Vietnam Journal of Food Control

A Scientific Journal of National Institute for Food Control
Journal homepage: https://vjfc.nifc.gov.vn

Research Article

Research on the treatment method and production process of high-purity calcium chloride from hatching poultry eggshells waste

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(Received: 23 Nov 2025; Revised: 25 Dec 2025; Accepted: 25 Dec 2025)

Abstract

The rapid growth of the poultry industry generates a significant volume of eggshell by-products from hatcheries, which pose biological hazards and are often discarded, causing environmental concerns. Eggshells consist of over 93% calcium carbonate ($CaCO_3$), representing a valuable natural source for calcium-based products, particularly calcium chloride ($CaCl_2$). This study investigated pre-treated methods to reduce microbial contamination, improve membrane separation, and recover eggshells rich in $CaCO_3$ for $CaCl_2$ production. Sterilization at 121°C for 60 min, followed by drying at 45°C for 4 h, achieved the best microbial reduction and membrane separation efficiency (93%), the purity of $CaCO_3$ reaches 98%. Conversion from $CaCO_3$ to $CaCl_2$ achieved 89% recovery and 100% purity, meeting the QCVN 4-9:2010/BYT standards, with technological conditions: demembraned eggshell with (particle size >3 mm) was mixed with a 5% HCl solution at a 1:15 (mass/volume) ratio for 3.5 h. These findings demonstrate the potential for industrial-scale $CaCl_2$ production from eggshell waste.

Keywords: *Calcium chloride; recycling; eggshell membrane separation; eggshell waste.*

1. INTRODUCTION

In 2022, approximately 87 million tons of hen eggs were produced worldwide, including 6.3 million tons in the European Union [1]. Currently, the majority of this eggshell waste-originating from both hatcheries and food processing facilities-is still treated as solid waste and disposed of in landfills, imposing significant economic and environmental burdens. The disposal cost is considerable and may incur over USD 100.000 annually in landfill fees for a single egg-breaking plant [2].

Eggshell waste (ESW) is rich in bioactive compounds and has therefore attracted increasing scientific interest for the development of value-added products with commercial potential. Chicken eggshells consist primarily of $CaCO_3$ (92%) and roughly 6% organic matrix, which contains proteins and proteoglycans that interact with the mineral phase to determine its microstructure and mechanical properties [3]. Therefore, eggshells can partially substitute commercial products such as limestone, purified $CaCO_3$, soil conditioners, bioceramic materials, food additives and supplements, cosmetic or pharmaceutical raw materials, as well as catalysts and wastewater treatment agents [2].

A promising application of eggshell waste is its conversion into $CaCl_2$, a compound widely used in the food, chemical, and environmental sectors. This process involves reacting eggshell-derived calcium carbonate with hydrochloric acid to produce $CaCl_2$ [4]. As a common strong acid, HCl is less hazardous than many other inorganic acids and rapidly dissolves eggshell-derived $CaCO_3$ to form soluble $CaCl_2$ while releasing CO_2. Simultaneously, it induces microstructural expansion within the calcite layer, enlarging pores and enhancing

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https://doi.org/10.47866/2615-9252/vjfc.4586

solvent permeability, thereby improving mineral release efficiency. This mechanism increases reaction rate, improves yield, reduces residual impurities, and simplifies the production process [5].

The overall reaction can be represented as:

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O$$

This study was conducted to investigate and optimize the conversion of post-hatching eggshell waste into high-purity $CaCl_2$ that meets stringent quality standards. The research focuses on developing an efficient, sustainable extraction process and characterizing the physicochemical properties and purity of the resulting product to assess its potential as a food additive.

2. MATERIALS AND METHODS

2.1. Materials

Hatchery ESW, originating from the GT1 and GT2 chicken breeds, were collected from the Thuy Phuong Poultry Research Centre, a facility under the National Institute of Animal Science (NIAS), Ministry of Agriculture and Environment. The shells' surfaces were contaminated with various materials, including feathers, faeces, soil, and other organic residues.

***Figure 1**. Eggshell waste*

2.2. Chemicals and reagents

Disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2O_8Na_2.2H_2O$), China; HSN (2-hydroxy-1-(2-hydroxy-4-sulfo-1 naphthylazo)-3-($C_{21}H_{14}N_2O_7S.3H_2O$), naphthoic acid, China, purity 99%; HCl, solution, concentration 37%, Germany; $Ca(OH)_2$, China, purity 95%, etc.

2.3. Experimental design

2.3.1. Initial quality assessment and pretreatment of eggshell waste after hatching

After collection, ESW were immediately transported to the Department of Food Engineering, School of Chemical and Life Sciences, for analysis. The untreated eggshell samples (ES0) were analyzed for moisture, protein, lipid, calcium ion (Ca^{2+}), $CaCO_3$ content and the numbers of microorganisms, including total viable count, *Enterobacteriaceae*, *Coliforms*, and *E. coli*.

Eggshells after incubation are quite dirty, often contaminated with soil, sand, mucus, etc., and especially susceptible to microorganisms from the poultry's gut such as *E. coli, Salmonella, Campylobacteria*, etc., which are pathogenic microorganisms that produce toxins quite dangerous to humans [6-8]. Therefore, in order to eliminate the above pollution risks, the eggshells were pre-treated according to the schematic diagram presented in **Figure 2**. The application of these treatment conditions produced 3 individual eggshell samples: ES75, ES100, ES121. The efficiency of the eggshell pretreatment was evaluated on the following:

Microbial reduction efficiency, including the total viable count of microorganisms, *Enterobacteriaceae*, *Coliforms* and *E. coli*.

Moisture content and organic matter content.

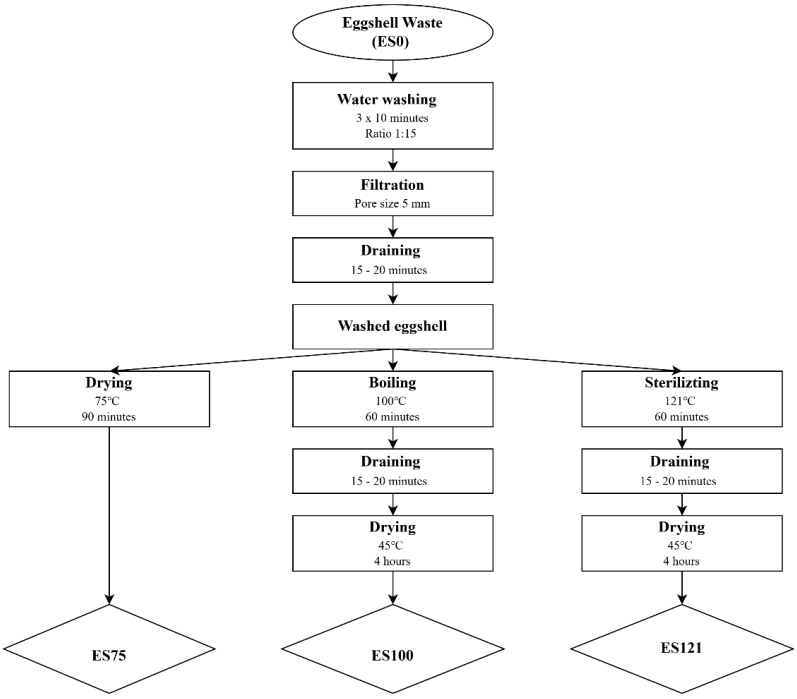


Figure 2. *Eggshell Pretreatment Process*

2.3.2. Separation of eggshell membrane and recovery of the high-$CaCO_3$ shell layer

The demembraned eggshell was conducted on the ES0 (untreated eggshell), ES75 (shell was washed and dried at 75°C for 1.5 h), ES100 (eggshell was washed, boiled at 100°C for 1 h and dried at 45°C for 4 h), ES121 (eggshell was washed, sterilized at 121°C for 1 h and dried at 45°C for 4 h) to evaluate the membrane separation efficiency and the $CaCO_3$ content of the shell obtained post-separation.

The eggshell membrane separation process was performed using combined method: ESW→ hydrolysis by 2% NaOH solution (70°C, 45 min)→wash to remove eggshell membranes and excess NaOH→hydrolysis by enzyme solution (0.5% Alcalase, 75°C, pH 9.5 for 1.5 h)→ remove membrane by washing→drying shell (80°C for 2 h)→demembraned eggshells (moisture < 2%) [9].

2.3.3. Production of calcium chloride from eggshell

Theoretical basis: The demembraned eggshells (primarily $CaCO_3$) react with HCl solution according to the following chemical equation:

$$CaCO_3\ (s) + 2HCl\ (aq) \rightarrow CaCl_2\ (aq) + H_2O\ (l) + CO_2\ (g)$$

The process for producing calcium chloride from eggshells is shown in **Figure 3**.

The factors investigated in this study include:

+ Eggshell/ acid solution ratio (solid-to-liquid ratio): 1/10, 1/15 and 1/20, corresponding to the theoretical $CaCO_3$/HCl molar ratio of 1/2.

+ HCl solution concentration: 4.5%; 4.75%; 5.0% and 5.25% (applied at the optimal eggshell-to-acid ratio determined in the prior experiment).

+ Eggshell piece size: >5 mm, 3-5 mm, 1.5-3 mm, 0.25-1.5 mm and <0.25 mm.

+ Reaction time: 2.5 h, 3 h, 3.5 h, 4 h and 4.5 h.

+ Influence of pH on the neutralization and organic matter flocculation step using $Ca(OH)_2$: 0.69 (pH of intinital solution), 5.0, 6.0, and 7.0.

The final product is stored in a sealed bag to prevent moisture absorption and is evaluated for the following parameters: $CaCl_2$ content and recovery efficiency relative to theory.

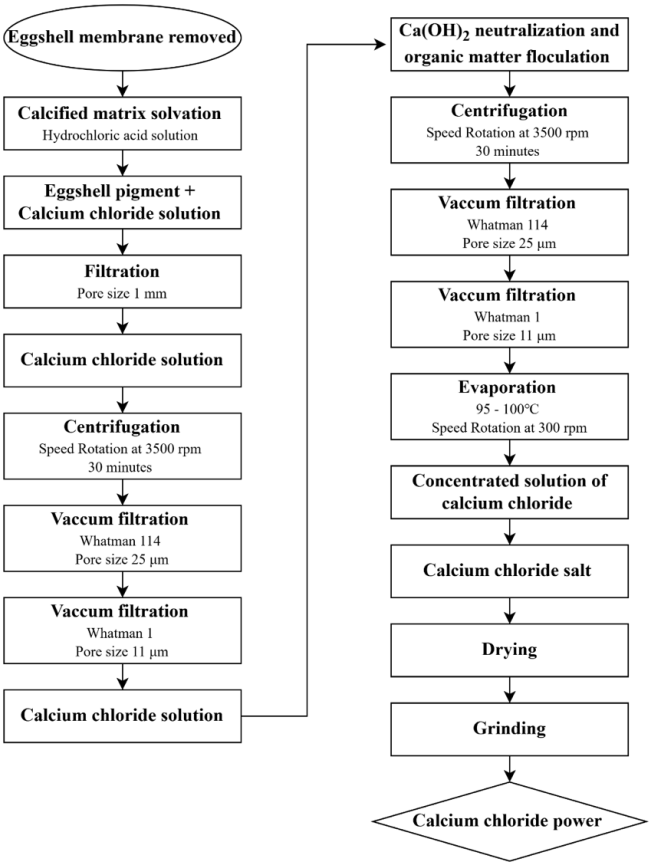


Figure 3. *The process for producing calcium chloride from eggshells*

2.4. Methods

2.4.1. Analytical methods

The total counts of aerobic microorganisms was determined according to TCVN 4884-1:2015 (ISO 4833-1:2013), *Coliforms* - TCVN 6848:2007, and *E. coli* - TCVN 7924-2:2008 (ISO 16649-2:2001). Enterobacteriaceae - TCVN 5518-2:2007.

The total protein content was determined according to TCVN 10034:2013.

The total lipid content of eggshell waste was determined by Soxhlet method [10].

The calcium ion (Ca^{2+}) content of the experimental samples was determined by complexometric titration according to TCVN 6198:1996.

The moisture content of the experimental samples was determined based on the procedures outlined in TCVN 10788:2015.

The content of heavy metals (lead, mercury, arsenic) were determined according to TCVN 10912:2015 (EN 15763:2009).

2.4.2. Determination of $CaCO_3$ content

The purity of calcium carbonate was accounted for by calcium:

$$Purity_{Ca} = \frac{C_{Ca(tt)}}{M_{sme}} \times 100\ (\%)$$

Where: $C_{Ca(tt)}$ is the calcium content (calculated as calcium carbonate) in the experimental samples determined by titration (based on the dry matter); M_{sme} is the mass of the experimental samples (based on the dry matter) [9].

2.4.3. Determination of membrane separation efficiency

The membrane separation efficiency was calculated as the ratio:

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

Where: $m_{theoretical\ membrane}$ is the theoretical total mass of the cuticle layer and the inner and outer membrane layers of the eggshell; $m_{separated\ membrane}$ is the mass of the eggshell before membrane separation – the mass of the eggshell after membrane separation (based on the dry matter) [9].

2.4.4. Determination of $CaCl_2$ content and recovery efficiency of $CaCl_2$

The $CaCl_2$ content of the experimental samples was determined by complexometric titration according to QCVN 4-9:2010/BYT.

The recovery efficiency of $CaCl_2$ was determined by the equation:

$$H\ (\%) = \frac{W}{W_0} \times Degree\ of\ Purity \times 100\%$$

Where: W is the actual mass of recovered calcium chloride powder; W_0 is the theoretical mass of recovered calcium chloride powder (based on dry matter).

2.4.5. Structural evaluation of $CaCl_2$ salt

Structural characterisation of the sample was performed by analysing characteristic functional group bands via FTIR spectroscopy on a ThermoFisher Scientific spectrometer (USA) over the wavenumber range of 4000–400 cm^{-1}. Focus on characteristic functional groups of hydration bonds (O-H, H-O-H) [11].

2.4.6. Determination of the lightness of $CaCl_2$ salt

Sample lightness was evaluated based on the L* value, measured with a Colorlife SPH860 instrument (Germany).

2.4.7. Data analysis

Each experiment was replicated at least three times. Data were analyzed using SPSS 18 software at the 0.05 significance level.

3. RESULTS AND DISCUSSION

3.1. Characterization of raw eggshell waste after hatching

Results of chemical composition and microorganisms of eggeshell was presented in **Table 1** and **Table 2**.

Table 1. *Chemical composition of eggshell*

Content (%)	Moisture	Protein	Lipid	Ca^{2+}	$CaCO_3$
ESW	2.47±0.12	6.50±0.17	0.54±0.03	36.98±0.03	92.45±0.09

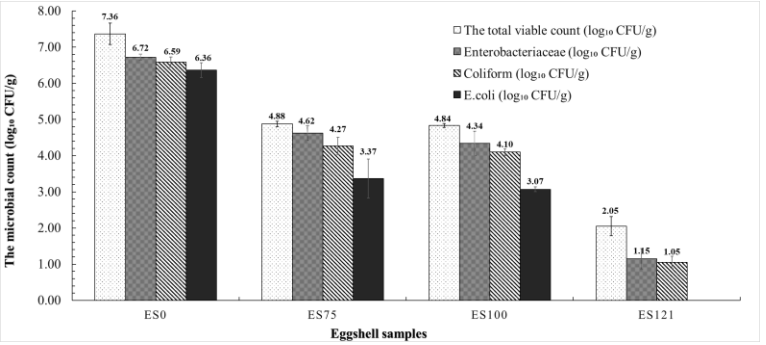
Table 2. *Microorganisms of eggshell*

Microorganisms	Total viable count (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Coliform* (CFU/g)	*E.coli* (CFU/g)
ESW	$2.65.10^7$ $\pm 1.48.10^7$	$5.35.10^6$ $\pm 1.09.10^6$	$4.01.10^6$ $\pm 1.37.10^6$	$2.49.10^6$ $\pm 1.21.10^6$

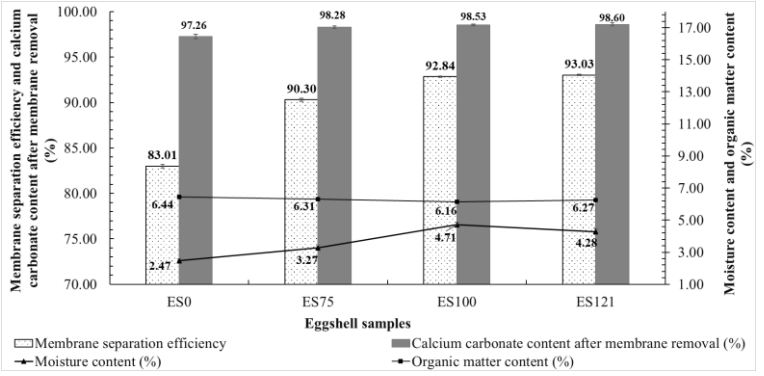
These compositions demonstrated that the eggshell was a rich source of calcium carbonate. In addition, the high level of biological contamination in unprocessed post-hatch eggshell waste may be attributed to faecal, feather, and gastrointestinal microorganism contamination during laying [12]. Therefore, it is essential to conduct a preliminary study to remove the eggshell membrane and improve the eggshell's hygiene to produce high-purity, safe $CaCO_3$.

3.2. Efficacy of different pretreatment conditions for eggshells

The efficiency of the different pretreatment conditions was demonstrated by physicochemical parameters, membrane separation efficiency, and microbial indicators of the eggshell samples processed under each condition. The results were presented in **Figure 4**.



a - Microbial reduction efficiency



b - Physicochemical indicators and membrane separation efficacy

Figure 4. *Efficiency of the treatment methods*

The organic matter content in the eggshell samples processed by different pre-treated conditions was lower than that of the initial eggshell samples (ES0). This reduction is attributed to the washing procedure, which leads to the removal of organic matter, primarily feathers and faeces of the eggshells, along with a small fraction of the eggshell membrane.

The membrane separation efficiency and $CaCO_3$ content obtained after separation were higher than those of the untreated eggshell sample (ES0), which clearly demonstrated the effectiveness of eggshell membrane removal by heat-humidity treatment. The highest membrane separation efficiency was 93.03±0.08%, and the $CaCO_3$ content was 98.60±0.16% for the sample treated at 121℃ for 60 min (ES121).

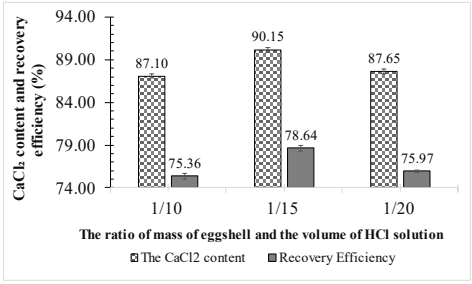
The microbial reduction efficacy was most evident in the eggshell sample treated by the sterilization method (ES121). This method demonstrated a significant reduction in the presence of microorganisms, specifically the numbers of *Enterobacteriaceae* were reduced from 7-logCFU of eggshell samples (ES0) to 1-logCFU of ES121, and Coliform was reduced from 6-logCFU of eggshell samples (ES0) to 1-logCFU of ES121.

Consequently, sterilisation at 121℃ for 60 min, followed by drying at 45℃ for 4 h, was selected as the optimal pretreatment method for raw eggshell waste.

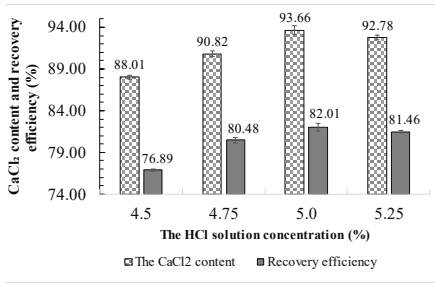
3.3. Production of high-purity $CaCl_2$ from demembraned eggshells

Theoretically, 10 g of $CaCO_3$ requires 7.3 g of HCl for complete reaction (corresponding to a $CaCO_3$:HCl molar ratio of 1:2, in accordance with the theoretical equation presented in Section 2.3.3). Experiments were conducted to investigate the factors influencing the $CaCl_2$ content and recovery efficiency by varying the following parameters: eggshell/acid solution (solid-to-liquid) ratio (w/v), HCl solution concentration, eggshell piece size, and reaction time. Each experiment was performed under continuous stirring at 300 rpm. The post-reaction liquid was pre-filtered through cloth to remove the initially remaining organic matter. Subsequently,

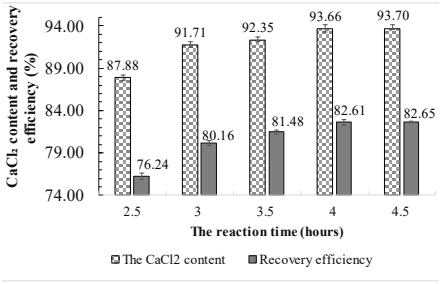
the solution was further purified by centrifugation to separate suspended solids, followed by double filtration using Whatman 114 and Whatman 1 filter papers. The filtered solution was concentrated and dried at 130°C to constant weight. The results of this investigation were presented in **Figure 5**.



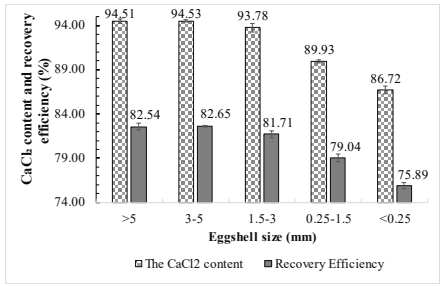
a - Effect of the reaction ratio



b - Effect of HCl concentration



c - Effect of eggshell piece size



d - Effect of reaction time

Figure 5. *Effect of specific factors on reaction efficiency*

The 1/15 (solid-to-liquid) ratio showed the highest $CaCl_2$ content (90.15%) and recovery efficiency (78.64%). While the 1/10 ratio, which theoretically promotes the reaction, led to excessive gas evolution that hindered the eggshell-acid interaction, reducing the $CaCl_2$ content. The 1/20 ratio suffered from a dilute acid concentration, which reduced reaction and product recovery efficiencies. Consequently, the 1/15 ratio was identified as optimal, aligning with similar optimization trends reported by Strelec et al [13], and also quite consistent with the results of Rosnah *et al*. (2021), $CaCl_2$ was prepared by extracting calcium from eggshells using a 4% (w/v) HCl solution for 3 h at a solid-to-liquid ratio of 1/15 (w/v). The resulting calcium chloride powder with $CaCl_2$ content was 87.38% (w/w), 0.3% protein, and 94.37% ash, pH - 5.27 [7].

Both $CaCl_2$ and recovery efficiency increased as the HCl concentration rose from 4.5% to 5%. The 5% HCl concentration was identified as optimal, achieving the maximum $CaCl_2$ content (93.66%) and recovery efficiency (82.01%). However, increasing the acid concentration to 5.25% resulted in a decrease in both $CaCl_2$ content and recovery efficiency. This decline was attributed to the excessive acid concentration, which diminished reaction efficiency. Furthermore, the significant excess of residual HCl drastically lowered the pH of the reaction medium.

Strelec *et al*. (2023) [3], the laboratory-scale production of $CaCl_2.H_2O$, the mineral fraction was dissolved via reaction with a 5% HCl solution at a solid-to-liquid ratio of 1:15 (eggshell to acid solution), corresponding to an estimated $CaCO_3$:HCl molar ratio of 1:2.55. From a starting material of 100 g of dried eggshells, the study yielded 108.74 g of $CaCl_2$. $2H_2O$, demonstrating a high conversion efficiency.

Both $CaCl_2$ content and recovery efficiency increased from 2.5 to 4 h. However, extending the reaction to 4.5 h did not yield a significant difference in these parameters compared to the 3.5 h sample and 4 h sample. Consequently, 3.5 h was selected as the optimal reaction time.

The reaction between HCl and the demembrane eggshell (>98% $CaCO_3$) was quite strong. This intensity leads to strong CO_2 release, causing significant foaming and covering the eggshell surface, thereby clearly affecting the HCl reaction's effectiveness, especially with small eggshell pieces (<0.25 mm). This leads to a large amount of excess acid after the reaction and a correspondingly large amount of unreacted eggshell, thereby reducing both the $CaCl_2$ recovery efficiency and the product purity (**Figure 6**).

a. >5 mm *b. 3-5 mm* *c. 1.5-3 mm* *d. 0.25-1.5 mm* *e. <0.25 mm*

Figure 6. *Effect of eggshell waste size on reaction efficiency*

The investigation results indicate that the highest product recovery efficiency and $CaCl_2$ content were achieved when demembraned eggshells (particle size > 3 mm) were mixed with a 5% HCl solution at a 1:15 (solid-to-liquid) ratio for 3.5 h.

3.4. Treatment process to increase the purity of $CaCl_2$ and quality assessment

The post-reaction solution (without neutralizing) exhibited a pH of 0.69, indicating the presence of residual acid after the reaction. The post-reaction solution was neutralised with $Ca(OH)_2$ powder while stirring at 500 rpm, ensuring complete dissolution of the powder. During this process, an acid-base neutralization reaction occurred, resulting in a change in the solution's pH.

$$Ca(OH)_{2\,(p)} + 2HCl_{\,(aq)} \rightarrow CaCl_{2\,(aq)} + H_2O_{\,(l)}$$

This pH shift led to the precipitation of certain proteins, which were simultaneously coagulated by the $Ca(OH)_2$ and subsequently settled at the bottom of the solution. It was observed that the coagulation process began at pH 5, when a small amount of precipitate formed, resulting in a slight change in solution colour. The neutralized solution was centrifuged and then filtered sequentially through Whatman 114 and Whatman 1 filter papers to remove precipitates. Various pH values were then investigated to compare differences in $CaCl_2$ content, recovery efficiency, and lightness (L* value) of the final product. The results of this investigation are presented in **Table 3**.

Table 3. *Effect of pH during the neutralization and organic matter coagulation process*

pH of solution after using $Ca(OH)_2$	**0.69 (initial solution)**	**5.0**	**6.0**	**7.0**
$CaCl_2$ content (%)	93.66±0.32^a	96.32±0.11^b	97.57±0.15^c	98.32±0.07^d
Recovery efficiency (%)	84.15±0.43^a	82.56±0.29^b	86.92±0.14^c	89.08±0.17^d
Lightness (L* value)	84.79±0.06^a	90.55±0.10^b	93.26±0.13^c	96.58±0.07^d

Values with different lower-case letters in a row are significantly different.

The $CaCl_2$ content, the recovery efficiency and lightness (L* value) of the final product increased with increasing pH. At pH 7, the final product parameters were significantly higher than those of the initial sample (**Figure 7**). The increase in $CaCl_2$ content is due to the $Ca(OH)_2$ powder denaturing and coagulating the protein remaining in the post-reaction solution. This facilitated their easy removal during filtration, thereby increasing the purity of the final product. The product recovery efficiency increased because additional $CaCl_2$ was formed from the reaction between the $Ca(OH)_2$ and the residual HCl in the solution. This addition contributed approximately 5% to the total recovery.

Concurrently, the residual alkalinity (calculated as $Ca(OH)_2$) of the product was found to be 0.11±0.02%, which meets the standards set by QCVN 4-9:2010/BYT. This result clearly demonstrates the efficacy of $Ca(OH)_2$ supplementation in the production of calcium chloride from eggshells.

Compared to the calcium chloride production process from food-grade eggshells by Ivica Strelec *et al.* [13], our procedure shows similarities regarding the eggshell-to-acid solution ratio and the concentration of the reacting acid solution. However, our study specifically examined the influence of $Ca(OH)_2$ during the neutralisation step on the final $CaCl_2$ content and product recovery efficiency, thereby making a distinct contribution to the field.

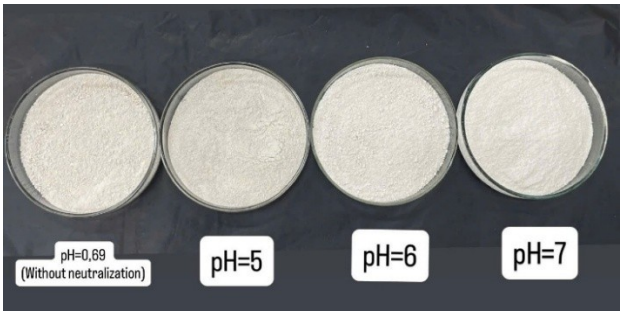


Figure 7. *The final product from solutions neutralized to various pH levels*

To further evaluate the potential of the calcium chloride product derived from eggshell waste (ESW-based $CaCl_2$), a comparison was conducted with a commercial sample using selected parameters. The results are presented in the following **Table 4**. The results demonstrate that the ESW-based $CaCl_2$ sample exhibits parameters comparable to those of the commercial one.

Table 4. *Comparative Analysis of eggshell-based and commercial calcium chloride*

Content	**Commercial $CaCl_2.2H_2O$**	**ESW-based $CaCl_2$**
$CaCl_2$ content (%)	≥98% (Calculated as $CaCl_2.2H_2O$)	100 (Calculated as $CaCl_2.2H_2O$)
Lightness (L* value)	97.31±0.38	96.58±0.07

The heavy metal content of $CaCl_2$ from eggshells has been determined: Pb (0.03 mg/kg), As (0.08 mg/kg), and with undetectable Hg, confirming compliance with the maximum residue limits established by QCVN 4-9:2010/BYT and EU standards [14]. This highlights the promising potential for the valorization of eggshell waste into calcium chloride for application as a food additive.

To compare the purity between ESW-based $CaCl_2$ sample and the commercial calcium chloride ($CaCl_2.2H_2O$), the FTIR (Fourier-Transform Infrared) analysis was performed to evaluate the vibrational characteristics. The results are presented in **Figure 8**.

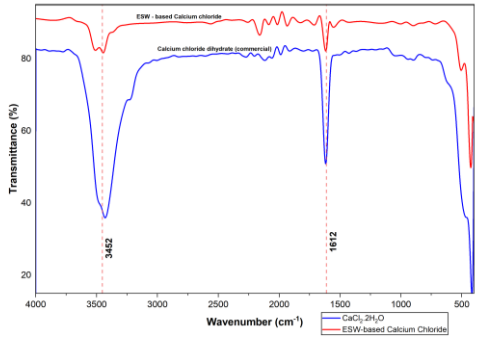


Figure 8. *FTIR Analysis of ESW-based $CaCl_2$ and commercial $CaCl_2.2H_2O$*

The FTIR spectra of both the ESW-based $CaCl_2$ sample and the commercial sample exhibited identical characteristic peaks in terms of position, which confirms the similarity in their chemical structure. Specifically, the ESW-based $CaCl_2$ and the commercial $CaCl_2.2H_2O$ sample showed identical peak positions at 3429, 3486, 3450 and 1616 cm^{-1}. Therefore, it can be concluded that the obtained product exists in the dihydrate form ($CaCl_2.2H_2O$). However, they differed in absorption intensity. The absorption intensity of the ESW-based $CaCl_2$ was weaker than that of the commercial $CaCl_2.2H_2O$ sample, which indicates a difference in the hydration state between the two samples. The maxima concentrated at 3486 cm^{-1} and 3450 cm^{-1} in the ESW-based $CaCl_2$ sample are attributed to the symmetric or asymmetric stretching vibration of the O-H bond in the crystal, and the maximum at 1612 cm^{-1} is the H-O-H bending vibration frequency in the crystal water [15]. These results are consistent with the natural hygroscopicity of $CaCl_2$ and demonstrate that the synthesised product achieves chemical purity equivalent to the commercial standard.

4. CONCLUSION

A process for treating post-hatch eggshell waste and converting it into $CaCl_2$ at the laboratory scale has been proposed. In this process, eggshell pretreatment via sterilization at 121°C for 60 min, followed by drying at 45°C for 4 h, yielded optimal efficacy in microbial reduction. This pretreatment also demonstrated hig efficiency in eggshell membrane separation (>90%) and in the recovery of high-calcium carbonate content (>98% $CaCO_3$). This treatment method not only eliminates the risk of microbial contamination but also helps increase the efficiency of membrane separation, thereby improving the extraction of $CaCO_3$ from eggshells.

The proposed process for producing calcium chloride ($CaCl_2$) from demembraned eggshells for that the highest product recovery efficiency (89%) and high $CaCl_2$ content (100%), meets the criteria of both QCVN 4-9:2010/BYT: demembraned eggshell with (particle size >3 mm) were mixed with a 5% HCl solution at a 1:15 (w/v) ratio for 3.5 h. The purity of the eggshell product was determined to be nearly equivalent to the commercial sample, thereby demonstrating the strong application potential of calcium chloride produced from eggshell waste as a food processing additive. Despite the promise of recovering high-value products from post-hatch eggshell waste, the recovery of by-products remains limited. Key challenges for future research include large-scale implementation and the recovery of eggshell membranes – a valuable source of biological protein would have many applications.

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