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Recent advances in edible bird's nests and edible bird's nest hydrolysates

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Abstract

Edible bird's nest is built by swiftlet during breeding using salivary gland secretions, which have rich nutritional value, such as anti-aging, immunomodulatory, and antioxidant activity. As a result, the global demand for edible bird's nests has increased significantly. Swiftlet farmers, consumers, traders, and authorities are increasingly concerned about obtaining safe and high-quality edible bird's nests. On the other hand, subject to the limitations of the number of swiftlet populations and the ecological environment, exploring new food processing technologies to improve the utilization efficiency of edible bird's nest nutrition has also become the research frontier. Therefore, this study introduced the origin and classification of edible bird's nests in detail, presented the current situation of the edible bird's nest industry and the corresponding review measures of various countries, reviewed the efficacies of edible bird's nest, and introduced the new form of edible bird's nest utilization: edible bird's nest hydrolysates and their efficacies.

Keywords: swiftlet; edible bird's nest; hydrolysate; review; efficacy.

Practical Application: Swiftlets, edible bird's nest (EBN) industry, EBN and EBN hydrolysates were reviewed.

1 Introduction

Swiftlets were first classified by Gray as early as 1840 as a genus of *Collocalia* (Gray, 1840). Subsequently, between 1937 and 1970, the classification of swiftlets was expanded with the discovery of some of the echo localization capabilities of swiftlets. Brook noted that swiftlets could be divided into three distinct genera: *Collocalia* (no echolocation), *Hydrochous* (consists only of giant swiftlets), and *Aerodramus* (echolocation) (Brooke, 1970, 1972). Currently, it is generally believed that edible bird's nest (EBN) is produced by seven species of swiftlets of the genus *Collocalia* and *Aerodramus* (Dai et al., 2021). During the breeding season, the sublingual salivary glands of these swiftlets secrete saliva rapidly. Swiftlets can nest in caves or vertical walls using sticky saliva alone or co-condensing with feathers or grass (Marcone, 2005). A complete EBN can reach 1-2 times the weight of the swiftlet to support female swiftlets and chicks. Once the chicks leave the nest, these discarded bird's nests will be picked by swiftlet farmers, go through a series of impurity removal steps, and be made into EBN.

At present, the origin of EBN is concentrated in Southeast Asia from 10° south latitude to 20° north latitude, such as Malaysia, Indonesia, Thailand, Vietnam, Cambodia, and parts of China's southeast coast. In Chinese historical records, including the Tang Dynasty, Yuan Dynasty, Qing Dynasty, etc., EBN has always been considered to have the effect of nourishing the body and is regarded as a high-grade health food and a status symbol because of its preciousness (Jiang, 2016). With the time and living standards developing, consuming EBN has become a healthy habit in Southeast Asia, and people's demand for available EBN is gradually increasing. At the same time, swiftlet farmers, consumers, traders, and authorities are increasingly concerned about obtaining safe and high-quality EBN. Therefore, it is necessary to summarize and review the existing EBN review methods to provide a basis for formulating more laws and regulations on EBN. Besides, some studies have found that EBN hydrolysates have a better ability to be digested and absorbed by the human body and may have better values than all-component EBN in some aspects (Fan et al., 2022). However, this part is not well summarized and presented.

Therefore, it is very important to further demonstrate the basic knowledge of EBN and review the research progress of EBN hydrolysates. It can allow people to further understand the basic knowledge of EBN and point out possible directions for EBN research and improve the efficiency of EBN nutrition utilization. Based on this, this study introduced the origin and classification of EBN, the current situation of the EBN industry, and the corresponding review measures of various countries, reviewed the EBN efficacies, and introduced the new form of EBN utilization: EBN hydrolysates and their efficacies.

2 Swiftlet and EBN

2.1 Swiftlet

Swiftlet is a small insectivorous bird. Twenty-four species of swiftlet have been recorded worldwide (Ibrahim et al., 2009; Medway, 1962; Sankaran, 2001). Brook noted that swiftlets could be divided into three distinct genera: *Collocalia* (no echolocation), *Hydrochous* (consists only of giant swiftlets), and *Aerodramus* (echolocation) (Brooke, 1970, 1972). Currently, it is generally believed that EBN is produced by seven species of swiftlets of the genus *Collocalia* and *Aerodramus* (Dai et al., 2021). They are

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Collocalia esculenta and *Collocalia linchi*; *Aerodramu fuciphagus*, *Aerodramu germani*, *Aerodramu maximus*, *Aerodramu unicolor, and Aerodramu francicus*. The most productive EBNs in Southeast Asia are *Aerodramu fuciphag* and *Aerodramu maximu*s (Chua & Zukefli, 2016).

Yifeng et al. (2018) reported that swiftlet growth requires a unique environment, about 90% air humidity, 28-30 °C temperature, and sufficient food sources (Yifeng et al., 2018). Therefore, swiftlets only survive in parts of the country that meet this condition, such as Malaysia, Indonesia, Thailand, Vietnam, the Philippines, and parts of the southeastern coast of China. Swiftlets have a small body (about 8-12 cm, 10 g), light wings, and soft and short legs. Interestingly, their soft and short legs cannot support their body weight (Zuki et al., 2012), so they must perch on the walls of caves or houses to gain enough speed to fly during the fall. It was widely believed that swiftlets only forage during the day and cling to the walls of natural caves, mines, tunnels, or houses to roost at night (Hoyo et al., 1992; Fullard et al., 2010). However, recently, there have been some reported observations of swiftlet's nocturnal activity (Price et al., 2005).

Although there is no clear understanding of the swiftlet's visual abilities, researchers determined that swiftlets could catch insects through echo localization for growth and reproduction (Brinkløv et al., 2013). During the rainy season, when food is abundant, swiftlet colonies enter an active breeding period, which can breed twice a year, and even reach three times a year. During the dry seasons, when food is scarce, swiftlet colonies breed only once. During the breeding season, the sublingual salivary glands of swiftlets secrete saliva rapidly, at which time the sublingual salivary glands of swiftlets reach their maximum secretion activity and increase from 2.5 mg to 160 mg (Clark, 1906; Lowe, 1939; Medway, 1962; Nakagawa et al., 2007). Before breeding, swiftlet couples use the sticky saliva alone or coagulate with feathers or grass to build a nest, providing a stable habitat for female swiftlets, eggs, and chicks, known as bird's nests. Nesting sites are usually chosen on the vertical walls of caves, mines, tunnels, or houses. The nesting time lasts about 35 d (Marcone, 2005). Complete bird's nests are generally 5-10 cm in diameter, 3-8 cm in-depth, and 4-15 g in mas. Generally, swiftlets start nesting 35 d before laying eggs. After 22-29 d of the egg period and 40-59 d of the brooding period, the whole process of nest-building, egg-laying, and brooding will be completed (Phach, 1996). That is, it will last 92-120 days from nesting to abandonment (Langham, 1980). After the chicks leave the nest, swiftlet farmers will pick the nests that swiftlets have discarded. These bird's nests are finally made into EBN through a series of steps such as impurity removal.

2.2 EBN

The bird's nests picked by swiftlet farmers after the chicks leave are called raw-unclean EBN, which are primarily processed (removed feces, soil, and general impurities), and are mold-free but without any added substances. According to the amount of feathers or grass mixed at the time of building the bird's nest, the raw-unclean EBN are classified into light-feathered, medium-feathered, and heavy-feathered bird's nests. Generally, raw-unclean EBN with a 10%-15% impurity ratio is classified as light-feathered raw-unclean EBN, raw-unclean EBN with a 20%-30% impurity ratio is classified as medium-feathered raw-unclean EBN, and raw-unclean EBN with more than 40% impurity ratio are classified as heavy-feathered raw-unclean EBN. Subsequently, raw-unclean EBN will be finely processed (including sorting, water soaking, cleaning, de-feathering, reshaping, heating, drying, and packaging), which are called EBN. Therefore, the general definition of EBN can be summarized as a kind of edible biological product secreted by the salivary glands of several species of swiftlets of the genus *Collocalia* and *Aerodramus*, inhabiting Southeast Asia and adjacent islands (Sheikha, 2021).

EBNs are classified according to various bases, as shown in Table 1. The long-term production and consumption activities determine the classification of EBN. A classic example is a transition from the consumption of cave EBN to the consumption of house EBN. Cave EBN refers to EBN Picked from stone walls, caves, caves, and cliffs. Influenced by the natural climate and natural environment, cave EBN is hard in texture, dark in color, absorbs more minerals, and is mostly beige or yellowish-brown. The picking process of cave EBN is often arduous and dangerous, which is affected by various factors such as the location of the nest in the cave or cliff and the height of the ground or water. The swiftlet farmers who pick EBN usually use tools such as fishing nets or bamboo- or iron-made temporary scaffolding to pick the EBN (Leh, 2001; Marcone, 2005). This process is not only accompanied by dangers but also the picking cycle is too long. It is gradually regarded as a backward production method with the development of house EBN. EBN picked from artificially built bird houses is called house EBN. The artificially built swiftlet house is only used as the habitat of swiftlets and does not restrict the activity of swiftlets. Swiftlet farmers lure swiftlets by simulating the calls of swiftlets and create a familiar habitat for the swiftlets by controlling the light (no light), temperature (28 °C), humidity (> 90%), and smell (bird droppings). This method fully considered the convenience of swiftlet farmers who pick EBN. At the same time, because of the better habitat environment, the house EBN has fewer impurities, and the cup shape is more complete. It thus has gradually become the mainstream source of EBN.

In addition, in the early days of the bird's nest industry, consumers could only buy dry EBN, which needed to undergo complicated steps of cleaning, soaking, and stewing before eating. This process is very dependent on manual labor, and it takes eight hours for one person to clean only about 10 EBN (Lim & Cranbrook, 2002; Leh, 2001). Therefore, in the early stage of the EBN industry, only consumers with a certain social status could hire specialized labor to treat EBN for consumption. In 2012, "Yan Palace" (a Chinese EBN company) launched the first open-box ready-to-eat EBN product named "ONENEST", which changed the traditional consumption process of EBN. The complicated steps before eating EBN (e.g., cleaning, de-feathering, foaming, stewing) are replaced by standardized factory processes. This advanced technology and solution have greatly enhanced consumers' interest in EBN. In recent years, with the development of food science and technology, EBN has also been developed into more and more end-consumer products: such as candy, jelly, beverages, effervescent tablets, nano-sized EBN

Table 1. Classification of the EBN.

Basis for classification	Category	Description
Nest Site	Cave EBN	Picked from caves
	House EBN	Picked from swiftlet houses
Color	White EBN	The color of EBN is white
	Yellow EBN	The color of EBN is yellow
	Red EBN	The color of EBN is red
	Red corner EBN	Only two corners of EBN are red
Quality	Imperial EBN	Most of the nest's ingredients are edible
	Feather EBN	Most of the nest's ingredients are feathers
	Grass EBN	Most of the nest's ingredients are grasses
Picking time	Phase I EBN	EBN that built for the first time in the year
	Phase II EBN	EBN that built for the second time in the year
	Phase III EBN	EBN that built for the third time in the year
Shape	Cup EBN	The shape is complete and is half-bowl; be built on the edge of the top of the walls
	Triangular EBN	The shape is triangular; be built at the corner between the ceiling and the top of the walls
	EBN corners	Corners of EBN; the "load-bearing beam" of the EBN
	bar-like EBN	The complete EBN cup is crushed during transportation or processing, unable to maintain the half-bowl shape, and becomes strips
	EBN fragments	Small fragments from the crushed EBN, which contains all parts of the EBN
	Reshape EBN	EBN that be reshaped into different shapes by models
Density	Dense EBN	The filaments of EBN are evenly distributed and the gaps are not obvious
	Sparse EBN	The filaments of EBN are unevenly distributed and the gaps are obvious
Impurities removal method	Dry picking EBN	Picking out impurities without using water
	Semi-dry picking EBN	Picking out impurities after sprinkling water locally
	Wet picking EBN	Picking up impurities after soaking in water
Processing method	Dry EBN	EBN that has not been picked, soaked, or stewed
	Ready to stew EBN/Pure EBN	EBN that have been picked, soaked, but not stewed
	Instant EBN	EBN that have been picked, soaked, stewed, and sterilized

particles, and EBN that with improved taste by adding ginseng or other ingredients (Dai et al., 2021; Xiujuan et al., 2016).

Although EBN is not produced locally in China, it has become an important luxury in China's mainstream society at the latest in the Ming and Qing dynasties. Its main source is the tribute and trade between neighboring countries and China. The EBN products in the Chinese market have always been imported from Southeast Asian countries. With the improvement of the national economy, people's demand for EBN has steadily increased. The China Academy of Inspection and Quarantine (CAIQ) reported the legal import volume of Chinese raw bird's nests (Figure 1), showing the blowout trend of China's EBN import and consumption (Chinese Academy of Inspection and Quarantine, 2020). It should be noted that Figure 1 does not include large quantities of EBN entering China through informal channels. China alone consumes more than 80% of EBN produced globally, making it the world's largest EBN consumer country (Sheikha, 2021). In 2020, the global EBN industry size was estimated to be around USD 10 billion (Sheikha, 2021). Thus, the EBN industry is becoming more and more huge.

2.3 Environmental protection and sustainability of EBN picking

The quality of the EBN is closely related to the environment in which the swiftlet nests and forages (e.g., soil, climate, pollutants, food sources, "insects") (Chen et al., 2015; Chua & Zukefli, 2016; Sheikha & Menozzi, 2019; Elfita et al., 2020; Kew et al., 2014; Kew et al., 2015; Norhayati et al., 2010; Quek et al., 2018). Chua et al. (2014) showed that the original EBN environment plays an important role in the EBN quality control (Chua et al., 2014). Huda et al. (2008) and Norhayati et al. (2010) reported that the nutrient composition of EBN varied by breeding location (Huda et al., 2008; Norhayati et al., 2010). Paydar et al. (2013) found that the nitrite content of cave EBN was significantly higher than that of house EBN (Paydar et al., 2013). Tan, Quek, and Yusuf measured the nitrite content of

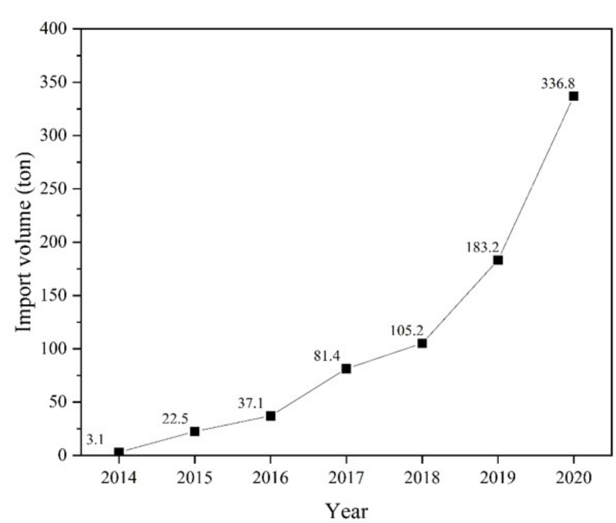

Figure 1. Imports of bird's nests in China.

EBN before and after cleaning, and the results showed that the nitrite content of uncleaned house EBN was 4.8-164.9 mg/kg, while the nitrite content of cleaned cave EBN was still 229 mg/kg-1175 mg/kg (Quek et al., 2018; Tan et al., 2020; Yusuf et al., 2020). The study by Guo et al. (2006) found that there were differences in inhibitory activity between EBN built by wild and captive swiftlets, which were considered to be related to the differences in saliva composition caused by different living environments (Guo et al., 2006). Therefore, on the one hand, minerals and other components in the site where swiftlets build their nests will penetrate into the bird's nest, and different kinds of microorganisms (caused by different environmental humidity or other reasons) will affect the quality of the bird's nest. On the other hand, the different types of food foraged by swiftlets will affect the composition of saliva secreted by swiftlets, resulting in differences in the EBN quality. Therefore, swiftlet farmers are paying more and more attention to protecting the swiftlet's foraging environment and reasonably controlling the swiftlet's nesting environment to obtain high-quality EBN.

Although the EBN industry has gradually changed from cave EBN to house EBN, it does not mean that bird's nest picking activities of swiftlet farmers will affect or damage the growth and reproduction of swiftlets, and it does not mean that humans can "domesticate" swiftlets. In contrast, the foraging, habitation, breeding, and other activities of swiftlets do not depend on the swiftlet farmers, and the role of the swiftlet farmers in the production of EBN is only as of the provider of the swiftlet's habitat and the picker of the abandoned bird's nest. At the same time, swiftlet farmers attach great importance to the protection of swiftlets. They made three no-picking principles: no-picking if there are eggs, no-picking if there are chicks, and no-picking if nesting is not complete. The picking process is carried out while the swiftlets are out foraging. During the picking process, swiftlet farmers will first use mirror reflection to confirm that there are no unhatched eggs in the bird's nest, the chicks have already flown away, and the bird's nest is discarded before picking. It should be noted that swiftlets do not reuse nests that have already been used but prefer to build new nests in the same location. If the abandoned bird's nests are not picked, there will be fewer and fewer places for swiftlets to build their nests, and many strange-shaped bird's nests will be produced. Therefore, picking the discarded bird's nests will not harm the swiftlets but provide them with a sustainable nesting environment, making the EBN industry sustainable.

3 Specification and standardization of EBN industry in various countries

Based on the background of the rapid development of the EBN industry, and in order to protect consumers' health, relevant departments in many countries have taken active actions. Malaysia issued a national standard MS 2334:2011 "Edible-bird nest (EBN)-Specification" in 2011 (Department of Standards Malaysia, 2010a), and Thailand issued a national standard TAS 6705-2014 "Bird's Nest" in 2014 (National Bureau of Agricultural Commodity and Food Standards, 2014). Indonesia does not have a special national standard for bird's nests, but the documents "Procedures of good manufacturing practices of the edible bird's nest" and "Animal quarantine actions for the import or export the bird's nest into or out of the territory of Republic of Indonesia" etc. stipulate the limit requirements for main indicators such as bird's nest microorganisms, foreign substances, feathers and dirt, and nitrite (Indonesia, 2013a, 2013b, 2006; Department of Standards Malaysia, 2010b). The inspection items and limit requirements of the above three countries for the EBN export are shown in Table 2.

Table 2. Inspection items and limit requirements for the EBN export in Malaysia, Thailand, and Indonesia.

Item	Malaysia	Thailand	Indonesia
Sensory	×	The natural color and smell of bird's nest, undyed, with no added fragrance ingredients	×
Physical hazard	×	×	No visible metal, wood chip
Feathers, impurities	×	No visible feathers and impurities	No visible feathers and impurities
Moisture content	15%	15%	×
Water activity	1.0	×	×
Sialic acid	Detected	×	×
Pb	2 mg/kg	×	×
As	0.05 mg/kg	×	×
Hg	1 mg/kg	×	×
Cd	1 mg/kg	×	×
Cu	1.0 mg/L	×	×
Fe	0.3 mg/L	×	×
Nitrite	30 mg/L	30 mg/kg	30 mg/kg
Hydrogen peroxide (for cave EBN only)	Must not be detected	×	×
Food additives	×	Must not be added	×
Pesticide residues	×	Compliant with Thai pesticide residue standards TAS 9002 and TAS 9003	×
Total number of bacterial colonies	2.5×10^6 CFU/g	×	1×10^6 CFU/g
Coliform bacteria	1100 MPN/g	×	100 CFU/g
Escherichia coli	100 MPN/g	100 CFU/g	10 CFU/g
Salmonella	Must not be detected	×	Must not be detected
Staphylococcus aureus	100 MPN/g	1000 CFU/g	100 CFU/g
Bacillus cereus	×	1000 CFU/g	×
Yeasts	10 CFU/g	×	×
Fungus	10 CFU/g	1000 CFU/g	×

Note: × indicates that there are no specific requirements.

From Table 2, it can be seen that the quality control of EBN in the three countries is relatively comprehensive, and the export quality control requirements for nitrite content are the same, but the other scopes and focuses involved are different. Malaysia is the first country that issue EBN standards. The standards cover bird's nest definition, general requirements, grading, packaging, labeling, sampling, compliance with standards, certification marks, factory requirements, legal requirements, etc. Inspection and quarantine items mainly focus on microorganisms, heavy metals, and minerals. Thailand covers the quality of EBN, food additives, pesticide residues, contaminants, hygiene, packaging, signs and symbols, analysis methods, and sampling methods in the standard. Indonesia stipulates the limit indicators of main indicators such as microorganisms, foreign substances, feathers, pollutants, and nitrite.

Besides, as the main consumer of EBN, China is gradually strengthening its control of EBN, although related standards were formulated later than the above three countries. In terms of import control, in 2012, China signed a memorandum of understanding on the inspection, quarantine, and hygiene requirements for imported EBN products with Malaysia and other governments, stipulating that every EBN imported into China must abide by the agreement, ensuring food safety (Yeo et al., 2021). In addition, China has also proposed traceability requirements for EBN and launched the "China Bird's Nest Traceability Management Service Platform" in 2013 (based on the China Academy of Inspection and Quarantine). In terms of domestic control, in 2014, China issued the first EBN industry-standard GH/T 1092-2014 "Grade of Edible Bird's Nest" (All-China Federation of Supply and Marketing Cooperatives, 2014), which covers the definition, shape, quality grade regulations, inspection rules, and sensory evaluation methods of EBN, etc. This is the beginning of the standardization development of China's EBN industry. In 2018, China issued the group standard T/CPCS 001-2018 "Instant Bird's Nest" (Chinese Medicine Culture Research Association, 2018), which stipulates the technical requirements, test methods, inspection rules, labels, signs, packaging, transportation, storage, and shelf-life requirements of instant EBN. Subsequently, EBN-related standards issued by China include group standard T/CPCS 001-2020 "Stewed Bird's Nest" (Chinese Medicine Culture Research Association, 2020), T/XMSSAL-2020 "Food for Xiamen - Instant Bird's Nest" (Xiamen Food Safety Work Federation, 2020), etc. In addition to the published standards, the Nation Health Commission of the People's Republic of China approved the establishment of "National Food Safety Standard for Bird's Nest and Its Products" (project number spaq-2018-002) in 2018, and the Ministry of Industry and Information Technology of the People's Republic of China approved the establishment of Chinese light industry standard "Bird's Nest Products" (project number 2019-1868T-QB) in 2019.

It can be seen that the EBN industry is becoming more and more standardized. Certainly, from the perspective of promoting the standardization of the EBN industry, establishing national standards cannot fully meet the needs of industrial development. It is also necessary to strengthen industry and enterprise standards, promote industry standardization, strengthen industry self-discipline, ensure product quality, and protect consumer benefits.

4 The efficacies of EBN

EBN is rich in protein, amino acids, sialic acid, and inorganic elements. Traditional Chinese medicine believes that EBN has the functions of moistening the lung, nourishing the spleen, stomach, and kidney, relieving the liver, clearing the eyes and tonifying the heart, and regulating fatigue (Fan et al., 2020). Eating EBN is a health-preserving culture in China and South Asia, recorded in the prescriptions, dietetics, and folk experiences in medical books of the past dynasties. Modern medicine's research on EBN focuses on composition research, authenticity identification, pharmacological effects, traceability, etc. In recent years, a large number of studies on the pharmacological effects and mechanisms of EBN have emerged, mainly focusing on whitening effects, antiviral effects, immunomodulatory effects, enhancement effects of intelligence and memory, improvement effects of neurodegenerative diseases, and antioxidant effects (Chen et al., 2017; Dai et al., 2021).

Whitening activities: The epidermal growth factor in EBN is a polypeptide hormone, which can affect cell proliferation and differentiation, improve UV-induced pigmentation, and significantly depigmentation effect on light-damaged skin (Kim et al., 2012a, 2021). Wong et al. found that the EBN simulated digestion product significantly inhibited intracellular melanin and tyrosinase activities (Wong et al., 2018). Chan et al. (2015) found that sialic acid inhibited tyrosinase activity, resulting in significant whitening activity (Chan et al., 2015).

Antiviral effects: The sialic acid at the end of the EBN glycoprotein can effectively inhibit some important antigenic sites and recognition marks on the cell surface, thereby protecting these glycoproteins from being recognized and degraded by the surrounding immune system. It is also a receptor for the influenza virus, the binding point of the influenza virus in mucosal cells. Haghani et al. (2016) reported that EBN has an antiviral ability and is positively correlated with the content of acetylated sialic acid in EBN (Haghani et al., 2016).

Immunomodulatory effects: Zhang et al. (1994) found that feeding mice with pearl-EBN extract promoted T lymphocyte transformation and increased serum IgM levels (Zhang et al., 1994). Zhao et al. (2016) established an immunosuppressive mouse model with cyclophosphamide and found that EBN can promote the proliferation and activation of B cells and enhance the secretion of B cell antibodies, thereby reducing intestinal immune damage in the model mouse (Zhao et al., 2016).

Enhancement effects of intelligence and memory: EBN is the food with the most sialic acid content (Dai et al., 2021). Sialic acid has been shown to be an important nutrient for infant brain development, playing an important role in ensuring synaptic connections, memory formation, and neuronal growth (Kiss & Rougon, 1997). Sialic acid is synthesized by the liver. The babies' livers are not fully developed and cannot synthesize enough sialic acid on their own, so they must obtain sialic acid from their mothers (Duncan et al., 2009). After childbirth, sialic acid levels in the mother's body tend to drop over time (Wei et al., 2011). Therefore, a mother's continued intake of sialic acid during pregnancy and breastfeeding can provide adequate sialic acid to the child and help maintain their sialic acid levels. An animal experiment showed that newborn mice with normal sialic acid intake during lactation performed significantly better on memory tests than newborn mice with deficient sialic acid intake (Oliveros et al., 2018). In addition, another animal experiment showed that oral administration of EBN to female mice during pregnancy or lactation could improve learning and memory function in their offspring. The possible mechanism is that ingested EBN increased the activities of superoxide dismutase and choline acetyltransferase but decreased the level of malondialdehyde and the activity of acetylcholinesterase (Xie et al., 2018).

Improvement effects of neurodegenerative diseases: Neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), are caused by the loss of neurons and/or their myelin sheaths, which can worsen and lead to dysfunction over time. Oxidative stress, inflammation, mitochondrial dysfunction, and the production of excitotoxins are common causes. EBN has been shown able to enhance memory significantly and able to exert neuroprotective effects by inhibiting the changing process of the nervous system. For example, the study by Careena et al. (2018) found that EBN can inhibit neuroinflammation and oxidative stress processes, exert neuroprotective effects and enhance memory (Careena et al., 2018). In animal experiments, Yew et al. (2019, 2018) found that EBN extract has neurotrophic properties, which can significantly improve the motor ability (walking distance and balance) of Parkinson's mice (Yew et al., 2019; Yew et al., 2018). This is thought to be achieved by enhancing antioxidant enzyme activity but inhibiting microglial activation, nitric oxide formation, and lipid peroxidation. Besides, Zhiping et al. (2015) and Hou et al. (2015) also found that EBN has protective effects on nerve damage caused by estrogen deficiency, and ready-to-eat EBN can be used as a new strategy for the clinical treatment of menopausal neurodegenerative diseases (Zhiping et al., 2015; Hou et al., 2015). These studies suggest EBN may be a viable nutritional option for improving neurodegenerative diseases.

Promoting cell proliferation: EBN can promote cell proliferation. For example, Roh et al. (2012) found that EBN extract can strongly promote the proliferation of human adipose-derived stem cells, which can be used for stem cell therapy (Roh et al., 2012). In addition, Kong et al. (1987) detected and partially purified epidermal growth factor (EGF) from EBN and found that it can increase skin cell metabolism and cell proliferation, improve skin texture, rejuvenate skin cells, and make skin whitened (Kong et al., 1987).

Antioxidant, anti-inflammatory, and anti-aging effects: EBN can enhance antioxidant capacity and reduce oxidative stress levels in non-pregnant rats (Albishtue et al., 2018). At the same time, through a comprehensive mechanism of maintaining antioxidant-reactive oxygen species balance and regulating related genes, EBN can protect and prevent the changes in reproductive system tissue morphology and function caused by lead acetate toxicity (Albishtue et al., 2019). EBN has also been shown able to inhibit the formation of tumor necrosis factor-alpha and nitric oxide (Vimala et al., 2012). Furthermore, EBN can also partially attenuate high-fat diet-induced oxidative stress and inflammation through transcriptional regulation of hepatic antioxidant and inflammation-related genes, better than simvastatin (Yida et al., 2015). Zhang et al. (1994) also found in animal experiments that EBN-pearl powder mixtures can significantly reduce lipid peroxidation in mouse brain tissue and increase the level of superoxide dismutase in rat erythrocytes, thereby delaying aging (Zhang et al., 1994). In addition, EBN can inhibit the expression of matrix metalloproteinase-1, showing anti-aging properties by down-regulating extracellular signal-regulated protein kinase/c-Jun N-terminal kinase and transcription factor activator protein-1 pathways (Kim et al., 2012b).

Improving bone strength, increasing dermis thickness, and promoting chondrocyte regeneration: In animal experiments, Matsukawa et al. (2011) fed EBN to ovariectomized rats and found that bone strength and cortical thickness of the rats were improved, while serum estradiol concentration was not affected (Matsukawa et al., 2011). Therefore, Matsukawa et al. (2011) believe that EBN can be used to prevent osteoporosis in postmenopausal women. In addition, Chua et al. (2013) found in cell experiments that EBN can effectively control the deterioration of arthritis and promote the regeneration of cartilage cells, suggesting the potential of EBN for the treatment of osteoarthritis (Chua et al., 2013).

Improving cardiovascular disease: Lee et al. (2020) reported that EBN has a relatively high ratio of unsaturated fatty acid/saturated fatty acid, which is beneficial for lowering serum cholesterol and preventing atherosclerosis and heart disease (Lee et al., 2020). Yida et al. (2015) found that EBN can alleviate high-fat diet-induced hypercholesterolemia and coagulation, preventing the deterioration of metabolic indicators and the transcriptional changes of insulin signaling genes (Yida et al., 2015). The mechanism is similar to simvastatin, which is partly regulated by transcriptional regulation of coagulation-related genes. Furthermore, it has also been reported that EBN can improve cardiometabolic disease in estrogen-deficient rats (Hou et al., 2015).

Regulating gut flora: Zhao et al. (2014) found that EBN can regulate the gut flora of mice by supporting beneficial gut bacteria and inhibiting harmful bacteria (Zhao et al., 2014). However, they indicated that the relationship between the regulatory effects of EBN on gut flora and its immune-promoting effects needs further study.

5 Frontier research direction of EBN

The frontier research direction of EBN can be divided into two parts, namely, the identification of EBN and the acquisition and efficacy evaluation of EBN hydrolysates.

EBN has always been expensive due to its limited supply and huge demand. Therefore, EBN source traceability and ingredient identification have always been important issues in the EBN supply chain (Hamzah et al., 2013; Ma & Liu, 2012; Shim et al., 2016). Researchers have developed various identification and detection methods. In addition to conventional methods, many unconventional identification and detection methods are effective under specific conditions, as well as the combination of various methods to facilitate comprehensive analysis and response to different conditions. Such as molecular biology identification

(Guo et al., 2013; Huang et al., 2011), and fingerprinting (Sheikha, 2021; Ma & Liu, 2012).

Bioactive peptides hydrolyzed from protein are the general term for 2-peptides to complex peptides composed of amino acids. Modern nutritional studies have pointed out that bioactive peptides hydrolyzed from protein can be more effectively absorbed by the body, thereby playing a specific role or improving the growth of intestinal flora to provide probiotic effects (Ji et al., 2022; Li et al., 2022; Tekle et al., 2022; Wei et al., 2022). For example, Ruann J. S. de Castro and Hélia H. Sato found that soy protein isolate hydrolysate had a 7-fold enhanced antioxidant activity compared with soy protein isolate (Castro & Sato, 2014). Toopcham et al. (2017) observed the anti-inflammatory activity in casein hydrolysate and pea protein hydrolysate (Toopcham et al., 2017). In addition, studies have clearly reported that polysaccharides and polysaccharide hydrolysates have excellent immunomodulatory activities, including effects on tumor cells, immune cells, cytokine secretion, and system immunity. Based on the same principle, EBN hydrolysates should also have the enhanced biological activity of general protein hydrolysates and polysaccharide hydrolysate. Therefore, the research on EBN hydrolysates has become the frontier research direction of EBN.

6 EBN hydrolysates and efficacies

6.1 EBN hydrolysates

As mentioned above, the research on EBN hydrolysates is currently a hot spot in the field of EBN. Similar to hydrolysates of other biological macromolecules, such as proteins, polysaccharides, and glycoproteins, the broad definition of EBN hydrolysates should be the small biomolecules that be decomposed from EBN by physical, chemical, or enzymatic treatment. However, the research on EBN hydrolysates has just begun, and currently, there are only EBN hydrolysates obtained by enzymatic treatment. With the exploration of the functional properties of EBN hydrolysates, the methods for obtaining EBN hydrolysates should also be expanded.

At present, the application of enzymatic technology on EBN has produced EBN hydrolysates with stronger biological activities. Studies have found that the proteins, glycoproteins, and polysaccharides in EBN that are poor in water solubility and not easily absorbed by the human body can be converted into simpler and more easily absorbed free peptides, glycopeptides, and free sugar chains (e.g., mannose, galactose, N-acetylgalactosamine, and N-acetylglucosamine) (Fan et al., 2022; Yan et al., 2022). In which the peptides are also named edible bird's nest peptides. In addition, the bioactive components with low bioavailability, such as conjugated sialic acid and N-acetylneuraminic acid in EBN, can obtain higher bioavailability through hydrolysis (Wong et al., 2018). Therefore, EBN hydrolysates are superior to EBN in some functional properties.

6.2 The efficacies of EBN hydrolysates

Antioxidant and Antiaging effects: Fan et al. (2022) compared the effects of stewed EBN without enzymatic hydrolysis and enzymatic hydrolyzed EBN on H_2O_2-induced oxidative damage in HepG2 cells (Fan et al., 2022). The study found that the viability of cells treated with H_2O_2 for 2 h dropped to 40%, while the viability of cells could be increased to 46.85% by adding 1 mg/mL stewed EBN without enzymatic hydrolysis, and the viability of cells could be increased to 57.37% by adding 1 mg/mL enzymatic hydrolyzed EBN. The results suggested that the enzymatic hydrolyzed EBN had significantly enhanced the protection against cellular oxidative damage ($p < 0.05$). Murugan et al. (2020) found that pepsin and trypsin mimetic digested EBN hydrolysates could modulate oxidative stress in mice and protect endothelial cells from oxidative damage (Murugan et al., 2020). Ling et al. (2020) used alkaline protease to hydrolyze EBN, and its DPPH free radical scavenging ability was significantly improved, suggesting its antioxidant activity was improved (Ling et al., 2020). Besides, it was also found that the components of EBN with a molecular weight of less than 3 kDa can increase the activity of antioxidant enzymes in Drosophila melanogaster and reduce the content of lipid peroxidation products, thereby delaying the aging of Drosophila (Ghassem et al., 2017).

Whitening activities: Tyrosinase is a copper-containing enzyme that exists in plant and animal tissues. It can catalyze the production of melanin and other pigments from the oxidation of tyrosine, so it is a rate-limiting enzyme that regulates melanin production. Therefore, the inhibiting test of tyrosinase is one of the common tools for screening skin lightening agents (Chen et al., 2005; Moon et al., 2010). Fan et al. (2022) used intracellular tyrosinase activity experiments to compare the whitening activity of stewed EBN without enzymatic hydrolysis and enzymatic hydrolyzed EBN. It was found that the intracellular tyrosinase inhibitory activity of enzymatic hydrolyzed EBN was significantly higher than that of stewed EBN without enzymatic hydrolysis (Fan et al., 2022). In the result, the EC50 value of stewed EBN without enzymatic hydrolysis for tyrosinase inhibitory activity was 18.74 mg/mL, and the EC50 value of enzymatic hydrolyzed EBN for tyrosinase inhibitory activity was 7.22 mg/mL. Fan et al. (2022) believed that such a significantly increased whitening activity was related to increased and more readily absorbed free sialic acid after digestion of EBN (Fan et al., 2022). By fully digesting the EBN protein into smaller peptides using simulated gastric juice under acidic conditions, Wong et al. (2018) found that the fully digested EBN has better whitening activity (Wong et al., 2018). Compared with EBN without enzymatic hydrolysis, enzymatic hydrolyzed EBN had stronger inhibitory effects on melanogenesis and tyrosinase enzymatic activity of B16 cells. Wong et al. (2018) put forward a different view in this regard, and they believed that the increased whitening activity is related to the release of conjugated N-acetylneuraminic acid in enzymatic hydrolyzed EBN (Wong et al., 2018). Therefore, the EBN hydrolysates have more free bioactive substances than the unhydrolyzed EBN seems to be the main reason for its stronger biological activity. Regarding whitening activity, EBN hydrolysates are suitable for developing a new generation of whitening products.

Promoting differentiation of bone cells: N-acetyl glucosamine is an active substance with the function of promoting bone cell differentiation, which is widely used in osteoarthritis health care products. Wong et al. (2018) found that EBN hydrolysates have stronger effects on promoting differentiation of bone cells than EBN, and the mechanism is speculated to be related to the released free small molecular carbohydrates after hydrolysis, especially N-acetylglucosamine (Wong et al., 2018).

Promoting cell proliferation: The ability to promote cell proliferation has always been one of the advantages of EBN. Interestingly, Gao et al. (2019) recently found that the EBN oligopeptides hydrolyzed by trypsin and pepsin after heat denaturation at 60 °C were more effective than EBN in inducing thymus-dependent lymphocyte proliferation (Gao et al., 2019).

Blood pressure-lowering effects: Ramachandran et al. found better antihypertensive activity in EBN hydrolysates. They believed that EBN hydrolysates prepared from algal enzymes, bromelain, and pancreatin could be classified as functional foods due to their significant blood-pressure-lowering effects (Ramachandran et al., 2018).

Antiviral effects: EBN hydrolysates (trypsin hydrolyzed) could neutralize influenza virus-infected MCDK cells and inhibit the coagulation of red blood cells (Guo et al., 2006).

Improving solubility: Although EBN has a strong water-holding capacity, the solubility of EBN protein is poor, it remains intact after stewing, and the solubility is only 5% (Wong et al., 2017). Existing studies have found that enzymatic hydrolysis can improve the solubility of EBN protein. For example, Fan et al. found that enzymatic hydrolysis and homogenization combined with enzymatic hydrolysis can significantly improve the solubility of EBN, and the solubility of EBN could reach more than 90% (Fan, 2022). Wang et al. (2021) reported that protein solubility of enzymatic hydrolyzed EBN increased from 13.85% to 47.23%, total sugar solubility increased from 7.49% to 39.02%, and sialic acid solubility increased from 18.69% to 44.24% (Wang et al., 2021). Such results are understandable. For biomacromolecules, smaller molecular weights tend to be accompanied by better solubility.

In summary, some efficacies of EBN hydrolysates are stronger than that of unhydrolyzed EBN, which may be related to more free bioactive components in EBN hydrolysates or may be related to more absorbable properties due to smaller molecular weights. In short, EBN hydrolysates are potential bioactive substances. At present, there are few studies in this area, and more in-depth research can be carried out in this area.

7 Conclusion and prospects

Edible bird's nest, a precious ingredient with high nutritional value, has been valued since ancient times. With the improvement of living standards and the restoration of cultural self-confidence in the major edible bird's nest consumer countries led by China, the consumption scale of edible bird's nest has grown rapidly in recent years. The development of more and more effective edible bird's nest laws, regulations, and review measures are of great significance. At the same time, the production of edible bird's nests cannot grow indefinitely due to the limitation of the swiftlet population and the ecological environment. It is of great significance to improve the utilization efficiency of bird's nest nutrition by exploring new food processing technologies. The edible bird's nest hydrolysates obtained by enzymatic hydrolysis have been proved to have more effective functional properties and can be used to improve the efficiency of bird's nest nutrition utilization, e.g., they are potential whitening products. In addition, the acquisition method of edible bird's nest hydrolysates is currently limited to enzymatic hydrolysis, so the expansion of the hydrolysis method is also an area that needs to be studied urgently.

Author contributions

Qunyan Fan: Writing – Review & Editing, Supervision, **Xuncai Liu**: Investigation, Writing – Original draft, **Yaxin Wang**: Writing – Original draft, **Dunming Xu**: Funding acquisition, **Baozhong Guo**: Conceptualization, Data curation, Investigation, Visualization, Writing – Original Draft.

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