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Research on Eggshell Membrane Separation for High-Purity Calcium Carbonate ($CaCO_3$) Recovery

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Abstract

Along with growing poultry farming, a large amount of eggshell waste is generated daily from food plants and hatcheries. This waste represents a potential biological hazard and is currently being disposed of as environmental pollution. The main composition of eggshells - calcium carbonate - can produce various types of calcium products, such as calcium citrate, calcium lactate, calcium gluconate, etc., which are beneficial to human health. However, eggshells contain a membrane composed of proteins like collagen and sulfur-rich proteins. These membranes have a fibrous and durable structure, making them difficult to separate from the hard shell layer. The separation of eggshell membranes facilitates the production of high-purity calcium products from calcium carbonate. This study employed three methods to investigate the separation of eggshell membranes: a chemical method using NaOH (1), an enzymatic method utilizing protease enzymes (2), and a combined chemical-enzymatic treatment (3). The results showed that the composition of the eggshells was 94.63% inorganic salts and 3.58% eggshell membranes. The membrane separation efficiency reached 90.63% within 1 hour using method (1). The membrane separation efficiency was relatively high, with Alcalase 2.5L reaching 84.75% and with Protamex reaching 75.62% after 3 hours using the enzymatic method. The combined method achieved a good separation efficiency of 95.04% within 2.25 hours, and the recovered calcium purity reached 99.69%. These results demonstrate the potential for developing an industrial-scale process to treat eggshells and produce high-purity natural calcium products as a valuable source of calcium supplementation for human consumption.

Keywords: Calcium carbonate, eggshell, eggshell membrane, protease enzymes.

1. Introduction

The global poultry egg production in 2020 was 92.967 million tones, of which 93.2% were chicken eggs, dominating poultry egg products. The proportion of eggshell in the egg structure is 10-12%, suggesting that 9.531 million tones of eggshell by-products were produced yearly worldwide [1]. Poultry eggshells are considered valuable livestock waste materials. In recent years, significant research has been conducted on using eggshells as a "cost-effective alternative source of expensive supplements" [2]. The hard-shell layer, containing over 94% natural calcium, can be transformed into organic calcium products such as gluconate, citrate, lactate, etc., which are highly bioavailable for human supplementation. The eggshell membranes, composed of fibrous proteins, glycoproteins, glycosaminoglycans, collagen, etc., have valuable applications in medical fields, such as materials for treating joint and muscle inflammation, wound dressing and more [2, 3].

Eggshells are divided into three layers [2, 4] from outermost to innermost, as follows:

- Cuticle: It is a protein layer on the surface of the eggshell's mineral component. It is approximately 10μm thick and contains two-thirds of the pigments on the eggshell's surface. The major pigment in eggshells of brown-egg laying hens was protoporphyrin IX, but traces of biliverdin and its zinc chelates are also present. The pigment was deposited onto all shell layers, including the shell membranes, but most of it was concentrated in the calcareous shell's outermost layer and the cuticle [5].

- Shell: This layer primarily consists of mineral compounds with calcium crystals. It is around 200 μm thick and has a porous, spongy structure with approximately 10,000-20,000 pores penetrating the shell.

- Eggshell membrane (ESM): It is a fibrous layer surrounding the egg white, containing 90% protein, 3% lipid, 2% sugar, etc. The eggshell membrane has a unique physical structure with disulfide bonds and cross-linking of lysine residues and di-vinyl chains, making it highly resistant to dissolution. The maximum solubility of EMS is only 62%, limiting its exploitation and utilization. The eggshell membrane is

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divided into two layers separated by an air chamber: the outer shell membrane, adjacent to the shell, with a thickness of 50-70 μm and 1-7 μm fibre membranes containing type I, X collagen proteins; and the inner shell membrane, with a thickness of 15-26 μm and 0.1-3 μm fibre membranes containing type I, X, V collagen proteins [1].

To reduce and utilize these wastes, eggshell wastes were transformed into calcium carbonate to use as raw materials for producing highly valuable calcium products. Low-purity calcium carbonate grade was used in the fertilizer industry, whereas high-purity calcium carbonate grade was used in mineral feed and food additive industries [6]. Various methods can be employed to extract high-purity calcium from these layers selectively. Chi *et al.* (2019) reported a mechanical method to separate eggshell membranes from eggshells using bubble blowing and a stirring tank. These methods rely on the differences in mechanical strength and density between the eggshell and eggshell membrane, but they require bulky equipment and have limited effectiveness [7]. Chunhao *et al.* (2023) [1] demonstrated that the most effective membrane separation was achieved using alkaline protease combined with papain for the highest efficiency. W. Bao-huan *et al.* [8] and Nurul Syazwina N.H. [9] provided favourable results using the Alcalase enzyme for membrane hydrolysis. Enzymatic methods offer high efficiency in membrane recovery and reduce protein denaturation but have higher costs. Chemical methods were also reported to involve chemical agents to disrupt the fibre bonds between the eggshell membrane and the outer mineralized shell layer [1]. Hydrochloric acid and acetic acid were commonly chosen. Using 3.68 M HCl resulted in a membrane recovery rate of 96.52%. However, this method dissolves the mainly calcium-containing insoluble salts in the hard-shell layer, making it unsuitable for recovering the entire hard shell. Alkali methods often involve using NaOH solution and heat to denature proteins, breaking disulfide and peptide bonds to obtain lower molecular weight proteins (gelatin form). Using NaOH and NaClO, calcium carbonate recovery reached over 98% [10].

Based on the reported results, the first step is to thoroughly separate the organic components within the eggshell to achieve the highest purity of recovered calcium. Therefore, a combination of chemical and enzymatic methods is a suitable approach to achieve the primary goal of maximizing calcium recovery.

2. Material and Methods

2.1. Materials

Industrial eggshells were collected from bakeries, thoroughly cleaned, and dried at 60 °C for 60 minutes before being crushed to a size of 3-5 mm for convenience in further research.

2.2. Methods

2.2.1. Determination of eggshell components

a- The total components of eggshell membranes and pigments were determined as follows: Crushed eggshells underwent a dissolution process in 5% hydrochloric acid (at room temperature, within 6-8 hours, ensuring reaction completion by the total dissolution of the hard shells). Subsequently, the resultant solution was subjected to filtration, followed by the drying of the residue until a constant weight was achieved. The final step involved the measurement of the dried residue [1, 7]. The experiment was done in triplicate.

b- The components of eggshell membranes were determined as follows: The large eggshell sample was subjected to solubilization in 5% hydrochloric acid under ambient temperature conditions for 6-8 hours, ensuring complete dissolution of the rigid shell components. Following this, the extracted membrane underwent a recovery process, including thorough washing with distilled water to remove residual impurities. Subsequently, the membrane was dried to a constant weight [1, 7]. The experiment was done in triplicate.

The moisture content of eggshell powder was according to TCVN 10788:2015.

2.2.2. Chemical membrane separation method

The crushed eggshell underwent a membrane separation by adding NaOH (solid form from Vietnam) or an enzyme solution (Protamex, Alcalase from Novozymes). Following this step, filtration and washing were conducted to isolate the membrane, after which the shell was dried to obtain calcium carbonate ($CaCO_3$). The eggshell/NaOH solution ratio was 30 g/100 mL for the chemical separation. The NaOH concentration (2.5%, 3%, and 3.5%), reaction time (45, 60 and 75 minutes) and reaction temperature (60 °C, 70 °C and 75 °C) were investigated to determine the recovery efficiency and the purity of calcium carbonate [10]. The experiment was done in triplicate (Fig. 1).

The EDTA titration method was used to determine the calcium content according to TCVN 6198:1996. The experimental procedure involved immersing the sample in a 2M HCl solution for an extended duration overnight, followed by filtration of the resultant solution. A mixture comprising 2 mL of the sample, 600 - 800 μL of NaOH, 50 mL of distilled water, and 0.2 g of the calconcarboxylic acid [2-hydroxy-1 (2-hydroxy-4-sulfonaphthylazo) (HSN, ($C_{21}H_{14}N_2O_7S \cdot 3H_2O$), powder, China) indicator (also known as the Patton-Reeder indicator) was prepared and subsequently titrated with an ethylenediamine tetraacetic acid disodium salt solution **(**EDTA ($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$) in white powder form, originating from China). Notably, the titration process

culminated in an endpoint characterized by a distinct alteration in colouration from pink to a vibrant and conspicuous shade of bright blue.

2.2.3. Enzymatic membrane separation method

The enzyme concentration (calculated based on percentage of substrate, total of cuticle, pigment, and membrane), reaction time, and hydrolysis temperature for Protamex and Alcalase enzymes separately were investigated for the enzyme membrane separation process. The eggshell/enzyme solution ratio was 30 g/200 mL [1]. Protamex enzyme in powder form (activity: 1.5AU/g, pH: 6-7 and temperature: 50-60 °C) and Alcalase 2.5L PF enzyme in liquid form (activity: 2.5AU-A/g, pH: 7-10 and temperature: 60-75 °C) were obtained from Novozymes. The experiment was done in triplicate (Fig. 1).

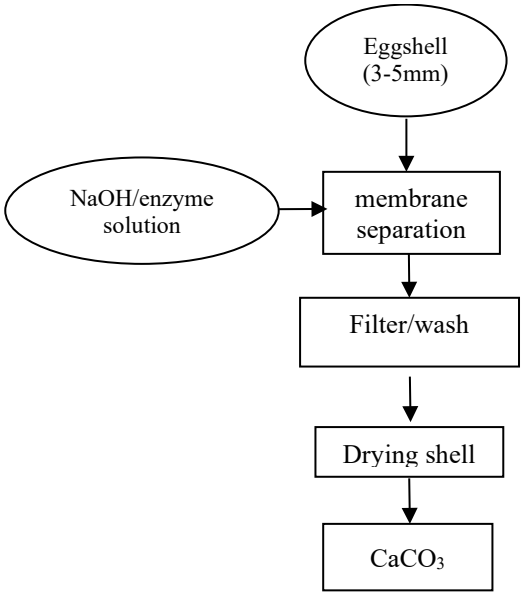


Fig. 1. Procedure for membrane separation using NaOH/or enzyme

2.2.4. Combined membrane separation method

NaOH concentration and treatment time were investigated first for the combined membrane separation process, followed by enzyme treatment (Fig. 2). The membrane separation efficiency and calcium content were determined. The experiment was done in triplicate.

The purity of calcium carbonate was accountable by calcium:

$$Purity_{Ca} = \frac{C_{Ca\,(tt)}}{Msme} \times 100\ (\%) \qquad (1)$$

where: $C_{Ca\,(tt)}$ is the calcium content (calculated as calcium carbonate) in the separated membrane eggshell sample determined by titration (based on the dry matter); M_{sme} is the mass of separated membrane eggshells (based on dry matter).

The membrane separation efficiency was calculated as the ratio of the actual separated membrane mass to the initial membrane mass with the same initial dry matter mass:

$$H\ (\%) = \frac{m_{separated\ membrane}}{s_{\ theoretical\ membrane}} \times 100 \qquad (2)$$

where: $m_{\ theoretical\ membrane}$ is the theoretical total mass of the cuticle layer and the inner and outer membrane layers of the eggshell (determined according to section 2.2.1-a); $m_{\ separated\ membrane}$ is the mass of the eggshell before membrane separation - the mass of the eggshell after membrane separation (based on the dry matter).

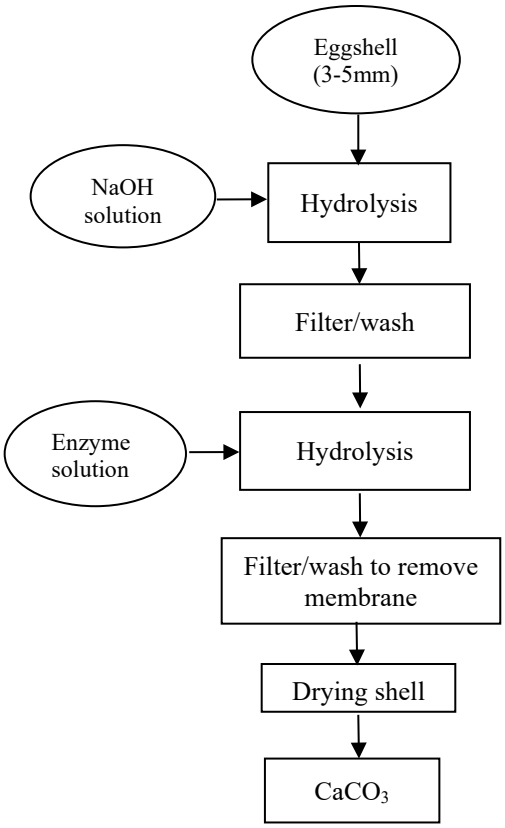


Fig. 2. Procedure for membrane separation using combined methods

2.2.5. Statistical analysis

Statistical analysis was performed using Minitab 19 software. Analysis of variance (ANOVA) was performed in Minitab and tested at a significant difference level with *p* smaller than 0.05.

3. Results and Discussion

3.1. Eggshell Components

After preliminary washing and drying, the eggshells were further ground to increase the reaction rate with 5% HCl acid according to section 2.2.1. This was done to determine the proportion of each layer and

the number of organic components (cuticle, pigment, and membrane). The residue obtained on the filter paper was dried to determine its mass, as shown in Fig. 3 and Table 1.

To determine the mass of the membrane layer, intact pieces of eggshells were immersed in a 5% HCl acid solution according to section 2.2.1. The solution was left until complete dissolution, while larger pieces of the membrane were cleaned of any residue, dried to a constant weight, and then compared with the weight of each layer (cuticle, pigment, and membrane) to determine the proportion of each layer of the eggshell, as shown in Fig. 4 and Table 1.

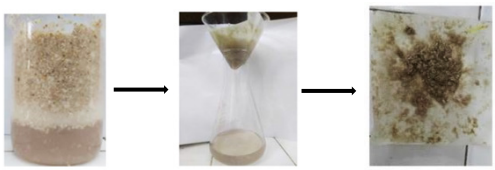


Fig. 3. Eggshell fluid and total membrane and pigment obtained after reaction

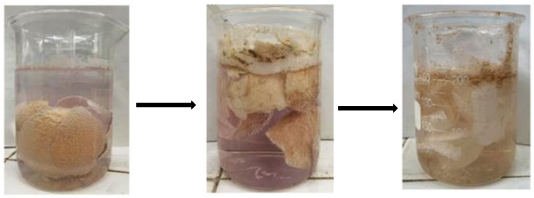


Fig. 4. The dissolution of eggshell in 5% HCl and remaining eggshell membrane after reaction

Table 1. Proportions of eggshell layers

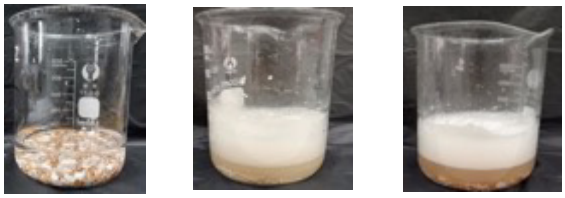
	Eggshell	Shell	Cuticle and pigment	Eggshell membrane
Dry matter (g)	30.00 ± 0.0	28.39 ± 0.15	0.54 ± 0.01	1.07 ± 0.03
Ratio (%)	100	94.63	1.79	3.58

The results obtained were in accordance with a previous study. The author showed that eggshells contained 95% minerals (93.5% $CaCO_3$) and 3.4% organic matter [11, 12].

3.2. Membrane Separation by Chemical Method

The process of chemically separating the eggshell membrane involves targeting the fibrous linkage between the outer eggshell membrane and the mineralized papillae of the eggshell. The eggshell membrane was separated by disrupting the interconnecting fibres using specific chemical reagents [1].

In this study, after being collected and processed according to section 2.2.2 (Fig. 1), the eggshells were investigated for three factors influencing membrane separation efficiency (NaOH 2.5-3.5%; temperature: 60-75 °C and time: 45-75 min). The investigation results on the separation of eggshell membranes by the chemical method using NaOH were shown in Fig. 5 and Table 2.



0 min 45 mins 60 mins

Fig. 5: The dissolution of eggshell membrane and pigment by NaOH over time

Table 2. Results of the investigation on the membranes separation efficiency and purity of $CaCO_3$ by the chemical method

NaOH (%)	Time (min)	Temperature (°C)	Membrane separation efficiency (%)	$CaCO_3$ Purity (%)
2.5	45	75	61.38 ± 3.13[a*]	97.74 ± 0.25[a]
	60	70	73.24 ± 2.21[b]	98.46 ± 0.43[b]
		75	85.66 ± 2.40[c]	99.09 ± 0.21[b]
	75	70	84.75 ± 2.55[c]	99.06 ± 0.32[b]
		75	89.56 ± 1.24[f]	99.16 ± 0.10[bc]
3	45	60	44.36 ± 3.02[d]	96.07 ± 0.16[d]
		70	49.78 ± 3.11[d]	97.24 ± 0.25[a]
		75	68.11 ± 1.76[e]	97.91 ± 0.34[ab]
	60	60	67.48 ± 1.39[e]	98.24 ± 0.42[b]
		70	90.63 ± 2.13[f]	99.31 ± 0.14[c]
		75	90.84 ± 1.41[f]	99.36 ± 0.13[c]
	75	60	88.21 ± 2.04[f]	99.15 ± 0.08[c]
		70	90.70 ± 2.36[f]	99.36 ± 0.13[c]
		75	90.88 ± 1.52[f]	99.39 ± 0.12[c]
3.5	45	70	69.41 ± 2.87[e]	98.24 ± 0.09[b]
	60	60	79.94 ± 0.45[h]	98.91 ± 0.37[b]
		70	92.26 ± 0.24[g]	99.58 ± 0.02[d]
	75	60	86.99 ± 2.56[c]	99.18 ± 0.04[c]

[*]Different lowercase letters within a column indicate significant differences ($p < 0.05$)

NaOH can break peptide and disulfide bonds to form protein molecules with lower molecular weight [1]. The study demonstrated that the chemical method showed the high membrane separation efficiency when NaOH concentration is greater than 3.0%, at temperature is higher than 70 ºC and within 60 minutes.

The organic matter content of the eggshell accounts for about 5-6% of the eggshell mass, so the mass of the membrane separated was relatively small compared to the mass of calcium carbonate. The membrane separation efficiency, calculated as the percentage of membrane separation ranging from 0-100%, was equivalent to the initial purity of calcium carbonate ranging from approximately 94% to 99.6%. Furthermore, as the membrane separation efficiency increases, the purity of calcium carbonate also increases within a narrower range.

Our study's results from Table 2 showed that the best membrane separation efficiency was achieved using NaOH at 3-3.5% concentration, a reaction time of 60 minutes at 70 °C, reaching a range of 90.63-92.26%. This treatment also exhibited the highest purity of $CaCO_3$, reaching 99.31-99.58%, which was higher than the results reported by Nurul Syazwina. *et al.* [9]. and T. Mark Daniel *et al.* [10]. Mark Daniel *et al.* [10] showed that when treating eggshells with 2.5% NaOH at 60 ºC for 30 minutes and further treated with 8% NaClO, the obtained calcium carbonate had a purity greater than 98 %.

However, utilizing alkali to separate the eggshell membrane yields a high recovery rate, but it could compromise the membrane's bioactivity. The introduction of additional chemical reagents escalates the cost of separation and contributes to environmental pollution.

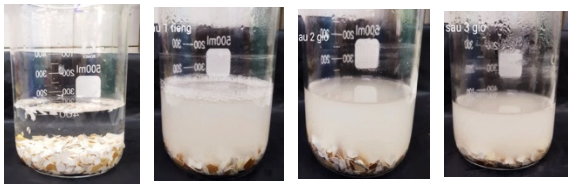
3.3. Membrane Separation by Enzymatic Method

3.3.1 Enzyme Protamex

After being collected and processed according to the recommended procedure in section 2.2.3 (Fig. 1), the eggshells were subjected to a fixed temperature of 55 °C and a pH range of 6-7, as recommended by the supplier. Two factors influencing membrane separation efficiency were investigated: enzyme concentration and hydrolysis time. The enzyme concentration was 1-2% (based on protein content in 30 g of eggshell), and the hydrolysis time ranged from 2-3 hours.

Fig. 6 and Table 3 show the evaluation results of membrane separation efficiency and the purity of calcium content in the separated membrane using the Protamex enzyme.

Based on the information provided, using the Protamex enzyme alone at a concentration of 2% did not yield high efficiency in membrane separation, achieving only 75.62% membrane separation after 3 hours. Therefore, exploring other types of protease enzymes was necessary to improve the membrane separation efficiency.



0h 1h 2h 3h

Fig. 6. The dissolution of eggshell membrane and pigment in solution by the enzyme Protamex over time

Table 3. Membrane separation efficiency and calcium content of white shell after treatment with Protamex enzyme

Enzyme content (%)	Time (hours)	Membrane separation efficiency (%)	$CaCO_3$ purity (%)
1.0	2 hours	33.00 ± 1.84[a*]	95.19 ± 0.11[a]
	3 hours	38.78 ± 1.39[b]	95.34 ± 0.26[a]
1.25	2 hours	50.89 ± 2.67[c]	96.07 ± 0.14[b]
	3 hours	59.62 ± 3.06[d]	96.57 ± 0.28[b]
1.5	2 hours	61.09 ± 2.12[d]	97.46 ± 0.43[c]
	3 hours	66.04 ± 2.83[e]	97.60 ± 0.68[c]
2.0	2 hours	69.44 ± 1.75[f]	97.83 ± 0.47[c]
	3 hours	75.62 ± 2.04[g]	98.54 ± 0.45[cd]
2.5	3 hours	75.64 ± 1.52[g]	98.62 ± 0.42[**cd**]

[*]Different lowercase letters within a column indicate significant differences ($p < 0.05$)

3.3.2. Enzyme Alcalase

After being collected and processed according to the procedure in section 2.2.3, the eggshells were subjected to a 3-hour reaction time. Three factors influencing membrane separation efficiency were investigated while maintaining a constant reaction time. The factors studied were:

- pH: 7-8 (7.5) and 9-10 (9.5);
- Hydrolysis temperature: 65-75 °C;
- Enzyme concentration: 0.5-0.75% (based on the amount of substrate).

The results were presented in Fig. 7 and Table 4.

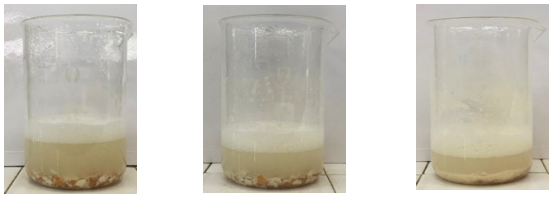


Fig. 7. The changes of the hydrolysis reaction of the eggshell membrane by the enzyme Alcalase over time at pH 9-10

Table 4. Results of the investigation of membrane separation efficiency and $CaCO_3$ purity of the method using Alcalase enzyme

pH	Enzyme content (%)	Temperature (°C)	Membrane separation efficiency (%)	$CaCO_3$ purity (%)
7.5	0.5	65	72.50 ± 1.07[a*]	97.33 ± 0.14[a]
		75	75.97 ± 0.35[b]	97.32 ± 0.35[a]
	0.75	60	59.67 ± 2.44[c]	96.56 ± 0.87[a]
		65	72.32 ± 2.43[a]	97.21 ± 0.23[a]
		75	74.99 ± 1.88[b]	97.22 ± 0.15[a]
9.5	0.5	65	75.69 ± 1.35[b]	98.59 ± 0.43[b]
		75	84.81 ± 1.49[c]	98.75 ± 0.58[b]
	0.75	65	75.07 ± 2.24[b]	98.22 ± 0.29[b]
		75	81.97 ± 2.16[c]	98.42 ± 0.61[b]

*Different lowercase letters within a column indicate significant differences ($p < 0.05$)

Alcalase and Protamex enzymes have different characteristics and protein hydrolysis capabilities. The research aims to identify the suitable and more effective enzyme for eggshell membranes. According to the results in Table 4, using the Alcalase enzyme at 75 °C, pH 9.5, resulted in the highest membrane separation efficiency of 84.75%, which was higher than the Protamex enzyme. This sample also exhibited the highest purity of $CaCO_3$, reaching 98.85%. Therefore, alkaline enzyme, Alcalase, was suitable for the eggshell membrane, providing better membrane separation efficiency. These results were consistent with the reports by W. Bao-huan [7] and Nurul Syazwina *et al.* [8] that highlighted the conditions and efficiency of Alcalase in hydrolysis.

In our study, increasing the temperature generally increased the reaction rate. Interestingly, when the enzyme content increases (0.75%), the membrane separation efficiency did not increase. It can be explained that all available enzyme active sites may be occupied by substrate molecules at a certain point. Adding more enzymes beyond this point did not increase the reaction rate because there were no additional active sites for the substrate to bind. Additionally, if substrate concentration was limited, increasing the enzyme concentration beyond a certain point may not lead to a proportional increase in product formation since there was not enough substrate available for all the enzyme molecules to act upon. In some cases, increasing the enzyme concentration excessively may lead to side reactions or the formation of inhibitory products, which can interfere with the desired reaction and reduce overall membrane separation efficiency. The membrane separation efficiency was slightly lower because the statistical results did not show a significant difference.

Comparing the membranes separated by chemical and enzymatic methods, the chemical method's separation time was fast, and the membrane was separated into large fragments. Meanwhile, the enzyme method requires more time, and the membrane was separated into a smaller and smoother form (Fig. 5, Fig. 6, Fig. 7).

Enzymatic approaches were reported to exhibit the highest separation efficiency, mainly achieved through the utilization of diverse enzymes to cleave the peptide bonds present within the fibrous connections of the eggshell membrane and the connections between the outer eggshell membrane and the mineralized layer of the eggshell.

3.4. Membrane Separation by Combined Method

The subsequent study will follow the proposed approach and involve membrane separation using a combination of NaOH and Alcalase enzyme to improve membrane separation efficiency further and minimize the use of NaOH and processing time.

After being collected and processed, the eggshells were treated with varying concentrations of NaOH ranging from 1-2% for 30-45 minutes. Subsequently, they were further processed using Alcalase enzyme at a fixed concentration of 0.5% (as determined in Section 3.3.2) at a hydrolysis temperature of 75°C and varying hydrolysis times ranging from 60-90 minutes. The results were presented in Fig. 8 and Table 5.

The results from Table 5 indicate that the membrane separation efficiency depends on the NaOH concentration and the enzymatic processing time. The best membrane separation approach was combining NaOH at a 2% concentration (70 °C/45 minutes) with Alcalase enzyme at a concentration of 0.5% (75 °C, pH 9.5) for 90 minutes. This approach yields the highest membrane separation efficiency and results in the highest purity of $CaCO_3$, reaching 99.63%.

Table 5. Results of the investigation of membrane separation efficiency and $CaCO_3$ purity of the method using NaOH combined with Alcalase enzyme

Sample	Membrane separation efficiency (%)	$CaCO_3$ purity (%)
NaOH 1% (45min) + 0.5% Alcalase (90 min)	85.91 ± 2.16[a*]	98.73 ± 0.29[a]
NaOH 1.5% (45min) + 0.5% Alcalase (90 min)	91.05 ± 1.24[b]	99.24 ± 0.13[a]
NaOH 2% (30min) + 0.5% Alcalase (90 min)	86.04 ± 3.47[a]	98.82 ± 0.43[a]
NaOH 2% (45min) + 0.5% Alcalase (90 min)	95.04 ± 2.25[c]	99.69 ± 0.11[b]
NaOH 2% (45min) + 0.5% Alcalase (75 min)	91.12 ± 1.39[b]	99.32 ± 0.28[b]
NaOH 2% (45min) + 0.5% Alcalase (60 min)	88.29 ± 2.53[b]	99.05 ± 0.13[a]

*Different lowercase letters within a column indicate significant differences ($p < 0.05$)

Separated membrane by NaOH (2%) | Separated membrane by Alcalase | De-membrane eggshell

Fig. 8. Membrane and de-membrane eggshell

4. Conclusion

In conclusion, the eggshell comprised calcium salts (94.63%), with the membrane containing 3.58% and cuticle and pigments 1.79% of the dry matter. The moisture content of the eggshell ranged from 1-1.2%. Various extraction methods exhibited distinct membrane separation efficiencies and calcium purities: a chemical process with 3.5% NaOH at 70 °C for 60 minutes yields 90.63% efficiency and 99.58% purity; a biological method using Protamex enzyme at 2% for 3 hours at 55 °C and pH 6.5 results in 75.62% efficiency and 98.54% purity; Alcalase enzyme for 3 hours at 75 °C and pH 9.5 attained 84.81% efficiency and 98.75% purity; a combined NaOH and Alcalase approach, with NaOH at 2% (70 °C for 45 minutes) and enzyme at 0.5% (75 °C, pH 9.5 for 90 minutes), achieved 95.04% efficiency and 99.69% purity. Therefore, the combined method showed the best effect to produce high-purity calcium carbonate from eggshells. The high-purity calcium carbonate obtained from this study can be a great source in mineral feed and food additive industries.

Acknowledgements

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