STRUCTURAL AND CHEMICAL ANALYSIS OF DIFFERENT TYPES OF RAW CLEANED (RC) EDIBLE BIRD'S NEST (EBN)

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Abstract. Edible Bird's Nest (EBN) are known to have high nutritional values and medicinal properties. Raw cleaned (RC) EBN is EBN that has undergone cleaning processes. In this study, five types of RC EBN products were characterized by structural and chemical analyses. These are different grades of RC EBN products, including four products from house nest and one product from cave nest. RC EBN products from house nests were cup- shaped EBN (RC_{ha}), white EBN Biscuits (RC_{hb}), yellowish fragments (RC_{hc}) and rejected RC EBN (RC_{hd}), from cave nests were cup- shaped EBN (RC_{cave}). The characterization includes physicochemical analysis (morphology, elemental composition, and color) and chemical analysis (nitrite concentration, antioxidant activity, total sialic acid content, total glycoprotein content and total polysaccharide content). Scanning electron microscope (SEM) images showed that no contaminants were found for all RC EBN products and that each RC EBN product has different structure. All RC EBN products have < 30 ppm nitrite and are export compliant. All RC EBNs showed antioxidant activity, DPPH and ABTS free radical scavenging activity, among which RC_{ha} and RC_{hb} had the highest ($P < 0.05$) antioxidant activity. RC_{cave} shows the lowest ($P < 0.05$) total sialic acid, total glycoprotein and total polysaccharide content among RC EBN products.

Keywords: Raw cleaned edible bird's nest, SEM, nitrite, sialic acid.

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Introduction

Edible bird's nest (EBN) is the nest built by swiftlet's' saliva of genus *Aerodramus* during breeding [1]. EBN basically produced in Southeast Asia and is known as nourishing food with delicious taste and high nutritional value among the Chinese community [2]. EBN can be harvested from cave (cave EBN) and swiftlet house (house EBN). There were differences in physicochemical properties, sialic acid content, nitrite content and mineral content between house nests and cave nests [3]. Recent studies showed the EBN nutritional values and pharmacological activities are including the body maintains and strengthens the immune system, promoting cell growth and cell division, enhanced antioxidant effect, anti-influenza virus, skin whitening, anti-aging, anti-inflammatory and wound healing (including corneal wound), prevents joint degeneration and protects cartilage from osteoarthritis, improving intelligence and memory functions of multi-generational mice, improvement of neurodegenerative disease (neuroprotection in Parkinson's Disease), anti-obesity and anti-hypertensive effects, improve cardiovascular disease and diabetic diseases [1,2]. Raw uncleaned (RUC) EBN is defined as EBN harvested from caves and swiftlet houses without any cleaning process, while raw cleaned (RC) EBN is EBN that has undergone cleaning processes such as sorting, soaking/ softening, removing feathers and foreign matter, shaping, drying, grading and packing [1].

EBN is made from viscous saliva and contains a high concentration of mucin glycoproteins, the main source of sialic acid. The sialic acids Neu5Ac and Neu5Gc are abundantly present in EBN (approximately 10%) [2]. Sialic acid is a signature element in EBN where consumers always refer to the presence and percentage of sialic acid to determine the purity and grade of EBN. Sialic acid is a biologically active compound for human nutrition and healthy development [4]. Nitrite (NO_2-) is an unstable, naturally occurring chemical in our food and water that consists of one nitrogen atom and two oxygen atoms. Nitrite may be beneficial to the body when it forms nitric oxide, on the other hand, it is harmful to the body when it forms nitrosamines, a carcinogenic compound [1]. In addition to the formation of nitrosamines, excessive daily intake of nitrites may have adverse effects. These adverse health effects include urinary tract cancer risk, thyroid cancer risk, and gastrointestinal cancer risk [5]. The acceptable daily intake for nitrite is 0.07 milligrams per kilogram of body weight per day [6]. In 2011, EBN products were banned from exporting to China due to high nitrite (NO_2-) levels, with the highest level detected in cave EBNs at 11,000 ppm [1]. China is the largest (82% of global trade) market for RC EBN products [7]. The Malaysian authorities had several bilateral discussions with the Chinese authorities, and a Memorandum of Understanding (MOU) on the Protocol on Inspection, Quarantine and Sanitary Requirements for Imported Bird's Nest Products was signed between Malaysia and China in September 2012 to continue the export of RC products to China. Every RC EBN exported to China must abide by the protocol to ensure food safety [1]. The requirements listed in the protocol for China include a requirement for nitrite content in EBN must be less than 30 ppm. The color of EBN is an important attribute and indicator when customers choose EBN [1]. Previous studies have shown that in swiftlet farms, nitrite, nitrate [8–11] and iron ions [12] can affect the color of bird's nests. In primary processing, high temperatures during drying can affect the color change of EBN [13]. Different grades of RC house EBN products are sold at different prices in the market. House and cave RC EBN are also sold at different prices.

This study aimed to understand the structural and chemical properties of different RC EBN products. RC EBN products including house EBN products with different grades (cup-shaped EBN, white EBN Biscuits, yellowish fragments and rejected RC EBN), and RC cave EBN products. These results allow us to learn more about the variability among RC EBN products in terms of morphology, color, nitrite content, antioxidant activity, total sialic acid content and total glycoprotein content.

Materials and Methods

Sample Collection

Figure 1 shows the raw clean (RC) EBN samples, including house and cave EBNs RC product. All house RC EBN was given by a privately owned company from 2019 to 2021. RC EBN included cup- shaped EBN (RC_{ha}), white EBN biscuit (RC_{hb}), yellowish fragment (RC_{hc}), and rejected RC EBN (RC_{hd}). The cup shaped cave RC EBN (RC_{cave}) was purchased from the market in 2022. RC_{ha} was higher grade RC product, and it was prepared through semi-dry picking of white raw uncleaned (RUC) EBN. Semi-dry picking is a cleaning method that only sprays water locally before removing impurities [14]. RC_{hb} was prepared using triangular or heavy feather white RUC EBN. RC_{hc} was the lowest grade RC product. The yellowish RUC EBN residue after picking was subjected to a second wash to obtain RC_{hc}. RC_{hd} was the rejected RC product due to the color (yellow- orange). RC_{cave} was red orange. Some of the RC_{hd} and RC_{cave} sample were double- boiled. The purpose of double-boiled samples is to obtain thinner EBN samples. After treatment, the RC_{hd} samples appeared to have 2 colors – light yellow and orange (Figure 1(e)). The RC_{cave} only showed one color which is red orange after double-boiled (Figure 1(g)).

Structural and Element Analysis

Scanning electron microscopy - energy dispersive X-ray microanalysis (SEM-EDX) was used to study the structural or morphology and element weight (%) of EBN samples. EBN sample was stick to the carbon tape and evenly coated with platinum. The sample was studied under a JEOL-JSM-7600F field emission SEM-EDX.

Nitrite Analysis

EBN sample was prepared by mixing 0.5 g of EBN powder with 40 mL of ultrapure water. Then the mixture was incubated in water bath at 70 ºC for 15 minutes and cooled down to room temperature. The mixtures were centrifuged at 8,000 rpm for five minutes. Then, the supernatant was taken and 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 x 250 mm) (Thermo Scientific, USA) with mobile phase 1.7 mM sodium bicarbonate at flow rate of 2.0 mL/min.

Color

The color of EBN sample was determined by using a calibrated hand-held colorimeter (CR-400 Chroma Meter, Minolta). The EBN must cover the whole lens when measurement was taken. The surface color parameters were expressed in L^* a^* and b^* value.

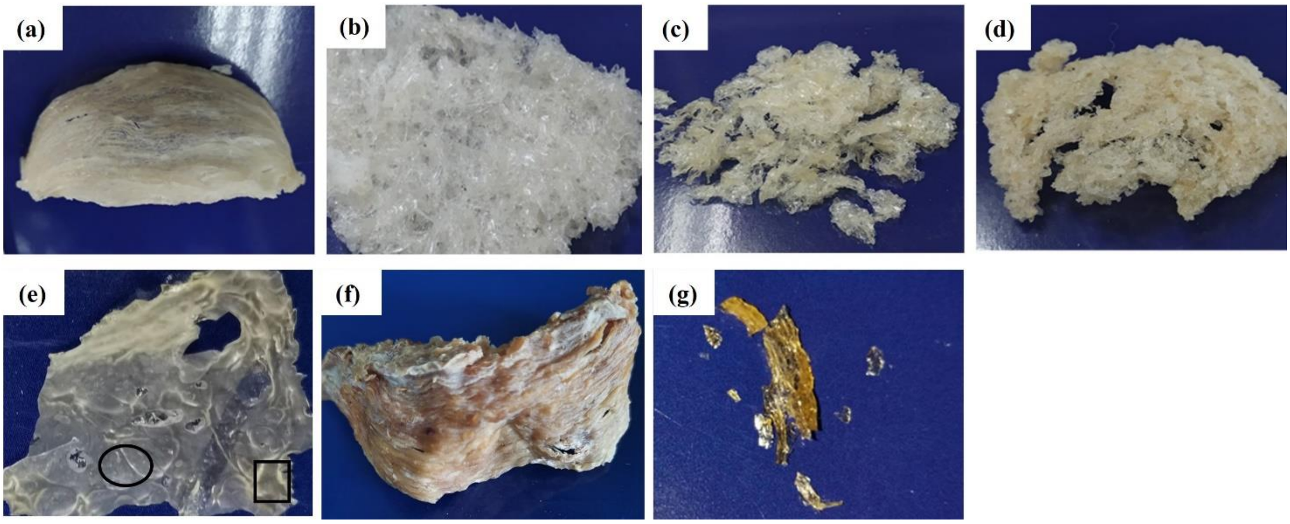

Figure 1: RC EBNs: (a) cup- shaped EBN (RC_{ha}), (b) white EBN biscuit (RC_{hb}), (c) yellowish fragment (RC_{hc}), (d) rejected RC EBN (RC_{hd}), (e) RC_{hd} after double- boiling: - $RC_{hd}DB_w$ light-yellow part of the sample (oval shape); and $RC_{hd}DB_j$ orange part of the sample (square), (f) cave RC EBN (RC_{cave}), and (g) RC_{cave} after double- boiling ($RC_{cave}DB$)

Antioxidant Activity

DPPH assay: 1 mL of EBN sample was added to 14 mL of DPPH reagent (0.036 mM). The mixture was incubated in the dark for 30 minutes. ABTS assay: 0.2 mL of sample was added to 1.8 mL of ABTS reagent (absorbance = 0.7 ± 0.2, at 734 nm). Later, the mixture was incubated in the dark for 10 minutes. For both assays, distilled water was used as blank, and for the control sample, distilled water was used instead of EBN for DPPH and ABTS assay. And for both assays the sample/control was filtered with a PTFE syringe filter (0.45 µm) after reaction and before reading absorbance. DPPH absorbance was read at 517 nm, and ABTS absorbance was read at 734 nm (SHIMADZU UV-VIS Spectrophotometer mini-1240, Japan).

Free radical scavenging activity (%) for DPPH/ABTS = [(DPPH/ABTS control absorbance -DPPH/ABTS sample absorbance)/ DPPH/ABTS control absorbance] * 100

Total Sialic Acid Content

The periodate-resorcinol assay [15] was used to analyse the total sialic acid content in the sample. In a test tube, 0.5 mL (EBN weight: 2.0 mg/mL) sample was mixed with 0.5 mL resorcinol reagent. Then the test tube was covered with chilled marble and incubated in boiling water (15 minutes). The sample was cooled to room temperature and 2.0 mL of 1-butanol was added to the sample after that. The sample mixture was vortexed vigorously to form a single-phase solution. The sample mixture was vortexed vigorously to form a single-phase solution. The samples were then incubated in a 37 °C water bath for three minutes to stabilize the color. After the sample returned to room temperature, the absorbance was measured at 580 nm (SHIMADZU UV-VIS Spectrophotometer mini-1240, Japan). N-Acetylneuraminic acid (analytical standard) was used as standard.

Total Glycoprotein Content

The Periodic Acid/Schiff (PAS) method [16] was used to determine the total glycoprotein content in the sample. The absorbance of the sample was read with visible spectrophotometer at 555 nm (SCILOX SCI- V1000)

Total Polysaccharide Content

The phenol-sulfuric acid method [15] was used to analyse the total polysaccharide content in the sample. In a test tube, 1.0 mL of sample (2.0 mg/mL) was mixed with 0.5 mL of 5 % phenol solution (w/w). Later 1.5 mL of concentrated sulfuric acid (H_2SO_4) was added to the test tube and shake gently. Then the sample was incubated at room temperature for reaction (10 mins). Sample absorbance was read at 490 nm (SHIMADZU UV-VIS Spectrophotometer mini-1240, Japan). Glucose monohydrate was used as standard.

Results and Discussion

SEM and Element Analysis

Three previous studies have used scanning electron microscopy (SEM) to study the structure of house nests, including: RUC EBN [17,18], RC (commercial) EBN [17-19] and enzyme treated RC EBN [18]. Previous studies have reported detection of mites, mite eggshells, mite fecal particles, feather filaments, fungal structures (yeast, hyphae, and fungal spores), bacteria, other arthropods, and some unidentified structures on the surface of RUC EBN [17,18]. Compared to RUC EBN, previous studies on RC EBN showed less [17,18] or no [19] contamination under SEM. The surface of RC EBN was found to be coated with a partially clear or transparent substance [17,19]. Another study showed different morphologies between houses and cave nests, but only at one magnification [20]. The surface of house nests has been reported to be relatively smooth, and the surface of cave nests has been reported to have clusters of nearly spherical particles [20]. The morphology or structure of different RC EBN products is shown in Figures 2 to 5, from magnification 50x to 25kx. Figures 2 to 5 show that no contaminants such as mites, fungal spores and plumes were found in any of the RC EBN products. Comparing RC_{ha} and RC_{hb}, both are structurally similar at lower magnifications of 50 x and 100 x. But RC_{ha} has a more complete structure and a rougher surface. A clear difference between these samples is the structural ordering, with RC_{hb} having a crystalline appearance at higher magnifications of 10k x and 25kx (Figures 3(d) and (e)). Figure 4 shows the structure of RC_{hc}, which has a smooth surface compared to RC_{ha} (Figure 2) and RC_{hb} (Figure 3). RC cave nest (RC_{cave}) have a relatively rough surface (Figure 5) and clustered grains on the surface (Figure 5(d)) compared to other house EBN RC products. The RC_{hd} sample was chosen for this study because RC_{hd} turns yellow-orange after primary processing. In this study, the colored RC EBN products (RC_{hd} and RC_{cav}e) were subjected to secondary boiling to obtain thinner samples for structural analysis. Figure 1(g) shows that the whole body of RC_{cave} is orange-red after the second boiling. However, RC_{hd} showed part light-yellow and part orange (Figure 1(e)). Figures 7(c) to (e) show that the orange part of the RC_{hd} is "three-dimensional" in appearance. More research is needed to understand this color change in RC products after primary processing and what causes this "three-dimensional" appearance.

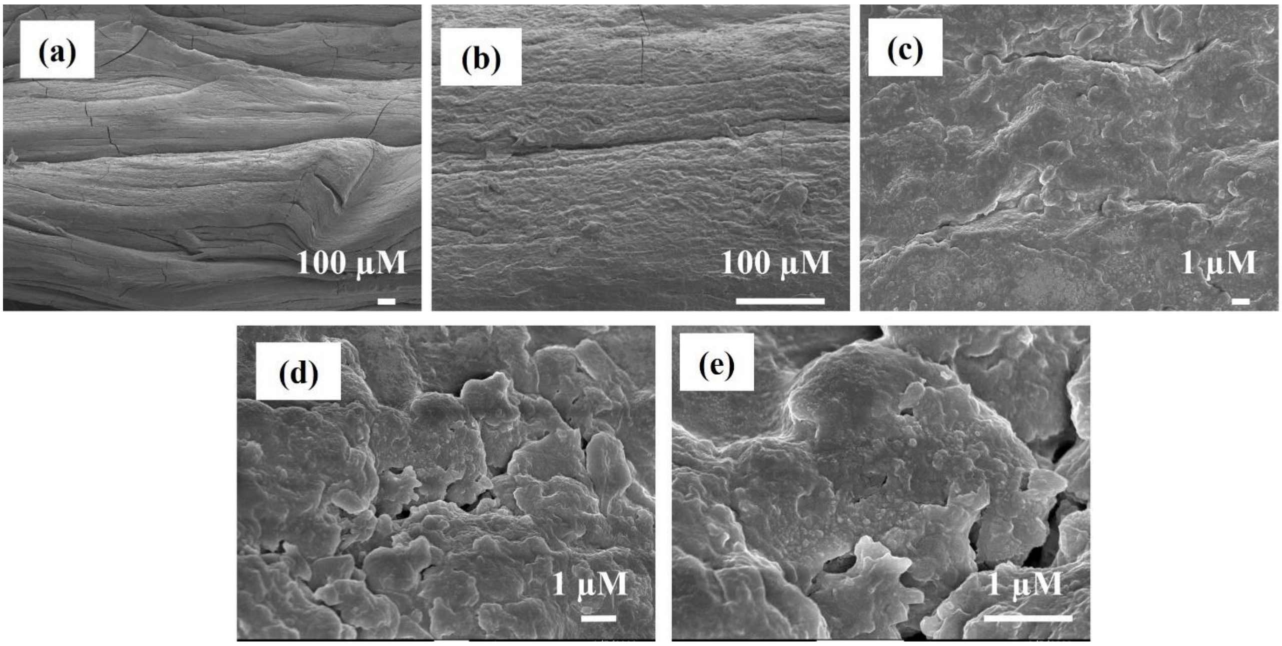

Figure 2: SEM micrographs of RC_{ha} at different magnifications

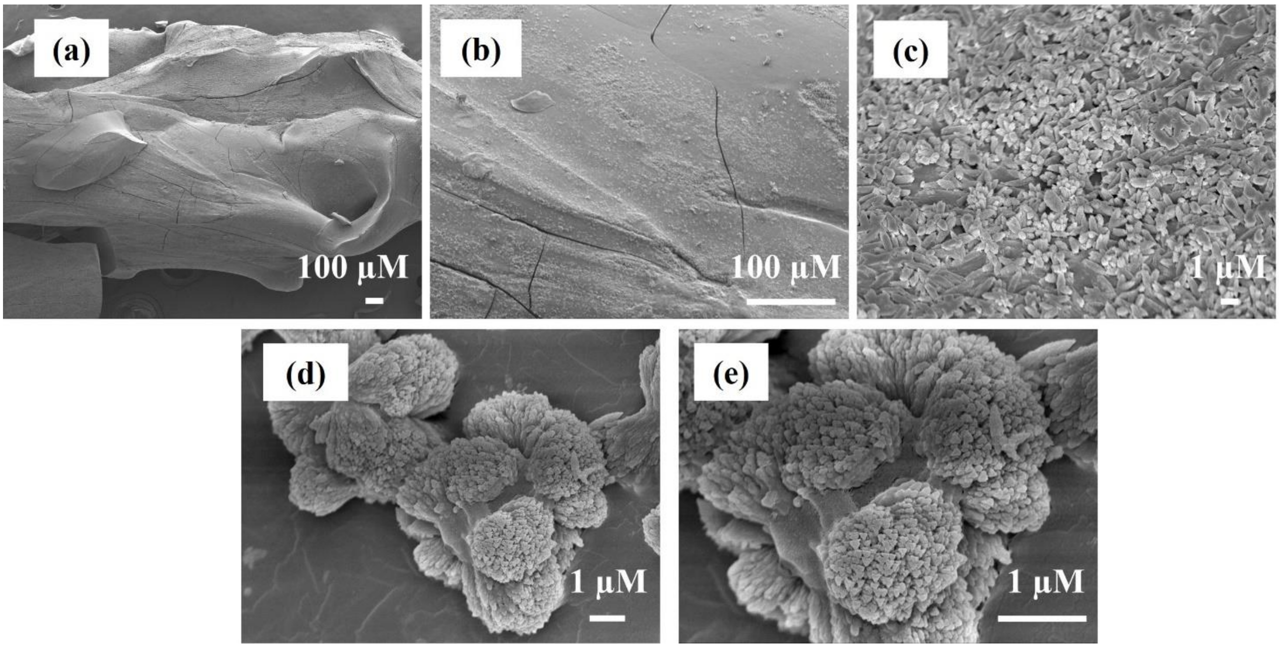

Figure 3: SEM micrographs of RC_{hb} at different magnifications

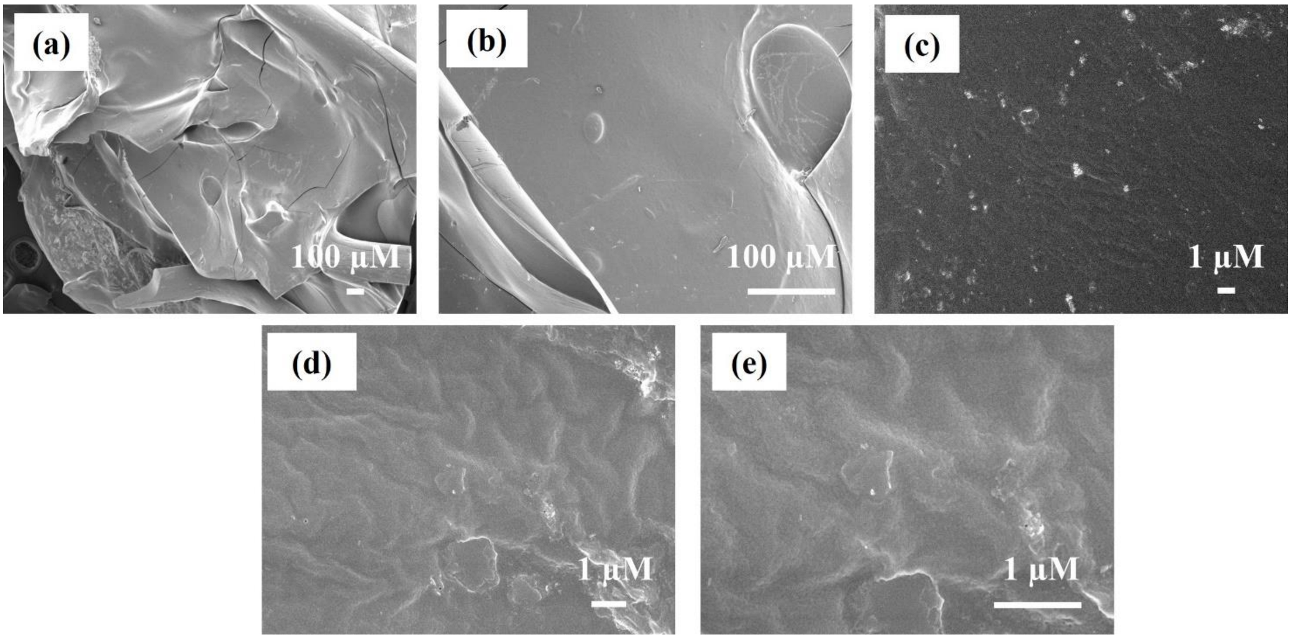

Figure 4: SEM micrograph of RC_{hc} at different magnifications

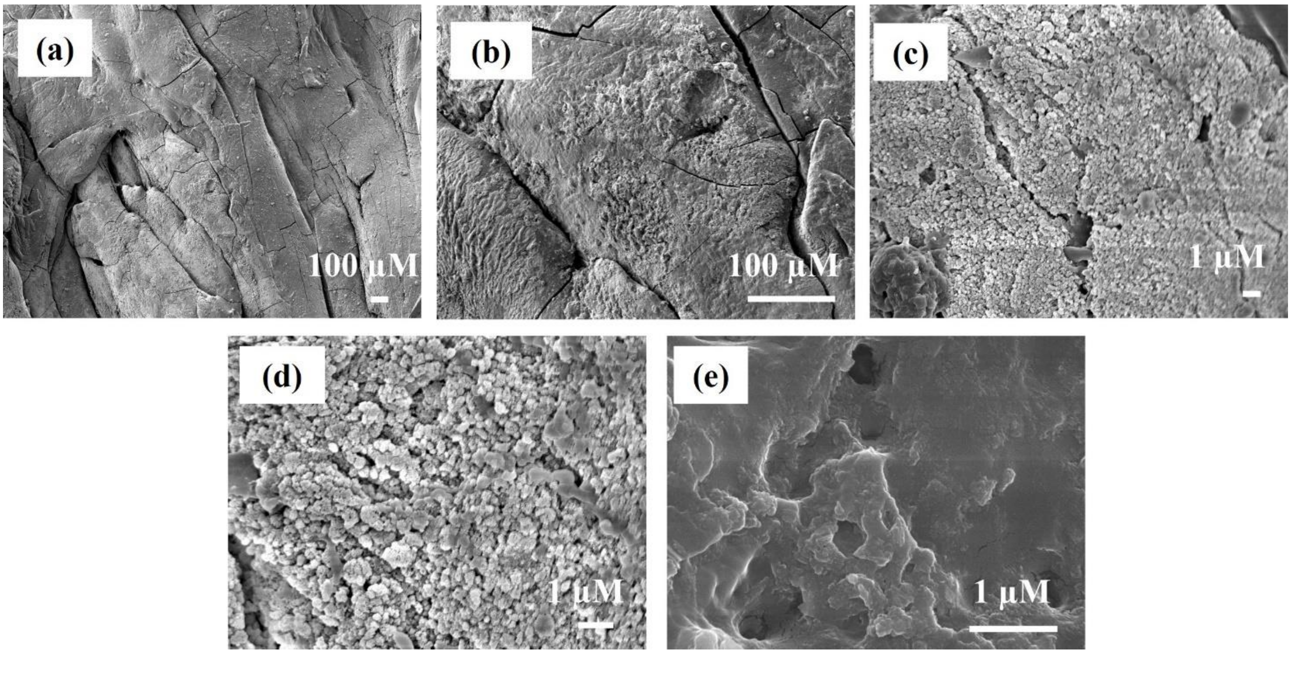

Figure 5: SEM micrograph of RC_{cave} at different magnifications

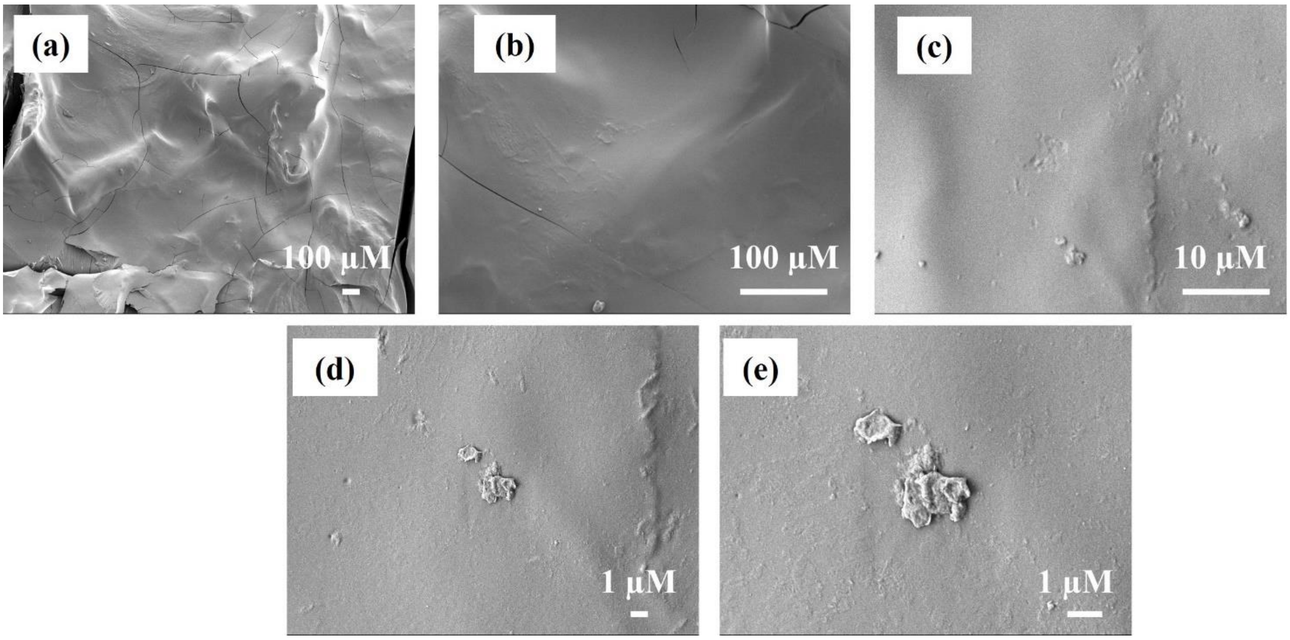

Figure 6: SEM micrograph of $RC_{hd}DB_w$ at different magnifications

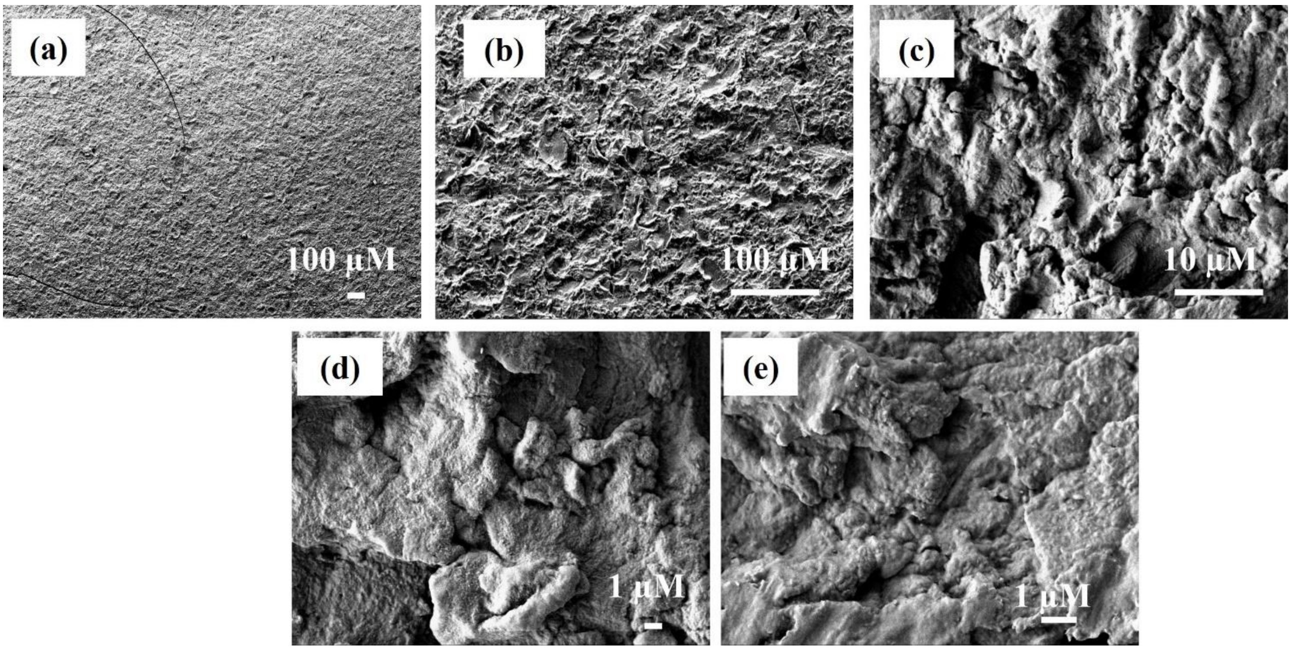

Figure 7: SEM micrograph of $RC_{hd}DB_j$ at different magnifications

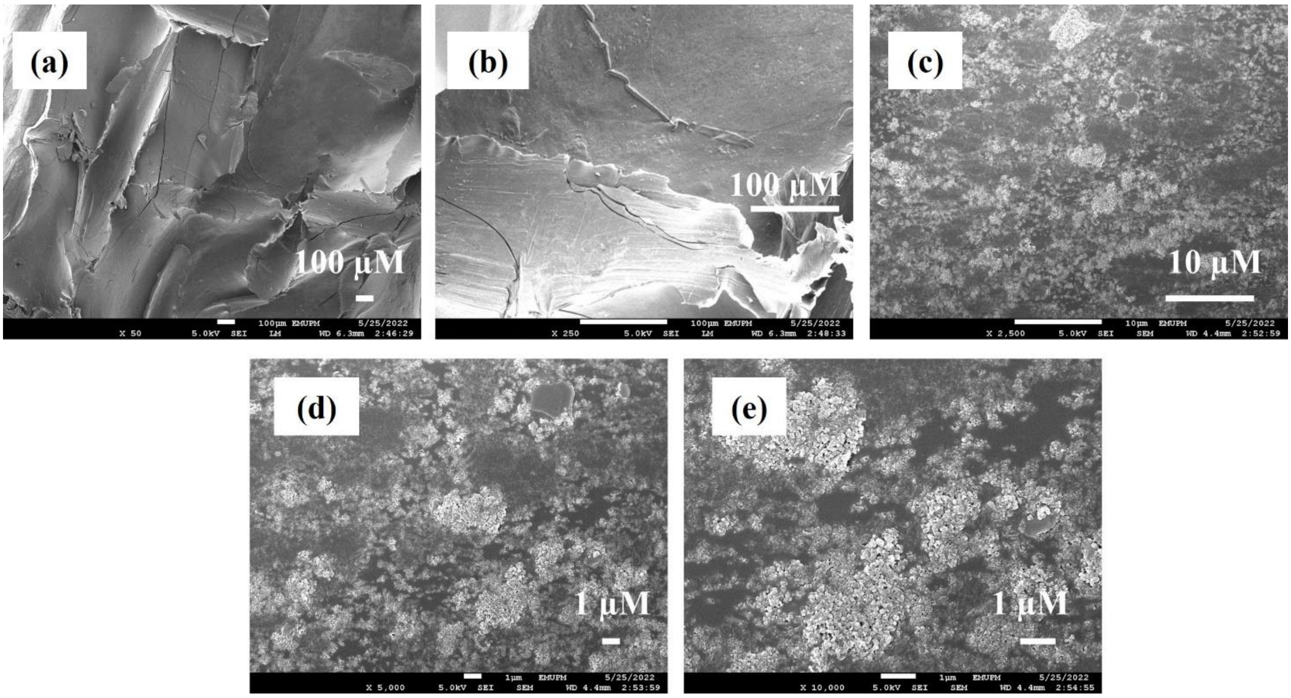

Figure 8: SEM micrograph of $RC_{cave}DB$ at different magnifications

Table 1 shows the elemental composition of the yellow- orange and orange-red RC EBN products detected by EDX. This study showed the presence of the elements C, O, Na, S, and Ca in both house (RC_{hd}) and cave (RC_{cave}) RC EBN. Element C and O are the main elements for the glycoprotein thus showed the highest weight % in the RC EBN product. This study showed elements
F, Mg, and Cl were not detected in the house nest. The element Al only detected after double-boiling at the light yellowish part ($RC_{hd}DB_w$) and with small percentage. Previous EDX's study [20] showed that element Al, Mg and Cl were detected in both house and cave nest. In this study, element N was not detected in cave nest. Element Ca showed significant higher ($p < 0.05$) in cave nest compared to house nest. This was suggested due to cave nests being harvested from caves (limestone walls). Calcium bicarbonate or carbonate in caves can leach and deposit on cave-nest cements, which may be the source of elemental Ca [20]. For house nest, element C, S, and Ca showed significant different ($p < 0.05$) before and after double- boiling for RC_{hd}. For the cave nest (RC_{cave}) no significant different ($p > 0.05$) for any elements in this study before and after double-boiling.

Table 1: Elemental composition (weight %) for red- orange RC EBN products, before and after double-boiling

Element	**RC_{hd}**	**$RC_{hd}DB_w$**	**$RC_{hd}DB_j$**	**RC_{cave}**	**$RC_{cave}DB$**
C	59.48 ± 5.53	67.02 ± 0.69	65.29 ± 0.96	55.05 ± 2.64	56.90 ± 5.87
O	33.42 ± 2.31	32.92 ± 0.67	33.60 ± 1.05	35.77 ± 0.88	34.18 ± 5.52
Na	0.10 ± 0.05	0	0	0.79 ± 0.09	0.07 ± 0.04
N	4.97 ± 3.23	0	0	0	0
S	1.54 ± 0.31	0.02 ± 0.01	0.05 ± 0.02	1.03 ± 0.16	1.24 ± 0.51
F	0	0	0	0.42 ± 0.42	2.94 ± 1.31
Ca	0.49 ± 0.17	0.01 ± 0.00	0.035 ± 0.08	5.04 ± 1.62	3.84 ± 0.94
Mg	0	0	0	0.45 ± 0.14	0.17 ± 0.08
Al	0	0.02 ± 0.02	0	0.36 ± 0.03	0.42 ± 0.17
Cl	0	0	0	0.71 ± 0.15	0.25 ± 0.22
Si	0	0.01 ± 0.01	0.65 ± 0.16	0.38 ± 0.22	0

Nitrite Content

Table 2 shows the nitrite content for all RC EBN products. All RC EBN products in this study contained < 30 ppm nitrite, meeting one of the requirements for exporting RC EBN to China. The RC_{ha} sample showed significantly ($P < 0.05$) the highest nitrite content compared to the other samples. This condition might be contributed by the cleaning method and storage time. RC_{ha} was cleaned using a semi-dry picking method with limited time exposure to water. Previous studies have shown that longer washing times can significantly reduce nitrite in EBN [21]. Increased nitrite levels in lettuce due to longer storage times [22], and increment of nitrite content in all RC samples after storage for 6 months [23] have been reported from previous studies. The RC_{ha} was received at 2019, and the rest of the samples were received after June 2020. Storage time for RC_{ha} was longer than other samples. The RC_{cave} sample (cave EBN) showed the lowest (significant, $P < 0.05$) nitrite content among the samples. Previous studies reported nitrite content in house nest was between 0.2-317.08 ppm [1,3,11], and cave EBN was between 0.4 – 843.8 ppm [1,3]. N element (Table 1) was not detected in cave EBN (RC_{cave}).

Color

Color for all the RC EBN products shows in Table 2. RC_{hd} had the highest *a** and *b** values ($p < 0.05$) among the products of RC House EBN. This is a rejected RC product, the color turns yellow- orange after processing, the reason was unknown. This study suggested that the color change after processing may not be affected by the drying process and nitrite concentration. Because RC_{hd} was dried under the same drying conditions as RC_{ha}, RC_{hb} and RC_{hc}, the nitrite concentration was the lowest among RC house EBNs products. We suggest that structural changes may also affect the color of EBN. SEM micrographs of $RC_{hd}DB_j$ (Figure 7) reveal an uneven surface structure not found in other samples. RC_{cave} with the highest redness (*a** value) but with the significant lowest nitrite and no N element detected in the sample. The relationship between color with other elements remains inconclusive.

Table 2: Nitrite concentration and color for different RC EBN products.

Sample	Nitrite (ppm)	Color L^*	a^*	b^*
RC_{ha}	21.93 ± 0.75^a	69.12 ± 0.45	2.64 ± 0.12	12.47 ± 0.71
RC_{hb}	5.01 ± 0.5	74.21 ± 0.21^a	0.19 ± 0.01^b	12.35 ± 0.23
RC_{hc}	4.21 ± 0.11	72.53 ± 0.35	0.77 ± 0.03	14.34 ± 1.81
RC_{hd}	4.02 ± 0.32	72.17 ± 0.07	5.31 ± 0.06	20.40 ± 0.32^a
RC_{cave}	2.77 ± 0.44^b	44.04 ± 0.03^b	8.91 ± 0.32^a	12.65 ± 0.55

*The superscript *a* indicates significantly the highest in the same column ($P < 0.05$), and the superscript *b* indicates a significantly the lowest in the same column ($P < 0.05$).

Antioxidant Activity

The antioxidant activity, DPPH and ABTS free radical scavenging abilities of samples RC_{ha} and RC_{hb} were significantly higher than those of samples RC_{hc}, RC_{hd} and RC_{cave} ($P < 0.05$). But there was no significant difference between RC_{ha} and RC_{hb}. Sample RC_{hc} had the lowest DPPH ($P > 0.05$) and ABTS ($P < 0.05$) free radical scavenging abilities.

Total Sialic Acid Content

Previous research has shown that, on average, house nests have higher sialic acid content than cave nest [3,24]. This study also shows (Table 3) the same outcome, the RC_{cave} sample had the significant ($P < 0.05$) lowest total sialic acid content. The total sialic acid content of RC house EBN products was 5.89 – 18.67 %, and RC cave EBN product was 2.69 %. The difference suggested was due to swiftlet species, habitat and food source [24]. Between the RC house EBN products, RC_{hc} had the significantly ($P < 0.05$) lowest total sialic acid content.

Total Glycoprotein Content

Among samples, sample RC_{ha} had the significantly ($P < 0.05$) highest and sample RC_{cave} had the significantly ($P < 0.05$) lowest total glycoproteins (Table 3). Total glycoprotein content for the samples RChb, RChc and RChd were not significant ($P > 0.05$) different.

Total Polysaccharide Content

Table 3 shows that RC_{cave} had a significant ($P < 0.05$) lowest total polysaccharide compared to the RC EBN product from the house nest. Previous studies reported carbohydrates (approximate analysis) of RC EBN products [3,24]. To date, studies on the total polysaccharide content of RC EBN are limited. These studies showed that the total polysaccharide content of RC products of house EBN was 1.51-14.6 % [15,25]. Total polysaccharide content has not been studied in any of the RC products of Cave EBN. Between the RC house EBN products, RC_{hc} had the significantly ($P < 0.05$) lowest total polysaccharide content.

Table 3: Chemical analysis results for different RC EBN products

Sample	DPPH Free Radical Scavenging Activity (%)	ABTS Free Radical Scavenging Activity (%)	Total Sialic Acid Content (%)	Total Glycoprotein content (%)	Total Polysaccharide Content (%)
RC_{ha}	28.14 ± 1.11	99.90 ± 0.10	13.49 ± 0.38	38.47 ± 0.57^{a}	7.15 ± 0.16
RC_{hb}	28.08 ± 1.66	99.19 ± 0.25	14.19 ± 0.18	22.67 ± 0.95	8.73 ± 0.11
RC_{hc}	10.10 ± 0.53	66.99 ± 1.81^{b}	5.89 ± 0.13	20.10 ± 0.96	2.34 ± 0.10
RC_{hd}	11.80 ± 0.81	86.03 ± 0.29	14.52 ± 0.30	20.71 ± 1.37	8.38 ± 0.42
RC_{cave}	14.93 ± 0.57	83.34 ± 1.08	2.69 ± 0.18^{b}	12.70 ± 1.33^{b}	1.74 ± 0.02^{b}

*The superscript *a* indicates significantly the highest in the same column ($P < 0.05$), and the superscript *b* indicates a significantly the lowest in the same column ($P < 0.05$).

The antioxidant activity, sialic acid content and total polysaccharide of EBN from this study were affected by the cleaning methods. RC_{hb} was exposed to water for longer durations than RC_{ha}, but there were no significant differences in the chemical analysis results. The difference in the cleaning process between RC_{hb} and RC_{hc} is that RC_{hb} was cleaned directly from the RUC EBN. The raw material for RC_{hc}, on the other hand, was residual EBN (fragmented EBN with feathers) from primary processing. Residual EBN during picking soaks/emerges in water and the residue takes the shape of chips. Then, these residues were washed and cleaned to obtain RC_{hc}. RC_{hc} was exposed to water for an extremely long time (2- 4 hrs), and in addition, the EBN was thinner and smaller when exposed to water. The antioxidant activity, total sialic acid content, and total polysaccharide content of EBNs were affected by the shape and duration of EBNs exposure to water.

The cup- shaped house RC EBN (RC_{ha}) shows the significantly ($P < 0.05$) highest total glycoprotein content but not the highest total sialic acid compared with RC_{hb}. Previous study had shown that sialic acid content in RC EBN was significantly reduced after a period of storage [23]. RC_{ha} has been stored longer time than the other RC products. Another possible reason for the significant difference between samples RC_{ha} and RC_{hb} is the detection method. The periodic acid Schiff assay was used to measure glycoprotein content but was inefficient in the measurement of glycopeptides. Previous studies have shown that the shorter the hydrolysis time of bird's nest, the higher the concentration of glycoprotein. Longer hydrolysis times yielded higher amounts of EBN glycopeptides [16]. In this study, RC_{ha} was thicker than RC_{hb} and exposed to less water during primary processing. This may result in less hydrolysis when samples are prepared prior to periodic acid Schiff analysis (double boiling/ heat treatment - 1 h). Therefore, RC_{ha} might contain higher amounts of glycoproteins, while RC_{hb} might contain higher amounts of glycopeptides.

The sample RC_{cave} in this study showed significantly low total sialic acid, total glycoprotein, and total polysaccharides. This may be related to swiftlet species, habitat, and food source, or it may be the limitation of the detection method. These methods quantify the content in liquid or supernatant of EBN samples. For sample RC_{cave} after pretreatment (double boiling - 1 h), solid EBN was still observed at the bottom of the bottle, while for other samples, EBN in gel form was observed. This may affect the quantity of sialic acid, glycoproteins, and polysaccharides in the supernatant.

Conclusions

This study uses high magnification of SEM images of various RC EBN products. Through these SEM images, each RC EBN product with a different structure can be observed. This may be due to the raw material (RC EBN) and different cleaning methods during primary processing. The chemical analysis in this study shows that: RC products from cave EBN were significantly different from those from house EBN, RC_{ha} and RC_{hb} were different grades of products in the market, but show similar antioxidant activity and total sialic acid content, on the other hand the lowest grade RC EBN product (RC_{hc}) compared to RC_{ha} and RC_{hb} had significantly lowest antioxidant activity and total sialic acid content, and the rejected product RC_{hd} showed significantly lower antioxidant activity but similar total sialic acid content compared to RC_{ha} and RC_{hb}.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

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