Review

A comprehensive review of edible bird nests and swiftlet farming

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ABSTRACT

Edible bird’s nest (EBN) is currently widely consumed by the Chinese community as tonic food and functional food, which is believed to have many medicinal benefits. Some studies have reported the biochemical compositions of EBN, graded on the basis of colour, nitrate and nitrite contents. Other studies have shown significant biological effects, while ongoing research is in progress to explore potential pharmacological applications. The high demand for EBNs in the global market has forced the local regulatory bodies to monitor swiftlet farming activities, including the EBN cleaning process. Furthermore, numerous techniques have been developed to authenticate EBN; proteomics is likely to be the most promising of these methods. However, there are limited numbers of relevant protein sequences deposited at the database. More research is needed at the molecular level to explore the mechanisms behind the biological functions, such as bone strength improvement, skin rejuvenation, epidermal growth factor activity and cell proliferation. The current and future prospects of EBN and swiftlet farming are critically reviewed in this article.

Keywords: edible bird’s nest; swiftlet farming; proteomics; authentication; Aerodramus


1 Introduction

Swiftlets (Apodidae; Collocaliini) are birds superficially similar to swallows (Hirundo rustica, Hirundinidae) and sparrows (Passer domesticus, Passeridae), but they are not closely related. Although swiftlets consume a wide range of aerial insects like other species, they can fly at higher velocity and have greater maneuverability.

Swiftlets are small in size with body weights between 6 to 40 g. They are predominantly found in the South East Asian countries from Andaman to Nicobar Island, in the Indian Ocean to the coastal regions of Malaysia, Thailand, and Vietnam, Palawan Island in the Philippines and in the South Eastern part of China. Swiftlets are from the Apodidae family. The Greek word apous means “without feet”, and refers to the swiftlets’ short legs and reluctance to settle voluntarily on the ground. They perch on the surfaces of cave walls or nesting planks where they built their nests. Swiftlets are grey brown in appearance and possess a short beak with a wide gape.

There are 24 species of swiftlets recorded in the world. They are divided into four genera, namely Aerodramus (echolocating swiftlets), Hydrochous, Schoutedenapus and Collocalia (non-echolocating swiftlets). Indeed, the Aerodramus genus is known to be made up of four different species of swiftlets which are A. fuciphagus, A.
maximus, A. germani and A. unicolor[7]. The taxonomy, morphology and characteristics of swiftlets have been critically reviewed by Looi and Omar[8]. The five most common species of swiftlets found in Malaysia and Borneo Island are Hydrochous gigas, Collocalia esculent (white belly swifts), Cypsiurus balasiensis (Asian palm swift), Aerodramus fuciphagus and Aerodramus maximus[12]. However, most edible bird’s nests (EBNs) produced in South East Asia are built by three common species of cave swiftlets: A. fuciphagus, A. maximus and C. esculent.

In 1937, Ernst regarded the classification of swiftlets as the most difficult task in the taxonomy of birds[13]. Swiftlets are difficult to be distinguished physically, and are best identified by their nests, which may include feathers and impurities. For example, 85%–90% of the nests from A. maximus are comprised of feathers, while the remaining 10%–15% are edible; whereas the nests of C. esculenta are comprised of 95%–98% feathers and other impurities, and the remaining 1%–2% are edible[14]. Because of the high impurity content of A. maximus and C. esculenta nests, they are not ideal for human consumption. However, the nests of A. fuciphagus are preferred, more valuable, and heavily exploited, as they are mainly composed of salivary secretion (70%–80%) with relatively small amounts of impurities (20%–30%), such as feathers and droppings[10].

Swiftlets exhibit colonial behaviour, and are usually present in large numbers at their nesting or hunting sites. They are able to produce echolocation calls which aid them in hunting insects and navigating in total darkness[11]. One of the most intriguing features of the birds is that they secrete a viscous mucus from their salivary glands and use it as construction material for the nests that protect their eggs and hatchlings[12]. Furthermore, swiftlets are observed to be faithful to their nesting sites, and rebuild their nests in the same site even after their nests are removed.

The most famous swiftlet nesting sites are Niah Cave and Gomantong Cave, which are located in Sarawak and Sabah, respectively, in Malaysia. There are also many nesting sites at the southern tip of Borneo Island in Indonesia. The caves come in various shapes and sizes, but they share a similarity of being dimly lit or in total darkness. The poor lighting conditions create an environment exclusive to swiftlets, providing a natural form of protection against their predators. The caves of swiftlets are also well-known for strong ammonia smell, generated from the massive amount of swiftlet droppings that accumulate on the ground. Although the droppings are a major deterrent to most creatures including human, they are a main source of nutrients for insects.

Here, a review on EBN and its biochemical composition is systematically presented, as well as a review of its in vivo and in vitro biological effects. The extraction and identification of proteins of EBN and the current and future prospects of swiftlet farming industry are also reviewed. Since high demand for EBN provides motivation for falsification of products, here we also review current methods for EBN authentication, including their advantages and limitations.

2 Edible bird’s nest

Edible bird’s nest, or “Yan Wo”, is a great delicacy and a traditional medicine, which is also considered to be a beauty enhancer. Currently, it is widely used as healthy food due to its high nutritional values and therapeutic benefits. The market value of EBN ranges from $1 000 to $10 000 per kilogram depending on its grade, type and origin[9].

EBN is composed of secretions from the swiftlet’s sublingual salivary glands, which is used as a cement in the nest. The secretions are produced at the greatest rate during nesting and breeding season. The sublingual salivary glands may increase their weights from 2.5 to 160 mg[13]. This sticky secretion is regurgitated and hardens once it is exposed to air, forming the bird’s nest. The nests are built into the walls of inland or sea-side caves, over a period of approximately 35 days, and mainly built by male swiftlets[14,15]. The glutinous secretions form a part of the nest, and bind together other materials, such as feathers, seaweed or mosses[16].

EBNs are built in a half-bowl shape; their curvature is made up of fine strands of solidified saliva and numerous feathers, while the ends are formed from thick and compact solidified secretions. Such structure enables the nest to withstand the weight of their eggs and hatchlings, and remain firmly attached to the walls throughout the course of a breeding season[16]. The unprocessed EBN has an approximate length of 5.0–10.0 cm and breadth of 3.5–6.0 cm. The thickness of the nest at the curvature is about 0.5–1.0 cm, while its thickness at the ends is around 1.0–2.5 cm, and approximately 4.0–8.0 g per piece in weight.

There are three different colours, white, orange and red, that are found in EBN in the market. The difference in colour is not known. One of the most popular hypotheses is that the red coloration is produced by swiftlets’ blood which is released during the construction of the nests. Some people believe that red nests are produced from natural fermentation in the bird house or cave. The colour of nests may change from white to yellow, and then slowly become orange or red[17]. Other theories suggest that the colour is related to the food source of the swiftlets, which is mainly insects, and may have high mineral or
iron content from the surrounding environment, such as deposits leached from the limestone of the cave walls\textsuperscript{[17]}. Oxidized iron may produce the red colour. Recently, a scientific explanation has been presented to explain observed colour difference of EBNs, suggesting that the red EBN is due to oxidation of nitrate in the swiftlets’ droppings after oxidation\textsuperscript{[19]}. Another group of researchers also reported the correlation between nitrite and nitrate contents, and the colour of EBN\textsuperscript{[17]}.

3 Biochemical compositions of EBN

The chemical composition of EBN is important to understanding the biological activity of EBN as a medicine and as a functional food. The earliest documentation of the composition of EBN was probably reported in 1921 by Wang\textsuperscript{[19]}. The macro-nutrient composition of EBN samples has been reported by Ma \textit{et al}\textsuperscript{[20]}, as well as Marcone\textsuperscript{[14]}, both of which found that protein and carbohydrate are known to be the major components, comprising 60\% and 30\% of the total mass, respectively. Therefore, it is not surprising that protein and carbohydrate are used as the target compounds for the quality assessment of EBN. EBN also contains fat (<2\%) and trace amount of minerals including sodium, calcium and magnesium\textsuperscript{[21]}. The micro-nutrients in EBN may be affected by seasonal variation and even breeding site. This is because EBNs are produced by swiftlets whose diet is composed of food from the local environment. Hence, it is recommended that more samples should be obtained at different seasons from a wide range of breeding sites for a more inclusive profile of EBN composition. The minor difference in biochemical composition of EBN might contribute to the variation of biological activity.

The average crude protein content of EBN reported by Marcone\textsuperscript{[14]} was 62\%–63\%, whereas 56.5\%–60.6\% reported by Xin \textit{et al}\textsuperscript{[22]} and 32.3\% reported by Kathan \textit{et al}\textsuperscript{[23]}. The major protein in EBN is glycoprotein which is defined as protein bound to a carbohydrate and its composition resembled salivary mucin\textsuperscript{[19]}. Numerous studies have been carried out to define the role played by oligosaccharides in the functionality of glycoproteins\textsuperscript{[24]}. The benefits of glycoproteins to human health are actively investigated and the glycoproteins in EBN may serve as lubricant and protective agent\textsuperscript{[19]}. Possibly, the unique structure of the glycoproteins found in EBN make it differ from other protein sources, such as chicken and fish, in terms of solubility, function and bioactive properties\textsuperscript{[25]}. EBN was found to have a common 77 kD protein with properties similar to those of ovotransferrin protein, found in chicken eggs\textsuperscript{[14]}. This protein may be partially responsible for the allergic reaction experienced among young children who consume EBN products\textsuperscript{[14]}.

Marcone\textsuperscript{[14]} compared the amino acid contents of white and red EBN samples. Interestingly, methionine was found in white, but not red EBN samples. A total of 18 amino acids were reported from EBN and the most abundant amino acids were serine (15.4\%), valine (10.7\%), tyrosine (10.1\%) and isoleucine (10.1\%). They are important for energy production, facilitating regulation of cell function and building up the immune system by producing immunoglobulins and antibodies. Recently, researchers analysed the composition of amino acids in EBN samples collected from different locations in Malaysia and Indonesia and found that tyrosine and glutamic acid were promising amino acid markers for the differentiation between house and cave nests\textsuperscript{[26]}. Carbohydrate is the second highest component in EBN, ranging from 27\%–58\%\textsuperscript{[27,14]}, and as low as 8.5\%–16.4\% in another study\textsuperscript{[28]}. In the latter study, EBN samples were collected from locations ranging from Malaysia (Perlis and Langkawi), Indonesia (Java, Balikpapan and Kalimantan), Thailand and Philippines. EBN from the Philippines showed the highest percentage of carbohydrate content. According to Kathan \textit{et al}\textsuperscript{[23]}, the carbohydrate component of EBN consisted of 9.0\% sialic acid, 7.2\% galactosamine, 5.3\% glucosamine, 16.9\% galactose and 0.7\% fructose. Sialic acid is often associated with neurological enhancement, brain development and intellectual advantages in infants, since it serves as a functional component of brain gangliosides\textsuperscript{[29–31]}. Furthermore, the neuroprotective effect of EBN has been shown by using neurotoxin 6-hydroxydopamine to induce oxidative stress on SH-SY5Y human neuroblastoma cells in an in vitro Parkinson’s disease model. The cytotoxicity test showed that crude extract of EBN did not cause SH-SY5Y cell death at concentrations up to 75 \(\mu\)g/mL, and the maximum non-toxic dose of this water extract was 150 \(\mu\)g/mL\textsuperscript{[32]}. Recently, EBN was found to have high potential for neuroprotection against estrogen deficiency associated senescence mainly because of the modification of the redox system and attenuation of advanced glycation end-products\textsuperscript{[33]}. Sialic acid is also considered to be an immune moderator that affects the ability of mucus to repel harmful microbes\textsuperscript{[34]}. However, the nutritional, functional and biological mechanisms of sialic acid in the human body are still under active investigation by researchers worldwide.

Marcone\textsuperscript{[14]} examined and compared the ash content of both white and red EBNs and found that they were virtually identical at 2.1\%. A higher ash content would usually indicate a greater amount of minerals in a sample. According to Nurul Huda \textit{et al}\textsuperscript{[27]}, there was a significant difference in the ash content of EBN samples harvested from house and cave nests, with house nests (2.75\% to 5.74\%; from Indonesia) having fewer ash content than
cave nests (7.53%; from Malaysia). A similar work was carried out by Saengkrajang et al.\(^\text{13}\), who analysed farmed EBN samples harvested from the seashore of Thailand. They found that the ash content of EBN samples ranged from 5.9% to 7.4%. The wide range of ash content in EBN may be explained by the variation in food sources among swiftlets. Based on the study of Marcone\(^\text{14}\), EBN contained sodium (0.65‰), potassium (0.11‰), calcium (1.298‰), magnesium (0.33‰), phosphorous (0.04‰) and iron (0.03‰). The elemental tests of EBN showed that the white EBN contained more calcium than red EBN, while red EBN contained more potassium, magnesium, and iron than white EBN\(^\text{14}\). These minerals are essential micro-nutrients, serving as cofactors to activate many enzymatic reactions in the body. Thus, it is important to preserve the mineral content of EBN through the steps of cleaning and processing for market.

4 Preparation of EBN for market

The raw EBN contains a lot of impurities such as swiftlet dropping, twigs and dirt. Therefore, the raw EBN needs to go through a series of cleaning processes in order to remove these impurities before further product development. The cleaned EBN is re-moulded into a half-bowl shape and dried prior to packaging. The detail of this process has been explained by Jong et al.\(^\text{36}\). They also introduced an advanced quality and risk assessment tool which is based on the fuzzy Failure Mode and Effect Analysis (FMEA) methodology. The assessment tool is useful to improve and modernize the processing of EBN, in addition to ensuring the quality of EBN products.

The raw EBN is first soaked in clean water until the soft and the glutinous material is partially loose (1–2 h). Usually, the impurities, such as dirt and feathers, will float and be removed. The EBNs are then scooped out of the water bath and the remaining feathers and dirt are removed manually using tweezers. This is the most tedious and time-consuming step in the cleaning process. A magnifying glass is used during this step to ensure the cleanliness of EBN. It is believed that the high price of EBN is partly due to this intensive cleaning process, in addition to its nutritional and functional values.

The cleaned EBN is then shaped into a half-bowl, with the aid of a plastic mould. After shaping, EBN is dried in an oven or with a fan. The cleaned and dried EBN is packaged and sent to market for sale.

It is important to note that there is no international standard method and/or guideline for cleaning and processing EBN. Recently, the Malaysian government enforced a regulation stipulating that the cleaning process of EBN must follow the protocols provided by the Department of Veterinary Services, Ministry of Agriculture and Agro-based Industries. The standards governing the practice of handling and processing EBN include Good Animal Husbandry Practice for EBN ranching and its premises (MS 2273:2010) and EBN specification (MS 2334:2011). The Ministry of Health of Malaysia has also taken the initiative to establish a set of standards, which were implemented on March 2012, to ensure safety and quality by monitoring and limiting the nitrite level in processed, raw-unclean and raw-clean EBNs. The establishment of these standards has increased the quality of EBN products from Malaysia and stimulated the global trade of EBN, especially export to high demand countries like China.

5 Extraction of EBN proteins

There is no universal extraction method to obtain all of the proteins found in EBN\(^\text{37}\). Conventional techniques for protein extraction include chemical, mechanical and enzymatic approaches\(^\text{39}\). The choice of the methods depends on the nature of the sample matrix. The chemical method involves the use of ionic, non-ionic or zwitterionic detergents to assist in breaking cells for the release of proteins. However, high concentration of detergent often results in protein denaturation. Non-ionic detergent concentrations below 0.1% have been reported to not be harmful to proteins\(^\text{39}\). Ionic detergents, like sodium dodecyl sulphate, are not suitable for functional studies because they have high-denaturing power.

The mechanical method uses physical force, usually grinding or sonication to break the cells. In contrast, enzymatic methods employ enzymes such as lysozyme to disrupt the cell membranes of samples, releasing contents of the cells\(^\text{39}\).

Since dried EBN is a thick and hard sample, mechanical force is required to break down the structure. EBN can be ground into a powder with a mortar and pestle before protein extraction. Protein extraction efficiency is strongly dependent on the fineness of EBN powder\(^\text{40}\). The finely ground powder issued normally for the preparation of samples for proteomics\(^\text{41}\).

It was found that aqueous extraction is the easiest and most commonly used method, especially for water soluble and non-thermally labile proteins. This is because EBN contains mostly water-soluble proteins. This method was applied by Zhang and his colleagues\(^\text{42}\), who used sonication to break apart the EBN fibres prior to protein extraction, and then dialysis. After dialysis, EBN was lyophilized for further analysis. Many studies have also used aqueous extraction which is similar to the preparation of EBN soup\(^\text{43–46}\). Chief differences among these methods included heating temperature and time.
6 Identification of proteins in EBN samples

Peptides and proteins in biological matrices have traditionally been quantified by immunological methods such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA)[47]. Previously, Zhang et al[49] developed a competitive ELISA, with a specific monoclonal antibody to quantify sialoglycoprotein in EBN products. In the subsequent year, the same group of researchers optimized the ELISA conditions to screen a large number of EBN samples with IC50 of 1.5 ng/mL, and coefficient of variation of 2.9%–5.8%[48]. However, these methods are insufficiently selective, and often unable to differentiate between the peptides and their derivatives or degradation fragments[49].

Mass spectrometry (MS), integrated with advanced bioinformatics, has become the preferred technique for many biological studies, including functional plant studies[50]. Furthermore, MS does not need specific antibodies for analysis. It is a powerful tool for the study of post-translational modifications and protein identification. A 2-dimensional electrophoresis combined with MS has proven to be the more powerful method for protein identification in sample mixtures[51]. Gel electrophoresis can be used to determine the molecular size of a protein[14,42,52,53]. The integration of gel electrophoresis and MS can improve the robustness and reliability for peptide quantification.

Currently, MS coupled to liquid chromatography (LC) has emerged as the primary tool for protein identification[54]. This hybrid system, utilizing the separation power of LC, and the identification power of mass analyser, is very useful for identifying components from complex mixtures of proteins. It is highly sensitive, selective and suitable, especially for protein identification from EBN samples. The identification of compounds is based on the fragmentation pattern of charged ions in term of their mass to charge ratios. In other words, peptide mass fingerprinting is used to match with the protein database for identification. Recent findings on the identification of α 2–3 linked and α 2–6 linked sialoglycoproteins in EBN samples have proven the advantage of this technique in protein identification[55]. Glycoproteins, especially sialoglycoproteins are rich in EBN and sialic acids (>10%) are also reported to have high biological and medicinal values. Based on their results, α 2–3 linked sialoglycoprotein was an acidic mammalian chitinase-like protein, and α 2–6 linked sialoglycoprotein was an acidic mammalian chitinase.

Although MS-based peptide identification is relatively new in the study of clinically significant proteins, quantitative analysis of proteins and peptides by LC-MS/MS is becoming a practical technique for clinical laboratories[49]. This is because the technique offers cost effectiveness, high throughput, multiplexed analysis and quantification. Moreover, MS-based techniques can be used to measure small molecules together with peptides and proteins on a single technology platform[49]. In the study by Chua et al[56], 15 metabolites were detected and tentatively identified from EBN samples. The metabolites ranged from fatty acid amides to vitamin D and lipids. These beneficial metabolites were first reported in EBN.

7 Biological activities of EBN

The medicinal use of EBN can be traced back to the Tang (about 907 AD) and Sung (960–1279 AD) dynasties[9]. Although the consumption of EBN is referred in centuries old Chinese literature, there is limited scientific documentation on the benefits of EBN. Wong[57] conducted a review of the recent in vitro and in vivo evidence-based discoveries, but the subtopics of the review are very limited. To our knowledge, none of the EBN studies have been based on clinical investigation.

EBN has been regarded as a delicacy in Chinese cuisine, and is eaten to maintain general health and radiant, youthful-looking skin. Traditionally, EBN is double boiled with rock sugar to make “bird’s nest soup” and has been used to replenish strength and revitalize energy. According to ancient Chinese literature, EBN is believed to enhance the skin complexion, alleviate asthma and strengthen the immune system[9]. In line with the observation of Hu et al[58], treating Drosophila melanogaster with EBN was found to have an anti-aging effect by increasing the activity of antioxidant enzymes, fecundity and lifespan, but decreasing mortality rate and lipid peroxidation.

7.1 Epidermal growth factor-like activity

Epidermal growth factor (EGF) is a low molecular weight polypeptide with 53 amino acids. It stimulates cell growth and proliferation, and was first isolated from the male mouse submandibular glands[59]. EGF is also present in milk, saliva, urine, blood platelet, macrophages, tears and semen[60]. Uchihashi et al[61] reported that the urinary excretion of human EGF decreased with age. It is suggested that replenishing EGF may benefit the regeneration and normalization of cell function lost to aging.

Further evidence of EGF-like activity was discovered and partially purified using Bio-Gel P-10 column from the aqueous extract of EBN[62]; the EGF-like activity included dose-dependent stimulation of DNA and thymidine synthesis in 3T3 fibroblasts in vitro.

EGF is one of several growth factors, including vascular endothelial growth factor (VEGF) and interleukine-6 (IL-6), that play an important role in cellular processes. They are intercellular mediators which regulate survival,
growth, differentiation and function of cells\textsuperscript{[62]}. Kong \textit{et al}\textsuperscript{[63]} reported that a protein from the EBN extract, which was partially purified by Sephacryl-200 superfine chromatography, was able to produce a mitogenic response in the human lymphocyte cells and potentiated the mitogenic response in concanavalin A-transformed lymphocyte cells. EGF plays a pivotal role in the proliferation, differentiation and survival of cells\textsuperscript{[64]}. Thus, this could explain EBN exhibiting the rejuvenating properties. However, no report on the purification of EGF from EBN has been made for more than two decades. The discovery of this EGF-like activity in EBN could lead to future work on its effects on cell proliferation and differentiation.

\subsection*{7.2 Anti-influenza virus and hemagglutination-inhibitory activities}

The extract of EBN was found to neutralize influenza virus in Madin-Darby canine kidney (MDCK) cells and inhibit the hemagglutination of human erythrocytes caused by influenza A viruses\textsuperscript{[65–67]}. Guo \textit{et al}\textsuperscript{[68]} reported that EBN extract showed potent inhibitory activity against infection of influenza viruses in a host range-independent manner, when it was hydrolysed by pancreatic enzyme (pancreatin F) from glycoproteins (>50 kD) to glycopeptides (10–25 kD). The effect was mediated by the N-acetyleneuraminic acid (NANA) residues of sialyl-sugar chains in the EBN extract. However, the EBN extract did not inhibit the activity of influenza virus sialidase. The active inhibitor in EBN was susceptible to the neuraminidase of influenza virus of all strains. \textit{Collocalia} mucoid could be a substrate for influenza virus sialidase\textsuperscript{[69]}. Therefore, the inhibitory activity could be destroyed by neuraminidase\textsuperscript{[69]}. EBN extract has a wide range of activities against influenza virus, indicating the presence of a mixture of inhibitory substances. NANA was shown to be the major sialic acid derivative found in EBN, by fluorometric high-performance liquid chromatography. NANA in EBN is believed to be responsible for the inhibitory activity against influenza virus\textsuperscript{[66,68]}. The EBN extract exhibited no side effect of haemolysis or cytolysis on erythrocytes or MDCK cells even at higher concentrations. The pancreatic F-digested EBN extract with compounds smaller than 25 kD could be an effective and safe material for antiviral medication\textsuperscript{[66]}. The bioactivity of NANA needs to be studied in detail. However, there are some differences between the inhibitory activity of EBN harvested from caves and houses birds. This may be due to the differences in their living environment and the degradation of O-acetyl sialic acid in EBN collected from caves\textsuperscript{[66]}

\subsection*{7.3 Cell proliferative effect of EBN}

There are few studies dealing with the proliferative effect of EBN using various cell lines. Aswir \textit{et al}\textsuperscript{[70]} investigated the effects of EBN on cell proliferation, using the human colonic adenocarcinoma cell line (Caco-2 cells), and the release of tumour necrosis factor-alpha (TNF-\(\alpha\)), using the mouse leukemic monocyte macrophage cell line (RAW 264.7). They used two types of commercially available EBN and four types of unprocessed EBN samples collected from four regions in Malaysia. Interestingly, the Caco-2 cells treated with all EBN samples showed a significant increase in the percentage of cell proliferation. The increase was ranged from 135\% to 215\% compared to the negative control. On the other hand, the RAW 264.7 cells treated with the two commercially available EBN and two unprocessed EBN demonstrated a reduction in the percentage of TNF-\(\alpha\) release. The remaining two unprocessed EBN samples, however, showed an increase in the release of TNF-\(\alpha\). These data demonstrate that EBN can influence the proliferation rate of Caco-2 cells and the release of TNF-\(\alpha\) in RAW 267.2 cells. The extent of the effects varied depending upon the source of EBN and the degree of processing. Nevertheless, suppression of TNF-\(\alpha\) production was also observed in experiments by Vimala \textit{et al}\textsuperscript{[71]}, who used lipopolysaccharide-stimulated RAW 264.7 macrophage cells treated with EBN. These authors suggested that EBN might have anti-inflammatory properties because they also noticed the inhibition of nitric oxide production using the Griess assay.

In addition, the effects of EBN on the proliferation of corneal keratocytes were also investigated. Zainal Abidin \textit{et al}\textsuperscript{[72]} demonstrated that a low concentration of EBN (0.05\% or 0.1\%) was adequate to enhance the cell proliferation of corneal keratocytes, derived from New Zealand white rabbits, in both serum-containing and serum-free media. The increased proliferation could be due to the presence of a hormone-like substance in the EBN, such as avian EGF. This EGF could stimulate cell division and regeneration as previously reported by Kong \textit{et al}\textsuperscript{[43]}. The finding is considered a breakthrough, since no effective treatment is available to promote the healing process in corneal wounds.

A study by Roh \textit{et al}\textsuperscript{[73]} investigated the mechanism of EBN extract in the proliferation of human adipose-derived stem cells (hADSCs). The proliferation was mediated by the productions of IL-6 and VEGF, which were induced by EBN extract through the activation of AP-1 and NF-\(\kappa\)B. The result supports the potential application of EBN as an inducer to proliferate hADSCs, which are increasingly accepted for stem cell therapy. In line with the finding of Wong \textit{et al}\textsuperscript{[74]}, several Chinese herbal medicines including EBN have been reported to exert proliferation effects on adult stem cells for tissue regeneration.

\subsection*{7.4 Bone strength and dermal thickness promotion}

Matsukawa \textit{et al}\textsuperscript{[75]} prepared EBN extract with pancreatin
In particular, EBN is rich in proteoglycans containing non-sulphated chondroitin GAG, which is one of the main components of bones. The EBN extract was orally administered to ovariectomized rats and the bone strength and mineral (calcium and phosphorous) content, as well as hydroxyl-proline concentration were measured. The results showed an increase in the concentrations of calcium, phosphorus and hydroxyl-proline in femurs, as well as an increase in femoral weight and its maximum breaking force. The histological evaluation on the dorsal skin indicated that the dermal thickness or the thickness of collagen fibrils in the skin was also increased. Therefore, the EBN extract appears to promote bone strength and the improvement of skin, and may have special application for post menopause women.

Chua et al. investigated the effect of EBN extract on the catabolic and anabolic activities of human articular chondrocytes isolated from the knee joint of osteoarthritis patients. The catabolic activity of the cultured chondrocyte was significantly reduced when an EBN-based supplement was applied, which may be caused by down-regulating matrix metalloproteinases, cytokines or expression of other catabolic mediators. Hence, it is suggested that EBN may be a potential anti-inflammatory or anti-degenerative agent in the treatment of osteoarthritis. The anti-inflammatory effect of EBN was also reported by Yida et al. who found that EBN could attenuate high-fat diet-induced oxidative stress and inflammation. This was because EBN could regulate hepatic antioxidant and inflammatory genes. The same group of researchers also reported that EBN could reduce insulin resistance and had anti-pro-coagulation effects induced by high-fat diet in rats. Similarly, EBN-based supplement also caused an increase in the anabolic activity of chondrocytes. However, longer term experimentation is necessary to demonstrate the significance of these changes before concluding that EBN-based supplements have a chondro-protective effect.

8 Current and future prospects of swiftlet farming industry

The history of the swiftlet industry is unique in the Malaysian agricultural sector. This industry has grown since the 18th century, when the majority of nests were collected from caves. Currently, the main product of this industry is EBN. The most expensive nest is white nest, which is produced by A. fuciphagus. Swiftlets construct their nests in caves located at or near the coastal region, or near tropical rainforests. The nests are built onto the smooth surface of the concave walls, located at least 2.5 m above the ground and at least one meter away from the cave entrance.

Rapid urbanization has reduced the availability of nesting sites for swiftlets; in the urban environment they may seek out unoccupied buildings for new nesting sites, for the dark, damp and cooling environment inside the buildings. The discovery of EBN in the buildings has prompted the local community to build more vacant buildings to increase the activity of EBN construction. Over the years, this kind of activity has grown into a sizeable industry, which is now known as swiftlet farming. Swiftlet farming is conducted in man-made buildings (bird houses) that imitate a cave-like environment in order to provide alternative nesting sites and attract the swiftlet birds. The ranchers do not control the birds’ movement, breeding or even their diets. Swiftlets move freely to hunt for insects, and they live naturally without any interference by mankind. However, the quality of these nests is subject to large variability of nutritional composition and especially mineral content. The variability is mainly due to seasonal and geographical factors, as well as diet.

The activities in swiftlet farming are illustrated in Figure 1. The activities include bird house construction, waiting for bird nesting, egg hatching and young bird growing and leaving their nests before farmers harvest the EBN. The growth of this industry has stimulated and offered many business opportunities, including consultancy services, swiftlet house construction, hardware supplies, transportation and logistics for building materials, as well as job opportunities for local communities in the EBN cleaning process.

However, swiftlet farming has also brought several problems to residents who live near swiftlet farms. The major problems are the strong unpleasant odour from the swiftlets’ dropping and the loud chirping sounds produced by the swiftlets. Because of these complaints, the swiftlet farms have been moved to the suburbs and their hygiene conditions are closely monitored by local authorities to ensure the safety and quality of nests. Mostly, the newly constructed swiftlet houses are in rural or agricultural land, and existing bird houses in more urban settings are closely monitored by local authorities for their compliance with regulations. It is also important to make sure that the swiftlet farming does not pose any hazard to the surrounding environment.

Malaysia is a very fortunate country because it has large regions of green grasses, pristine forests, farmlands, rice fields and crop plantations. These areas provide plenty of food for swiftlets. Hence, this industry has tremendously expanded in the last few years, and it is expected that the future of the industry includes continued growth. Currently, Malaysia is not only trying to catch up with well performing EBN producers from Indonesia and
Thailand, but also facing growing competition from other neighbouring countries such as Vietnam, Myanmar and Cambodia.

Recently, the global demand for EBN has markedly increased. Demand is highest in Asia, where most of the estimated 160 tons per annum of EBN produced is consumed, and most are consumed by China\(^\text{[85]}\). This phenomenon has further promoted swiftlet farming in Malaysia\(^\text{[86]}\), even though the authority from China has set more stringent screening tests for EBN products; for instance, the nitrate level in EBN must be lower than 5 μg/g. Some countries, such as Australia and Canada, restrict the import of EBN products into their market, as EBN is considered vulnerable to avian flu infection. However, based on a study of highly pathogenic avian influenza (H5N1), of 137 EBN samples collected from different regions in Malaysia, none of the samples showed positive results for H5N1 infection\(^\text{[87]}\). Furthermore, EBN extract has been shown to have antiviral properties against influenza viruses in a host-range independent manner\(^\text{[66]}\). The import restriction does not keep farmers from their EBN businesses. Great efforts have been made by farmers to reduce the contamination level in bird houses, and to improve the cleaning process in order to meet the standards for nitrite and nitrate contents of EBN. It is expected that the future of the EBN industry is very promising and encouraging. This is because the medicinal benefits of EBN are being slowly demonstrated by scientific research. Many EBN-based products are entering the market as beauty and skincare products, as well as health supplements. Therefore, there is an urgent need to have standardized benchmarks and quality assurance practices from regulatory authorities, which ensure the EBN products from Malaysia are safe for human consumption.

According to the Food and Agriculture Organization of the United Nations and World Health Organization, the acceptable daily intake of nitrite and nitrate in EBN are 30 and 5 μg/g, respectively. Recently, Quek et al\(^\text{[17]}\) investigated the nitrite and nitrate contents of 4 house nests and 4 cave nests, using an ion chromatography system. The results showed that the nitrite and nitrate contents of cave nests were significantly higher than house nests. The nitrate content was about 5.7 μg/g for the house nests and 843.8 μg/g for the cave nests, whereas the nitrate contents of the house and cave nests were 98.2 and 36 999.4 μg/g, respectively. Chan et al\(^\text{[88]}\) reported that up to 98% of nitrite and nitrate could be removed through the process of soaking raw EBNs in water. The high nitrite and nitrate levels may be due to anaerobic fermentation by bacteria, in the presence of ammonia in EBN, but is not from food processing methods\(^\text{[89]}\). Nitrite has been used as a food-preservative and anti-botulinal agent in the food processing industry.

It is estimated that the trade of EBN earns up to several hundred million dollars in foreign exchange, making its production important to the economies of major suppliers, like Indonesia, Thailand and Malaysia. The trade value of EBN has increased dramatically from approximately $170 million in 1989 to $380 million in 2004, and it is expected to grow further with the rising demand from East Asian countries\(^\text{[90]}\). According to Kuan et al\(^\text{[91]}\), Malaysia is the third largest supplier of EBN after Thailand and Indonesia. Malaysia contributes approximately 10% of the 210 tons of EBN produced annually, which is worth up to 4 billion dollars in total. The current top buyers are mostly from Thailand,
China. Based on the Malaysian Federation of Bird’s Nest Merchants Association records, the annual production of EBN in Malaysia has reached a value of 1 billion ringgits ($290 million). In order to benefit from such trade growth, the Malaysian government has recently announced in the Malaysia Economic Transformation Programme that the EBN industry will receive prioritized public investment and policy support for its development.

9 Establishment of authentication technique for EBN

The authentication of EBN can be carried out using many approaches, including physical examination\(^{[92]}\), DNA and proteomics analysis\(^{[5,93,94]}\) and glycan profiling\(^{[96]}\). Table 1 compiles the existing methods used by researchers for EBN authentication. Each method has its advantages and limitations. Currently, there is no standard method for quality assurance and authentication.

The physical examination of EBN is mainly based on the appearance and structural characteristics, specifically, the pattern and tension of the salivary strands under microscopic evaluation\(^{[92]}\). Sam et al\(^{[93]}\) observed the EBN fibre array through scanning electron microscopy. Marcone\(^{[14]}\) examined the physical properties through X-ray microanalysis. The advantages of this physical examination are that it is non-destructive, provides fast screening result, and requires no extraction procedure. The only limitation is that this screening technique relies on qualitative examination, and is unable to evaluate the quality of EBNs in term of their nutritional values\(^{[14]}\).

It is noted that DNA fragments have also been used for EBN authentication by previous researchers\(^{[5,93]}\). The DNA sequence in EBN is likely to be unique and difficult to falsify. However, DNA authentication would still not provide information on nutritional composition of EBN. Moreover, the resolution power of DNA authentication is not adequate to classify EBN according to established grading categories\(^{[94]}\). This may explain the establishment of a combined analysis that uses DNA-based PCR and protein-based two dimensional gel electrophoresis for highly reliable identification of EBN products\(^{[33]}\).

Recently, MS-based proteomics have been developed to determine the quality of EBN with high reliability. Usually, the technique includes one dimensional\(^{[90]}\) and two dimensional polyacrylamide gel electrophoresis\(^{[45]}\) to separate complex protein into individual protein bands based on their molecular size and isoelectric point. Also recently, an antibody raised against an EBN glycoprotein was employed for authentication\(^{[42]}\). In 2013, the same group of researchers developed monoclonal antibodies and a quantitative sandwich-ELISA for the characterization of sialoglycoprotein of EBN\(^{[48]}\). However, the database for EBN is still being developed, and only a few proteins have been matched to the database for identification\(^{[12,110]}\).

Surprisingly, glycan analysis is seldom carried out for EBN samples\(^{[44,75,111]}\). There were five sugar constituents, including mannose, galactose, N-acetylgalactosamine, N-acetylglucosamine and total NANA detected in EBN\(^{[69]}\). The limit of glycan analysis may be the highly complex oligosaccharides and polysaccharides in EBN. The fingerprint of oligosaccharides in EBN was established by Yang et al\(^{[112]}\) using gas chromatography and MS. It is noteworthy that glycan is proposed to be responsible for the biological functions of EBN, for example, anti-influenza effect\(^{[66]}\).

The establishment of authentication technique is of great importance to ensure the quality and safety of EBN products in the market. The high medicinal and nutritional values, and the high price of the EBN have led to widespread counterfeiting or adulteration of EBN products. The commonly used materials for counterfeiting include Tremella fungus, karaya gum, red seaweed, gelatin, agar and starch\(^{[13,20]}\). There is another group of researchers who specifically focused on the detection of porcine gelatine in EBN samples\(^{[95,113]}\). A fast and reliable analytical technique should be developed to protect EBN consumers and to ensure the sustainability of swiftlet farming. Nevertheless, there have been few studies on the safety issue of EBN consumption. To the best of our knowledge, Chen et al\(^{[96]}\) investigated fungal contamination of EBN, using culture and molecular techniques. Further study on the mycotoxin level in EBN is of great priority to ensure the quality of EBN products and the safety of consumers.

10 Conclusion

EBN has been considered a great delicacy for the Chinese community since ancient times. The long history of EBN in Chinese cuisine has been expanded to include it as a contemporary functional food. This phenomenon is mainly due to the remarkable medical effects of EBN reported in recent scientific literature. Studies have supported the traditional belief that EBN may confer medical benefits including improved immune function, skin rejuvenation and improved bone strength. Numerous EBN-related products are available in the market today.

The high demand, particularly from mainland China and Hong Kong indicates that EBN’s potential health benefits are being actively sought. This has led to the fast growth of swiftlet farming, especially in the region of South East Asia, where swiftlets can find abundant food sources and suitable climate. Since swiftlet farming is one of the major incomes of these countries, the local authorities have implemented regulations to monitor the hygiene and
safety of the farming area, as well as the quality of EBN products.

11 Conflict of interests

None.

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