialic Acid Content (%)	Total Polysaccharide Content (%)
FD EBN_{rh}	12.02 ± 0.43^a	19.48 ± 0.63^a	99.14 ± 0.61	19.13 ± 0.43	15.48 ± 0.21^a
FD EBN_{ch}	6.62 ± 0.10	10.81 ± 0.62^b	96.39 ± 0.87	18.67 ± 0.20	11.04 ± 0.17^b
SD EBN_{ch}	$< UDL^b$	17.50 ± 0.41	97.34 ± 0.22	17.47 ± 0.21	13.75 ± 0.55

UDL: under detection limit. Superscript a means significantly higher in the same analysis ($P < 0.05$), while superscript b means significantly lower ($P < 0.05$).

Table 2. Comparison of the total sialic acid and total polysaccharide content with published literature

Sample/ parameters	RC EBNs (from primary processing) [25]	EBN hydrolysate [11, 16, 26]	This study
Sialic acid (%)	2.69 - 14.52	15.00 – 22.40	17.47 – 19.13
Total Polysaccharide (%)	1.74 – 8.73	9.74 – 26.50	11.04 – 15.48

Table 1 shows the chemical properties of EBN hydrolysates. Table 2 shows the comparison of EBN quality between the EBN hydrolysates from this study and those from previous studies [11, 16, 26], as well as the comparison between the EBN hydrolysates from this study and the RC EBNs from a previous study [25]. In EBN, sialic acid serves as a distinctive component that consumers consistently use to assess the purity and quality grade of EBN. **Table 2** shows the EBN hydrolysates produced in this study had higher total sialic acid content and total polysaccharide content compared to RC EBN reported in previous studies and were comparable to the EBN hydrolysates produced in those previous studies. The RC EBNs was from various grades of EBN products produced from primary processing.

Table 1 shows that the sialic acid content in FD EBN_{ch} was higher but not significantly different ($P > 0.05$) in comparison to that of SD EBN_{ch}. This finding is similar to that of Chong et al. where there was no significant difference in the total sialic acid content of EBN with different drying methods [11]. They suggested that this may be due to the thermal stability of sialic acid. DPPH and ABTS radical scavenging activity were used to study the antioxidant activity of EBN hydrolysate. FD EBN_{rh} has the highest antioxidant activity (DPPH and ABTS free radical scavenging activity). Compared with FD EBN_{ch}, the DPPH radical scavenging activity of SD EBN_{ch} was significantly higher ($P < 0.05$), while there was no significant difference in ABTS radical scavenging activity ($P > 0.05$) between the two samples. This finding is similar to that of Dong et al., that showed that the DPPH radical scavenging activity of SD fish skin hydrolysate (SPH) was significantly ($P < 0.05$) higher than that of FD SPH, while there was no significant difference in the radical scavenging activity of ABTS between the 2 samples [24].

Gan et al. showed the same results for ABTS radical scavenging activity, where SD EBN hydrolysate was slightly higher than FD EBN hydrolysate, but not significantly different [27]. However, results were different for DPPH radical scavenging activity, where studies showed that FD EBN hydrolysate was significantly higher ($P < 0.05$) than SD EBN hydrolysate. More research needs to be done to draw conclusions about the antioxidant activity after different drying methods. Nitrite content < 30 ppm in EBN is one of the quality requirements for raw RC EBN exported to China [1]. FD EBN_{rh} showed the highest nitrite content (12.02 ppm) but was below 30 ppm. This may be because the raw material for the production of FD EBN_{rh} was RUC EBN, and RUC EBN had not undergone any cleaning process such as primary processing before enzymatic treatment. RUC EBN washed with distilled water for only a few minutes before enzymatic treatment may retain a high nitrite content. EBN by-products, on the other hand, were the residual EBN after primary processing and may retain low nitrite content. Therefore, it is not surprising that EBN_{rh} has higher nitrite content than EBNch. FD EBNrh was of better quality compared to hydrolysates produced by EBN by-product (FD EBNrh and SD EBNrh) due to higher antioxidant activity, sialic acid, and polysaccharide content.

4. Conclusion

This study showed that EBN hydrolysates produced from different raw materials: RUC EBN or by-products of EBN; or EBN hydrolysates dried by different drying methods: freeze-dried or spray-dried, also free of contaminants; exhibited antioxidant activity and high sialic acid content; and nitrite content below the regulatory limit. Therefore, enzymatic hydrolysis can be used to clean EBN, and enzymatically processed EBN products show potential in the development of nutraceuticals.

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References

[1] B. H. Yeo, T. K. Tang, S. F. Wong, C. P. Tan, Y. Wang, L. Z. Cheong, & O. M. Lai. (2021). Potential residual contaminants in edible bird's nest. Frontiers in pharmacology. 12: 631136.

[2] C. T. Guo, T. Takahashi, W. Bukawa, N., Yagi, H. Takahashi, K. Kato, ... & Y. Suzuki. (2006). Edible bird's nest extract inhibits influenza virus infection. Antiviral research. 70 (3): 140-146.

[3] A. Haghani, P. Mehrbod, N. Safi, N. A. Aminuddin, A. Bahadoran, A. R. Omar, & A. Ideris. (2016). In vitro and in vivo mechanism of immunomodulatory and antiviral activity of Edible Bird's Nest (EBN) against influenza A virus (IAV) infection. Journal of ethnopharmacology. 185: 327-340.

[4] E. Hwang, S. W. Park, & J. E. Yang. (2020). Anti-aging, anti-inflammatory, and wound-healing activities of edible bird's nest in human skin keratinocytes and fibroblasts. Pharmacognosy Magazine. 16 (69): 336-342.

[5] O. P. Mahaq, M. A. Rameli, M. Jaoi Edward, N. Mohd Hanafi, S. Abdul Aziz, H. Abu Hassim, ... & H. Ahmad. (2020). The effects of dietary edible bird nest supplementation on learning and memory functions of multigenerational mice. Brain and behavior. 10 (11): e01817.

[6] S. K. A. Khalid, A. Abd Rashed, S. A. Aziz, & H. Ahmad. (2019). Effects of sialic acid from edible bird nest on cell viability associated with brain cognitive performance in mice. World Journal of Traditional Chinese Medicine. 5 (4): 214-219.

[7] K. H. Chua, T. H. Lee, K. Nagandran, N. H. Md Yahaya, C. T. Lee, E. T. T. Tjih, & R. Abdul Aziz. (2013). Edible Bird's nest extract as a chondro-protective agent for human chondrocytes isolated from osteoarthritic knee: in vitro study. BMC complementary and alternative medicine. 13 (1): 1-9.

[8] M. Y. Yew, R. Y. Koh, S. M. Chye, I. Othman, T. Soga, I. Parhar, & K. Y. Ng. (2018). Edible bird's nest improves motor behavior and protects dopaminergic neuron against oxidative and nitrosative stress in Parkinson's disease mouse model. Journal of Functional Foods. 48: 576-585.

[9] A. S. Zulkifli, A. S. Babji, S. J. Lim, A. H. Teh, N. M. Daud, & H. A. Rahman. (2019). Effect of different hydrolysis time and enzymes on chemical properties, antioxidant and antihyperglycemic activities of edible bird nest hydrolysate. Malaysian applied biology. 48 (2): 149-156.

[10] T. H. Yan, S. J. Lim, A. S. Babji, M. H. Rawi, & S. R. Sarbini. (2021). Enzymatic hydrolysis: Sialylated mucin (SiaMuc) glycoprotein of edible swiftlet's nest (ESN) and its molecular weight distribution as bioactive ESN SiaMuc-glycopeptide hydrolysate. International journal of biological macromolecules. 175: 422-431.

[11] P. K. Chong, S. L. Mun, L. S. Chang, A. S. Babji, & S. J. Lim. (2022). Fractionation of edible bird's nest glycoprotein hydrolysates: characterization and antioxidative activities of the fractions. Food Science and Human Wellness. 11 (4): 886-894.

[12] A. M. Amin, K. Din, & K. C. Hui. (2019). Optimization of enzymatic hydrolysis condition of edible bird's nest using Protamex to obtain maximum degree of hydrolysis. Asian Journal of Agriculture and Biology. 7 (1): 1-9.

[13] J. W. A. Ling, L. S. Chang, A. S. Babji, & S. J. Lim. (2020). Recovery of value-added glycopeptides from edible bird's nest (EBN) co-products: enzymatic hydrolysis, physicochemical characteristics and bioactivity. Journal of the Science of Food and Agriculture. 100 (13): 4714-4722.

[14] Q. Fan, X. Liu, Y. Wang, D. Xu, & B. Guo. (2022). Recent advances in edible bird's nests and edible bird's nest hydrolysates. Food Science and Technology. 42.

[15] Q. Fan, J. Lian, X. Liu, F. Zou, X. Wang, & M. Chen. (2021). A study on the skin whitening activity of Digesta from edible bird's nest: a mucin glycoprotein. Gels. 8 (1): 24.

[16] T. H. Yan, S. L. Mun, J. L. Lee, S. J. Lim, N. A. Daud, A. S. Babji, & S. R. Sarbini. (2022). Bioactive sialylated-mucin (SiaMuc) glycopeptide produced from enzymatic hydrolysis of edible swiftlet's nest (ESN): degree of hydrolysis, nutritional bioavailability, and physicochemical characteristics. International Journal of Food Properties. 25 (1): 252-277.

[17] M. A. Amiza, D. Khuzma, P. S. Liew, M. Salma Malihah, & N. M. Sarbon. (2019). Effect of heat treatment and enzymatic protein hydrolysis on the degree of hydrolysis and physicochemical properties of edible bird's nest. Food research. 3 (6): 149.

[18] H. S. M. Noor, R. M. Ariff, L. S. Chang, X. Y. Chai, H. Y. Tan, A. S. Babji, & S. J. Lim. (2022). Enzymatic recovery of glycopeptides from different industrial grades edible bird's nest and its by-products: nutrient, probiotic and antioxidant activities, and physicochemical characteristics. Food Science and Human Wellness. 11 (6): 1555-1564.

[19] M. H. Nurfatin, I. K. Etty Syarmila, D. Nur'Aliah, M. K. Zalifah, A. S. Babji, & M. K. Ayob. (2016). Effect of enzymatic hydrolysis on Angiotensin converting enzyme (ACE) inhibitory activity in swiftlet saliva. International Food Research Journal. 23 (1).

[20] J. H. Zhao, L. S. Liu, S. S. Sablani, Y. J. Peng, H. W. Xiao, J. Bai, & H. Guo. (2020). Comparison of the thermal transitions of spray-dried and freeze-dried egg whites by differential scanning calorimetry. Food and Bioprocess Technology. 13: 1329-1343.

[21] G. D. Santos, R. I. Nogueira, & A. Rosenthal. (2018). Powdered yoghurt produced by spray drying and freeze drying: a review. Brazilian Journal of Food Technology. 21: e2016127.

[22] G. W. Jourdian, L. Dean, & S. Roseman. (1971). The sialic acids: XI. A periodate-resorcinol method for the quantitative estimation of free sialic acids and their glycosides. Journal of Biological Chemistry. 246 (2): 430-435.

[23] M. DuBois, K. A. Gilles, J. K. Hamilton, P. T. Rebers, & F. Smith. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry. 28 (3): 350-356.

[24] Y. Dong, W. Yan, & Y. Q. Zhang. (2022). Effects of spray drying and freeze drying on physicochemical properties, antioxidant and ace inhibitory activities of bighead carp (Aristichthys nobilis) skin hydrolysates. Foods. 11 (14): 2083.

[25] B. H. Yeo, Y. Rukayadi, O. M. Lai, S. F. Wong, & C. P. Tan. (2023). STRUCTURAL AND CHEMICAL ANALYSIS OF DIFFERENT TYPES OF RAW CLEANED (RC) EDIBLE BIRD'S NEST (EBN). Malaysian Journal of Microscopy. 19 (1): 76-89.

[26] S. R. Ng, H. S. Mohd Noor, R. Ramachandran, H. Y. Tan, S. E. Ch′ ng, L. S. Chang, ... & S. J. Lim. (2020). Recovery of glycopeptides by enzymatic hydrolysis of edible bird's nest: the physicochemical characteristics and protein profile. Journal of Food Measurement and Characterization. 14: 2635-2645.

[27] J. Y. Gan, L. S. Chang, N. A. M. Nasir, A. S. Babji, & S. J. Lim. (2020). Evaluation of physicochemical properties, amino acid profile and bioactivities of edible Bird's nest hydrolysate as affected by drying methods. *LWT* - Food Science and Technology. 131: 109777.