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Review Article

A Review on the Edible Bird's Nest Quality and Manufacturing Standards of the Three Largest Exporting Countries in the World

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Edible bird's nest (EBN) is an animal product with the world's highest market price due to its value. The nests are made exclusively from the saliva secreted by swiftlet, a species of bird native to Southeast Asia. For over a century, EBN has been consumed in many parts of the world as a nutritious food. The high economic value of EBN attracts people to invest and engage in the bird nest industry. Currently, China is the largest importer of EBN, while Southeast Asian countries, namely, Indonesia, Malaysia, and Thailand, are the three largest exporters of EBN. An analysis of EBN's compositions from most previous studies revealed that protein, carbohydrate, moisture, fat, and ash in EBN from the three central producing countries did not have apparent differences in their origins and were comparable to each other. Before 2011, EBN trade with China was unregulated. Consequently, the industry encountered problems due to the high nitrite content in EBN. Since then, these three countries have taken great measures to deal with this food safety issue and formulated a standard operating procedure (SOP) to meet the specific criteria listed for exporting EBN to China. Hence, this review discusses the quality and safety standards of EBN from the three countries and China's standards for EBN importation.

1. Introduction

EBN refers to the nest created usually by male swiftlets. In contrast to other birds that build their nests out of materials such as grass, twigs, sticks, and mud, the swiftlet is distinguished through its nest-building behaviour as it produces EBN from saliva. Two enlarged sublingual salivary glands under the tongue of the male swiftlet secrete the saliva during nesting and breeding seasons, which then wound into a translucent, half-cup shape to tolerate its eggs and nestlings [1]. The swiftlets are a small bird species that requires specific environmental conditions for growth and reproduction, such as humidity of about 90% and temperature of 28°C~30°C [2]. Hence, they can be abundant throughout Southeast Asian and South Pacific countries such as Malaysia, Thailand, Indonesia, Vietnam, China, and the Philippines.

More than 24 species of swiftlets are reported globally. However, a few of the many swiftlets identified worldwide are notable for producing valuable EBN commercially. The most harvested and exploited is from the *Aerodramus* genus (echo-locating swiftlets species), which are *A. fuciphagus* (white-nest swiftlet) and *A. maximus* (black-nest swiftlets) [1]. These two species commonly live in sea caves in the Indian Ocean and along the coastlines of Southeast Asian countries [3]. Naturally, swiftlets prefer to nest in dark and highly humid caves. Nevertheless, extreme humidity can lead to fungal growth and inhibit EBN swiftlets from

creating their nests. Furthermore, a nesting site with a higher, inwardly sloping, smooth, and concave surface is chosen over one with a low and rugged cave wall to ensure higher breeding success. A smooth and concave surface offers better support for nest construction and more remarkable nestling survival, whereas steeper and inwardly sloping regions protect eggs and nestlings from predators [4]. EBN built exclusively by a swiftlet in natural stone walls or limestone caves is commonly known as cave EBN. The nest exhibits mostly beige, orange-red, brown, or yellow colour with redhead and has a rugged, solid texture as it absorbs natural minerals from the caves. Conversely, the colour of artificial EBN ranges from white to yellow and brown [5]. Historically, the natural nesting habitat of swiftlets originated from limestone caves. However, over the century, EBN house farming has been implemented to support these birds in nesting away from caves. Due to the high demand for EBN and the risk of harvesting EBN from caves, swiftlets are domesticated and bred to increase the production of EBN. In EBN farming, people have transformed buildings or created structures that closely imitate their native cave environment and attract swiftlets using recorded swiftlet audio. As a result, EBN farming practices have been developed in Indonesia (60%), Thailand (20%), and Malaysia (10%), and the number is increasing every year as it can earn a lucrative return.

EBN, referred to as the "caviar of the East," has been highly consumed by the Chinese community, mainly in China, Taiwan, Singapore, and North America, followed by the new growing market in the Middle East, Japan, and Korea [6]. EBN is known as "Yan Wo" in the Chinese literature, and soup made from EBN is a great delicacy in the Chinese cuisine and a health supplement [7]. The consumption of EBN soup is known to have purported multiple health beneficial effects. Previous studies revealed that the supplementation of EBN contributes to improved cell proliferation, promotes hair growth, and enhances bone strength [2, 7, 8]. Several reports have attributed these health benefits to the many bioactive compounds of EBN, such as sialic acid and antioxidant activity in EBN. EBN is a prominent source of sialic acid. As reported previously, sialic acid makes up approximately 10% of the weight of EBN [9]. Sialic acid is one of the key components of EBN for brain development in infants and plays an essential role in synaptic connections, neuronal growth, memory formation, and immunity enhancement [10, 11]. Sialic acid from EBN was also observed to improve cognitive performance in mice and increase cell viability [12]. Besides, EBN also possesses antioxidant and antiageing effects due to its high antioxidant activity. An in vitro study by Hu in 2016 evaluated the antioxidant capacity of EBN in flies (*Drosophila melanogaster*) [13]. Results show that EBN can inhibit the formation of malondialdehyde in flies, which is associated with the ageing process. Hu et al. [13] also reported significant antioxidant activity in EBN-treated flies, as indicated by the improved catalase and superoxide dismutase (SOD) actions. EBN contains many high-density lipoproteins, thus making it an essential feature of high antioxidant activity [14]. Nonetheless, the positive impacts of EBN are contingent upon the quality of EBN itself, which various factors, including nesting seasons, can influence harvesting windows, habitat conditions, food resource availability, and the specific species origin of the swiftlets involved.

Consequently, the rapid growth of EBN consumption due to its various health and medicinal benefits has led to strong global demand, especially in China, for the prized delicacy and thus raised the market price of the bird nest's commodities. EBN is categorized as one of the most expensive food items globally, alongside Kopi Luwak and argan oil derived from argan nuts that have undergone digestion in goats' digestive tracts [15]. In addition, EBN is placed among humans' most expensive animal products, retailing at prices ranging from US$1000.00 to US$10,000.00 per kilogram [16]. EBN prices are determined based on the colour, shape, and curve of the EBN. In addition, raw-clean (RC) EBN has a higher market price than raw-unclean (RUC) EBN, as it involves a lengthy cleaning process. In the primary producing nations, such as Malaysia, Indonesia, and Thailand, EBN quality requirements focused on sensory indices, water content, and microbiological and nitrite limits. Meanwhile, the EBN quality requirements in China were based on sensory index, size, moisture, protein, and sialic acid [4]. Based on Malaysian Standard (MS), raw EBN can be categorized into two-part, namely, raw-unclean EBN and raw-clean EBN [17]. RUC is EBN harvested from a cave or swiftlet's house without a cleaning process with visible feathers and impurities, while RC is EBN that has undergone a meticulous cleaning process. The exact definition of RC EBN applies under Indonesian regulation with physical observation (naked eye) at a distance of 20–30 cm as it is free from feathers and any visible impurities [18].

China has been the leading importer of EBN worldwide, with a demand of 80 metric tons a year. Meanwhile, Indonesia was recorded as the world's largest exporter of EBN, exporting around 2000 tons per year, accounting for 70%–80% of the global market of EBN. Other top producers and exporter countries of EBN also include Malaysia (600 tons per year) and Thailand (400 tons per year). In 2009, Indonesia's contribution to the global EBN supply exceeded 100 metric tons, constituting 60% of the world's EBN production valued at RM 500 million [1]. Conversely, Thailand generated revenues of 10 billion Baht annually from trading houses and cave EBN, amounting to 400 tons [19]. Malaysia is the third largest EBN producer, supplying approximately 10% of the global EBN output. China stands out as the largest importer of RC EBN, accounting for 82% of the worldwide trade, which amounted to 105.2 tons in 2019. Furthermore, China dominates the EBN market, consuming over 90% of the total EBN.

Unfortunately, in August 2011, the EBN industry faced problems as a safety food scandal broke out when China received reports of contamination of EBN products imported from overseas. The abrupt decline in the prices of EBN occurred following China's imposition of a ban on EBN imports from Malaysia and Indonesia. This ban was prompted by detecting excessively high nitrite content, measuring 4,400 mg/kg, in EBN products from these regions, significantly surpassing the national limit of 70 mg/kg.

Consequently, there was a notable reduction in the demand by 20–30% for EBN, resulting in a 60% drop in the selling price of grade "A" RC EBN, plummeting from RM 4,000/kg to a range of RM 1,200 to RM 1,500/kg [20]. The nitrate scandal did not exclusively impact EBN exporters in Malaysia and Indonesia; it also affected Thailand. Consequently, immediate research efforts were undertaken to mitigate nitrite content and elevate the overall quality of EBN. In response, each country took proactive measures by amending various standard operating procedures (SOPs) to ensure the highest EBN quality and safety standards for human consumption. Notably, in September 2012, Malaysia and China signed a protocol governing EBN exports to China, focusing on the examination, quarantine, and hygiene of EBN. Lastly, in December 2013, China lifted its trade ban on EBN imports from Indonesia and Malaysia after both governments signed memoranda of understanding to reinstate this lucrative trade relationship. This review provides a comprehensive summary of the following aspects: (1) quality distinctions in EBN, including a comparative analysis of proximate characteristics of EBN from the three largest producing nations, variations between RC and RUC EBN, and distinctions between house and cave EBN; (2) levels of nitrite and nitrate found in EBN; (3) factors related to the grading of EBN; and (4) the standards, regulations, and SOPs put in place to meet consumer demands, whether for domestic distribution or export to China.

2. Quality of EBN

2.1. Proximate Analysis

2.1.1. Protein. Over the years, the fundamental research has emphasised the analysis of the compositions of EBN. This is crucial in understanding the biological action of EBN as functional food, thus creating translational research. Ultimately, in-depth studies on the properties of EBN yielded significant findings in facilitating the development of functional food products and determining the value and quality of EBN. Based on previous works of the literature, the proximate composition analysis found that the main components of EBN are protein, carbohydrate, moisture, ash, and fat.

The average protein content in EBN ranged from 50 to 55%, which is compared to carbohydrates, moisture, ash, and fat [2]. As the major component and bioactive in EBN, protein will most likely be the primary focus when screening EBN's quality. The bioactive peptides derived from EBN hydrolysates can have various biological and nutritional properties, such as angiotensin-converting enzyme (ACE) inhibitory peptides that reduce blood pressure. Moreover, proteins in EBN, particularly lactoferrin and ovotransferrin, have been found to reduce H_2O_2-induced cytotoxicity and lower radical oxygen species in human SH-SY5Y cells via enhanced scavenging activity [21]. The high protein level of EBN also demonstrates its potential use as a protein supplement for human diets.

Commonly, the determination of protein in EBN is performed using the Association of Official Analytical Collaboration (AOAC) standard method via the Kjeldahl method and calculated using 6.25 as the protein conversion factor [1]. According to the Thailand Standard (TAS 6705-2014), the Kjeldahl method (AOAC 930.29) must be explicitly used in the analysis of EBN's protein to detect the authenticity of RC EBN [22]. Accordingly, procedures such as Bradford protein assay, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and liquid chromatography-tandem mass spectrometry can be employed to analyse and quantify proteins extracted from EBN [23]. Based on Table 1, most of the published works of the literature were conducted using the Kjeldahl method, except for the report from Hamzah et al. [22]. Instead, they conducted a combustion method by using a CHNS elemental analyser to determine the percentage of nitrogen and applied protein factor of 6.25, resulting in high protein content (59.8–60%). These results were comparable with the Kjeldahl method and can be justified by the previous study from Marcó [32], as it obtained high precision and accuracy results with a 95% confidence level.

The compositional studies of EBN collected from different regions in Malaysia, Indonesia, and Thailand are summarised in Table 1. Based on the data compiled, it is evident that the crude protein content is the highest among all the elements tested, as many researchers reported the protein content ranging between 24.4% and 68.0% of the total composition. A proximate analysis of EBN from different regions in Indonesia showed that protein is the largest constituent of EBN, from 53.4% to 55.9% [29], which is comparable to that reported by Hamzah et al. [22] (59.8% to 60.0%) in Malaysia. These results correlate with the previous report by Saengkrajang et al. [5], as the protein content of EBNs from Thailand is within the range of the protein content of EBNs from surrounding countries. Similarly, Norhayati et al. [28] found that the highest component of EBN obtained from different states of Peninsular Malaysia is protein, accounting for 61.8 to 65.2%. It is also reported that although significant differences between the regions are not determined, seasonal variations were observed in the quantity of protein present in the EBNs. The difference in harvesting seasons of the EBNs from the different locations may explain this. In fact, EBN harvested during the rainy season has a higher protein level than other seasons. The rainy season is the most suitable time for swiftlets to breed, as an abundant food supply provides a suitable environment for insects to proliferate [28]. Furthermore, EBN harvested during the rainy season is high quality as it is large, thick, highly swollen, and has fewer impurities [33]. Meanwhile, EBN harvested during the dry season might impact the protein content as EBN is found to be dirty with feathers and other impurities [34].

Although most of the data analysed showed protein content approximately above 50%, few outliers were observed in determining protein in EBN. A low amount of protein was exhibited in EBN 35.8% and 24.4% collected by the authors in [14, 27], respectively. This may be attributed to the difference in environment as both EBNs were obtained from Johor and Penang, Malaysia, which regions are known to be developed as industrial areas. Rapid deforestation for the development of the industrial area may lead to a decline

Table 1: Proximate composition of edible bird's nest (EBN) samples in Malaysia, Indonesia, and Thailand.

	EBN types	Origin	Protein (%)	Carbohydrate (%)	Moisture (%)	Ash (%)	Lipid (%)	Reference
House EBN								
Malaysia	RC	Johor	55.3	21.5	17.7	5.1	0.3	[24]
		Kelantan	56.3	23.1	15.3	5.0	0.3	
		Perak	54.2	22.4	17.6	5.4	0.3	
		Sarawak	55.2	20.1	16.7	5.4	1.9	
	RC	Kedah	54.3	29.7	10.8	2.8		[25]
		Sarawak	53.9	30.5	12.4	2.7		
		Pahang	54.3	28.3	12.2	3.1	0.1	
		Selangor	55.1	30.3	12.6	2.5		
	RC	Johor	54.4	28.6	14.0	2.9		[26]
		Pahang	58.6	22.3	15.9	2.6	0.7	
		Terengganu	55.5	25.8	15.9	2.6	0.3	
	RC	Penang	24.4	58.2	13.8	2.8	0.5	[27]
	RC	North	60.3–63.6	—	—			[28]
		South	61.8–65.2					
		East coast	57.9–61.2					
	RC	—	58.6	22.3	15.9	2.6	0.7	
	RC	Perlis	60.0	12.0	12.8	2.0	0.01	[22]
	RC	Johor	35.8	46.5	11.3	5.2	1.5	[14]
Indonesia	RC	Sumatra	55.9	20.1	18.5	6.0	0.3	[29]
		Java	53.4	20.7	19.6	5.8	0.6	
		Kalimantan	53.5	23.0	17.7	5.5	0.4	
		Sulawesi	54.4	20.2	19.6	5.6	0.2	
Thailand	RUC	Songkhla	58.6	24.4	10.9	3.1	0.01	[30]
	RC	Trat	66.9	25.4	24.3	6.8	0.8	[3]
		Phetchaburi	61.0	31.0	17.8	6.7	1.1	
		Nakhon Si Thammara	60.9	30.4	19.2	7.4	1.3	
		Satun	61.5	31.4	19.0	5.9	1.2	
		Narathiwat	62.6	30.1	18.8	6.8	0.4	
Cave EBN								
Malaysia	RC	Langkawi	59.8	11.0	13.9	3.0	0.1	[22]
	RUC		—	9.8	9.38	9.0	0.1	
	RC	Sarawak Sabah	57.4	19.1	18.6	7.9	0.1	[7]
	RC	Sarawak Sabah	67.3	22.5	18.2	6.6	0.2	[31]
Indonesia	RC	North Sumatra	45.5	29.4	15.7	7.6	0.5	[27]

RC, raw-clean EBN; RUC, raw-unclean EBN.

in the diversity of insects and the food supply of swiftlets [35].

On the other hand, it can also be noted from Table 1 that the protein content for house EBN is slightly higher than cave EBN (more than 60% protein content). The difference in environmental conditions between a cave and house EBN may contribute to such findings. This includes temperature, relative humidity, light intensity, and feeding behaviours [31], such as extensive swiftlet ranching practices that have been implemented by ranchers and encouraged by the Malaysia government. In addition, the Malaysian government has also provided documented guidelines for adopting these practices to ensure continuous improvement and sustainable production of EBN (MS 2273:2012) (No. 484/Kpts/OT.160/L/4/2012).

(1) Amino Acid. Besides protein, amino acid in EBN was also explored properly [14, 26, 36]. EBN is a rich source of both essential and nonessential amino acids. A modern analysis of amino acid compositions has been reported in various pieces of the literature. Eighteen out of twenty types of amino acids were detected in EBN. This includes nine essential amino acids (phenylalanine, valine, threonine, histidine, tryptophan, isoleucine, methionine, lysine, and leucine) and nine nonessential amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine, and tyrosine). The AccQ-Tag method has been used to characterize amino acid profiles. It is a chromatographic-based technique that involves the use of an amino acid-specific derivatizing reagent and consists of three main steps, namely, (1) hydrolysis of individual amino acids from the protein backbones under acidic, alkaline, or oxidation pathways; (2) separation of individual amino acid using chromatographic procedure; and (3) detection and quantification of the separated amino acid commonly using fluorescent or UV-Vis detection [14, 37, 38].

The amino acid composition is important in protein as it affects the nutritional value (essential amino acids) and functional properties in EBN, such as cysteine and arginine. Cysteine, specifically, is abundant and plays a significant role in the unique scent and taste of EBN. Glycine, proline, and lysine, which are amino acids, play a role in both the

nutritional value and potential therapeutic benefits of EBN [39]. According to the previous research, there were discrepancies in the amino acid compositions [40]. These differences might be attributed to the diverse origins and species of the EBN samples [31]. Furthermore, variations could have arisen from processing and potential adulteration, as well as the absence of standardized processing and cleaning protocols. In another study, Kilthan and Weeks determined three nonessential amino acids (aspartic acid, glutamic acid, and proline) and two essential amino acids (threonine and valine) in EBN [36]. This result agrees with the finding reported by Hun et al., in which the amount of serine, aspartic acid, threonine, tyrosine, and proline were significant in EBN samples [14]. Halimi et al. conducted an amino acid analysis to quantify the amino acid content in EBN. Results showed that major amino acids in EBN were glutamic acid (9.61%), aspartic acid (6.34%), lysine (5.44%), and leucine (5.30%) [26].

2.1.2. Carbohydrate. Besides protein, EBN is also rich in carbohydrates and is described as one of the main constituents of EBN. Carbohydrate parts of the EBN glycoprotein are commonly classified into monosaccharides, oligosaccharides, and polysaccharides. Studies identified major carbohydrates in EBN: sialic acid, mannose, galactosamine, glucosamine, galactose, and fucose [2]. Among the myriad carbohydrates present, *n*-acetylneuraminic acid (sialic acid) is most abundant in EBN, accounting for 9% of total carbohydrates. Sialic acid is classified as a group of carboxylated monosaccharide acylated derivatives with nine carbon atoms commonly found as oligosaccharides, glycolipids, or glycoproteins [2]. As a major carbohydrate of EBN, the highly nutritious sialic acid is referred to as EBN acid and exhibits potential as a functional ingredient. Recently published works found that sialic acid offers multiple beneficial therapeutic effects, including promoting cognitive function, enhancing immune systems, improving cell proliferation, and boosting skin complexion [9, 12].

The amount of carbohydrates in EBN was generally quantified based on the AOAC method (AOAC 986.25) by subtracting the percent of all other components (protein, moisture, ash, and crude fat) from the total weight. Meanwhile, other methods, such as adding crude fibres and nitrogen-free extractives, have also been employed in a few studies to obtain the carbohydrate value. On the other hand, different techniques have been developed to isolate carbohydrates: hot water extraction, acid hydrolysis, combined enzymatic and acid hydrolysis, and ion-exchange chromatography [1].

Based on the results listed in Table 1, it is apparent that EBN is a significant source of protein and carbohydrates. This is evident as carbohydrates exhibited the second highest components of EBN, as shown from the proximate analysis in Table 1. The carbohydrate content ranged approximately from 9.8% to 58.2%. A recent study [30] revealed that the carbohydrate composition in Songkhla is 24.4%. Similar findings were reported by Halimi et al. [26], Tan et al. [25], and Zulkifli et al. [24] that the carbohydrate value of house EBN obtained from different parts of Malaysia was in the range between 20.1% and 28.6%. This is in concordance with the carbohydrate composition of cave EBN reported by the authors in [7, 31] (22.5% and 19.1%, respectively). The variations in the amount of carbohydrates detected in EBNs from Thailand were the smallest (24.4% to 31.4%) compared to those of the EBNs collected from Malaysia and Indonesia, which were found to be 9.8% to 58.2% and 20.1% to 29.4%, respectively. When comparing the house EBN and cave EBN, the lowest carbohydrate content was obtained by cave EB in Langkawi (9.8%). Conversely, the highest level of EBN was documented in the EBN in Penang (58.2%). The variation in the carbohydrate level detected may be attributed to the swiftlet species nested in the area, the habitat, and the geological location [4]. However, overall, the rest of the carbohydrate composition of EBN in Malaysia, Thailand, and Indonesia is close within the range, perhaps due to similar climate and environment.

2.1.3. Moisture. Measurement of moisture content is essential in determining the stability and quality of EBN, where the tolerance level is set below 15% by the Thailand Standard (TAS 6705-2014) and Malaysian Standard (MS 2334:2011). This is because high moisture content may negatively impact the compositions of other nutritional compounds in EBN. Furthermore, low moisture also plays a vital role in minimizing fungal growth [41]. Moisture content can be determined using a direct method, and AOAC has been recognized due to its high reproducibility and accuracy [42]. Based on Table 1, most of the literature studies have used the official AOAC method by drying EBN at 105°C until a constant weight was obtained [25]. Moisture determination by using the gravimetric method (7.5–21.5%) is comparable to the weight loss from the thermogravimetric analysis (TGA) (12.6%) at 25–200°C due to physical dehydration of free water [2].

It is noteworthy from Table 1 that RC and RUC EBN have a range of moisture levels between 9.4% and 24.3%. Previous studies also found that there were no apparent differences between different sources of EBN (cave and house) [22, 31]. However, in contrast, few articles have reported the significant difference in moisture levels between different regions of EBN. This may be attributed to the variation of the environment and humidity in the diverse natural habitats of swiftlet birds and the microclimate of bird houses [27, 29]. Several research studies have also shown a relatively high moisture content of bird nests, exceeding the tolerance limit, for instance, Indonesia (Kalimantan, Sulawesi, Java, and Sumatra), ranging between 17.7% and 19.6% [29] and EBN from Malaysia (Sarawak and Sabah), which was from 16.7% to 18.6% [7, 24, 31]. Such results may be explained by the typical wet, warm climate with high relative humidity. In addition, Kalimantan, Sulawesi, Java, and Sumatra were also known as the most consistent damp areas in Indonesia as they are located near the sea. Besides that, the high amount of moisture in RC EBN was most likely influenced by the series of cleaning and drying procedures of EBN involving the removal of impurities, fine feathers, and

nitrite and nitrate compounds. Cleaning of EBN is a time-consuming process as raw EBN needs to be soaked with water for 6 to 48 hours, and it can increase the moisture content by 31% to 91% [43]. Nevertheless, the high moisture content is favourable during the transportation of EBN as it is less dry and fragile [2]. According to MS 2273:2012, EBN houses should be capable of maintaining an adequate microclimate of EBN as in natural habitat [44]. Each house should have a ventilation system that maintains the recommended temperature and humidity to prevent overheating or chills in young and adult swiftlets by installing an air humidifier and water pool inside the house. The humidity level should be in the range of 80–85% to obtain the highest quality EBN.

2.1.4. Ash. Ash refers to the inorganic residue remaining after the complete oxidation of organic matter in the presence of air. It is another essential quality attribute for EBN, as ash content can influence the physicochemical properties of the product, which is a part of the proximate analysis required for nutritional evaluation. Subsequently, the determination of ash in the EBN can assure food safety by verifying the presence of toxic minerals. The amount of ash is highly correlated to the amount of minerals in EBN, which is one of the elements for restoring body function [45]. A higher ash content indicates a higher concentration of minerals within a food sample. Studies revealed that the most abundant minerals in EBN are sodium, magnesium, potassium, and calcium. These elements can be detected using atomic adsorption analysis (AAS) [24]. The high nutritional mineral of EBN can be attributed to the swiftlet's diet, which eats airborne flying insects and ingests sand and mineral-rich water from the river [2]. Based on the AOAC method, ash content was evaluated by the dry ashing method in a furnace at 550°C for 18 hours [3, 7, 26]. The technique is widely utilized in measuring ash content as it is relatively simple and allows the complete destruction of organic matter. Based on the thermal gravimetric analysis, EBN experiences notable mass and chemical deterioration reduction within the temperature range of approximately 200 to 735°C. This degradation process is attributed to the decarboxylation and breaking of bonds within the protein structure of the glycoprotein backbone, resulting in the formation of smaller fragments, which predominantly occur around 300°C. This observation provides a valid rationale for the susceptibility of EBN to complete incineration into ash at temperatures below 550°C [42].

As listed in Table 1, the average ash content of EBN samples is between 2.6% and 9.0%. Several proximate compositional studies revealed that ash remained one of the lowest constituents of EBN. Based on the table, the highest ash content in RC EBN was reported by Zulkifli et al. [24], while the lowest value was shown by Hamzah et al. [22]. It can be summarised that there is no significant difference between the ash levels in different regions as the results are quite similar between countries. Although no significant differences were noted, variations in the ash may be influenced by the presence of foreign matter, such as feathers and eggshells that are trapped in the hardened part of the bird nest [46]. In contrast, the low ash content detected from *A. fuciphagus* may be associated with the bird nest's structures that consist of saliva, which are easily digested [47]. Although most of the studies agreed that there is no significant difference between the ash content in different regions of EBN, according to Quek et al. [31] based on their study, the amount of ash measured in EBN significantly varies between species, location, and type of EBN collected. This is probably associated with the different environmental conditions, which in turn affect the swiftlet diets, contributing to a considerable impact on marker variables and influencing EBN differentiation by the geographical origin [31].

2.1.5. Lipid. Apart from the major proteins and carbohydrates, lipids have been determined to be one of the minor components of EBN. Correspondingly, the lipid content is reported to be the lowest constituent compared to other compositional properties of EBN, which indicates that EBN is a low-fat functional food. Lipid content in EBN is commonly determined via the Soxhlet method by using nonpolar solvents such as petroleum ether or *n*-hexane. It is the common and recommended dry extraction method as it is straightforward, has a shorter extraction time, and utilizes a low volume of solvent for its extraction process [48]. Besides that, the Soxhlet method is also recognized as one of the AOAC standard methods based on the AOAC method 920.39 [49].

Based on Table 1, studies on the nutritional composition of EBN discovered that the crude fat content in EBN is generally below 1%, regardless of its sources. This is in line with the results shown by Elfita et al. [29] as they discovered that the lipid level between different regions ranges from 0.2% to 0.6%, with an average value of 0.33%. These amounts of lipid content are quite similar to the findings that those regions in Malaysia (Pahang and Terengganu) recorded (an average of 0.48%) [26] but relatively lower than those from Thailand (an average of 0.96%) [3]. According to Halimi et al. [26], their study demonstrated the lipid extraction from the hydrolysed EBN sample and found that the content is still comparable to that of other previous studies that employed the Soxhlet method. The low-fat content in EBN may be attributed to the bird's distinct eating habit, as it only feeds on specific groups of insects. This consists of *Hymenoptera* (winged ants, wasps, and bees), *Coleoptera* (small beetle), *Homoptera* (froghoppers), and *Ephemeroptera* (dragonflies) [30]. Besides, it can be reflected in the composition of EBN made from highly digestible saliva [7].

2.2. Nitrite and Nitrate Contents. Nitrite and nitrate are inorganic compounds that exist in the natural environment, such as soil and water. Dietary nitrate is derived primarily from vegetables, particularly green leafy and root vegetables; processed meat, fruits, and vegetables, on the other hand, are major sources of nitrite. Unfortunately, studies revealed that exposure to high nitrate levels may cause harmful effects on the body as nitrate can be converted to nitrite by bacterial or microbial reduction. Nitrite can convert into nitrite oxide, which provides potential cardiovascular health benefits.

Conversely, it can also pose detrimental health effects when nitrite reacts with dietary amines under acidic conditions to produce *N*-nitroso compounds that are considered carcinogens [5, 50]. Given the potential benefits and toxicology effects associated with nitrite and nitrate intakes, the World Health Organization (WHO) has established an acceptable daily intake (ADI) for nitrate (3.7 mg/kg body weight per day) and nitrite (0.07 mg/kg body weight per day) [51].

Besides vegetables and processed meats, nitrite and nitrate are also available in EBN. These contaminants are believed to come from the fermentation process of bird soil and guano [52]. Consequently, this issue has increased worries among the public regarding EBN's food safety. Implementing rules for EBN inspection and quarantine under the Chinese Academy of Inspection and Quarantine (CAIQ) has set the limit of nitrite to below 30 mg/kg for dried EBN [53]. In order to comply with the standard listed by China, the same amendment has been established by the Malaysian government (1985 food regulation and MS 2334: 2011), Thailand government (TAS 6705-2014), and Indonesian government (No. 395/kpts/OT.160/l/4/2014), respectively [54–57].

The nitrate and nitrite levels in EBNs are influenced by the microenvironment of the EBNs, whether the EBNs are produced from the swiftlet house or cave nest. Generally, the uncontrollable external environment in the limestone cave leads to elevated levels of both compounds derived from ammonia through anaerobic fermentation [30]. Poor ventilation, as well as natural ecological elements (air, mineral, water) around the cave, can also be the factors that contribute to the penetration of nitrite into EBN through rain dripping. Besides that, the high nitrite and nitrate content are attributed to the contamination of bird soil due to guano, which is rich in nitrite and nitrate materials, and irregular cleaning of swiftlet farms. Meanwhile, the differences in nitrite levels of EBN products are influenced by many factors, particularly the harvesting time, contamination during harvest, and various cleaning procedures for the collected EBNs. External environmental factors such as humidity, temperature, pH, and climate also affect the differences in contaminants' concentration between swiftlet houses and caves' EBN [58]. Hence, regular cleaning and appropriate management of swiftlet farms and cave nests are required to minimise contamination of nitrite and nitrite into EBN products.

Table 2 lists the nitrate and nitrite data from previous studies. Based on the table, cave EBN possesses a significantly higher nitrate and nitrite concentration than house EBN. According to Paydar et al. [59], cave EBN showed a high reading of the nitrite level, 147-fold higher than house EBN. The nitrite content obtained from cave EBN also exceeded the permissible limit as stated in the Malaysian Standard MS 2334:2011, which is 28% higher. Meanwhile, nitrate content was 105-fold higher, more stable, moderately reactive chemical, and can be generated from nitrite oxidation [63]. Similarly, Quek et al. [7] reported that nitrite and nitrate are the highest among cave EBN, *A. maximus,* and East Malaysia. On the contrary, the low nitrite and nitrate (<1 mg/kg) is probably due to the EBN being harvested from house farming that underwent frequent cleaning processes [61].

Malaysia, Thailand, and Indonesia (Regulation No. 832/kpts/OT.140/L/2013) have established guidelines to maintain the cleanliness of the swiftlet's houses, free from disease, and swiftlets guano that might affect the nitrite and nitrate content in EBN [64, 65]. Both nitrite and nitrate concentrations can be significantly reduced by softening and cleaning processes as it is highly soluble in water. Hamzah et al. [22] reported that the concentration of both compounds can be reduced by 98% after cleaning. Thus, the nitrite level can be an indicator of the management level in producing and processing clean EBN.

Nitrite and nitrate determination is crucial to ensure the EBN products are safe to eat. There are two commonly employed techniques for the detection of nitrite and nitrate in EBN, i.e., ion chromatography (IC) and spectrophotometry. According to Sirenden et al. [61], using spectrophotometry to assess nitrite and nitrate levels is considered less reliable than the IC method, as it is an indirect measurement approach. However, as indicated in Table 2, the quantities of nitrite and nitrate detected in EBN using these two methods do not exhibit a significant difference. This convergence in results can be attributed to the amendments made in the methods accepted by regulatory bodies in China (GB 5009.33-1010), Malaysia (MS 2509:2012), and Indonesia (832/kpts/OT.140/L/2013). These regulations now permit the utilization of both IC and spectrophotometry methods for determining nitrite and nitrate levels in EBN [44, 52, 65].

3. Grading of EBN

Traditional grading of EBN depends on specific physical determinants such as colour, cleanliness, shape, and size of EBN. However, the grading system is subjective and hard to implement due to the different seasonal harvesting periods; various grading and trading mainly involve lump-sum traders [34]. As the problem in grading the high-priced commodity arises, Malaysia has established MS 2334:2011 and SIRIM/DVS 3:2015 to standardize the grading of RUC and RC EBN based on the length and contact planes of EBN [54, 66]. These standards are developed to ensure the quality and the food safety of EBN for human consumption. Moreover, the grading system establishes a uniform method for evaluating quality and the price of EBN; the higher the grade is, the more expensive the EBN is. Unfortunately, the adulteration of EBN products by unethical suppliers for greater financial gain results in only 44.19% of producers using MS grading, 22.66% of traders trading in bulk buying, and 33.14% using a traditional grading system [67]. Over the years, more sophisticated techniques to determine grades, authenticity, and identification of EBN have been developed with the advancement and availability of technology. This includes microscopic inspection, automation (radio frequency identification), and analytical methods (genetic and observational analysis), and not only depend solely on the physical appearance of EBN [68]. However, this method has its own major advantages and limitations, making it unsuitable for establishing the level of EBN.

Table 2: Nitrite and nitrate levels from the previous literature.

	EBN types	Origin	Nitrite conc. (mg/kg)	Nitrate conc. (mg/kg)	Method	Reference
House EBN						
Malaysia	RUC	Johor, Selangor, Penang, and Sarawak	5.7	98.2	IC (MS 2509: 2012)	[52]
	RC	Selangor	0.09	1.1	UV-Vis spectrophotometer	[54]
		Kedah	10.1	24.9	IC (MS 2509: 2012)	[25]
		Sarawak	10.4	39.4		
		Rompin, Pahang	8.4	52.6		
		Jerantut, Pahang	10.3	31.1		
		Kuala Selangor, Selangor	15.8	47.0		
		Port Klang, Selangor	11.4	35.9		
		Johor	11.0	41.5		
	RUC	—	14.3	83.5	UV-Vis spectrophotometer (AOAC method 973.31)	[59]
Indonesia	RC	West Sumatra	13.6	1049.3	UV-Vis spectrophotometer	[29]
		South Sumatra	4.0	713.2		
		West Java	3.1	798.0		
		West Kalimantan	6.3	1051.1		
		Central Sulawesi	18.3	1027.4		
		Southeast Sulawesi	5.1	650.1		
	RUC	Wajo Regency	32.4–65.1		UV-Vis spectrophotometer	[60]
		Parepare city	4.7–23.6			
		Pinrang regency	48.4–164.4			
	RUC	Jawa	10.2		IC	[22]
		Balikpapan	8.5			
	RC	Jawa	0.4			
		Balikpapan	0.5			
	RUC	North Kalimantan	10.0		UV-Vis spectrophotometer	[61]
	RUC	South Kalimantan	93.1		UV-Vis spectrophotometer (AOAC method 973.31)	[62]
	RC		30.9			
Cave EBN						
Malaysia	RUC	Gua Gamantong, Sabah Gua Niah, Sarawak Gua Subis, Sarawak	843.8	36 999.4	IC (MS 2509: 2012)	[52]
	RUC	Langkawi	28.4	349.3	IC	[22]
	RC		0.4	1.2		
Southeast Asia	RUC		212.9	2128.6	UV-Vis spectrophotometer (AOAC method 973.31)	[59]

RC, raw-clean EBN; RUC, raw-unclean EBN; IC, ion chromatography; MS, Malaysian Standard; AOAC, Association of Official Analytical Collaboration.

3.1. Colour. Food with visually appealing colours is important in offering high-quality products with pleasant appearances to attract customers. A colour measurement system (colourimetry) has been used in the food industry to measure a broad range of food products. This includes EBN since it represents a consumer's first quality assessment and influences their preference and choices. In general, there are three distinct colour variations of EBN (Figure 1): white EBN derived from the white-nest swiftlet (*A. fuciphagus*), black EBN sourced from the black-nest swiftlet (*A. maximus*), and red EBN, which is essentially white EBN that has undergone a colour transformation [70]. Red EBN has the highest market price (five times the cost of white EBN) as it is relatively rare [71]. The colour of EBN showed a major difference based on the origin (cave or house) due to different nitrite and nitrate concentrations present in the environment and types of minerals contained in the swiftlet's diet [72]. EBN colour can be detected using the International Commission on Illumination (CIE) L∗a∗b∗ system, where L∗ is for lightness, a∗ for redness or greenness, and b∗ for yellowness or blueness [7].

As for a∗ (redness or greenness) and b∗ (yellowness or blueness), cave EBN tends to have dull, brownish, or orange and red colour [1]. According to the previous works of the literature, there are many factors affecting the formation of red EBN, such as the presence of blood during bird nest construction, swiftlets' diet (lotus seeds, seaweeds, or molluscs), iron oxidation caused by limestone dripping, the formation of aryl-C-N and NO2 side group on aromatic amino acid, and presence of albumin in EBN [1, 6, 71]. The red EBN is caused by the nitrifying bacteria that are present in the EBN reacting with ammonia vapours from the bird soil containing guano dropping [6]. However, red EBN has a lower nitrite level than brown EBN and lower protein extraction than white and yellow EBN [59, 73]. Besides, red EBN has a higher total lipid content, nine times greater than white EBN [73]. Red EBN has a higher market price than white EBN; thus, some EBN traders began treating white EBN with ammonia, sodium nitrate, or redding agents to produce more expensive red EBN [74].

3.2. Shape and Size. Even though all EBNs provide a potent nutritional value, each type of EBN comes in different shapes and sizes. These two factors are specifically emphasised when grading EBNs are based on the standard. Related to both attributes, there are four different types of shapes and sizes of EBN: (1) cup, whole nest from large to extra-large size, (2) triangle, medium size, (3) strips, broken pieces from whole EBN, and (4) cakes, tinny bird nest that is shaped into the shape and size of a biscuit. Based on MS 2334:2011 and SIRIM/DVS 3:2015, EBN can be categorized into Grades A, B, C, and others [54, 66]. Others include EBN in filament, fragment, fine grain, horn, and biscuit types. Each grade of EBN has a different market value. The EBN of grade A offers improved quality, followed by grades B and C. Thus, higher-grade EBN products have better quality attributes and greater profit. Therefore, compliance with the national bird nest industry standards demonstrates the best practices to create the ultimate quality EBN in tandem with market demand. Table 3 summarises the size grading for RUC EBN and RC EBN.

There are various shapes of EBN cups depending on the location where they were harvested. Based on the reference angle for EBN grading, the most sought-after shape of EBN is the perfect half cup that is large without holes and not too thick. However, the production of a half cup (Grade A, α 180) is lower as swiftlets prefer to construct a nest at the corner of swiftlets' house due to less material required [34]. Similarly, the shape of grade B EBN is the same as grade A EBN, but they are inclined 135° when placed on a flat surface. Grade C EBN has a 90° inclination when put on a stable, level surface. Traditionally, the size of EBN was determined by using the width of an adult finger as Grade A (3 cm × 6 cm) and Grade B (2.5 cm × 5 cm). However, this method is unsuitable for grading EBN as it is inconsistent for different individuals. Thus, there were studies on automated inspection using the Fourier-based shape separation (FD) method to grade the shape and size of EBN to help standardize the quality evaluation of EBN [68].

3.3. Cleanliness. Cleanliness is also considered one of the main factors determining the value of EBN. Regarding cleanliness, EBN can be categorized based on the feather or impurities content in raw EBN. In the market, EBN with lesser content of impurities and feathers can be considered as a high-value, premium grade EBN. Conversely, EBN that contains more impurities is classified as low quality. A lower grade of EBN requires a prolonged, diligent cleaning process that affects the cost of cleaning and the quality of EBN. The Ministry of Health Malaysia has introduced a point of control (POC) in the cleaning process of EBN to minimise contamination in EBN products, particularly the lethal nitrite and nitrate compounds, to avoid the exportation of EBN products from being banned. The birdhouse is the first POC, which needs to be cleaned regularly. Guano should be removed, hazardous substances need to be stored away, and cleaning records should be kept. In addition, transport vehicles shall comply with the Malaysian Food Hygiene Regulations 2009. All EBN products must be properly transported to prevent cross-contamination of nitrite and nitrate with trucks, crates, and other products. Exposure to surrounding and pests can also result in contamination in EBN.

The third POC is the cleaning process of raw EBN. The conventional cleaning of EBN includes a series of pretreatments. Firstly, the raw harvested EBN will be soaked with at least two bowls of clean water and cleaned by brush to remove impurities (fine feathers and nitrites) on the surface of EBN. The wet EBN is held stiffer while forceps are used simultaneously to remove the feathers [75]. All tools (i.e., forceps and stiffers) must be subjected to frequent cleaning and disinfection during each working day and at the end of each shift or each working day to avoid contamination in EBN [73]. During the cleaning process, EBN will expand five times bigger than its original size, making it hard to reshape and reduce the quality and nutritional value

White nests Black nests Red blood nests

Figure 1: An overview of white (a), black (b), and red (c) edible bird's nests [69].

Table 3: Grading system of raw-unclean edible bird's nest (RUC EBN) and raw-clean edible bird's nest (RC EBN) based on length and reference angle (α) [57].

α (reference angle)	α 180	α 165 to α 135	α 90
RUC EBN			
Length >4.5 cm	A large	A large	A large
3 ≤ Length <4.5 cm	B medium	B medium	B medium
Length <3 cm	C small	C small	C small
RC EBN			
Length >3.5 cm	A large	A large	A large
2 ≤ Length <435 cm	B medium	B medium	B medium
Length <2 cm	C small	C small	C small

of EBN. Besides, the cleaning process using forceps may create holes in EBN, and this causes 62.5% weight loss of EBN [29]. Some EBN producers use vegetable oil to float the feathers and hydrogen peroxide to bleach the EBN colour to remove the remaining black feathers. Due to the rapid procedure, applying hydrogen peroxide can significantly reduce labour costs. Unfortunately, there are certain adverse effects in employing hydrogen peroxide as a bleaching agent in the bird nest industry. It bleaches the bird nest's colour to white and decolorizes the black feathers (impurity). As a result, even though the nests still have some remaining feathers, they will appear clean, which may contribute to food hazards. According to Fan et al. [76], based on the Malaysian Standard for exportation to China, a trace of hydrogen peroxide must not be detected in the EBN products, and the usage of food-grade hydrogen peroxide is applicable only for cave EBN.

4. Indonesia, Malaysia, Thailand, and China Standards on EBN

4.1. Indonesia. As one of the largest importers of EBN products, Indonesia produces approximately 80% of the world's EBN, and 71% has been exported to China, accounting for 0.5% of Indonesia's gross domestic product (GDP). In the first half of 2019, Indonesia imported 58% (39.8 tons) of EBN to China, and the value will be increased annually by 32% [77]. The lucrative amount of EBN export to China resulted in various agreements between both governments to boost EBN export activities.

The government of the Republic of Indonesia has issued several regulations on EBN from three different sources: (1) the Ministry of Trade, (2) the Agricultural Quarantine Agency, and (3) the Drug and Food Agency. Under the Ministry of Trade, SOP No. 51/M-DAG/PER/7/2012 for exporting EBN to China was amended to ensure the effective quality monitoring and sustainability of Indonesia's export of EBN [78]. On the other hand, the guidelines from the Drug and Food Agency, HK.03.1.5208.12.5545:2012 stipulates that the maximum level of nitrite in EBN from *Collocalia* sp. should have a permissible limit of 200 mg/kg to be imported and circulated in the Indonesian territory [79]. Meanwhile, there are six decrees by the Head of the Agricultural Quarantine Agency, including (1) No. 484/KPTS/OT.160/L/4/2012, guidelines for requirements and procedures for determining the quarantine installation of swiftlet's nest and Sriti animal products [45], (2) No. 374/Kpts/KH.210/L/5/2020, technical guidelines for handling and inspection of swiftlet's nests and Sriti-procedures for laboratory examination, (3) No. 41/Permentan/OT.140/3/2013, quarantine measure for the import and export of EBN from the Republic of Indonesia, (4) No. 832/kpts/OT.140/L/2013, guidelines for quarantine requirements and measures. Animals on the expenditure of swiftlet's nests from the territory of the Republic of Indonesia to the People's Republic of China [80], (5) No. 406/Kpts/OT.160/L/4/2014, guidelines for heating wallet nests for expenditure to the state of the people's Republic of China, and (6) No. 395/kpts/ot.160/l/4/2014, quarantine monitoring guidelines on the issue of swiftlet's nests to the state of the people's Republic of

China [64–66, 79]. The detection limit for each of the safety, quality, and authenticity of EBN to exports to China was summarised in Table 4.

4.2. Malaysia. In 2011, the bird nest industry in Malaysia, Thailand, and Indonesia suffered a heavy loss when the Chinese government decided to impose a ban on trade and import from these countries due to the high nitrite content found in EBN. Consequently, the food safety issue has severely affected the countries involved as prices have dropped by at least 20%. Nevertheless, this has served as a wake-up call for them to set better quality control and regulate the quality of imported EBN. Given its economic value and export potential of EBN to Malaysia`s gross national income (GNI), Malaysian government has set an ambitious vision of expanding the scale of the bird nest industry. This vision has led to the development of several strategies to support the industry's sustainable growth, including increasing the production of EBN to 860 metric tons by 2020, developing and imposing industry guidelines, introducing good production practices and industry standards, and emphasising research and development activities [67].

In line with the government strategies to develop a competitive EBN industry that complies with the China Protocol of Inspection, Quarantine, and Hygiene Requirements for the Importation of Bird Nest Products, and the Department of Standard Malaysia (DSM) has established various standards for the EBN sector. The standards are introduced to regulate and facilitate domestic and international trade. Adherence to the standards is essential to maintain a good image of the country, furthering international cooperation between Malaysia and other countries. The national standards that are introduced consist of (1) MS 2273:2012: Good animal Husbandry Practices-Edible-Cave Edible nest Swiftlet Ranching, (2) MS 2333: 2010: Good Manufacturing Practice (GMP) for Processing Raw-unclean and Raw-clean Edible-Bird Nest, (3) MS 2334: 2011: Edible-Bird Nest (EBN)-Specification, (4) MS 2503: 2012: Good Animal Husbandry Practices-Edible-Cave Edible nest Swiftlet Ranching, and (5) MS 2509:2012: Test method for EBN-Determination for nitrite and nitrate contents by using spectrophotometric and ion chromatographic methods. The establishment of various standards shows that the Malaysian government is taking proactive and competent development. This ensures food safety and only the finest quality of EBN is produced. In addition, these standards are also employed as a reference for global EBN operators and traders from outside Malaysia. Other Malaysian standards, such as MS 2273:2012 Good Animal Husbandry Practice-Edible-Nest Swiftlet Ranching and its premises, also include guidelines for raising EBN swiftlets.

In the EBN industry, MS 2273:2012 and MS 2503:2012 are important as key references. They provide several guidelines to minimise the risk of contamination in EBN products. They emphasised the basic principle of swiftlets' management to produce high-quality cave and house EBN. Besides, both standards stated the obligation to have a radio frequency device (RFID) for both cave and house EBN with country code 458 [64, 75]. This required legal proof of ownership and should be stored at the Animal Repository Centre, stationed at the Malaysia Communication and Multimedia Commission (MCMC). MS2273:2012 listed a few principles in EBN's ranching operations, including the design and maintenance of the ranch, premises hygiene, and swiftlet illness symptoms. Meanwhile, MS 2333:2010 provides guidelines for EBN manufacturers to construct processing facilities to prevent cross-contamination and operational control procedures. It also showed the requirement for sorting, grading, cleaning, and packaging of RUC and RC EBN for safety and quality [73]. On the other hand, MS 2334:2011 is applied to regulate and monitor the safety, quality, and authenticity of both RUC and RC EBN (Table 4) for domestic and international markets [54]. Besides that, MS 2334:2011 introduces the grading, packaging, and labelling system according to the provisions of the Malaysian Food Act 1983 and Food Regulations 1985, as well as premises' requirements, personal hygiene, certification marks, and legal requirements. The analysis for safety, quality, and authenticity should be done by the accredited laboratory under the National Laboratory Accreditation Scheme of Malaysia (SAMM) [54]. Apart from DSM, other competent authorities such as the Ministry of Health (MOH), Standard and Industrial Research Institute of Malaysia (SIRIM), and Department of Veterinary Services (DVS) have produced, which plays a role in boosting Malaysian's reputation as a supplier of high quality and safe EBN to China.

Under SIRIM and DVS, SIRIM/DVS 3:2015, specification for RUC EBN: A Code of Veterinary Practice (CoVP) prescribes a good manufacturing practice (GMP) and the requirement of RUC EBN from caves or houses [66]. In adherence to the Food Act 1983, Food Regulations 1985, Food Hygiene Regulations 2009, and importing countries' requirements, the Food Safety and Quality Division (FSQD) under the Ministry of Health (MOH) establishes food safety over the EBN products' supply chain to ensure that edible bird's nest products are safe for human consumption [5]. Meanwhile, DVS has embarked on three different SOPs related to RUC EBN export to China: (1) SOPRUCEBN-1, SOP for registration and traceability system for RUC EBN export to China, (2) SOPRUCEBN-2, SOP for operational of RUC EBN primary processing establishment export to China, and (3) SOPRUCEBN-3A, DVS certification scheme: Malaysian Good Agricultural Practice (myGAP) Certification of EBN swiftlet [75]. DVS has also introduced the Good Animal Husbandry Practices (GAHP) and Veterinary Health Mark (VHM) quality assurance programs schemes for swiftlet farms and EBN processing factories to outline the fundamental principles of animal management. These standards act as guidelines for EBN farmers to strike a balance between swiftlet sustainability, EBN production, and disease management [4]. Apart from that, the Malaysian government established a Swiftlet Centre of Excellence (COE) in 2011 by electing Universiti Putra Malaysia (UPM) as a national COE centre and is dedicated to conducting various research and development (R&D) related to swiftlets and EBN to increase the production of EBN [82].

Table 4: Edible bird's nest (EBN) safety, quality, and authenticity requirements from Malaysian Standard, Indonesia Regulation, Thailand Standard, and China Standard.

		Malaysian Standard MS 2334: 2012 and MS 2509: 2012 [44, 54]		Indonesia Regulation, no. 832/Kpts/OT.140/3/2013 [80]		Thailand standard TAS 6705-2014 [22]		China standard CAIQ-RZ-2015002 [81]	
	Parameter	Detection limit	Method	Detection limit	Method	Detection limit	Method	Detection limit	Method
Safety	*E. coli*	≤100 MPN/g	Microbiological analysis in food product	$<\times 10^{1}$ cfu/g	Microbiological analysis	Not exceed 100 cfu/g	CPC ISO 4832:2006	≤100 cfu/g (house EBN) ≤1000 cfu/g (cave EBN) ≤100 cfu/g It cannot be detected	
	Staphylococcus aureus	≤100 MPN/g		$<1 \times 10^{2}$ cfu/g		Not exceed 1000 cfu/g	CPC BAM (2001), chapter 12		
	Coliforms	≤1100 MPN/g		$<1 \times 10^{2}$ cfu/g					
	Salmonella sp.	NIL		Negative					
	Total plate count	≤2.5 × 106 cfu/g		$<1 \times 10^{6}$ cfu/g					
	Yeast and mold	≤10 cfu				Not exceed 1000 cfu/g	CPC BAM (2001), chapter 18	≤10 cfu/g (house EBN) ≤1000 cfu/g (cave EBN)	
	Bacillus cereus					Not exceed 1000 cfu/g	CPC BAM (2012), chapter 14		

Table 4: Continued.

Parameter		Malaysian Standard MS 2334: 2012 and MS 2509: 2012 [44, 54]		Indonesia Regulation, no. 832/Kpts/OT.140/3/2013 [80]		Thailand standard TAS 6705-2014 [22]		China standard CAIQ-RZ-2015002 [81]	
		Detection limit	Method	Detection limit	Method	Detection limit	Method	Detection limit	Method
	Nitrite	<30 mg/kg	Spectrophotometry/ion chromatography MS 2509:2012	<30 mg/kg	Spectrophotometry/HPLC	<30 mg/kg	Flow analysis -spectrometric detection ISO 13395:1996	<30 mg/kg	
	Moisture Water activity	<15% ≤1.0	AOAC hot air oven method			<15%	Gravimetry	≤15%	
	Protein	Presence of sialic acid	AOAC HPLC method			—	Titrimetry (Kjeldahl method) AOAC 930.29 Ninhydrin test for proteins	Presence of sialic acid	
Quality	Physical inspection			Negative presence of metal, wood, feathers, and dirt	Visual inspection by the naked eye at a distance of 20–30 cm	Natural colour and odour, no off-colour and odour Clean and free of visible foreign matters	Visual inspection		
	Lead (Pb) Arsenic (As) Mercury (Hg) Cadmium (Cd) Other metals	2 ppm 1 ppm 1 ppm Refer to Malaysia Food Act 1985 and Food Regulation 1985	AOAC AAS method					≤2 ppm ≤1 ppm ≤0.05 ppm ≤1 ppm	
	Copper (Cu) Iron (Fe)	1.0 mg/L 0.3 mg/L							
	Hydrogen peroxide (∗FOOD GRADE) (cave nest only)	Nil							
Authenticity	Fourier transform spectroscopy						FTIR spectrometer		

MPN, most probable number; AOAC, Association of Official Analytical Collaboration; AAS, atomic absorption spectrophotometer; MS, Malaysian Standard; HPLC, high-performance liquid chromatography; CPC, conventional plate count; BAM, biological analytical manual, FTIR; Fourier transform infrared.

4.3. Thailand. After China's trading ban, Thailand conducted a few risk analyses and consultations with the Chinese authorities. As a result, a memorandum of understanding (MoU) on China Protocol of Inspection, Quarantine, and Hygiene Requirements for the Importation of Edible Bird Nests in China from Thailand was signed and sealed. Under the Ministry of Agriculture and Cooperatives of Thailand, the National Bureau of Agricultural Commodity and Food Standards has introduced a voluntary standard, TAS 6705-2014, to improve the safety, quality, and authenticity of EBN products to gain consumer trust for domestic and international use that comply with the protocol. Besides regulating and monitoring the access of RC Thai White EBN, the document also includes setting on the traceability methods and recall procedures. In compliance with the protocol, all products should obtain a "Plant and Animal Quarantine Permit" through quarantine inspection and veterinarian credit and certificate of origin for quality assurance. Limit detection for each analytical method is described in Table 4, which contains specific principles [55]. The Thai government stipulates that every EBN exported should have a traceability system, nitrite content not exceeding 30 mg/kg, and moisture level below 15%.

This standard is based on MS 2334:2011 and only applies to RUC EBN. However, there is little difference between MS 2334:2011 and TAS 6705-2014, as TAS 6705-2014 requires a physical examination of each RC EBN and determination of protein content that requires the use of the Kjeldahl method (AOAC 930.29). Besides, as compared to Malaysia's MS 2334:2011 and No. 832/Kpts/OT.140/3/2013, Thailand's TAS 6705-2014 guideline is distinct as it imposed the EBN suppliers to perform an EBN authenticity test using a Fourier transform infrared (FTIR) spectrometer.

4.4. China. As the largest import market of EBN, with 105.2 tons of dried EBN imported in 2018 [43], the Chinese government has issued two regulations under a national public institute of the Chinese Academy of Inspection and Quarantine (1) CAIQ-RZ-2015001, Bird's nest product processing enterprise, hygienic technical specification and (2) CAIQ-RZ-2015002, Bird's Nest Product Certification, Implementation Rules [53, 83]. CAIQ-RZ-2015001 emphasised the guidelines of management requirements and technical specifications of the EBN processing plant and handling the processing of EBN products. Meanwhile, CAIQ-RZ-2015002 provides details about the certification that must be obtained, the procedure by the certification body, and the detection limit in EBN, as listed in Table 4. However, the document does not specify the required method for each parameter. The CAIQ authority has also taken the approach of setting up a traceability management service platform (QR code) to provide information such as birdhouse identity document (ID), processing plant ID, and weight of each piece of EBN from registered companies in Southeast Asian countries for raw EBN only as shown in Figure 2. Source: http://ebn.caiq.org.cn/index.

4.5. Comparison of Malaysia, Thailand, and Indonesia Standards for EBN Trading to China. There are no apparent differences in the standards and regulations from the three largest EBN exporter countries in determining the quality and safety aspects of EBN. In summary, the emphasis parameter is based on the nitrite content (Table 4), where all three countries adhere to the same nitrite levels in compliance with the China requirement [53]. This is to prevent another unfortunate EBN trading ban that occurred in 2011 due to the high nitrite content. Naturally, the elevated nitrite level is mostly attributed to external environmental factors. Fortunately, regulated sanitation management and proper cleaning procedures at processing plants can be employed to overcome this issue. They thus can be used to determine the level of cleanliness and capability of an EBN processing plant. Besides, detecting nitrite and nitrate contamination has become significant in the industry as more Malaysian suppliers were able to discover the contaminants in their EBN products before exportation to China [27]. In addition, the Malaysian and Thai governments also emphasise the moisture content as it will affect the storage, shelf-life, and transportation of EBN to China.

4.6. Regulatory Improvement. The lack of harmonized national standards across the major exporting and importing countries makes it difficult to establish uniform requirements. In Malaysia, Rabu and Nazmi [67] found that more than half of the farmers do not follow the grading system prescribed in the national standard. Many industry players still use traditional grading systems based on factors such as colour, shape, and curvature rather than the official standards [23]. This reluctance of industry players to fully adopt the established standards poses a challenge to implement uniform practices.

While harmonized international standards for edible bird nest products have not been fully implemented across exporting and importing countries, several regulatory improvements can be considered. These improvements include implementing a standardized equivalence assessment and review process for exporting countries' food safety management systems to ensure compliance with major importing countries' standards. In addition, the registration scope should be expanded to include companies involved in processing and storing EBN for export to China, ensuring all entities adhere to strict regulations. Furthermore, stringent quality control measures, including on-site inspections, laboratory testing, and compliance with international sanitary standards for imported raw bird nests, should be enforced. Finally, collaborative protocols should be fostered between exporting countries and China through joint consultations on inspection and quarantine requirements, leading to the signing of protocols to harmonize standards and procedures. By implementing these regulatory improvements, exporting countries can enhance the standardization of regulations for edible bird nest exports to China, ensuring food safety, quality, and compliance with Chinese standards across the supply chain.

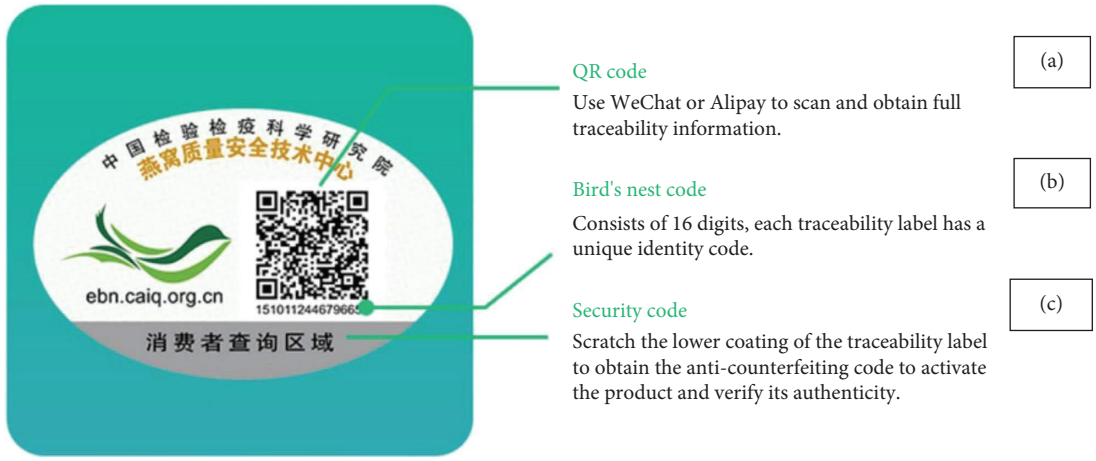

Figure 2: QR code for traceability, where (a) QR code source, (b) 16-digit code, and (c) authenticity code.

5. Identification and Authentication of EBN

5.1. Adulteration of EBN. EBN has become susceptible to adulteration over the years due to its high market price and limited supply of genuine EBN, thereby threatening consumers' interests and market competition. They are often adulterated with similar but less expensive ingredients to boost their weights and market value or deliberately mislabelled with false information regarding their origin and nutritional contents. These unscrupulous acts of fraudulent suppliers contributed to the deterioration of EBN quality and undermined genuine business as the boundaries of trade expanded globally. For instance, this dishonest practice is reflected by the addition of natural red colourants such as karaya gum, red seaweed, or *Tremella* fungus to white bird nests to mimic the premium-grade red bird nest, which commands a higher market price. Therefore, prevalent issues have become a growing concern as consumption of unnoticed adulterated EBN may pose serious health risks and allergies to consumers.

According to Shim et al., adulterants utilized in edible bird's nests (EBN) can be categorized into two groups [84]. Type I adulterants consist of solid materials externally added to EBN, such as karaya gum, *Tremella* fungus, coralline seaweed, agar strips, fish bladder, and pork skin. In contrast, Type II adulterants are water-soluble substances added internally to EBN, including glucose, sucrose, hydrolysed collagen, and monosodium glutamate. In addition, bleaching and staining of EBN are also routinely incorporated to simplify the tedious and labour-intensive cleaning process [59]. Nevertheless, current technological advancement enables scientists to actively address these food integrity challenges by developing innovative techniques. However, currently, no standard methods are used for EBN authentication and quality assurance. Various literature reviews have explored different approaches, each of which has benefits and drawbacks. Conventional methods such as physical and chemical evaluation have previously been employed to identify adulterants in EBN [2]. Recently developed scientific methods using advanced technologies and high-end analytical instruments are recognized for identification and authentication of EBN. Among these methods are chromatography, spectroscopy, molecular biology identification, and multiconcerted methods. Although most of these methods are simple, fast, and convenient, each has its limitations.

5.2. Authentication Method of EBN. Previously, the initial approach for identification involved using conventional techniques such as physically examining morphological traits such as colour, shape, taste, and smell [15]. This method is a pragmatic strategy that strongly depends on the visual characteristics and structural attributes of the samples. Morphological identification and physicochemical identification technology represent the culmination of knowledge and expertise from previous predecessors in the field of identification technology. Distinctive patterns of certain EBNs that are strange can be promptly examined. When experimental detection conditions are constrained, mastering the identification methods for morphological and physicochemical characteristics of EBN becomes essential. Nevertheless, these two approaches have certain drawbacks, notably their unreliability and inability to accurately detect adulterants [2, 85].

5.2.1. Spectroscopy. The spectroscopic method has been commonly implemented in the verification of food authenticity, as it offers rapid detection, minimal sample preparation, low cost, and is suitable for use by nonexpert technicians. Various spectroscopic techniques have been successfully established over the years, such as infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and Raman spectroscopy. IR spectroscopy analyses specific functional groups or distinct spectral regions through the measurement of infrared light absorption by chemical bonds, offering information about the molecular composition and structure of molecules. The chemical composition (fingerprints) and absorption peaks of the primary nutrients, such as protein, amino acids, and polysaccharides, are excellent indicators for the differences in the infrared spectra of genuine and adulterated EBN [2].

Hamzah et al. determined the spectra of different grades of raw and processed EBN along with six different types of adulterated EBN using Fourier transform infrared (FTIR) spectroscopy [86]. They found that different grades of pure EBN had similar fingerprint regions, and the main compounds present in EBN are carbohydrates and proteins. In addition, the spectra of different adulterated EBNs reflected their distinct infrared spectra and can be clearly discriminated from authentic EBNs. According to Sun et al., the authenticity of EBN can be determined by analysing the variations in wave numbers, locations, and relative peak intensity of the absorption peaks [87]. Guo et al. employed an FTIR system with chemometric analysis to authenticate EBN [88]. Based on the analysis of FTIR spectra data, it was found that authentic EBN from Malaysia exhibited distinct spectral characteristics compared to the four adulterated forms: *Tremella* fungus-EBN, agar-EBN, fried pigskin-EBN, and egg white-EBN. Specifically, the authentic EBN spectrum displayed characteristic absorption peaks for polysaccharides (1047 cm^{-1}) and proteins (1655 and 1517 cm^{-1}). In contrast, the *Tremella* fungus-EBN spectrum was dominated by a polysaccharide absorption peak at 1046 cm^{-1}. On the other hand, EBN adulterated with pigskin showed a protein peak with a special shape (1642 cm^{-1}) and two distinct lipid peaks (2855 and 1745 cm^{-1}), which were absorbed significantly. Similar findings have been reported in the study previously mentioned, where two additional absorption peaks representing lipids are observed in pork skin adulterated EBN [86]. Meanwhile, although the spectrum of authentic EBN exhibited similar protein peaks with EBN adulterated with egg white, the intense polysaccharide peak observed in pure EBN was absent in the egg adulterant. Further analysis involved utilizing chemometric techniques such as principal component analysis (PCA), linear discriminant analysis (LDA), support vector machine (SVM), and one-class partial least squares (OCPLS) to establish a rapid authentication method for commercial EBN products based on FTIR spectrum fingerprinting. The results indicated that the chemometric analysis effectively classified authentic EBN from its adulterants with high prediction sensitivity and specificity, thereby facilitating reliable identification and discrimination of EBN and its adulterants [88]. Chemometrics is a range of methods that utilise mathematical and statistical fundamentals to analyse plentiful and complex chemical data from various analytical instruments, thus making it a powerful tool for challenging analytical problems in food science [89, 90]. Chemometric tools allow for a rapid and efficient way to extract, predict, and discriminate valuable information from noise and unveil hidden relationships in complex and large instrumental data beyond multivariate dimensionality. Undoubtedly, the employment of chemometric approaches contributes significantly to food analysis, including authenticity, functionality, bioactivity, and safety [91, 92].

Besides FTIR spectroscopy, nuclear magnetic resonance (NMR) spectroscopy is proven to be effective in detecting various adulterants in EBN [90, 93]. NMR technology uses the magnetic properties of atomic nuclei, providing detailed information on compounds' chemical composition and structure [94]. Although the equipment cost of NMR technology is high, the limitation of NMR spectroscopy is counterbalanced by its unparalleled advantages, such as being nondestructive and highly reproducible, requiring minimal sample preparation and providing a comprehensive adulterant profiling with its high resolution and structural information [94, 95]. Xing et al. implemented the potential use of high- and low-field NMR spectroscopy for the detection of origin and adulteration of EBN [93]. Results obtained confirmed the provenance of EBN from Indonesia (leucine, glutamic acid, and N-acetyl glycoprotein), Malaysia (isocaproate), and Vietnam (citric acid) through the identification of specific compounds serving as geographical markers. They also found that the OPLS-DA analysis using T2 spectra effectively distinguished genuine EBN from samples contaminated with gelatine, starch, and agar. Yong et al. have also successfully demonstrated the potential application of untargeted 1H-NMR metabolomic fingerprinting along with chemometric methods, notably principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA), in identifying EBN adulteration with a perfect accuracy rate of 100% [90].

On the other hand, Raman spectroscopy has facilitated the identification of genuine EBN. Raman spectroscopy is a molecular characterisation technique that utilizes the Raman effect. It is a fast, direct, and nondestructive method that provides molecular information about a sample without the need for sample preparation. It offers good sensitivity and specificity. In Yu's study, Raman spectroscopy was employed to identify the genuine EBN in various locations and brands [96]. The analysis revealed six distinctive peaks at wavelengths of 828 cm^{-1}, 1000 cm^{-1}, 1237 cm^{-1}, 1330 cm^{-1}, 1448 cm^{-1}, and 1668 cm^{-1}. The findings indicated that geographical and species variations did not impact the distinctive peaks, and it was possible to detect samples that were contaminated with a 5% adulteration level. Shim and Lee obtained Raman spectra of different EBN samples from West Malaysia, East Malaysia, and Indonesia analysed by Raman spectroscopy [97]. The unique Raman spectra of EBN produced by the same swiftlet species from various geographic regions were found to overlap, suggesting that these EBN samples are composed of identical materials and can serve as a benchmark for authentication. Moreover, Raman microspectroscopy can differentiate between various substances: polysaccharides such as *Tremella* fungus, agar, and coralline seaweed, which lack amide bands; polypeptides such as pork rind and fish bladder, characterized by strong amide bands; and EBN, a glycoprotein featuring amide and saccharide bands. Although microscopic examination of water-soluble adulterated EBN yielded a composite image similar to that of unadulterated EBN, the Raman spectroscopy analysis revealed spectra differing from the unique Raman spectrum of unadulterated EBN. This proved that Raman spectroscopy is a promising tool in identifying genuine EBN from its adulterants.

5.2.2. Chromatography. A broad range of analytical methods to detect food adulteration has been reported. This includes the chromatography analysis such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), and liquid chromatography (LC). Chromatography is a biophysical method that isolates and identifies individual components in a mixture. In addition, this technique also allows for the determination of the concentration of specific compounds present in the matrix, hence enabling both quantitative and qualitative analyses. Chromatographic methods provide data on peak intensity (measured as area or height), peak locations (measured as retention time), and, if combined with mass spectrometry (MS), peak mass-to-charge ratio (m/z). Alternatively, instead of using specific peak information, one can utilise the entire profile.

GC is commonly employed for the analysis of volatile and thermally stable compounds. Conversely, LC is an ideal technique for the analysis of nonvolatile samples that degrade at high temperatures. Typically, a mass spectrometric (MS) detector is often linked to either a gas chromatograph (GC) or a liquid chromatograph (LC), resulting in the hyphenated procedures known as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS). Hyphenation is mostly done to ionize substances and subsequently determine their masses. Chromatographic techniques, particularly those hyphenated with MS, have provided some of the most powerful tools for the detection and identification of chemical markers for precise verification of food authenticity. Various chromatographic methods have been established over time to ensure the identification and authentication of EBN. EBN is primarily identified using chromatography by detecting carbohydrates and amino acids qualitatively or quantitatively [2].

Chua et al. proved that GC/MS and LC/MS are reliable analytical tools for the classification of EBN samples according to their production, species, and geographical origin using metabolic profiling, subsequently preventing mislabelling and counterfeits [98]. This method, in combination with chemometric models (PCA and OPLS), also effectively determined the metabolite profiling of the EBN along with its relationship with its medicinal properties. Furthermore, significantly varying metabolites responsible for the differences in the EBN samples were discovered, suggesting that the natural habitat significantly influences the categorization of EBN. This technique clearly revealed certain metabolic elements present in EBN samples. On the other hand, Lee et al. classified EBN samples based on their regions by comparing the amino acid profile of EBN from East Malaysia (EM) and West Malaysia (WM) using HPLC in combination with a multivariate approach [99]. A total of 18 amino acids were identified from EBN samples, and data obtained were analysed using different multivariate tools. Based on the analysis of data using the OPLS-DA model, reliable potential markers (glycine, cysteine, tryptophan, and aspartic acid) to accurately distinguish EBN from different regions in Malaysia were identified.

In another research study, composition of protein in EBN was measured by Wong et al. using HPLC [100]. The distinctive peptide peaks were collected and identified by LC-MS/MS. According to the results of the principal component analysis, the fingerprints exhibited the same level of discriminatory power regarding the production sites (cave/house) of EBN and its colour (white/red). In comparison to pure EBN, the quantities of peaks produced by EBN mixed with counterfeit adulterants were found to be significantly lower. Furthermore, the findings from protein identification indicated that the predominant protein in EBN was the Muc-5AC-like protein. Hence, the authenticity of EBN can be discerned based on the overall composition of peptide profiles demonstrated by HPLC fingerprinting. Zhang employed high-performance thin-layer chromatography and HPLC techniques to analyse the levels of taurine, vitamin B, and biotin in EBN [101]. This analysis was conducted to provide a benchmark for determining the authenticity of the sample.

5.2.3. Molecular Biology Identification. In recent years, many studies have been performed to detect food adulteration in EBN using diverse techniques. One such approach is using molecular biology identification involving 2-dimensional gel electrophoresis and PCR technology to authenticate EBN. These sophisticated techniques are widely employed in molecular biology identification of import and export EBN due to their combination of high sensitivity, stability, and specificity, making them promising and ideal to use [102]. Moreover, with the advent of molecular markers in improving PCR methods and analysis, they can identify DNA-level differences that are stable and detectable in all tissue types, regardless of growth conditions, developmental stage, or differentiation status [103]. In addition, these competitive tools can determine species from level kingdom to subspecies and address the issues caused by glycoprotein interference, low abundance, and external contamination. Furthermore, molecular biology identification techniques such as PCR are known to provide excellent results in detecting food fraud and are used with preference to address unethical food adulteration associated with animal origin in food products/foodstuff.

Wu et al. utilized DNA-based PCR and protein-based two-dimensional gel electrophoresis (2DGE) techniques to rapidly and accurately identify authentic EBN samples collected from different countries [104]. The PCR technique was employed for rapid detection of the presence of EBN, while 2DGE was used to identify heterogeneous proteins from potential adulterants. Results showed that this approach has proven to be effective in deciphering minute levels of adulteration, as it could detect as low as 0.5% of EBN in EBN-*Tremella* fungus mixture. Meanwhile, Guo et al. developed a TaqMan-based real-time polymerase chain reaction (PCR) assay capable of uniquely identifying components of EBN and four common adulterants, including white fungus, agar, pigskin, and egg white [88]. Their results indicated minimal detection limits of 0.001%, 0.5%, 0.001%,

and 1%, respectively, proving the effectiveness and precision of this method in identifying adulterants in EBN samples.

In another research report, Quek et al. innovatively demonstrated the application of forensically informative nucleotide sequencing and SYBR green I-based real-time PCR to identify the production origins of EBN [105]. This approach involves using mitochondrial and nuclear DNA molecular markers, which is useful for overcoming EBN fraud and confirming the authenticity of commercial EBN. Besides that, other approaches such as lateral flow immunoassay have also been applied for the detection of EBN-specific glycoprotein as potential biomarkers to identify EBN products' geographical sources and for verification of porcine in adulterated EBN using EBN protein-specific antibodies as EBN recognition elements [106, 107]. On the other hand, Lee et al. verified the EBN origin effectively and systematically using a loop-mediated isothermal amplification (LAMP) assay within 1 hour. Results also showed it had no cross-reaction with EBN adulterants, including white fungus, egg white, and pig skin, thus making it a promising tool to authenticate EBN and detect EBN DNA for the presence of adulterants [108].

Lin et al. established a novel method for distinguishing the genetic information of EBN based on PCR-RFLP assay, utilizing the cytochrome b (cytb) gene found in mitochondrial DNA [109]. The mitochondrial cytb sequence was employed due to its larger copy number and greater stability in comparison to nuclear DNA [110]. Sample sequences were aligned with swiftlet sequences in GenBank to construct phylogenetic trees for genetic identification, specifically to ascertain the origin of EBN samples. The findings revealed that 11 EBN samples and instant EBN soup originated from *A. fuciphagus*, while the Huaiji EBN sample originated from *Apus nipalensis*. In 2020, Liu et al. conducted DNA barcoding using mitochondrial cytb genes for sequencing analysis and phylogenetic classification, initially determining EBN sample origins from Vietnam, Indonesia, Malaysia, Thailand, and China. Further PCR-RFLP assay analysis identified the Af/g-486bp-F/R primer and Taq I enzyme as specific for identifying EBNs created by *A. fuciphagus* and discriminating them from other species. In addition, a subsequent analysis with the cytb592bp-F/R primer and BamH I enzyme effectively differentiated *A. fuciphagus* from its subspecies, *A. germani*, indicating the PCR-RFLP assay as a competitive tool for rapid authentication of EBN origins [110].

Tukiran et al. successfully discovered an indirect ELISA technique using antipeptide polyclonal antibodies (PABs) as a potential biomarker of porcine marker peptides for detection of porcine gelatine in adulterated EBN [111]. Results showed that these antibodies possessed acceptable affinity towards spiked samples and allowed the detection of at least 0.05% porcine gelatine in EBNs. Similarly, two polyclonal and monoclonal antibodies unique to EBN glycoprotein were evaluated by Zhang et al. to establish a sensitive ELISA method for the identification of EBN. The developed antibodies exhibited high specificity and sensitivity towards characteristic sialo glycoprotein in EBN, which can be used as a suitable biomarker for the quality check of EBN [101]. On the other hand, Wong et al. developed monoclonal antibodies using the hybridoma technique to specifically detect acidic mammalian chitinase (AMCase)-like protein in EBN [112]. The antibodies were then confirmed using immunoprecipitation and subsequent LC/MS/MS analysis. The Western blot and ELISA analyses demonstrated the specificity of chitinase-like protein in EBN, indicating its potential as an authentication marker for quality surveillance of EBN.

5.2.4. Concerted Methods. Recent studies on the combination of multiple methods for the identification of EBN have been emerging to improve the reliability of EBN identification. This is because the progression of the adulteration process and diversification of EBN products have rendered many individual techniques unable to determine the authenticity of EBN effectively.

For instance, Yang et al. established a concerted multitechnic approach for the authentication and quality assurance of EBN. This novel analytical system involves using gas chromatography-mass spectrometry (GC–MS) to analyse oligosaccharides, environmental scanning electron microscopy (ESEM) to study microstructure and immunoblotting to detect epidermal growth factor (EGF) in EBN [113]. Similarly, Hun et al. employed a combined strategy involving gel electrophoresis and liquid chromatography to differentiate genuine cave and house EBN from common adulterants [114]. They compared the protein and amino acid profiles of cave and house EBN with those of white fungus, jelly, fish swimming bladder, and egg white. The protein profiling studies revealed that cave nests exhibited ten bands, with two prominent bands at 30 and 35 kDa. House nest proteins displayed nine bands, with major bands at 120 and 140 kDa. White fungus exhibited three faint bands at 22, 35, and 75 kDa. Egg white proteins were found to have two predominant bands at 35 and 75 kDa. Meanwhile, the fish swimming bladder exhibited significant streaking of protein bands upon dilution, but the protein profile of the jelly did not display any bands. Interestingly, the protein profile of the jelly did not show any bands. On the other hand, EBN was discovered to have 17 different amino acids, with aspartic acid, arginine, histidine, and leucine being the primary amino acids in cave nests, while aspartic acid, glutamic acid, histidine, and leucine were the major amino acids in house nests. The analysis of amino acid profiles revealed that white fungus included 16 different types of amino acids, albeit in lesser amounts compared to EBN samples. Meanwhile, the fish swimming bladder has shown to be abundant in 6 amino acids. The composition of egg white consists of 16 different amino acids, including aspartic acid, glutamic acid, leucine, and lysine, which are present in significant amounts. In general, both analytical techniques yielded fingerprint profiles of the protein and amino acid compositions of the cave and home nests that were distinct from the adulterants. Overall, the implementation of both analytical methods revealed distinct fingerprint profiles for the protein and amino acid compositions of cave and house nests compared to the adulterants.

6. Conclusion and Future Improvement

Over the century, EBN created by the swiftlet bird has been consumed widely in Asian countries, particularly China, as a traditional medicine and prized delicacy in the Chinese cuisine. In recent years, concerted efforts in research and development of the EBN have supported the belief in the health benefits and therapeutic effect of EBN as a functional food. With the fast growth of technologies, the industry has remarkably expanded to become one of the major income sectors to many countries in Southeast Asia, as the swiftlets are native to this region. As the number of swiftlet farms increases, imperative action has been taken to regulate and monitor the quality assurance and safety of consumers, farms, and the environment. The standards and guidelines were established in compliance with the China protocol to ensure that only the finest quality products were produced, thus improving the national and international trade in this competitive market.

The bird's nest industry, however, has been unstable over the past few decades due to numerous unresolved problems, such as quality assurance standards, including food adulteration, allergic reactions, and the banning and integrity of halal and haram. Therefore, the three exporting countries (Malaysia, Indonesia, and Thailand) highly emphasise EBN authenticity. Many traders take advantage of high EBN commodities' prices by staining, whitening, and falsifying EBNs with cheaper materials such as gum, red seaweed, and pigskin. The use of these materials is based on similarities in texture, colour, and taste; this is to increase the size and weight of EBN. The use of pigskin in the EBN will pose other problems related to halal issues for Muslim consumers. Ideally, methods based on genetics, immunochemistry, and thermal analysis can be implemented in the SOP for each country as it can differentiate between authentic EBN with counterfeit materials and different grades of EBN. In addition, it is possible that kit technology can be recommended and widely used as it can provide accurate results rapidly. It is suggested that nutritional and chemical profiles coupled with the pattern recognition analysis are viable approaches to rapidly determine EBN origins for food safety, quality control, traceability, and authenticity. Furthermore, competent authorities should keep on improving the quality standards reasonably for import validation and market supervision for this commodity. In addition, food safety awareness training should be provided to the entrepreneurs, emphasising GMP, HACCP, Food Act, and Regulations to minimise issues related to this sector.

Data Availability

The data that support the findings of this study are available within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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