Sains Malaysiana 52(12)(2023): 3421-3435
http://doi.org/10.17576/jsm-2023-5212-06

Physicochemical Properties of Raw Cleaned Edible Bird's Nest after Different Primary Processing Including a New Cleaning Method

(Sifat Fizikokimia Sarang Burung Boleh Dimakan Mentah yang Dibersihkan Selepas Pemprosesan Utama Berbeza Termasuk Kaedah Pembersihan Baharu)

Bee-Hui Yeo[1], Yaya Rukayadi[1], Shew-Fung Wong[2], Chin-Ping Tan[3] & Oi-Ming Lai[1,4,*]

[1]*Natural Medicines and Products Research Laboratory (NaturMeds), Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*School of Medicine, International Medical University, 57000 Kuala Lumpur, Malaysia*
[3]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4]*Department of Bioprocess Technology, Faculty of Biotechnology & Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Received: 28 May 2023/Accepted: 17 November 2023

ABSTRACT

Primary processing is the cleaning process of raw uncleaned (RUC) edible bird's nest (EBN) into raw clean (RC) bird's nest. In this study, three primary processing methods were used, including two methods commonly used in industry (semi-dry and wet methods) and a newly proposed method (semi-wet method). EBNs before and after primary processing were characterised by structural and chemical analysis. The RC EBN samples after initial processing were divided into two categories, with moulding (cup-shaped) and un-shaped. Scanning Electron Microscopy-Energy Dispersive X-ray Microanalysis (SEM-EDX) was used to study the morphology and element composition of EBN samples. The EBN sample were also tested on their colour, moisture content, water activity, nitrite content, nitrate content, total sialic acid content, total glycoprotein content, and total polysaccharide content. The semi-wet method yielded > 75% cup- shaped RC EBN for six different batches of RUC EBN, outperforming wet cleaning method (68.76 - 82.92%). Micrographs showed structural similarity between RC EBN samples even with different cleaning methods; but showed structural difference between RUC and RC EBN samples. Elements Na and Cl were significantly ($P < 0.05$) higher in RUC EBN. After cleaning, RC EBNs showed nitrite reduction at 34.78 - 60.72%; and nitrate reduction at 33.62 - 72.35%, respectively. No reduction of antioxidant activity, total sialic acid content, total glycoprotein content and total polysaccharide were observed for EBNs after primary processing. The nitrate and glycoprotein contents in EBNs after moulding were lower than those before moulding.

Keywords: Edible bird's nest; glycoprotein; primary processing; sialic acid

ABSTRAK

Pemprosesan primer ialah proses pembersihan sarang burung walit (EBN) mentah (RUC) kepada sarang burung walit mentah bersih (RC). Dalam kajian ini, tiga kaedah pemprosesan utama telah digunakan, termasuk dua kaedah yang biasa digunakan dalam industri (kaedah separa kering dan basah) dan kaedah yang baharu dicadangkan (kaedah separa basah). EBN sebelum dan selepas pemprosesan utama dicirikan oleh analisis struktur dan kimia. Sampel RC EBN selepas pemprosesan primer dibahagikan kepada dua kategori dengan pengacuan (berbentuk cawan) dan tidak berbentuk. Mikroskop elektron pengimbas - X-ray Penyerakan Tenaga Microanalisis (SEM-EDX) digunakan untuk mengkaji morfologi dan komposisi unsur sampel EBN. Sampel EBN juga diuji warna, kandungan lembapan, aktiviti air, kandungan nitrit, kandungan nitrat, jumlah kandungan asid sialik, jumlah kandungan glikoprotein dan jumlah kandungan polisakaridanya. Kaedah separa basah menghasilkan > 75% RC EBN berbentuk cawan untuk enam kelompok RUC EBN yang berbeza, mengatasi kaedah pembersihan basah (68.76 - 82.92%). Mikrograf menunjukkan persamaan struktur antara sampel RC EBN walaupun dengan kaedah pembersihan yang berbeza; tetapi menunjukkan perbezaan struktur antara sampel RUC dan RC EBN. Unsur Na dan Cl adalah ketara (P <0.05) tertinggi dalam RUC EBN. Selepas pembersihan, RC EBN telah menunjukkan pengurangan nitrit pada 34.78 - 60.72%; dan pengurangan nitrat pada 33.62 - 72.35%. Tiada pengurangan aktiviti antioksidan, jumlah kandungan asid sialik, jumlah kandungan glikoprotein dan jumlah polisakarida diperhatikan di EBN selepas pemprosesan primer. Kandungan nitrat dan glikoprotein dalam EBN selepas pengacuan adalah lebih rendah daripada sebelum pengacuan.

Kata kunci: Asid sialik; glikoprotein; pemprosesan primer; sarang burung walit

Introduction

Edible bird's nests (EBN) are nests made from the salivary secretions of male and female swiftlets (*Aerodramus fuciphagus* or *Aerodramus maximus*) during the nesting and breeding season (Jamalluddin et al. 2019). Current scientific studies have demonstrated the therapeutic potential of EBN through various pharmacological activities, including anti-photoaging effects, anti-inflammatory, would healing/cell scratch repairing, neuroprotective effects (as a cognitive enhancer), prevention of cardiovascular disease, renal protective effect, skin whitening and moisturizing effect, anti-wrinkle effect, and increasing male reproductive health (Bai et al. 2023; Fan et al. 2022; Hwang, Park & Yang 2020; Jaffar et al. 2021; Kim et al. 2022; Lai et al. 2021; Lim et al. 2021; Loh, Cheng & Mohamed 2022; Murugan et al. 2020).

Raw uncleaned (RUC) EBN is feathered bird's nest harvested from caves and swiftlet houses without any cleaning. RUC EBN exhibits varying degrees of cleanliness contingent upon the presence of feathers and other foreign matters such as eggshells, swiftlet droppings, sand particles, mites, and other contaminants (Dai et al. 2021). In Malaysia, most RUC EBNs are nests composed mainly of swiftlets *Aerodramus fuciphagus* (white-nested swiftlet) rather than nests composed of *Aerodramus maximus* (black-nested swiftlet). Raw cleaned (RC) EBN is EBN that has been cleaned by primary or biotechnological processes so that it is free or minimal of feathers (fines) and foreign matters. In Malaysia, factories producing RC EBN is mainly of primary processing types. The primary cleaning process includes the following steps: sorting, surface cleaning, soaking/softening, picking/removing impurities, moulding/shaping, drying, grading and packaging (Yeo et al. 2021), and then sold as RC EBN. Generally, there are three different methods in primary process used in most of the factories in Malaysia, with the difference at the picking/ impurity's removal step. The three cleaning methods are: (i) dry method- without using water when picking out impurities; (ii) semi- dry method – sprinkling water locally (limited use of water) before picking out impurities; and (iii) wet method – soaking the EBN in the water when picking out impurities (Dai et al. 2021).

A key factor impacting the primary processing industry is the skilled workforce and until today the EBN cleaning process requires an incredible amount of manpower (Goh et al. 2017). The results of interviews with industry insiders agreed that improving the skills and attitudes of workers can improve the quality and product recovery rate of RC EBN. People in the industry also mentioned that workers' skills and emotions will affect work efficiency, and the industry often suffers from labor shortages, with many workers resigning after acquiring skills (Tan 2022). An alternative approach to prevent this labour shortage is to transform the EBN into powder form before cleaning takes place which is more effective than removing each and every impurity manually during cleaning process. This is known to be more effective, not to mention, efficient in purifying the nest. But an important criterion that determines the selling price of RC EBN is the shape of the nest. The price of RC EBN in powder or chip form or biscuit form has dropped significantly. Therefore, the industry still expects the shape of EBN to remain unchanged during the cleaning process (Goh et al. 2017).

Several alternative cleaning methods were explored to address the labour problem associated with EBN processing. These methods include: (i) treating EBN with keratinase, an enzyme that destroys the feathers attached to the EBN, eliminating the need for manual picking (Utomo et al. 2018); (ii) implementing an automated system combining machine vision arm and robotic arm for EBN cleaning (Subramaniam, Yeong & Ming 2015); and (iii) utilizing three-dimensional (3-D) printing of biotic material from swiftlet edible bird's nests, enabling the printing of EBN stripes through the machine (Chee 2018). Utomo et al. (2018) investigated the glycoprotein profile before and after washing with keratinase and reported no significant changes. The automated system introduced by Subramaniam, Yeong and Ming (2015) had a drawback. It required specimens to be soft (soaked for 12 h), flat, and with a thickness less than 2 mm, making it challenging to maintain the original shape of the EBN.

To date, there have been only a few studies examining the impact of primary processing steps, particularly using conventional water treatment, on EBN. One of these studies investigated the influence of washing frequency on the reduction of nitrite levels in EBN (Susilo, Latif & Ridwan 2016). Another study focused on understanding the effects of various soaking times (5 min and 8 h) and soaking temperatures (40, 60, 80 °C) on the sialic acid content of EBN (Lian, Fan & Li 2017).

The objective of this study was to examine the impact of primary processing on cup-shaped RUC EBN. Three different primary processing methods were employed to clean the EBN: two commonly used industry methods (semi-dry and wet), and a newly proposed method (semi-wet). The newly proposed method was designed with the consideration of the majority of processing companies in Malaysia being small and medium enterprises (SMEs), aiming to keep costs low. Additionally, this method adheres to Malaysia's stringent process standards for producing RC EBN products, eliminating the need for enzymes. The main goal of the new method is twofold: to reduce reliance on skilled labour and to maintain the EBN's shape after processing. The study investigated both structural and chemical changes in EBN before and after primary processing, encompassing the stages before and after the EBN shaping/moulding step.

Materials and Methods

MATERIALS

The raw material was raw uncleaned (RUC) edible bird's nest (EBN). The cup-shaped RUC EBN was purchased from a middleman in Segamat, Johor, Malaysia. Subsequently, it underwent a rigorous Quality Control (QC) step/screening process before use. RUC EBN with visible fungus-like contamination was rejected. Analytical grade of regents and solvents were used in the laboratory analyses. The chemicals were purchased from Sigma-Aldrich (phenol, *N*-acetylneuraminic acid, resorcinol, Schiff's fuchsin sulfite reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH)) and Thermo-Fisher Scientific (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate, methanol).

NEW METHOD DESIGN CONSIDERATION

The primary objective of the new cleaning process/method is to address the existing challenges in the primary processing industry. Among these challenges is the heavy reliance on skilled labour to recover cup-shaped RC EBN products, as the presence of feathers and dirt on the RUC EBN significantly affects the recovery process. The current methods, namely semi-dry (SDM) and wet (WM) cleaning methods, require experienced workers. The SDM necessitates workers to accurately gauge the water quantity to spray and the softening time for feather removal, both of which impact the final product's shape and recovery rate. Similarly, the wet method's effectiveness relies on the proficiency and 'emotion' of the workers, influencing the time taken for feather removal in water and subsequently affecting the shape and recovery rate of RC EBN. Moreover, the SDM is only suitable for RUC EBN with a lower feather content (< 10%) and minimal dirt.

To address these limitations and streamline the cleaning process, a new cleaning workflow termed semi-wet cleaning method (SWM) with a defined Standard Operating Procedure (SOP) has been developed and a new cost-effective device, known as the water outlet pressure device, was employed to aid the semi-wet cleaning process. This device comprises a 60W submersible pump (SOBO WP5000, China), integrated with L-shaped PVC pipes. This method can be implemented in primary processing industries, requiring less skilled/experienced workers, and it can effectively clean all types of RUC EBN. Importantly, the new method offers the advantage of lower processing costs as it does not entail significant equipment expenses.

PRIMARY PROCESSING – THREE DIFFERENT CLEANING METHODS

The same batch of RUC EBN (cup-shaped) was used for primary processing. Three distinct cleaning methods were employed to produce RC EBN: semi-dry, wet, and a newly designed method called semi-wet. For the semi-dry cleaning method, RUC EBNs with fewer feathers were selected. In contrast, RUC EBNs were randomly chosen for the semi-wet and wet cleaning methods. After cleaning, half of the samples were shaped back into cup-

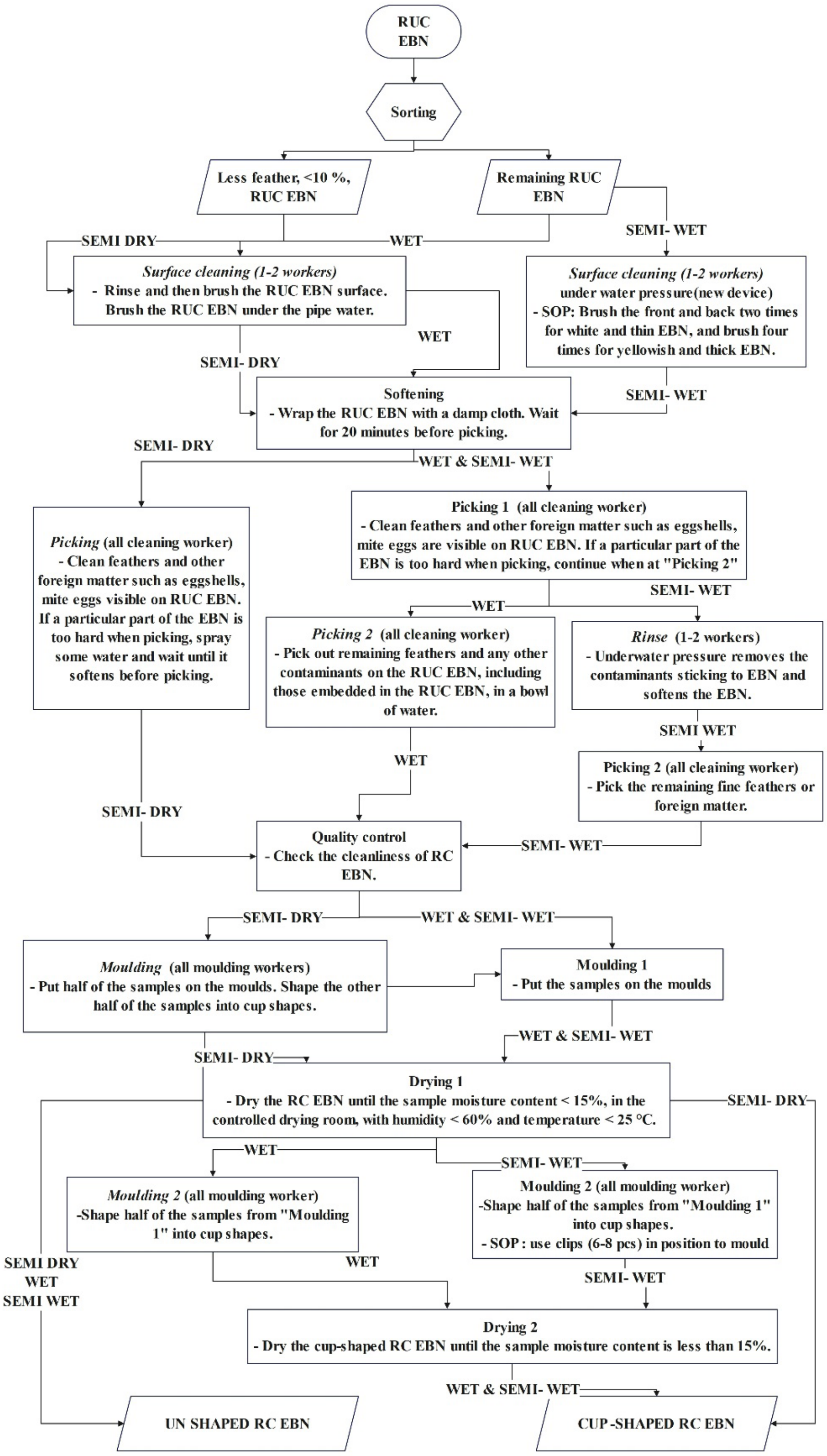

FIGURE 1. Process flow of semi- dry, wet, and semi- wet (new proposed method) cleaning methods. The steps in italic are steps that require skilled/experience worker

shaped form, while the other half remained unshaped or loosely shaped. Prior to any analysis, all samples were crushed. Each cleaning method involved two different samples, one in cup-shaped (A) and the other un-shaped/loosely shaped (B). Throughout all cleaning methods, filtered water was used in each step. Figure 1 illustrates the process flow for the semi-dry, wet, and semi-wet cleaning methods.

PRODUCT RECOVERY

The new method was taught to a private company. Subsequently, two workers from the company were assigned to process six batches of RUC EBN using this new method and their old method (wet cleaning). Dry weight of the final products including cup-shaped EBN and the fragment were weighed, and product recovery was calculated according to the following equations.

Cup-shaped RC EBN recovery rate (%) = [cup-shaped product / RUC EBN] *100 (1)

Fragment RC EBN recovery rate (%) = [Fragment product / RUC EBN] * 100 (2)

Total RC EBN recovery rate (%) = [Total products / RUC EBN] * 100 = (1) + (2) (3)

STRUCTURAL AND ELEMENT ANALYSIS

Scanning Electron Microscopy-Energy Dispersive X-ray Microanalysis (SEM-EDX) (JEOL JSM 6400; JEOL Ltd., Tokyo, Japan) was used to investigate the structure or morphology and elemental weight percent of RC EBN samples. The EBN sample was stuck on carbon tape and evenly coated with gold.

ANTIOXIDANT ACTIVITY ANALYSIS

DPPH and ABTS assays (Yeo et al. 2023) were used in antioxidant activity analysis. EBN sample (40 mg/mL) was used in DPPH assay while 2 mg/mL EBN sample was used in ABTS assay. Visible spectrophotometer (SCILOGEX SCI- V1000, USA) was used to read the DPPH absorbance (517 nm) and ABTS absorbance (734 nm). The free radical scavenging activity (%) for DPPH and ABTS was calculated according to the methods in Yeo et al. (2023).

TOTAL SIALIC ACID CONTENT ANALYSIS

The periodate-resorcinol assay (Yeo et al. 2023) was used to study the total sialic acid content in the sample. EBN sample (2 mg/mL) was used in this analysis and visible spectrophotometer (SCILOGEX SCI- V1000, USA) was used to read the absorbance (580 nm). *N*-acetylneuraminic acid (analytical standard) was used as standard.

TOTAL GLYCOPROTEIN CONTENT ANALYSIS

The Periodic Acid/Schiff (PAS) assay (Tan et al. 2021) was used to analyse the total glycoprotein content in the sample. A total of 50 μg/mL EBN sample was used in this assay. Sample was oxidized by periodate by mixing 2.0 mL of sample with 0.2 mL of periodic acid solution and incubating at 37 °C for 2 h. Ten μL of 50% periodic acid and 10 mL of 7% acetic acid were mixed to prepare the periodic acid solution. After incubation, samples were allowed to stand at room temperature for 10 min. Then, 2.0 mL of Schiff's fuchsin sulfite reagent was added to the sample. The mixture was incubated at room temperature for 30 min. The sample was centrifuged at 8,000 rpm for 10 min before reading the absorbance. Horseradish peroxidase (150 U/mg, analytical standard) was used as glycoprotein standard. The absorbance of the sample was read with visible spectrophotometer at 555 nm (SCILOGEX SCI- V1000, USA).

TOTAL POLYSACCHARIDE CONTENT ANALYSIS

The phenol-sulfuric acid assay (Yeo et al. 2023) was used to analyse the total polysaccharide content in the EBN sample (2 mg/mL). Visible spectrophotometer (SCILOGEX SCI- V1000, USA) was used to read the absorbance at wavelength 490 nm. Glucose monohydrate (1.0 mg/mL) was used as total polysaccharide standard.

COLOUR ANALYSIS

A calibrated handheld colorimeter (CR-400 Chroma Meter, Minolta) was used to analyse the colour of EBN samples. The entire lens of the colorimeter was covered with the EBN sample prior to reading. The EBN sample's surface colour parameters (L^*, a^*, and b^*) values were recorded.

NITRITE AND NITRATE CONTENT ANALYSIS

EBN sample (0 .5 g) was soaked in 40 mL ultrapure water. Then, the sample was incubated in water bath at 70 °C for 15 min. The samples were centrifuged at 8,000 rpm for 5 min after cooling down to room temperature. The supernatant was filtered through 0.45 μm filter and put in polyvial. Twenty μL sample was injected into ion chromatography (IC) Dionex IonPac AG4A-SC IC column (4 mm × 250 mm) (Thermo Scientific, USA) with mobile phase 1.7 mM sodium bicarbonate at flow rate of 2.0 mL/min.

WATER ACTIVITY ANALYSIS

The water activity meter (Aqualab Water Activity Meter, USA) was used to determine the water activity of EBN sample. EBN sample (1 g) was placed in a sample cup and the sample should completely covers the bottom of the cup. Then, the sample cup was placed in water activity meter and reading was recorded when meter's alarm sound.

MOISTURE CONTENT ANALYSIS

Oven drying method was used to analyse moisture content in EBN sample. Sample (around 0.5 g) was dried in a drying oven (Binder FD 115, Tuttlingen, Germany) at 105 °C for at least 24 h until no changes of weight were detected. Sample weight before and after drying were recorded. The difference of weight was calculated as water content in the EBN sample.

STATISTICAL ANALYSIS

The data were presented as the mean of three replicates ± standard error (SE). The p-value ($P < 0.005$) was computed using Microsoft Excel (Microsoft 365, version 2395) to assess the significant differences between the samples.

RESULTS AND DISCUSSION

NEW CLEANING METHOD- SEMI WET METHOD

In this study, a novel and cost-effective device called the water outlet pressure device (Figure 2) was utilized to assist in the semi-wet cleaning method. This device is a combination of an aquarium 60W submersible pump (SOBO WP5000, China) and L-shaped PVC pipes. Different submersible pump models (40W/60W/80W) were tested to determine the optimal water pressure for EBN cleaning. It was found that the 60W pump provided the best pressure, effectively flushing away foreign matter during the 'Surface cleaning' step. The 40W pump lacked sufficient power to perform this step adequately, while the 80W pump was too strong, potentially damaging the EBN's shape during the 'Rinse' step before 'Picking 2'. Various water outlet options, such as spiral nozzles and round pipe outlets, were tested, but they were not suitable for EBN cleaning as they lacked precise control over spraying. Instead, a flattened pipe with a lateral and thin final outlet was chosen, which proved highly suitable for EBN cleaning. The pipe is flattened to 2-3 mm and the final outlet is lateral and thin. This outlet design is particularly effective, one of the reasons is that the cup-shaped structure of RUC EBN is composed of many horizontal EBN stripes. The primary purpose of this new device is to use water pressure to clean the surface of RUC EBN, efficiently removing contaminants during the 'Surface cleaning' step. With this device's aid, workers only need to focus on picking up feathers from the RUC EBN, while other foreign objects like mites, mite eggs, and eggshells can be easily washed away using water pressure. Another application of this device is to rinse the EBN with water pressure before the 'Picking-2' step. This rinsing process aims to remove feathers from the EBN surface after 'Picking 1' and further soften and swell the EBN. Consequently, embedded fine feathers can be more easily removed during 'Picking 2'. For a visual representation of the 'Picking 2' step in both the wet method and semi-wet method, refer to Figure 3.

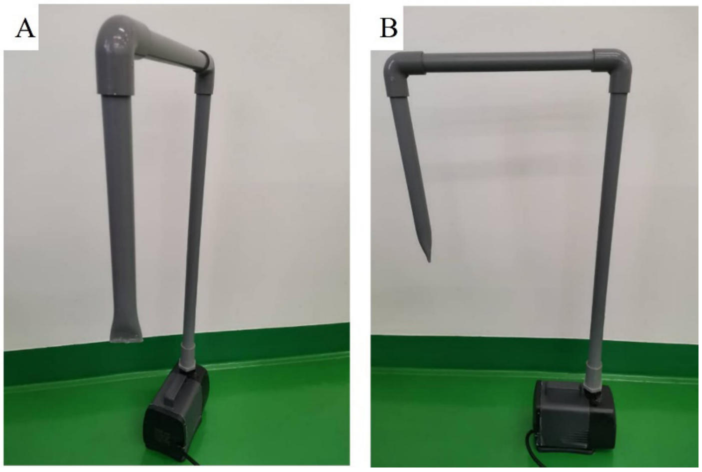

FIGURE 2. Water outlet pressure device: (A) front view; and (B) side view

In this study, two standard operating procedures (SOPs) were developed and implemented in the semi-wet cleaning method. The first SOP, termed the 'Surface cleaning' step, involves brushing the front and back of the EBN two times for white and thin EBN, and four times for yellowish and thick EBN. This SOP was designed to regulate the contact time of the EBN with water. In contrast, the traditional method, which heavily relies on the worker's experience, may lead to increased EBN fragmentation and excessive softening, making it challenging to proceed to the subsequent step. The second SOP, known as the 'Moulding 2' step, utilizes 6-8 clips positioned at the mould to shape the EBN into a cup-shaped form. Following this SOP, new workers can easily achieve a well-shaped RC EBN product. On the other hand, in the traditional method, workers use their hands, spoons, and moulds for EBN moulding, and obtaining a good shape for the product is heavily dependent on the worker's experience.

In the semi-wet method, only a few skilled workers are needed to operate the water outlet pressure device during the 'Surface cleaning' and 'Rinse' steps. Skilled workers are needed in these steps because each batch of RUC EBN varies in thickness and surface contamination, requiring skilled workers to adeptly control the moisture content of EBN, water pressure, and the correct cleaning angle of EBN while using the device. This precise control helps avoid excessive contact between EBN and water. The major advantage of the new method lies in its reduced skill requirements compared to the semi-dry and wet cleaning methods. With this change, even new and inexperienced workers can maintain the shape of EBN and ensure a high product recovery rate. Unlike the semi-dry method, which uses a limited amount of water, the semi-wet method is suitable for all RUC EBNs, as surface contaminants (not embedded) can be easily removed with the aid of pressure. In the semi-dry method, some contaminants may remain after the surface cleaning step, necessitating workers to pick them out one-by-one, resulting in a time-consuming process. The semi-wet method, on the other hand, streamlines the cleaning process by effectively removing surface contaminants, thereby reducing the manual effort required.

Previous studies aimed at enhancing the traditional cleaning method has included two approaches: (i) optimization of the cleaning process involving brushing, bubble, and microbubble techniques (Divean & Tan 2022); and (ii) implementation of the best value approach (BVA) to establish guidelines for EBN cleaning (Tan 2022). During the optimization process, two types of bubble and microbubble diffusers, along with a brushing arm, were developed. Cleanliness tests were conducted, and the best setting for EBN was determined to be a cleaning time of 7 min, achieving 66.18% cleanliness. The cleanliness was calculated as a percentage using the formula: % of cleanliness = [(weight loss after microbubble and brushing / total weight loss after microbubble, brushing, and manual cleaning) * 100% * 90%], taking into account a 10% loss in weightage of EBN dissolved in water (Divean & Tan 2022). In the proposed new cleaning process by Tan (2022), brushing and soaking/softening EBN steps were incorporated. The crude protein content of EBN was evaluated using both the traditional (31.16%) and new (50.25%) cleaning

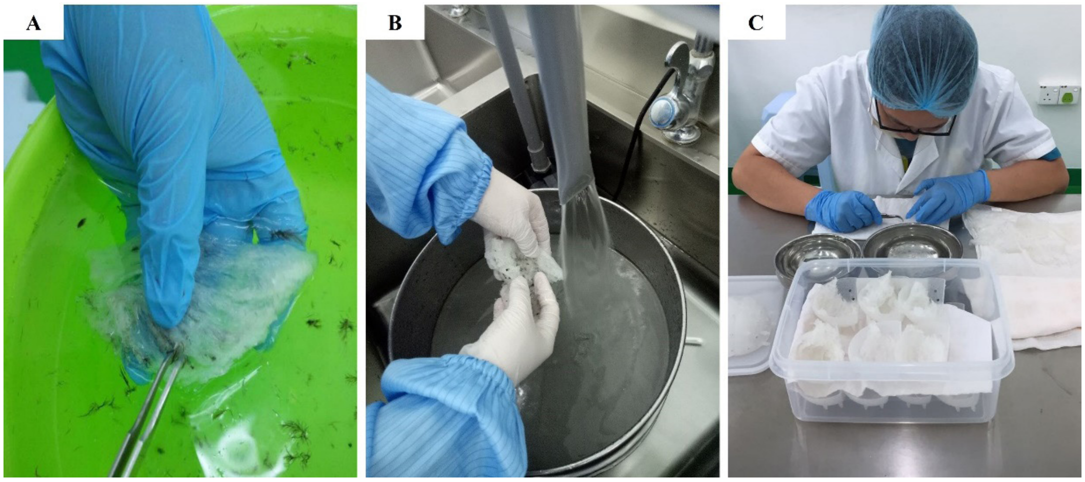

FIGURE 3. Process step: (A) Picking 2 step at wet method; (B) Rinse step and (C) Picking 2 step at semi-wet method

methods. In comparison to the study conducted by Divean and Tan (2022), our research achieved a higher product recovery, as demonstrated next. The newly proposed process flow chart, outlined in Tan (2022), closely resembled the semi-dry process employed in our study. The following sections (Physical and Chemical Properties) discusses the comparison between the methods employed in Tan's study (2022) and those utilized in our research.

PRODUCT RECOVERY

Table 1 shows the RC EBN recovery rate. Cup-shaped RC EBN have much higher market price than RC EBN in biscuit or fragment style. The price difference between them can vary from RM 2000 to RM 4000 per kg. The wet cleaning method produced slightly higher total RC EBN products. However, for any batch of RUC EBN regardless of its feather amount and cleanliness, semi-wet cleaning method yielded > 75% cup- shaped RC EBN, outperforming wet cleaning method (68.76 - 82.92%). The average percentage for biscuits or fragments produced by the wet cleaning method are higher, and the percentages produced by different RUC EBN batches were very different, the lowest is only 0.69%, and the highest is 12.45%. This may be due to the cleanliness of the raw materials and the 'condition' of the workers that day. Relatively, there was little difference in the percentage of biscuits or fragments (1.84 to 4.91%) between batches of RUC EBN cleaned using the semi-wet method. The results showed that the semi-wet cleaning process was relatively less dependent on raw materials and worker conditions.

STRUCTURAL AND ELEMENT

The RC EBN samples cleaned using the semi-dry method are denoted as RC-SDM, while those cleaned using the wet method are referred to as RC-WM. The RC EBN samples subjected to the semi-wet cleaning method are labelled as RC-SWM. After moulding the samples into a cup-shaped form (Figure 4(A)), the character (A) was added to their names. Conversely, the character (B) was added to the sample names before moulding, indicating their un-shaped state (Figure 4(B)).

Table 2 shows the elemental composition of RUC EBN and RC EBNs (after moulding). Six elements were detected in all EBN samples, and they were carbon (C), oxygen (O), sodium (Na), magnesium (Mg), chlorine/chloride (Cl) and calcium (Ca). Among the six elements, C and O were the two with the highest composition (%) in EBN samples, and these two elements are the main elements of glycoproteins (Yeo et al. 2023). Previous study showed the presence of elements Al and S in house EBN (Shim & Lee 2020), but these elements were not detected in this study. Elements Na and Cl were significantly ($P < 0.05$) higher before processing (RUC EBN). Sodium chloride (NaCl) is a compound that chemically combines sodium (Na) and chlorine (Cl). This study shows that NaCl on RUC EBN is washed away during washing, resulting in RC EBN with lower NaCl content. The semi-dry method sample had higher percentage Na and Cl due to the shorter contact time and volume of water compared to the other two methods.

TABLE 1. RC EBN recovery rate after cleaning the RUC EBN with wet and semi- wet methods

	Recovery (%)					
	Wet Method			Semi-wet Method		
Batch RUC	Cup shaped	Biscuit/ Fragment	Total	Cup shaped	Biscuit/ Fragment	Total
1	79.04	2.86	81.90	78.74	2.26	81.00
2	68.76	11.74	80.50	75.66	3.28	78.94
3	82.92	0.69	83.61	81.71	1.84	83.55
4	70.60	12.45	83.05	78.11	4.91	83.02
5	78.18	8.13	86.31	80.35	3.13	83.48
6	69.37	9.16	78.53	77.30	3.83	81.13
Mean ± SE	74.81 ± 2.44	7.51 ± 1.95	82.32 ± 1.09	78.64 ± 0.88	3.21 ± 0.45	81.85 ± 0.74

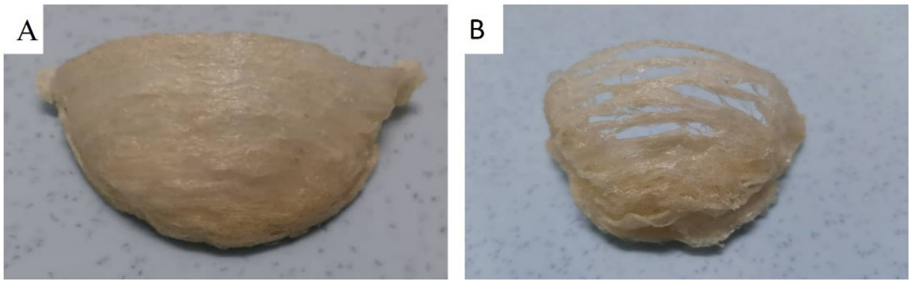

FIGURE 4. RC EBN: (A) cup- shaped RC EBN; and (B) un-shaped RC EBN

TABLE 2. Elemental composition (weight %) for RUC EBN and RC EBNs after moulding

Sample	C	O	Na	Mg	Cl	Ca
RUC EBN	52.74 ± 3.26	36.66 ± 2.01	3.56 ± 0.16^{a}	0.47 ± 0.08	3.39 ± 0.29^{a}	3.18 ± 1.02^{a}
RC-SDM (A)	48.99 ± 2.11^{b}	41.20 ± 1.40	2.10 ± 0.12^{ab}	0.73 ± 0.25	1.33 ± 0.21^{ab}	5.64 ± 1.75
RC-WM (A)	47.26 ± 4.31^{b}	40.61 ± 2.06	1.08 ± 0.12^{abc}	0.74 ± 0.14	0.38 ± 0.04^{ab}	10.13 ± 3.28^{ac}
RC-SWM (A)	54.27 ± 1.07	40.57 ± 0.62	1.66 ± 0.10^{abc}	0.82 ± 0.28	0.52 ± 0.12^{ab}	2.17 ± 0.67^{c}

*Different alphabet superscript (*a-c*) indicates significant difference (P < 0.05) between within the same column

Figure 5 shows the SEM images of the EBN samples, and the photomicrographs show the magnifications of the EBN samples from 50x to 5kx. Figure 5(a) to 5(d) shows the structures of RUC EBN samples. Figure 5(e) to 5(r) shows the structures of RC EBN samples with different cleaning methods. Figure 5 shows structural similarity between RC EBN sample even with different cleaning method; but shows structural difference between RUC and RC EBN sample. Previous studies have shown contaminants on RUC EBN like mite, feather filaments, fungal spores and eggshells (Kew et al. 2014; Tai et al. 2020), but in this study no contaminant was observed. This may be related to sample selection. In this study, only clean stripes (without visual contamination) were selected as samples. A recent study showed that different RC products (house EBN: cup- shaped EBN, white EBN biscuit, yellowish fragment, and rejected RC EBN biscuit and cave cup- shaped EBN) had different microstructure (Yeo et al. 2023). On the other hand, this study shows (Figure 5) structural similarity between the RC products (all was cup-shaped EBN). This may be related to the cleaning process and raw materials, including the area of the bird's nest in contact with water during cleaning, the time and amount of water in which the bird's nest is in contact with water. Cup-shaped RC EBN presented in Yeo et al. (2023) was cleaned using a semi-dry method.

The micrographs of this sample more closely resembled the RUC EBNs in this study (Figure 5(a) to 5(d)) than the cup-shaped EBNs (Figure 5(e) to 5(h)) cleaned in the same way. This was thought to be likely due to the time and amount of water exposure when using the same cleaning method.

PHYSICAL PROPERTIES

Table 3 shows the colour, water activity and moisture content for the RC EBNs. RC-WM EBNs had the highest water content but was not significantly (P > 0.05) different from RC-SDM and RC-SWM EBNs; these samples showed similar water activities to the EBN samples from the other two methods. RC-SDM EBNs show the two highest *b** values (yellow/blue coordinate). The differences observed between the samples before and

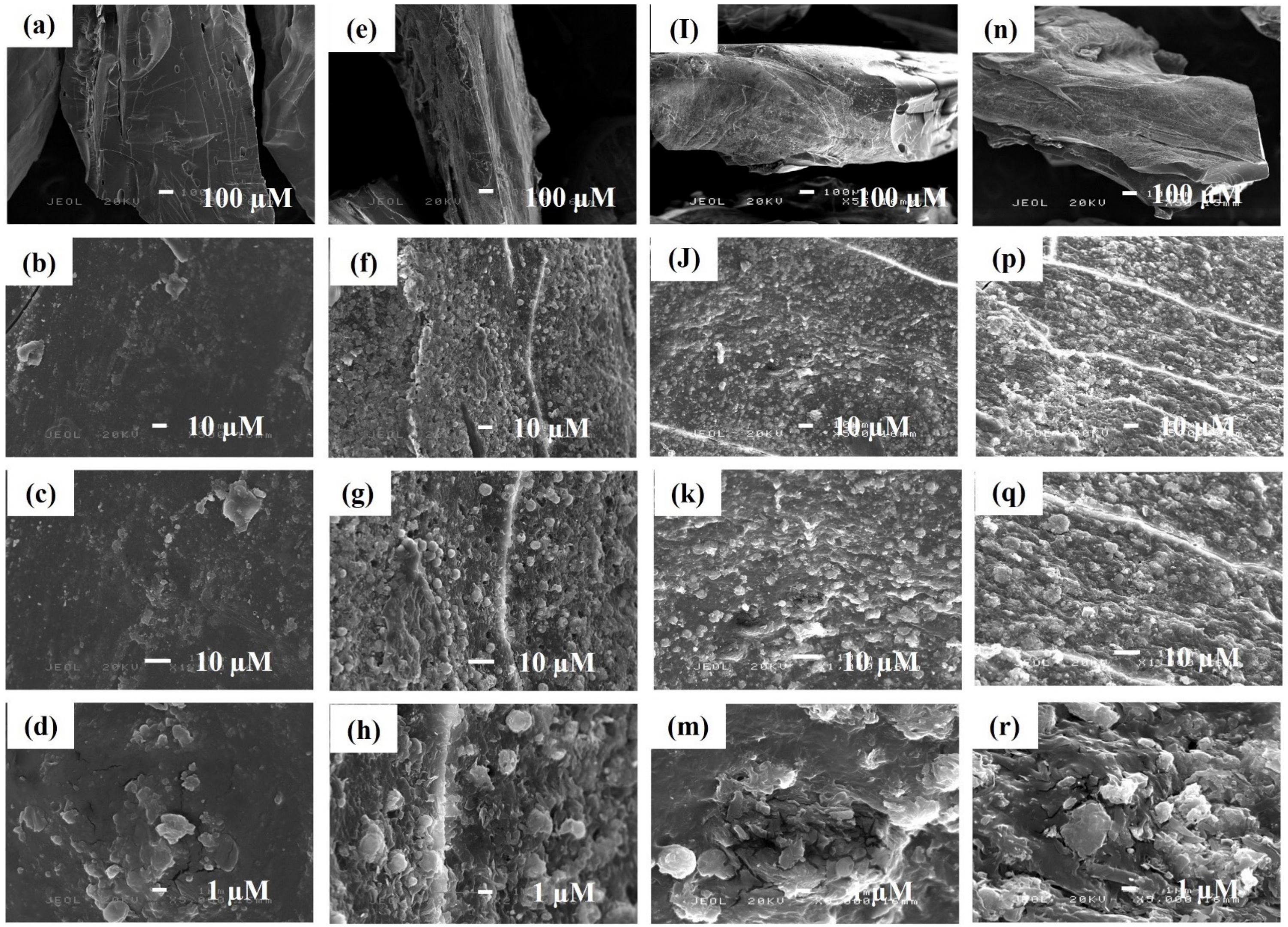

FIGURE 5. SEM micrograph of RUC EBN at magnification of (a) 50x, (b) 500x, (c) 1kx, (d) 5kx; RC- SDM (A) at magnification of (e) 50x, (f) 500x, (g) 1kx, (h) 5kx; RC- WM (A) at magnification of (I) 50x, (J) 500x, (k) 1kx, (m) 5kx; and RC-SWM (A) at magnification of (n) 50x, (p) 500x, (q) 1kx, (r) 5kx

after moulding: (a) there was no significant difference in the L^* values for the samples from all the cleaning methods; (b) there was no significant difference in the a^* values for samples from the wet method. However, there was a significant difference ($P < 0.05$) for samples from the semi-dry and semi-wet methods, with both showing significantly higher values after moulding; (c) there was no significant difference in the b^* values for samples from the wet and semi-wet methods. However, for samples from the semi-dry method, there was a significant difference ($P < 0.05$); (d) there was no significant difference in the water activity for samples from the semi-dry method. However, there was a significant difference ($P < 0.05$) for samples from the wet and semi-wet methods; and (e) there was no significant difference in the moisture content for the samples from all the cleaning methods. Based on the above observations, no significant conclusions can be drawn from these different physical properties of the samples before and after moulding, as there were no clear trends.

CHEMICAL PROPERTIES

Table 4 shows the nitrite and nitrate content in the EBN samples. All EBN samples in this study contained less than 30 ppm nitrite, meeting the requirements for export to China (Yeo et al. 2021). The previous studies reported that the nitrite reduction after washing with: (a) water was 29.93 - 80% (Ningrum, Candra & Wardhani 2023; Susilo, Latif & Ridwan 2016); (b) ascorbic acid solution was 88.89% (Utomo et al. 2016); and (c) *Citrus aurantiifolia* Swingle and sea salt solution was 86.0% (Ningrum, Candra & Wardhani 2023). Ascorbic acid solution and *Citrus aurantiifolia* Swingle and sea salt solution reduced nitrite levels more than water treatments. The two studies did not include antioxidant activity and sialic acid changes, suggesting more research on these two potential cleansing agents before replacing traditional water treatment methods. This study shows the nitrite reduction is 34.78 - 60.72%; and the nitrate reduction is 33.62 - 72.35%. RC EBNs subjected to the semi-dry method exhibited a lower percentage reduction of nitrite and nitrate. This outcome could be attributed to the reduced contact time and water amount during the cleaning process of RUC EBN. This observation aligns with the findings of a previous study, which demonstrated that higher frequency of washing leads to greater nitrite reduction (Susilo, Latif & Ridwan 2016). The nitrate concentrations of all three samples after moulding were lower than those before moulding. RC EBN samples after semi-dry and wet molding were significantly ($P < 0.05$) lower, while semi-wet samples were lower but not significantly different.

TABLE 3. Physical properties of RC EBN cleaned by different methods

Sample	Colour			Water activity	Moisture content (%)
	L*	***a****	***b****		
RC-SDM (A)	63.55 ± 0.32^{a}	1.86 ± 0.01^{a}	10.00 ± 0.13^{a}	0.600 ± 0.002	14.67 ± 0.18^{cd}
RC-SDM (B)	63.15 ± 0.24^{b}	2.34 ± 0.00^{ab}	9.59 ± 0.07^{ab}	0.604 ± 0.001^{b}	14.48 ± 0.06^{cd}
RC-WM (A)	60.16 ± 0.72^{ab}	2.07 ± 0.05^{abc}	8.28 ± 0.31^{ab}	0.606 ± 0.001^{c}	15.95 ± 0.10^{c}
RC-WM (B)	59.19 ± 0.34^{ab}	2.09 ± 0.04^{abd}	8.07 ± 0.19^{abd}	0.560 ± 0.015^{bcd}	15.63 ± 0.03^{d}
RC-SWM (A)	59.88 ± 0.24^{ab}	1.82 ± 0.01^{bcde}	8.61 ± 0.09^{abd}	0.596 ± 0.001^{bce}	14.55 ± 0.24^{cd}
RC-SWM (B)	60.16 ± 0.47^{ab}	2.01 ± 0.03^{abe}	9.18 ± 0.22^{ad}	0.604 ± 0.001^{de}	14.46 ± 0.14^{cd}

** Different alphabet superscript (*a-e*) indicates significant difference ($P < 0.05$) between within the same column

TABLE 4. Nitrite and nitrate content of RUC and RC EBNs

Sample	Nitrite (ppm)	Nitrate (ppm)	Nitrite reduction (%)	Nitrate reduction (%)
RUC EBN	9.63 ± 0.10^{a}	66.66 ± 0.72^{a}	-	-
RC-SDM (A)	5.65 ± 0.28^{ab}	37.21 ± 0.16^{ab}	41.33	44.18
RC-SDM (B)	6.28 ± 0.40^{ac}	44.25 ± 0.39^{abc}	34.78	33.62
RC-WM (A)	$4.61 \pm 0.04a^{bcd}$	18.43 ± 0.38^{abcd}	52.11	72.35
RC-WM (B)	4.17 ± 0.41^{abc}	23.17 ± 1.26^{abcde}	56.70	65.24
RC-SWM (A)	4.70 ± 0.17^{abcf}	27.86 ± 0.78^{abcde}	51.18	58.20
RC-SWM (B)	3.78 ± 0.20^{abcdf}	31.04 ± 1.68^{acde}	60.72	53.44

**Different alphabet superscript (*a-g*) indicates significant difference (P < 0.05) between within the same column

Table 5 shows antioxidant activity, total sialic acid content, total glycoprotein content and total polysaccharide content of RUC and RC EBNs in this study. The antioxidant activity of EBN samples in this study was investigated by free radical scavenging activity. RUC EBN in this study had 40.56% DPPH and 20.69% ABTS free radical scavenging activity. RC EBN in this study had 47.67 - 58.87% DPPH and 17.51 - 22.98% ABTS free radical scavenging activity. The effect of biotechnological processing on the antioxidant activity of bird's nest has been studied. These studies indicated that heat treatment and enzymatic hydrolysis enhanced the antioxidant activity of bird's nest (Babji et al. 2018; Ramachandran, Babji & Wong 2017). This is the first study to show changes in the antioxidant activity of bird's nest after primary processing, and no definitive conclusions could be drawn when comparing samples before and after the cleaning process. However, RUC EBN exhibited a significantly lower DPPH radical scavenging activity (P < 0.05), though this outcome was not consistent in the ABTS assay. Furthermore, no conclusive results can be drawn regarding the antioxidant activity of the samples before and after moulding, as the same trend was not observed in the samples from these three cleaning methods before and after moulding. This study showed that the antioxidant activity of RC-SWM EBNs was significantly (P < 0.05) higher than that of RC-SDM EBNs.

A study reported that total sialic acid content for RUC house EBN was 12.52% (Lian, Fan & Li 2017). Previous studies have shown that the total sialic acid content of RC house EBN ranges from 3.28% to 14.52% (Chantakun et al. 2022; Lian, Fan & Li 2017; Quek et al. 2018; Yeo et al. 2023). The content of total sialic acid varies greatly, which may be related to swiftlet species, regional differences (Quek et al. 2018), cleaning methods (Yeo et al. 2023), and experimental methods. In this study, the total sialic content for RUC EBN was 10.79% and RC EBNs was 10.56% - 12.02%. Compared with RUC EBN, RC-WM and RC-SWM EBNs had significantly (P < 0.05) higher total sialic acid content, but not RC-SDM EBN. According to Lian, Fan and Li (2017), RC EBN demonstrated higher total sialic acid content compared to RUC EBN, which the authors attributed to the impurity/ash content present in RUC EBN. Additionally, Quek et al. (2018) showed that the group with lower ash content exhibited higher sialic acid content. It is possible that RC-SDM samples washed with less water may retain more ash in the EBN, consequently leading to lower total sialic acid content. There was no significant difference (P < 0.05) in the total sialic acid content of RC EBN samples before and after moulding. This study shows that the total sialic acid content of RC-SWM EBNs was significantly (P < 0.05) higher than that of RC-SDM and RC-WM EBNs.

TABLE 5. Antioxidant activity, total sialic acid content, total glycoprotein content and total polysaccharide content of RUC and RC EBNs

Sample	DPPH Free radical scavenging activity (%)	ABTS Free radical scavenging activity (%)	Total sialic acid content (%)	Total glycoprotein content (%)	Total polysaccharide content (%)
RUC EBN	40.56 ± 0.61^{af}	20.69 ± 0.29^{acg}	10.79 ± 0.30^{a}	22.45 ± 1.20	9.13 ± 0.06^{a}
RC-SDM (A)	47.67 ± 0.59^{abfg}	20.25 ± 0.41^{cg}	10.64 ± 0.23^{b}	21.55 ± 0.63^{g}	8.96 ± 0.51^{b}
RC-SDM (B)	49.11 ± 1.17^{afg}	17.51 ± 0.39^{cg}	10.56 ± 0.32^{c}	23.16 ± 0.81	7.03 ± 0.35^{abc}
RC-WM (A)	52.30 ± 1.04^{abd}	19.40 ± 0.24^{acg}	11.49 ± 0.01^{bcd}	21.14 ± 0.83^{g}	10.49 ± 0.51^{acd}
RC-WM (B)	48.39 ± 0.13^{adfg}	20.14 ± 0.44^{cg}	11.76 ± 0.06^{abcde}	22.27 ± 0.18^{g}	9.81 ± 0.21^{ac}
RC-SWM (A)	58.87 ± 2.27^{afg}	19.56 ± 0.37^{cg}	12.02 ± 0.09^{abcde}	20.84 ± 0.65^{g}	8.98 ± 0.33^{cd}
RC-SWM (B)	53.64 ± 0.20^{afg}	22.98 ± 0.36^{cg}	11.99 ± 0.11^{abcd}	24.59 ± 0.88^{g}	10.63 ± 0.96^{c}

** Different alphabet superscript (*a-g*) indicates significant difference ($P < 0.05$) between within the same column

EBN has a unique glycoprotein structure that differentiates it from other protein sources such as fish and chicken in terms of solubility, functional properties, and bioactive compounds (Babji et al. 2015). After enzymatic analysis (biotechnological processing), the glycoprotein content in bird's nest increased significantly (Tan et al. 2022), while after keratinase cleaning (primary processing), the glycoprotein content decreased slightly (Utomo et al. 2014). This is the first study to show changes in glycoprotein content in bird's nest after water treatment (primary processing). In this study, the glycoprotein content of RUC EBN was 22.45%; that of RC EBN was 20.84% - 24.59%. There was no significant difference between samples before and after cleaning treatment. The glycoprotein content of all three samples after moulding were lower than those before moulding. RC EBN semi-dry and wet samples after molding were not significantly ($P > 0.05$) lower, while semi-wet sample was significantly ($P < 0.05$) lower. The present study showed that the total glycoprotein content of RC-SWM EBN was not significantly different from that of RC-SDM and RC-WM EBN ($P > 0.05$).

After enzymatic analysis (biotechnological processing), the total polysaccharide content in bird's nest increased significantly (Tan et al. 2022). A previous study indicated that different RC EBN products possess varying total polysaccharide content. The observed difference was attributed to the utilization of different cleaning methods (Yeo et al. 2023). The total polysaccharide content for RUC EBN in this study was 9.13%; and RC EBNs was 7.03- 10.63%. No conclusive results can be drawn on the total polysaccharide for the samples before and after cleaning and moulding as the same trend was not observed in the samples of these three cleaning methods before and after moulding. Unlike Yeo et al. (2023), in this study, all samples were taken from the same raw material and produced the same RC EBN product - cup-shaped EBN, so no significant differences were shown between the samples, unlike Yeo's study. In the previous study, fragment EBN (2.34%) exhibited significantly lower total polysaccharide content compared to cup-shaped EBN (7.15%). This difference was attributed to the fact that the raw material for fragment EBN was the residue EBN from primary processing. Additionally, the cleaning method used for this residue EBN involved an extremely long exposure time (2-4 h) to water, potentially impacting the total polysaccharide content of the EBN. This study shows that the total polysaccharide content of RC-SWM EBNs was significantly ($P < 0.05$) higher than that of RC-SDM EBNs.

A limitation of this study is that the working steps of the semi-dry and wet methods used may not be consistent among all industry players. Variations may exist, with some manufacturers using less water in the semi-dry method and more water in the wet method. For future research, it is suggested to collaborate with manufacturers and utilize the same batch of RUC EBN, along with the cleaning methods employed in their respective production processes of RC EBN. By conducting physical and chemical analyses on these RC EBNs, it will be possible to assess whether different cleaning steps within the same categorized method have an impact on the quality of RC EBNs.

CONCLUSIONS

The primary processing, comprising semi-dry, semi-wet, and wet methods, effectively reduces nitrite and nitrate concentrations while preserving the functional properties and bioactive compounds of EBN. RUC EBN exhibits a distinct microstructure compared to RC EBN, possibly due to the removal of NaCl during primary processing. Notable differences between samples before and after moulding were observed mainly in terms of nitrate and glycoprotein content. The moulded samples displayed lower nitrate and glycoprotein levels than the un-moulded EBN samples. This observation indirectly suggested that the 'Moulding 2' and 'Drying-2' steps may not significantly impact EBN's antioxidant activity and total sialic acid content. The newly introduced cleaning method, the semi-wet method, demonstrated promising results in RC cup-shaped EBN recovery and exhibited significantly higher antioxidant activity, total sialic acid content, and total polysaccharide compared to the semi-dry method. Moreover, it exhibited chemical properties equivalent to the wet method. As a result, this newly designed method is recommended for industry use, as it requires less skilled labour for picking and moulding, thereby addressing the scarcity of skilled labour in the industry.

ACKNOWLEDGEMENTS

The authors acknowledge the support given by Department Veterinary Service, Malaysia and Mr. Sia Beng Lai. The funding for this study was supported by National Industry-UPM Edible Birdnest Processing Research Group Scheme (Project Code 997).

REFERENCES

Babji, A.S., Nurfatin, M.H., Etty Syarmila, I.K. & Masitah, M. 2015. Secrets of edible bird nest. *Agriculture Science* 1: 32-37.

Bai, W., Liu, X., Fan, Q., Lian, J. & Guo, B. 2023. Study of the antiaging effects of bird's nest peptide based on biochemical, cellular, and animal models. *Journal of Functional Foods* 103: 105479.

Chee, B.M. 2018. Three dimensional printing of biotic material originated from swiftlet edible bird nest. China CN108260839A, filed January 3, 2018, and issued July 10, 2018. https://patents.google.com/patent/CN108260839A/en.

Chantakun, K., Kishimura, H., Kumagai, Y. & Benjakul, S. 2022. Physicochemical properties of house and cave edible bird's nest from Southern Thailand. *ScienceAsia* 48: 136-143.

Dai, Y., Cao, J., Wang, Y., Chen, Y. & Jiang, L. 2021. A comprehensive review of edible bird's nest. *Food Research International* 140: 109875.

Divean, S. & Tan, C.S. 2022. Optimization of brushing, bubble, and microbubble techniques using Taguchi method for raw edible bird nest cleaning purpose. *Pertanika Journal of Science & Technology* 3(2): 1273-1288.

Fan, Q., Lian, J., Liu, X., Zou, F., Wang, X. & Chen, M. 2022. A study on the skin whitening activity of digesta from edible bird's nest: A mucin glycoprotein. *Gels* 8(1): 24.

Hwang, E., Park, S. & Yang J-E. 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.

Jaffar, F.H.F., Osman, K., Chua, K.H., Zulkefli, A.F. & Ibrahim, S.F. 2021. Edible bird's nest supplementation improves male reproductive parameters of Sprague Dawley rat. *Frontiers in Pharmacology* 12: 631402.

Jamalluddin, N.H., Tukiran, N.A., Ahmad Fadzillah, N. & Fathi, S. 2019. Overview of edible bird's nests and their contemporary issues. *Food Control* 104: 247-255.

Kew, P.E., Wong, S.F., Lim, P.K. & Mak, J.W. 2014. Structural analysis of raw and commercial farm edible bird nests. *Tropical Biomedicine* 31(1): 63-76.

Kim, H.M., Lee, Y.M., Kim, E.H., Eun, S.W., Sung, H.K., Ko, H., Youn, S.J., Choi, Y., Yamada, W. & Shin, S.M. 2022. Anti-wrinkle efficacy of edible bird's nest extract: A randomized, double-blind, placebo-controlled, comparative study. *Frontiers in Pharmacology* 13: 843469.

Lai, Q.W.S., Guo, M.S.S., Wu, K.Q., Liao, Z., Guan, D., Dong, T.T., Tong, P. & Tsim, K.W.K. 2021. Edible bird's nest, an asian health food supplement, possesses moisturizing effect by regulating expression of filaggrin in skin keratinocyte. *Frontiers in Pharmacology* 12: 685982.

Lian, J-M., Fan, Q-Y. & Li, H-W. 2017. Influence of different processing technology on sialic acid content of edible bird's nest products. 食品工业科技1: 265-268.

Lim, C.T.S., Norhafizah, M., Sani, D. Tan, S.N., Lim, C.W., Kirby, B.P., Ideris, A. & Stanslas, J. 2021. Edible bird nest protects the kidney from gentamicin induced acute tubular necrosis. *Frontiers in Pharmacology* 12: 726005

Loh, S-P., Cheng, S-H. & Mohamed, W. 2022. Edible bird's nest as a potential cognitive enhancer. *Frontiers in Neurology* 13: 865671.

Goh K.M., G.K., Lai, W.K., Ting, P.H., Koe, D. & Wong, J.K.R. 2017. Size characterisation of edible bird nest impurities: A preliminary study. *Procedia Computer Science*, *Knowledge-Based and Intelligent Information & Engineering Systems: Proceedings of the 21st International Conference*, KES-20176-8 September 2017, Marseille, France. 112: 1072-1081.

Murugan, D.D., Zuhaida, M.Z., Choy, K.W., Nor Hisam, Z., Choong, M.J., Lim, Y.M. & Mohd Rais, M. 2020. Edible bird's nest protects against hyperglycemia-induced oxidative stress and endothelial dysfunction. *Frontiers in Pharmacology* 10: 01624.

Ningrum, S., Candra, A. & Wardhani, H.C. 2023. The potency of *Citrus aurantiifolia* swingle and sea salt solution as a cleansing agent for edible bird's nests. *Makara Journal of Science* 27(1): Article 4.

Quek, M.C., Chin, N.L., Yus Aniza, Y., Law, C.L. & Tan, S.W. 2018. Characterization of edible bird's nest of different production, species and geographical origins using nutritional composition, physicochemical properties and antioxidant activities. *Food Research International* 109: 35-43.

Ramachandran, R., Babji, A. S. & Wong, I.P. 2017. Effect of Heating on Antioxidant Activity on Edible Bird Nest. *The 7th international seminar on tropical animal production* (ISTAP): 380-386.

Shim, E.K-S. & Lee, S-Y. 2020. Calcite deposits differentiate cave from house-farmed edible bird's nest as shown by SEM-EDX, ATR-FTIR and Raman microspectroscopy. *Chemistry - An Asian Journal* 15(16): 2487-2492.

Subramaniam, Y., Yeong, C.F. & Ming, E.S.L. 2015. Edible bird nest processing using machine vision and robotic arm. *Jurnal Teknologi* 72(2): 85-88.

Susilo, H., Latif, H. & Ridwan, Y. 2016. Application of washing method under running water to reduce nitrit level of edible bird's nest. *Jurnal Kedokteran Hewan - Indonesian Journal of Veterinary Sciences* 10(2): 95-97.

Tai, S.K., Hamzah, Z., Ng, Q.H. & Tan, C.S. 2020. Surface morphology study on unclean, commercial and bromelain treated edible bird nest (EBN) using scanning electron microscope. *IOP Conference Series: Materials Science and Engineering* 932(1): 012013.

Tan, H.Y., Lim, S.J., Abdul Salam, B., Muhamad Hanif, R. & Shahrul Razid, S. 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.

Tan, H.Y., Mun, S.L., Lee, J.L., Lim, S.J., Nur Aliah, D., Abdul Salam, B., Muhamad Hanif, R. & Shahrul Razid, S.L. 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.

Tan, K.H. 2022. Applying best value approach in establishing guideline for edible bird nest cleaning process. PhD Dissertation, UTAR (Unpublished).

Utomo, B., Rosyidi, D., Radiate, L.E. & Purnomo, H. 2016. Metode penurunan kandungan nitrite dengan pencucian menggunakan asam askorbat pada tiga jenis sarang burung walet asal Indonesia. *Efektor* 3(1): 104-107.

Utomo, B., Rosyidi, D., Radiati, L.E., Tri Puspaningsih, N.N. & Proborini, W.D. 2018. Use of keratinase to maintain pre-washing glycoprotein profiles of edible bird's nest. *Drug Invention Today* 10: 2986-2990.

Yeo, B.H., Rukayadi, Y., Lai, O-M., Wong, S-F. & Tan, C-P. 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.

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

*Corresponding author; email: omlai@upm.edu.my