LABORATORY SCALE EGGSHELL MEMBRANE SEPARATION AND HYDROLYSIS

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Abstract: Eggshell membrane (ESM) has a variety of nutrients including fibrous protein and glycosaminoglycans. The ESM can be separated and hydrolysed from Eggshell (ES) prior to further usage. However, there are not many reviews on the separation and hydrolysis methods of ESM so far. Hence, the present review compiles the ESM separation and hydrolysis studies reported to date. The literature search in this review was conducted by looking into a Web of Science (WoS) database. Most of the papers reported the use of manual peeling as a separation method followed by acid separation (hydrochloric acid and acetic acid) and mechanical separation (foam separator and stirred tank). The mechanically stirred tank is reported to be the most efficient separation method with the highest yield of ESM (80.2% to 90.9%). In addition, the ESM hydrolyses reported were predominantly done by using alcalase. The hydrolyses were done with various hydrolysis duration (1 – 12 hours) and temperatures (50°C – 55°C). In conclusion, most of the current separation of ESM prior to further usage was still focussing on manual hand peeling, while a large amount of the reported ESM hydrolysates was done by using alcalase.

Keywords: Eggshell membrane, separation techniques, parameters, hydrolysis, hydrolysate

1. Introduction

Eggshell membrane (ESM) is a thin layer that exists between the calcified eggshell (ES) and the egg albumen. Furthermore, the ESM structure was made up of an inner and outer membrane which was discovered by using scanning electron microscopy (SEM). According to the result of SEM, the outer layer has a thickness of 50-70 µm, and the inner layer has a thickness of 15-30 µm (Park et al., 2016).

Consequently, the ESM contains several nutritional components such as sulphated glycoproteins (Görögh et al., 2017), glycosaminoglycan (polysaccharide), hyaluronic acid, and fibrous protein (collagen types I, V, and X) which 88–96% contribute to its dry weight (Baláž, 2014; Hewlings, Kalman, & Schneider, 2019). Apart from that, other proteins including osteopontin, sialoprotein, and keratin, have also been discovered in ESM (Torres et al, 2010; Baláž, 2014). These nutritional components could be received after a few processes of ESM such as ESM separation from the ES and ESM hydrolysis. Firstly, the ESM should be separated from ES prior to further analysis. Next, the ESM yield was collected and proceeded to the hydrolysis process. The complex structure of the protein from ESM should undergo a hydrolysis process in order to obtain ESM protein hydrolysate.

The aim of this paper is to examine the ESM separation and hydrolysis methods of ESM, and discuss the specific parameters involved in procedures. Despite the fact that ESM's nutritional potential has been recognised, while several ESM separation and hydrolysis processes have been carried out, the current trends of ESM separation and hydrolysis for research investigation are seldom discussed. Hence, the present review compiles the ESM separation and hydrolysis studies recently developed.

2. Discussion

2.1. Eggshell membrane separation

A literature search from WoS database indicated several separation techniques were available for ESM namely, manual separation, mechanical separation, and acid separation. Firstly, the manual peeling technique was predominantly used by most of the researchers (Lee & Huang, 2019; Li et al., 2018; Pant et al., 2017; Makkar et al., 2015; Abdolmohammad-Zadeh & Talleb, 2014; Shi et al., 2014a). Even though manual peeling was the preferred ESM separation method, however, all of these articles did not report the ESM yield from the peeling process. Thus, the efficacy of this technique is unknown.

Next, there are 2 types of mechanical separation methods that have been reported, namely, foam separator and stirred tank. The stirred tank recorded the highest ESM yield is in the range of 80.2 % - 90.9% (Chi et al., 2019) while the foam separator between 34 % - 77 % (Bayraktar et al., 2021).

It is worth noting that the manual ESM peeling process is usually coupled with acid treatment. The reported acids used to assist ESM peeling process are hydrochloric acid (Zhang et al., 2021; Andreassen et al., 2020; Moreno Araújo Pinheiro Lima & de Oliveira, 2020; Peigneux et al, 2020; Jyothi et al., 2018), acetic acid (Mahmoodi et al., 2019; Santana et al., 2016; Cheng et al., 2011) and ethylene diamine tetraacetic acid (EDTA) (Zhang et al., 2016).

2.2. Eggshell membrane hydrolysis

Subsequently, the separated dried ESM was hydrolysed to produce ESM hydrolysates. The ESM protein hydrolysates were produced either by using chemical hydrolysis (Ruff et al., 2015; Yoo et al., 2014) or enzymatic hydrolysis (Jain & Anal, 2016; Andreassen et al., 2020) process. The hydrolysis processes were used to break up the polypeptide bonds in the ESM protein structure. The products from this disruption process will produce amino acids and peptides (Baharuddin et al., 2016).

The chemical hydrolysis was performed using sodium hydroxide (Ruff et al., 2015; Yoo et al., 2014). However, one of these hydrolysis methods has utilised ethanol together with sodium hydroxide. The ethanol was used to catalyse the breaking of a peptide bond in the ESM via a nucleophile substitution process by the addition of water (Speight, 2018). There are not many reports available for ESM chemical hydrolysis, hence, more research could be done to explore further for its abilities to produce ESM hydrolysates.

Literature work shows that most of the ESM hydrolysate studies utilised enzymatic hydrolysis. The alcalase enzyme was the most used enzyme for ESM hydrolysis. To date, a study has started the investigations on the ESM hydrolysed by using several enzymes. The enzymes combination such as alcalase with different types of proteases (Shi et al., 2014a). It was found that enzyme

combination in hydrolysis increases the degree of hydrolysis (DOH). The ESM enzymatic hydrolysis studies reported were predominantly done by using alcalase enzyme with the duration ranged from 1 to 12 hours (Jain & Anal, 2016; Santana et al., 2016; Shi et al., 2014a) with temperature ranged within 50ºC – 55ºC (Andreassen et al., 2020 Shi et al., 2014b; Shi et al., 2014a). ESM chemical hydrolysis should be widely discovered because the research reported so far is insufficient to make any conclusion either this method is better than enzymatic hydrolysis or not.

3. Conclusion

In conclusion, laboratory separation of ESM from the ES for the ESM related research works were mostly based on the hand manual peeling due to its potential to preserve the protein content in ESM structure, while the usage of the mechanical stirred tank can improve the production of ESM, though it was not a commonly used laboratory method for ESM separation. The alcalase enzyme usage in hydrolysis was the most common one with a better DOH. In addition, the usage of multiple enzymes in ESM hydrolysis can improve DOH.

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