EDIBLE BIRD'S NEST (EBN) SAMPLE PREPARATION FOR RESEARCH STUDIES

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Abstract: Edible Bird's Nests (EBN) research works have seen a growing trend. Most of the EBN related studies involved solubilisation and solid-liquid separation before further analysis. However, there was a lack of examination on the solubilisation and separation techniques for EBN. Hence, this paper reported the examination of the EBN solubilisation methods and comparison of the solid-liquid separation techniques that were carried out previously. The collected data were based on the electronic literature search of Web of Science (WOS) and ScienceDirect. The literature search in the databases indicated the majority of the EBN processing related studies show the use of enzymatic solubilisation. It is a preferred solubilisation method and likely more effective than the use of non-enzymatic solubilisation. It is interesting to find that the data presentation for EBN solubility between enzymatic and non-enzymatic solubilisation was distinct. The solubility studies of enzymatic solubilisation were usually reported in the form of degree of hydrolysis (DH) while the non-enzymatic solubilisation reported the protein solubility. Besides that, it was found that the use of filter paper (Whatman No.1 and No.4) was the most frequently used solid-liquid separation method in EBN studies. In conclusion, enzyme solubilisation and filter paper filtration have emerged as preferable solubilisation and solid-liquid separation methods for EBN related research.

Keywords: Edible bird's nest, non-enzymatic solubilisation, enzymatic solubilisation, protein, separation

1. Introduction

Since the 16th century, Edible Bird's Nests (EBN) has been considered an important health supplement in traditional medicine to the Chinese. People consume EBN to enhance the immune system, promote metabolism, reduce skin complexion, and for rejuvenation (Looi & Omar, 2016). The distribution of EBN can be in several breeds of swiftlets which are *Aerodramus*, *Collocalia*, and *Hydrochous* (Dai, Cao, Wang, Chen, & Jiang, 2020; Looi & Omar, 2016).

White-nest swiftlets (*Aerodramus fuciphagus*) and black-nest swiftlets (*Aerodramus maximus*) are harvested for commercial purposes because these nests are able to produce saliva secretion contain mucin-like glycoproteins with high protein content (Looi & Omar, 2016). The cup-shaped nest produced by the swiftlet was made after the hardening of the high-protein glutinous secretion (Chua & Zukefli, 2016; Saengkrajang, Matan, & Matan, 2013). These hardened nests are harvested and cleaned into consumable EBN. Recently, there is a trend of increasing research in EBN studies.

Before carrying out the research studies, the EBN samples require solubilisation before proceeding to EBN-related analyses.

The cleaning process of EBN is crucial because the salivation of *Aerodramus swiftlet* species comprises about 5% to 10% of feathers and dirt (Ling, Chang, Babji, & Lim, 2020; Noor, Babji, & Lim, 2018; Nurfatin et al., 2016). Thereafter, solubilisation and solid-liquid separation will need to be completed before starting the EBN related research. Since there was also a lack of intensive review on the methods of solubilisation EBN and separation techniques for solubilised EBN. This study aims to summarise the different solubilisation techniques for EBN and review several methods of solid-liquid separation for EBN.

2. Discussion

2.1. Enzymatic solubilisation

Enzymatic solubilisation is an inventive method that used proteolytic enzymes to break down the protein network of EBN. In the end, protein solubilisation in EBN was measured using Degree Hydrolysis (DH). DH value can be influenced by several variables including concentration, pH, time, and temperature. According to the previous studies, there are a few enzymes that have been used to solubilise EBN such as Alcalase, Pancreatin, Protamex, and Papain (Amin, Mubeen, Ahmad, Aziz, & Arif, 2019; Amiza, Oon, & Norizah, 2019; Khushairay, Ayub, & Babji, 2014; Nurfatin et al., 2016).

However, the Alcalase enzyme has been reported by many research reports due to its broad specificity and has often been utilised to modify functional properties of EBN protein (Amiza et al., 2019; Khushairay et al., 2014; Nurfatin et al., 2016). The optimum pH reported was commonly 8, while the duration of solubilisation was usually within 60 to 180 minutes with the temperature between 60 to 90°C (Muhammad, Babji, & Ayub, 2015; Nurfatin et al., 2016; Ramachandran, Babji, & Sani, 2018). Meanwhile, DH reported in the literature was ranged from 82.7 to 84% (Khushairay et al., 2014; Nurfatin et al., 2016; Ramachandran et al., 2018). The value of DH increases with time due to Alcalse able enzyme to operate on the protein more thoroughly (Nurfatin et al., 2016; Zulkifli et al., 2019).

Moreover, most of the studies applied 60°C because temperatures above that have the potential for enzyme denaturation which affects enzyme activity and EBN protein solubilisation (Amiza et al., 2019). Overall, Alcalase is a widely used enzymatic solubilisation method for EBN, most probably due to its higher DH for EBN samples.

2.2. Non-enzymatic solubilisation

Apart from enzyme solubilisation, the non-enzymatic solubilisation method was proved to successfully solubilise the EBN, and the methods including water extraction, acid, and alkaline solubilisation methods. Previous studies claimed that the water extraction method is convenient since EBN contains mainly water-soluble protein and is widely used for protein extraction (Chua & Zukefli, 2016; Zukefli, Chua, & Rahmat, 2017).

Therefore, this method is widely used for EBN protein solubilisation. Water extraction methods reported were used in the temperature between 80 to 100°C with solubilisation time between 1 to 4 hours (Daud et al., 2019; Wong et al., 2018; Zamri et al., 2020). Apart from that, acid and alkaline solubilisation were also managed to solubilise EBN by disintegrating the structure to release

protein. The works of literature show that acid and alkaline solubilisation methods were commonly carried out at temperature 80 to 110°C and 25 to 45°C, respectively with different solubilisation time (Chantakun, Nuthong, & Benjakul, 2020; Noor et al., 2018; Tong, Lee, Cheong, & Lim, 2020).

Generally, the water extraction method exhibits the highest protein solubility of EBN among non-enzymatic solubilisation as reported in the previous studies ranged from 65 to 90% (Fan et al., 2020; Wong et al., 2018; You et al., 2015). Meanwhile, the acid and alkaline solubilisation method was commonly used as a pre-treatment to solubilise EBN prior to subjected analysis.

2.3. Solid-liquid separation methods

Both enzymatic and non-enzymatic solubilisation was required to perform separation on EBN prior to specific analysis and usage. There are several methods that currently exist to separate EBN which are centrifugation, gravity filtration, and ultrafiltration process (Babji et al., 2018; Muhammad et al., 2015; Ramachandran et al., 2018)**.**

Most of the studies applied centrifugation prior to other separation methods which are gravity filtration and ultrafiltration. Gravity filtration was effectively employed in enzymatic solubilisation of EBN using filter papers Whatman No.1 and Whatman No.4. Meanwhile, ultrafiltration method is applicable to solubilisation methods including enzymatic and non-enzymatic through different molecular weight cut-off (MWCO) membranes.

However, the ultrafiltration method is not extensively documented in EBN investigations as this method is mainly used to determine the properties of each fraction isolated from solubilised EBN.

3. Conclusion

In conclusion, the finding from this literature examination shows that enzymatic solubilisation is the most preferable processing method in solubilising EBN. This finding is supported by the majority of previous studies that used enzymes especially Alcalase prior to analysis of EBN. The implementation of separation methods from the previous studies synthesis that gravity filtration using filter papers Whatman No.1 and Whatman No.4 is effective to segregate the solubilised EBN.

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