Effect of Calcium Chloride Extracted from Poultry Eggshells on the Physicochemical Properties of Iranian High-Fat White Cheese

Jalali, Nasrin
Department of Food Science and Technology, Najafabad Branch, Islamic Azad University, Najafabad, I.R. IRAN

Goli, Mohammad[*+]
Department of food science and technology, Laser and Biophotonics in Biotechnologies Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, I.R. IRAN

Sheikhan, Nafisehsadat
Department of Food Science and Technology, Najafabad Branch, Islamic Azad University, Najafabad, I.R. IRAN

Shahi, Sharifeh
Department of Medical Engineering, Laser and Biophotonics in Biotechnologies Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, I.R. IRAN

Kermani, Shabnam
Department of Food Science and Technology, Najafabad Branch, Islamic Azad University, Najafabad, I.R. IRAN

ABSTRACT: *The rising global population necessitates increased urbanization and food waste management. Eggshells, a significant portion of this waste, present an opportunity for sustainable resource recovery and value-added product development. This research aims to mitigate global food waste by utilizing poultry eggshell waste from chickens, ducks, and ostriches as a cost-effective source of calcium chloride ($CaCl_2$). The eggshells were treated with hydrochloric acid and subjected to different drying methods—spray, freeze, and oven drying. This study evaluates the efficacy of the extracted $CaCl_2$ in the cheese-making industry, specifically for Iranian high-fat white cheese, and compares its performance to a standard Merck sample. The highest yield of $CaCl_2$ (92.46%) was achieved from chicken eggshells using the oven drying method, while the lowest yield (75.46%) occurred with ostrich eggshells via spray drying, with the purity of extracted $CaCl_2$ comparable to the Merck standard. Maximum cheese yield (12.69%) was obtained using unpasteurized milk with Merck $CaCl_2$, whereas the lowest yield (11.02%) was observed with pasteurized milk without it. The purity of $CaCl_2$ significantly influenced lipolysis and proteolysis during cheese-making. While no significant pH differences were noted among cheeses made with eggshell-derived $CaCl_2$, the N$CaCl_2$-NP sample exhibited the highest pH, resembling the Merck sample. Moisture content variations aligned with pH trends, whereas syneresis exhibited an inverse relationship, highlighting the role of poultry eggshell-derived $CaCl_2$ in cheese texture*

*To whom correspondence should be addressed.
+ E-mail: mgolifood@yahoo.com & m.goli@khuisf.ac.ir
1021-9986/2024/12/4443-4455

and quality. Despite no significant differences in the L index or whiteness among cheeses produced from different drying methods, those made with chicken eggshells, particularly via oven and freeze drying, exhibited the lowest color quality. The textural properties of high-fat white cheese made with poultry eggshell-derived $CaCl_2$ were generally comparable to the control (Merck-P), suggesting its viability in maintaining cheese texture. This study underscores sustainable waste management and promotes a circular economy through effective utilization of eggshell waste.

KEYWORDS: *Sustainable resource recovery; Food waste management; Spectroscopic analysis; Comparative drying methods; Textural properties.*

INTRODUCTION

The growing global population demands resources beyond basic food and housing, leading to increased urbanization and higher food demand [1-2]. This has increased food waste generation, exacerbated by slow waste management development. Food waste, a reservoir of nutrients, can be used for value-added products [3-4]. Eggshells, a valuable commodity, constitute a significant portion of food waste, making the issue even more challenging [5]. Egg consumption has increased due to increased income and dietary acceptance, particularly in developing countries. The US Department of Agriculture predicts that egg consumption will reach 8,917 million dozen by 2028. Countries such as Japan, Paraguay, and China exhibit the highest annual consumption rates, with individuals consuming an average of 320, 309, and 300 eggs per year, respectively [6]. Egg production is expected to remain steady or modestly progress globally, with Asia experiencing a four-fold expansion in the past 30 years. India reported a 30-year high production yield of 83 billion eggs in 2016. These large volumes are used for domestic and industrial food production, generating significant eggshell waste. China led in global eggshell production in 2019 with 24.8 billion kilograms and over 35 million metric tons in 2020 [7]. Eggshell waste is ranked as the 15th major food industry pollution problem by the Environmental Protection Agency. Improper disposal can cause health hazards due to fungal growth on eggshells. Non-effective waste disposal is critical for human survival and ecosystem health. Eggshell waste is mostly discarded in landfills, which have reached their capacity. Landfill owners avoid eggshells due to their protein-rich membranous part, which attracts rats and vermin [8-9].

The circular economy aims to eliminate food waste and use resources sustainably, such as using waste as a renewable resource for value-added products.

Research on plant fiber reinforced composites and inorganic food waste has gained interest. Egg production worldwide is estimated to reach 90 million tons by 2030, with China being the largest producer. Eggshell $CaCO_3$ powder can replace conventional limestone-derived $CaCO_3$ in final products, making recovery of food waste eggshells an ideal recycling strategy [10]. A wide range of applications, including the removal of hazardous metals from polluted water and soil, the manufacture of cement, cosmetics, and polymer and metal composites, are covered by the documented research on the utilization of eggshell waste in new materials [11]. Over the past five years, research on eggshell waste has seen a surge in interest, with studies focusing on its potential applications in various industries. Eggshells, which make up 9-12% of the total egg weight, are composed of 98% dry matter and 2% water. The dry matter is mostly ash and crude protein. Eggshells are composed of protein fibers, calcium, magnesium carbonate, and calcium phosphate crystals, along with water. The utilization of eggshells in various products, including food commodities and industrial applications, is primarily dependent on its main component, calcium carbonate [12-14].

Eggshells present significant potential as a valuable byproduct in the food science industry. Modified eggshells can be utilized in various applications, including as a source of calcium in food products and dietary supplements for both humans and animals. Their natural composition makes them suitable for incorporation into functional foods aimed at enhancing calcium intake. Additionally, eggshells can serve as a low-cost adsorbent for treating wastewater, which is especially relevant for food processing facilities that need to manage effluents containing contaminants. Moreover, the use of eggshells in composite materials can extend to food packaging, where they can enhance the mechanical properties of biodegradable materials.

In biodiesel production, chicken eggshells can also play a role by acting as catalysts for converting waste frying oil into biofuel, thus contributing to sustainable practices within the food industry. Overall, the valorization of eggshells offers a multifaceted approach to waste management while providing valuable resources for the food science sector [15-16]. Calcium chloride ($CaCl_2$) is an inorganic salt extensively used across various industries, including food, where it serves as a food-grade additive. Calcium chloride is a key component in cheese production, enhancing coagulation, enhancing texture, and influencing yield. It increases calcium ion concentration in milk, promoting rennet activity and curd formation. $CaCl_2$ also contributes to firmer curd structures, making cheese more desirable. It also influences flavor development, stabilizes pH during coagulation and aging processes, and prevents fat separation in cheeses. Its presence also influences cheese yields, flavor development, pH stabilization, and fat separation prevention, making it essential for cheeses with specific moisture content [17]. A significant alternative source of calcium carbonate is eggshell waste (ESW), which accounts for an estimated 8.6 million tons generated worldwide, with around 1.1 million tons produced in the EU alone. Given that 30% of ESW arises from egg-breaking plants, there is a pressing need to implement cost-effective strategies for its disposal. Transforming ESW into low-value products like fertilizers or animal feed can mitigate disposal costs and contribute to sustainable waste management. Recent studies from research group have explored the chemical treatment of ESW to produce valuable calcium salts and eggshell membranes, suggesting innovative pathways for zero-waste utilization of this by-product [18].

The novelty of this research lies in its innovative approach to recycling poultry eggshell waste into a valuable product, addressing both food waste management and sustainable resource recovery. Our findings suggest that eggshell-derived calcium chloride can serve as a cost-effective and environmentally friendly alternative in dairy applications, demonstrating comparable quality to commercially available calcium chloride. This study investigates the potential of using calcium chloride derived from poultry eggshells—an underutilized agricultural waste product—as a sustainable alternative for the dairy industry. We compared various drying methods (spray, freeze, and oven drying) to optimize the yield and purity of calcium chloride extracted from chicken, duck, and ostrich eggshells. The efficacy of the extracted calcium chloride was evaluated in the context of cheese-making, specifically for Iranian high-fat white cheese, by comparing its performance with standard Merck calcium chloride.

EXPRIMENTAL SECTION

Materials

Fresh eggs, including chicken, duck, and ostrich, were purchased from a local farm (Simorgh LTD, Isfahan, Iran). Poultry eggshells were washed, sun dried, and crushed to 0.25 cm pieces. They were dried in a tray dryer at 85°C for 4 h and ground to 0.2 mm particles by a hammer mill (Bastak Brand 1900, China). The crushed and dried eggshells were packed in plastic bags and stored in a desiccator at room temperature. They were used as raw material for the experiment [19]. Holstin-cow milk was provided from a local farm (Ghiam LTD, Isfahan, Iran). Hydrochloric acid, calcium chloride, and the other chemical reagents were of analytical grade (Merck, Germany). Lactococcus lactis is a kind of bacteria. In the manufacturing of Iranian high-fat white cheese, Lactococcus lactis Subsp. lactis and Lactococcus lactis Subsp. cremoris (Delvo®Cheese CT-type starter culture, DSM's Delvo®, France) and fungal coagulants from Rhizomucor miehei (DSM's Fromase®, France) were utilized.

Calcium chloride extraction from poultry eggshells

Crushed poultry eggshells were combined with a 4% hydrochloric acid solution to produce calcium chloride. This mixture was stirred periodically using ultrasound (JY99-IIDN Ultrasonic Transmitter, China), employing a titanium probe with a diameter of 1 cm and a frequency of 20 kHz at a power of 300 kW for 1 min, until no gas bubbles were visible. Following this, the mixture was centrifuged for 10 min at 1774 × g, and the supernatant was collected for drying. The calcium chloride was then dried using three methods: oven drying (DHG-9023A, China) at temperatures of 110–115°C, spray drying (Xiamen Ollital Technology Co., Ltd, Xiamen, China) with inlet and outlet temperatures set at 140 °C and 80 °C, respectively, and freeze drying (Parseh, Iran) at -50 °C under an air pressure of 0.04-0.06 mbar [19]. The following Eq. (1) illustrates the percent yield calculation. Each treatment was conducted in three duplicates.

$$\text{Yield of } CaCl_2\ (\%) = \frac{\text{Weight of crystals obtain from drying}}{\text{Wieght of crushed poultrysell used}} \times 100 \quad (1)$$

Calcium chloride purity from poultry eggshells

100.0 g of sample were weighed and washed with water in a 1000-mL volumetric flask. It was swirled in 10 mL of HCl (1 + 3) to dissolve the material. The mixture was allowed to cool to room temperature before being made to volume with water and mixed. A 20-mL aliquot was pipetted into a 500-mL volumetric flask, diluted to volume, and thoroughly mixed. A 100-mL aliquot of the produced solution was pipetted into a 500-mL Erlenmeyer flask and diluted with water to about 200 mL. Then, 10 mL of hydroxylamine hydrochloride solution and 3 g of sugar were added in that order. To dissolve, the container was swirled. Additionally, 40 mL of NaOH solution (80 g/L) was swirled in to combine. Subsequently, 0.1 g KCN was swirled in to dissolve and blend. Approximately 0.2 g of calcein indicator was added. A 0.1 M EDTA solution was used to titrate until the green to purple indicator changed. When α-hydroxynaphthol blue indicator was used, the solution was titrated to a blue end point after 0.4 g was added. The calcium chloride concentration was calculated as follows Eq. (2) [20]:

$$\text{Calcium chloride purity } (\%) = [\frac{A \times B}{C}] \times 100\text{-D} \quad (2)$$

A = mL of EDTA solution required for titration of the sample, B = calcium chloride equivalent of the EDTA solution, g/mL, C = mass of sample in the aliquot used, and D = percentage of calcium hydroxide expressed as calcium chloride.

Iranian high-fat white cheese production

High-fat cow's milk (3.5% fat) is pasteurized at 75°C for 15 seconds, and then it is inoculated with fungal coagulants, calcium chloride 0.015%, and direct vat set (DVS) starting culture. With gentle stirring, the curds and whey are cooked to a temperature of around 38–40°C. Using a cheese press, more pressure is applied to the curds for 12 hours at a rate of 0.9 kg/cm2. After that, the cheese curds are cut into pieces of 10 × 10 × 10 cm and put inside polypropylene containers. The sealed samples are stored at 25°C for 24 h following the addition of 10% sodium chloride brine. To complete the ripening process, they are then kept at a low temperature (5-8°C) for 60 days. Ultimately, sixty days following production, the cheeses' physicochemical, colorimetric, and textural characteristics are assessed [20].

Determining the physicochemical properties of the Iranian high-fat white cheese 60 days after production time

The 60-day period was selected based on industry standards and previous research indicating that this timeframe allows for a comprehensive assessment of cheese quality, including flavor development, texture changes, and microbial stability. This duration also aligns with typical storage practices for high-fat cheeses, enabling us to observe significant changes that occur during the aging process [16]. The pH measurements were carried out by an ATC pH meter (EDT instruments, GP 353, Kent UK) [21]. Free moisture, unbound to food components, contributes to food weight, microbial growth, spoilage, and loss during cooking or processing, and can be easily removed through evaporation was assessed according to the Zonoubi and Goli's method [21]. The cheese syneresis assessment protocol involved selecting representative samples of cheese and cutting them into uniform pieces. The initial weight (W_1) is weighed, and the cheese is incubated under controlled conditions. After the incubation period, the cheese is removed, blotted with a clean material, and the final weight (W_2) is weighed. The syneresis is calculated as the percentage of weight loss during incubation using the following Eq. (3):

$$\text{Syneresis } (\%) = [(W_1 - W_2) / W_1] \times 100 \quad (3)$$

The Kjeldahl distillation apparatus (Blaubrand, Wertheim, Germany) was used to determine the various nitrogen fractions in the cheese samples [22]. Total nitrogen (TN) and water-soluble nitrogen (WSN) were measured in cheese samples. The WSN of the cheese samples was given as a TN percentage (g/100 g). The computed protein content of the cheese samples was 6.38 × (TN - NPN). The portion of TN soluble in 12 g/100 mL trichloroacetic acid is known as nonprotein nitrogen, or NPN. The proteolysis index (PI), additionally referred to as the ripening index (RI) was also computed as a percentage of the WSN to TN ratio. The quantity of free fatty acids created during the aging process of cheese, usually represented in milliequivalents (meq) of oleic acid per gram (g) of cheese, was used to determine the lipolysis index [23]. The Hunter laboratory machine (UltraScanvis, US-Vis 1,310, USA) was used to assess the L*, a*, and b* values for cheese. L* measures brightness and ranges from zero (black) to 100 (white), a* measures tonality and ranges from + a (red) to -a (green), and b* ranges from + b (yellow) to -b (blue). The following equations were used to determine the Whiteness Index (WI), which indicates the purity of white color, and ΔE [24]:

$$\text{Whiteness index} = 100 - \sqrt{(100 - L)^2 + (b)^2 + (a)^2} \quad (4)$$

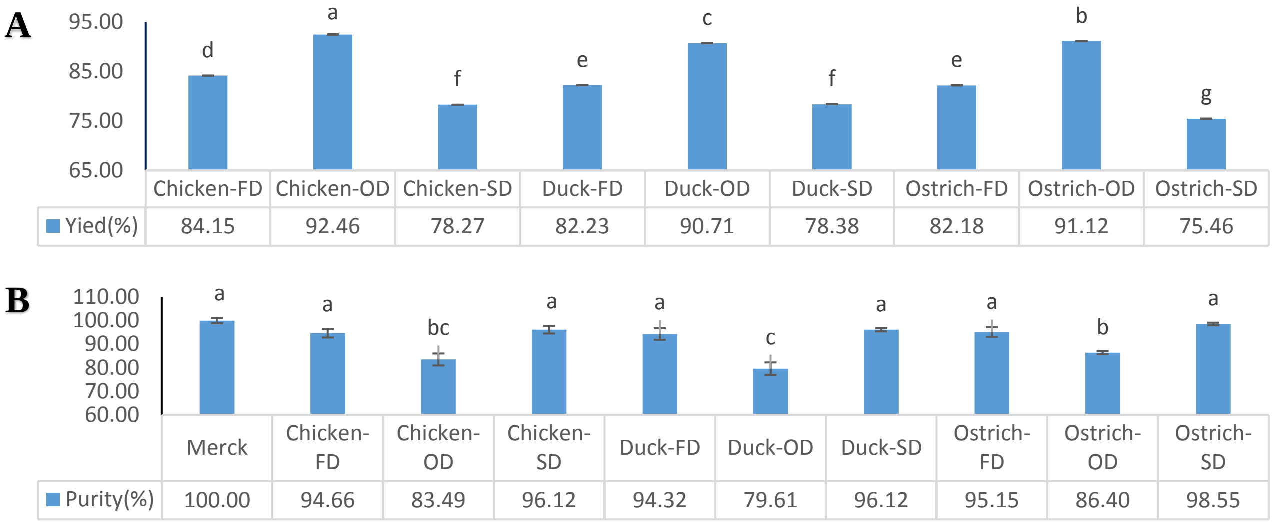

Fig. 1: Investigating the state of yield and purity of calcium chloride produced of different poultry eggshell waste. FD: Freeze drying, OD: Oven drying, SD: Spray drying.

Changes in color (ΔE) =

$$\sqrt{(b_{60th} - b_{1st})^2 + (a_{60th} - a_{1st})^2 + (L_{60th} - L_{1st})^2} \qquad (5)$$

The texture profile including hardness, cohesiveness, springiness, gumminess and chewiness properties was assessed by texture profile analyzer (Santam, model STM-5, Iran) as the Eqs. (6-10) [24]. Cylinders of 25 20-mm cheeses were cut from cheese blocks in the center. Specimens were maintained in a refrigerator at 4°C overnight before determining the textural profile. Textural characteristics were measured with an Instron universal testing machine (model 5564, Instron Ltd.). The samples were compressed to 50% of their original heights using a 100-N load cell with a flat plunger and a crosshead movement of 10 mm/min and all of analyses were carried out in triplicate [25].

Hardness (N) = the highest peak force measured during first compression (6)

$$\text{Cohesiveness} = \frac{\text{The area underneath the second compression curve}}{\text{The area underneath the first compression curve}} \qquad (7)$$

$$\text{Springiness} = \frac{\text{Cheese recovery height}}{\text{Cheese original height}} \qquad (8)$$

Gumminess (N) = Hardness (N) × Cohesiveness (9)

Chewiness (N) = Hardness (N) × Cohesiveness × Springiness (10)

Statistical analysis

All tests were conducted using SPSS software version 16 (SPSS Inc., Chicago, IL, USA) in a completely randomized design. The independent variables included the type of poultry eggshells used (chicken, duck, and ostrich) and the method of drying employed (spray, freeze, and oven drying). The dependent variables were assessed in white cheese on the 60th day after production, with three replications for each treatment. At the 95% confidence level, Duncan's multiple range test indicated statistically significant differences ($P<0.05$).

RESULTS AND DISCUSSION

Yield and purity of $CaCl_2$ in poultry eggshells

For each type of poultry eggshell, the highest and lowest yields of production belonged to the oven and spray drying methods, respectively ($P<0.05$). For the oven drying method, the highest and lowest yield of production belonged to chicken and duck egg shells, respectively, and for freeze drying, the highest yield belonged to chicken egg shells and the lowest to duck and ostrich egg shells, which of course showed a non-significant difference between the two ($P>0.05$). For the spray drying method, the $CaCl_2$ production yield was the highest from chicken and duck eggshells, and no significant difference was seen between them ($P>0.05$). The lowest production yield was reported from the ostrich eggshell. In total, the highest production yield of $CaCl_2$ belonging to the oven drying method for chicken eggs was 92.46%, and the lowest production yield belonging to the spray dryer method for ostrich eggshell was 75.46% (Fig. 1A). The findings in Fig. 1B demonstrate that the purity of calcium chloride obtained by the freeze and spray drying process from all three types of poultry eggshells is not substantially different from the Merck sample ($P>0.05$).

The shell of the duck egg had the lowest amount of purity belonging to the oven drying technique of 79.61% while the shell of the ostrich egg had the highest amount of purity belonging to the spray drying method of 98.55% (Fig. 1B). Calcium chloride ($CaCl_2$) production from chicken, ostrich, and duck eggshells is a widely used process due to their widespread availability. The yield of $CaCl_2$ is typically expressed as the percentage of calcium chloride obtained relative to the weight of the eggshells used. However, the yield can vary significantly depending on the specific conditions and techniques employed. Chicken eggshells are the most commonly used due to their widespread availability, with a yield of 70% to 90% under optimal conditions. Ostrich eggshells are larger and thicker, requiring additional processing steps to enhance extraction efficiency. The unique morphology of ostrich eggshells may lead to greater moisture retention during pre-drying processing, which can lower the yield when using methods like spray drying that depend on rapid moisture removal. Duck eggshells are also commonly used, with a yield of comparable to or slightly lower than chicken eggshells. Optimizing the extraction process and conditions can improve the yield of $CaCl_2$ production from eggshells, as parameters such as acid concentration, reaction time, and temperature can influence the efficiency of calcium carbonate dissolution and calcium chloride formation [26-27]. The purity of calcium chloride extracted from eggshells can be impacted by factors such as eggshell quality, extraction process, and purification stages used. Impurities in eggshells can include proteins, lipids, residual pigments, and trace minerals such as magnesium, phosphorus, and manganese. These impurities, although present in small quantities, can affect the overall purity of the eggshells [19].

As previously stated, further purification processes may be required to get high-purity calcium chloride. These purification techniques may involve filtration, precipitation, or recrystallization methods to eliminate contaminants and improve the purity of the final product, particularly when using the oven drying method.

Yield, lipolysis, and proteolysis indices of high-fat white cheese

Fig. 2A demonstrates that the highest and lowest cheese yields occurred in the Merk-NP (unpasteurized milk - use of Merck calcium chloride) and $NCaCl_2$-P (pasteurized milk - no use of Merck calcium chloride) treatments, with 12.69% and 11.02%, respectively. The data in Fig. 2A is congruent with the information in Fig. 1B, indicating that increasing the purity of calcium chloride affects cheesemaking yield. With the exception of the Ostrich-SD treatment, all of the $CaCl_2$ obtained from the three different types of poultry eggshells and the three drying techniques studied produced a lower yield than the two cheese samples that were made with Merck calcium chloride (pasteurized and unpasteurized); however, they produced a higher yield when compared to the $NCaCl_2$-P treatment (pasteurized milk - without the use of Merck calcium chloride) ($P<0.05$). Conversely, samples of cheeses made from pasteurized milk had a lower yield than samples made from unpasteurized milk ($P<0.05$). Calcium chloride is a key component in cheese-making, primarily used for coagulating milk proteins and forming curds. It enhances the stability and reactivity of protein casein, facilitating the efficient separation of curds and whey. The cheese-making yield, which is the quantity of cheese obtained from a given amount of milk, is influenced by factors such as milk composition, processing techniques, and the presence of coagulating agents like $CaCl_2$. $CaCl_2$ can improve coagulation, resulting in a firmer curd formation, higher cheese yield, increased moisture retention, and a firmer, more compact cheese. It also influences the texture and structure of the cheese, contributing to a higher yield due to reduced losses during processing [28]. Pasteurization of milk can decrease cheese yield due to two factors. First, it can cause denaturation of proteins, particularly whey proteins, which affects the stability of the curd during cheese making. This results in less moisture retention, reducing cheese yield. Second, pasteurization can alter the stability of calcium ions and casein micelles in milk, leading to changes in coagulation properties and affecting cheese yield [29].

The impact of several research treatments on the lipolysis and proteolysis indices is shown in Fig. 2B and 2C, respectively. As can be seen, both figures follow a similar general trend as Fig. 2A, demonstrating the significant effect that the purity of the calcium chloride generated has on these two indices. The relationship between lipolysis and proteolysis index in cheese making and the purity of calcium chloride is a topic of limited research. Calcium ions stabilize milk fat globules, preventing coalescence and aggregation during processing and storage. They also influence lipase activity, either enhancing or inhibiting it. The specific impact depends on factors like the type of lipase, its source, and the concentration of calcium ions.

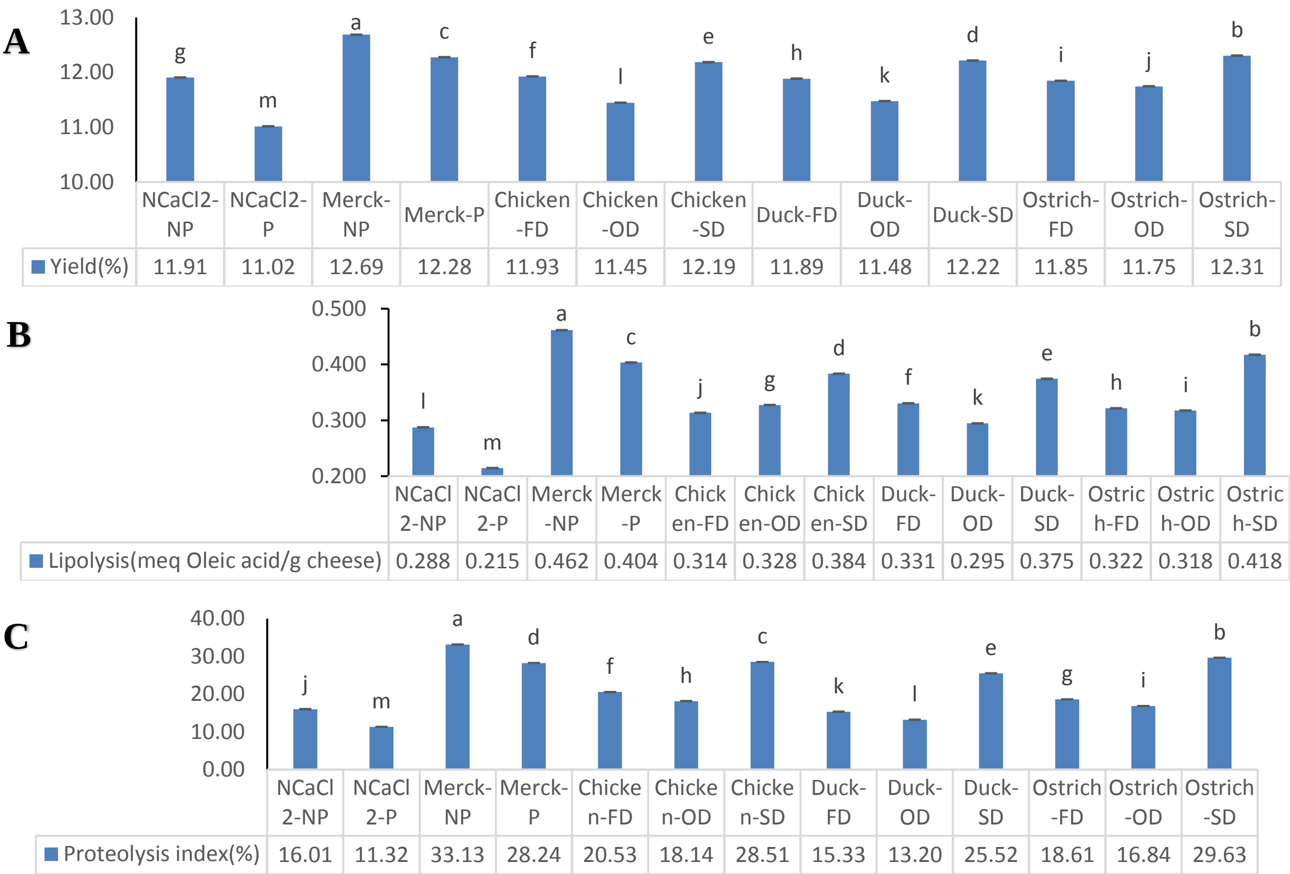

***Fig. 2:** Investigating the state of yield, lipolysis and proteolysis of Iranian white cheese resulting from the type and method of using calcium chloride on the 60th day after production. $NCaCl_2$-NP: Non-$CaCl_2$ & non-pasteurized milk, $NCaCl_2$-P: Non-$CaCl_2$ & pasteurized milk, Merck-NP: Merck-$CaCl_2$ & non-pasteurized milk, Merck-P: Merck-$CaCl_2$ & pasteurized milk, FD: Freeze drying, OD: Oven drying, SD: Spray drying.*

Research has shown that calcium ions can activate some lipases, leading to increased hydrolysis of milk fat triglycerides. This stability helps maintain a stable emulsion during processing and storage [28]. Calcium ions, found in $CaCl_2$, can enhance the activity of proteolytic enzymes. They can activate enzymes like plasmin, a milk protease, which aids in the breakdown of caseins during cheese ripening. Additionally, calcium ions stabilize proteolytic enzymes, preventing their denaturation or inactivation, thereby maintaining their activity throughout the cheese-making process, ensuring adequate proteolysis [28]. Lipolysis and proteolysis are crucial processes in cheesemaking, so temperature influences these processes. However, excessive heat treatment, like pasteurization, can denature these enzymes, reducing lipolysis and proteolysis. Heat can denature proteolytic enzymes during pasteurization, but residual proteases may still exist in milk, and starter cultures can contribute to proteolysis [29].

The pH, moisture and syneresis of high-fat white cheese

Fig. 3A depicts the results of the pH fluctuations of cheeses on the 60th day after manufacturing. The results show that there was no significant difference in pH in all three types of cheese resulting in poultry eggshells ($P>0.05$), but there was a significant difference in spray drying methods in all three poultry eggshell samples ($P<0.05$), as well as Merk-p, Merk-NP, and $NaCl_2$-P treatments ($P<0.05$). The $NCaCl_2$-NP sample had the greatest levels of pH of cheese, and the closest values to the Merk-P sample (the standard procedure of the world's dairy companies) may be attributed to the spray drying process for all three types of poultry eggshells employed in this study. Fig. 3B studies variations in moisture content in cheese sixty days after production, and the trend of changes is remarkably similar to the trend of pH changes in Fig. 3A. Fig. 3C depicts the variations in the amount of syneresis in cheese sixty days after production, so that the trend of changes in syneresis is the inverse of the trend of changes in pH (Fig. 3A) and moisture (Fig. 3B).

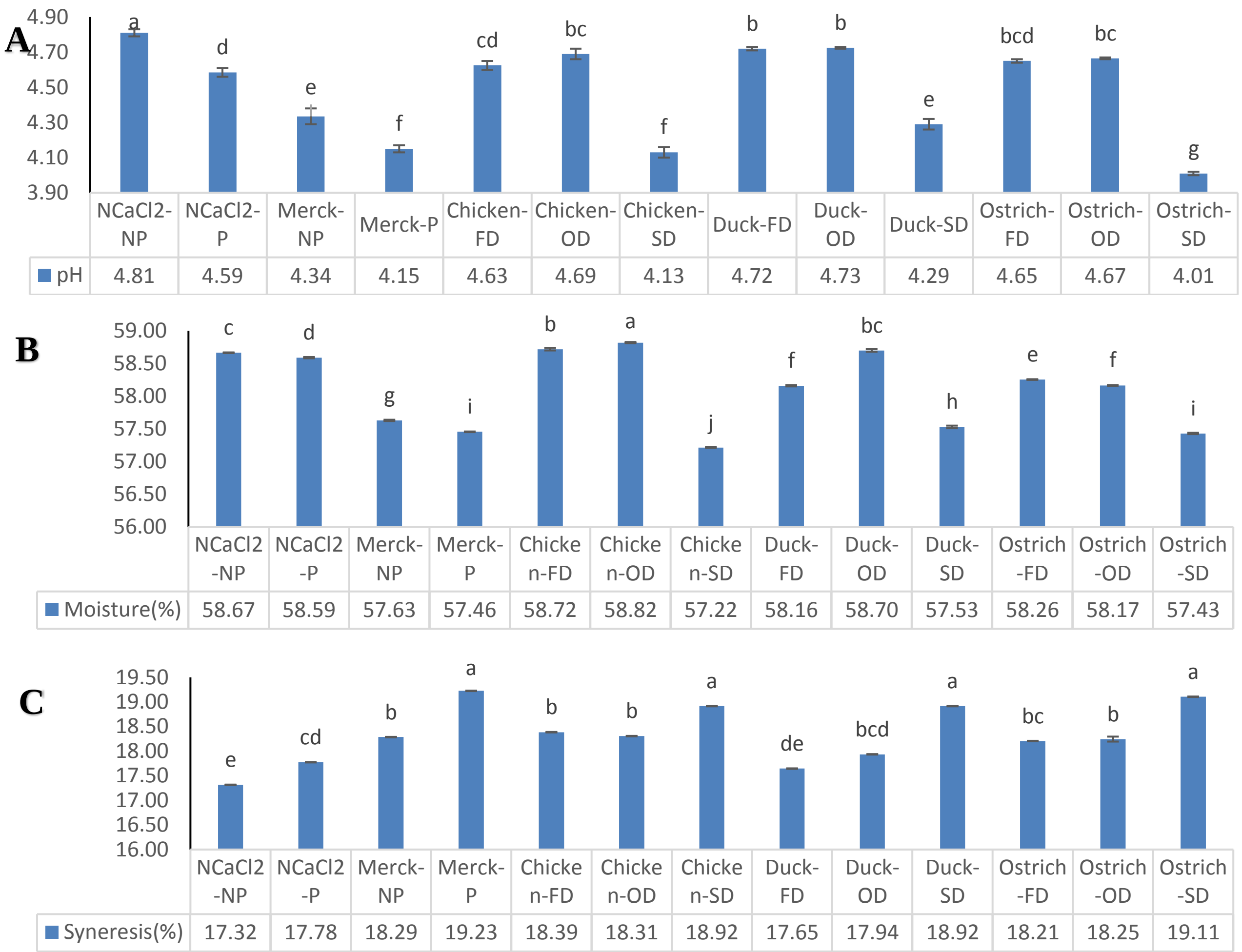

Fig. 3: Investigating the state of pH, moisture and syneresis of Iranian white cheese resulting from the type and method of using calcium chloride on the 60th day after production. NCaCl$_2$-NP: Non-CaCl$_2$ & non-pasteurized milk, NCaCl$_2$-P: Non-CaCl$_2$ & pasteurized milk, Merck-NP: Merck-CaCl$_2$ & non-pasteurized milk, Merck-P: Merck-CaCl$_2$ & pasteurized milk, FD: Freeze drying, OD: Oven drying, SD: Spray drying.

The common practice of adding $CaCl_2$ to milk promotes rennet coagulation through three beneficial changes: an increase in [Ca^{2+}], an increase in the concentration of colloidal calcium phosphate, and a decrease in pH (the addition of $CaCl_2$ to 0.02% (0.2 g/L), i.e., 1.8 mM Ca, reduces the pH by 0.05-0.1 units, depending on protein level). Due to the fact that rennet coagulation of milk depends on pH, rennet coagulability is influenced by various factors that affect milk's pH, including the type and quantity of starter added, the addition of $CaCl_2$, the ripening of milk, the addition of acid or acidogen to adjust pH, and the pH of the milk itself as a result of mastitis or lactation stage [28]. Calcium ($CaCl_2$) is added to milk to correct lactation stage variations and balance changes due to refrigeration or heat treatment. It improves milk coagulation properties and is used in cheese technology to offset heat treatment effects and enhance curd rheological properties. Calcium chloride induces changes in salt distribution, leading to the formation of calcium phosphate ($CaHPO_4$). This low solubility insolubilizes inside casein micelles, reducing their zeta potential and thermal stability. Additionally, some calcium ions decrease the level of hydration in the micelle. Overall, calcium is widely used in cheese technology to enhance curd properties [30]. Poultry eggshell calcium chloride can adjust milk pH, moisture content, and syneresis in cheese making. It lowers the pH, creating a favorable environment for rennet activity and curd formation. This affects the texture and quality of the cheese. Additionally, it influences the moisture content, potentially reducing moisture loss during aging. It also promotes syneresis, the process of whey expulsion from the curd.

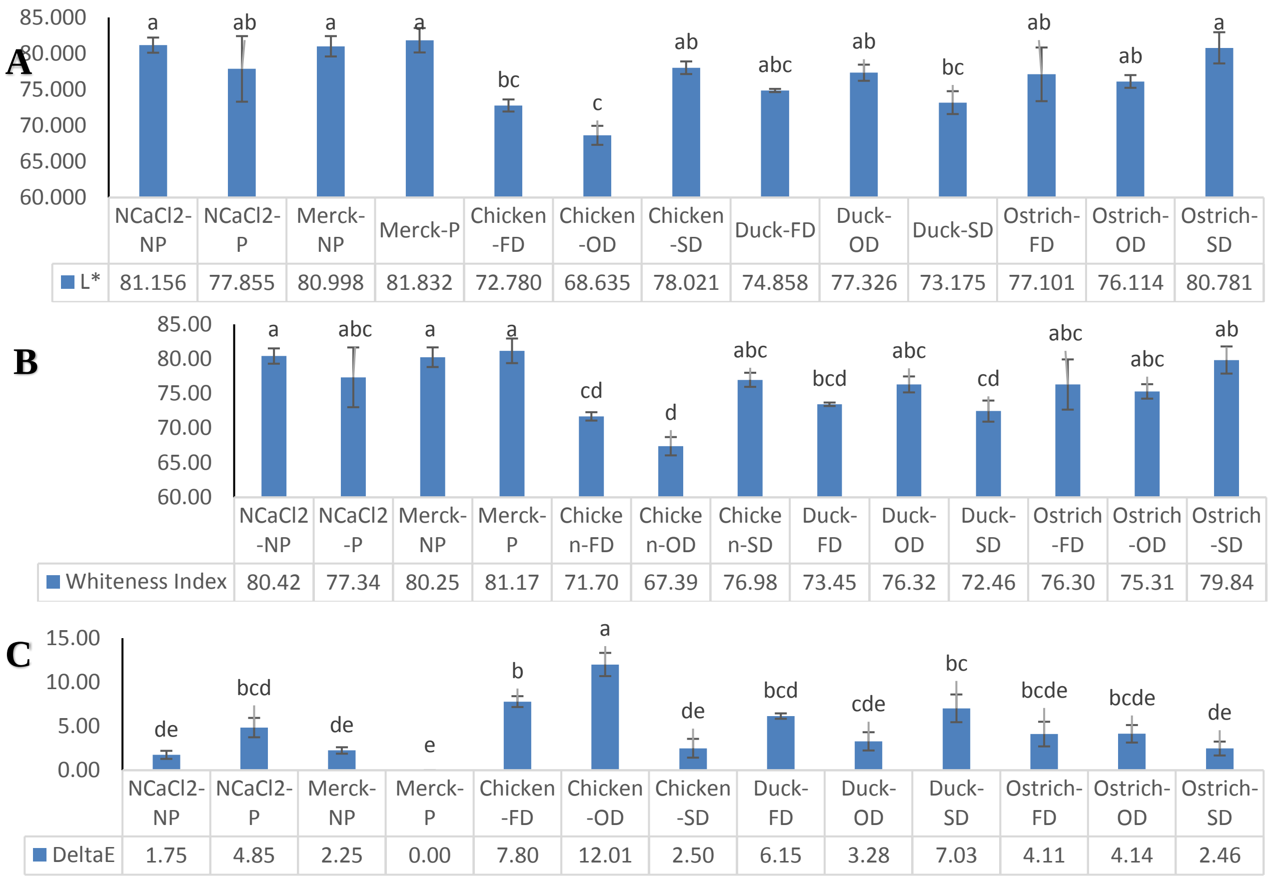

Fig. 4. Investigating the state of L*, whiteness index and ΔE of Iranian white cheese resulting from the type and method of using calcium chloride on the 60th day after production. NCaCl$_2$-NP: Non-CaCl$_2$ & non-pasteurized milk, NCaCl$_2$-P: Non-CaCl$_2$ & pasteurized milk, Merck-NP: Merck-CaCl$_2$ & non-pasteurized milk, Merck-P: Merck-CaCl$_2$ & pasteurized milk, FD: Freeze drying, OD: Oven drying, SD: Spray drying.

Whiteness, L, and ΔE indices of high-fat white cheese

Fig. 4A and 4B show the L index and the produced cheeses' whiteness, respectively. According to the data, there is no significant difference ($p<0.05$) between the L and whiteness index for each type of poultry egg shell for any of the three drying processes. The cheese samples that were made using $CaCl_2$ from chicken eggshells, particularly when using the oven and freeze-drying procedures, had the lowest color quality, while the samples that utilized Merck's $CaCl_2$ had the greatest color and whiteness index. The cheeses made from $CaCl_2$ made from chicken eggshells—particularly those dried by oven or freeze drying—as well as $CaCl_2$ made from duck eggshells that were dried by spray drying showed the greatest changes in color (ΔE) when compared to the standard sample (Merk-P) (Fig. 4C). The color changes, L index, and whiteness index were not significantly affected by the pasteurization process ($P<0.05$) as shown in Figs. 4A, 4B, and 4C.

The relationship between moisture content and light scattering and reflection in cheese is influenced by moisture content and water distribution. Lower water (Fig. 3B) content or higher synereses (Fig. 3C) leads to a denser matrix, and resulting in a lighter appearance [31]. This finding is in accordance with earlier research that showed a negative association between the L* and whiteness and moisture levels in cheese samples [32].

Textural properties of high-fat white cheese

Fig. 5A demonstrates that, with the exception of the Chicken-FD and Duck-OD treatments, which generated the least hardness ($P<0.05$), the other treatments did not vary significantly from the control treatment (Merck-P) ($P> 0.05$). Fig. 5B shows that, with the exception of the Ostrich-FD and Ostrich-SD treatments, which had the highest cohesiveness ($P<0.05$), all the rest of the treatments were not substantially different from the control treatment (Merck-P) ($P> 0.05$).

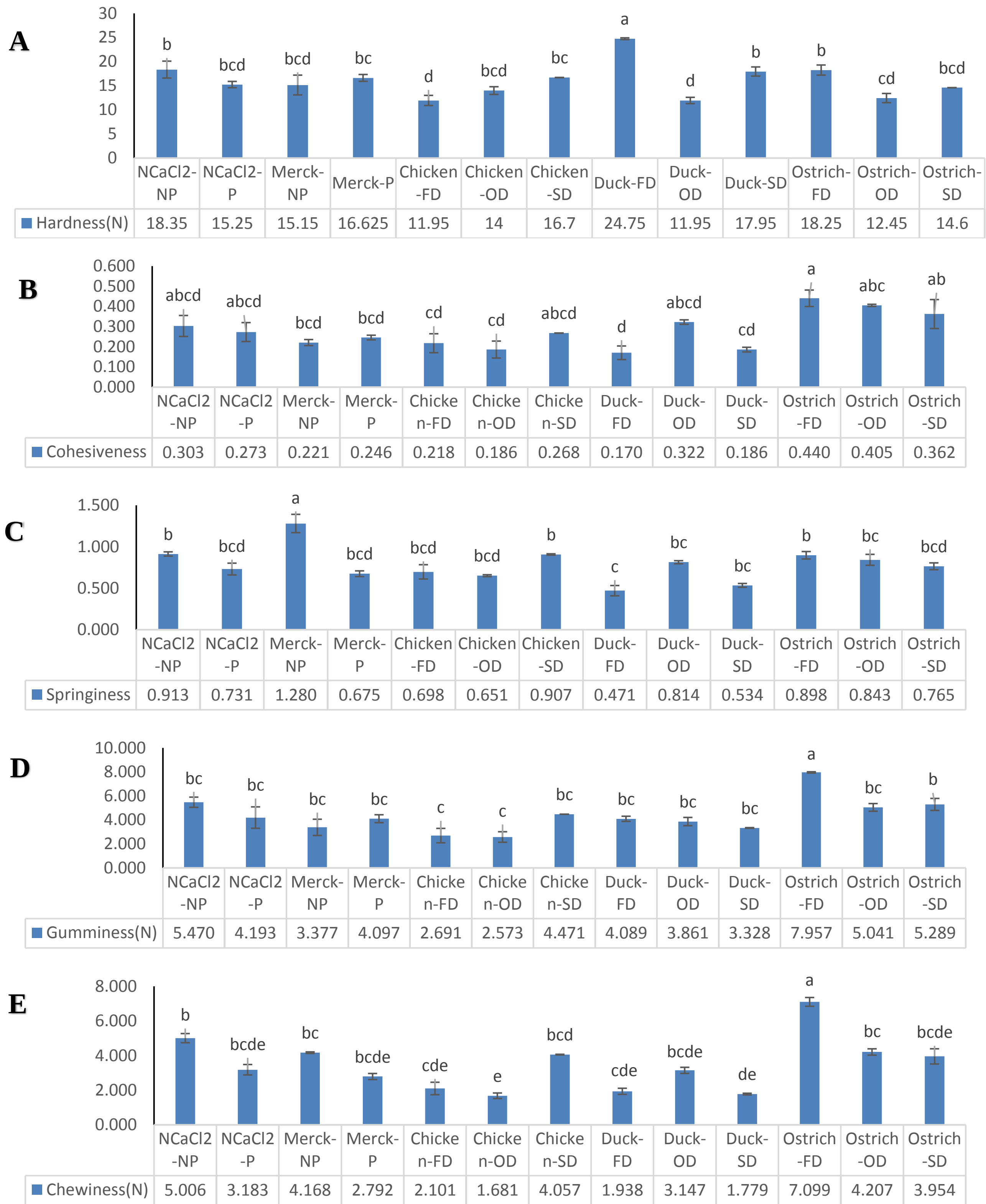

Fig. 5. Investigating the state of hardness, cohesiveness, springiness, gumminess and chewiness of Iranian white cheese resulting from the type and method of using calcium chloride on the 60th day after production. $NCaCl_2$-NP: Non-$CaCl_2$ & non-pasteurized milk, $NCaCl_2$-P: Non-$CaCl_2$ & pasteurized milk, Merck-NP: Merck-$CaCl_2$ & non-pasteurized milk, Merck-P: Merck-$CaCl_2$ & pasteurized milk, FD: Freeze drying, OD: Oven drying, SD: Spray drying.

The springiness features of the cheeses shown in Fig. 5C reveal that, with the exception of the Merck-NP treatment, which had the highest springiness ($P<0.05$), the other treatments were not substantially different from the control treatment (Merck-P) ($P>0.05$). Fig. 5D and 5E demonstrate that, with the exception of the Ostrich-FD treatment, which has the highest gumminess and chewiness ($P<0.05$), the other treatments have no significant differences compared to the control treatment (Merck-P) ($P >0.05$). Calcium chloride, derived from poultry eggshells, plays a crucial role in the textural properties of cheese. It stabilizes and strengthens the protein network in curds, resulting in a firmer texture. The addition of calcium chloride can enhance curd formation and contribute to a more compact and cohesive structure. Calcium chloride influences the texture of cheese by affecting interactions between proteins, calcium ions, and water, enhancing the cheese's ability to retain moisture and maintain its structure. During cheese aging, calcium chloride impacts proteolysis, the breakdown of proteins by enzymes. This can influence the development of flavor compounds and texture changes over time. The presence of calcium ions from poultry eggshell $CaCl_2$ can contribute to the characteristic texture and flavor profiles of aged cheeses [28-29]. $CaCl_2$ is commonly added to milk, particularly late lactation milk, to improve rennet coagulation properties. However, few studies have examined its effect on cheese yield. A study on Swiss-type cheese found that adding $CaCl_2$ to milk yielded insignificant increases in fat and non-fat milk solids recovery but increased cheese yield. This suggests that the positive effects of $CaCl_2$ on fat and protein recovery and cheese yield likely result from enhanced casein aggregation. Aggregation of renneted micelles is promoted by adding $CaCl_2$ or reducing the pH [33]. In terms of affecting the textural properties of Iranian high-fat white cheese, calcium chloride produced from three different sources of poultry eggshells, including chicken, duck, and ostrich, and dried by three different methods, including oven, freeze, and spray, had no discernible difference from Merck's calcium chloride ($P<0.05$). The uncertainty analysis conducted in this study identifies and quantifies the potential sources of variability that may impact the reliability of our results. Key sources of uncertainty include the intrinsic variability in raw material properties, such as the composition of poultry eggshells, which can affect the yield and purity of calcium chloride. Instrument precision is another critical factor, specifically concerning the calibration and measurement accuracy of the pH meter and texture analyzer used in our assessments. Environmental factors, including temperature fluctuations during drying and cheese aging, also contribute to variability. To quantify these uncertainties, we calculated standard deviations and confidence intervals for key measurements, including pH, color metrics (L*, a*, b* values), and textural properties. The analysis indicated that these uncertainties could influence the interpretation of physicochemical properties of the cheese, emphasizing the importance of considering variability in future studies. We recommend that subsequent research incorporate more comprehensive uncertainty analyses to enhance the robustness of findings and facilitate a deeper understanding of the factors influencing cheese quality.

CONCLUSIONS

This study investigated the potential of poultry eggshell waste as a sustainable source for calcium chloride ($CaCl_2$) extraction, with a focus on its application in the production of Iranian high-fat white cheese. The novelty of our research lies in the utilization of eggshells from chickens, ducks, and ostriches, transforming a waste product into a valuable resource for the dairy industry. We utilized hydrochloric acid treatment and explored various drying methods—spray, freeze, and oven drying—to extract calcium chloride from poultry eggshells. The primary parameters assessed included yield, purity of extracted $CaCl_2$, pH, moisture content, syneresis, and textural properties of the resulting cheese.

- The highest yield of $CaCl_2$ (92.46%) was obtained from chicken eggshells using the oven drying method, while ostrich eggshells yielded the lowest (75.46%) via spray drying.
- The purity of the extracted calcium chloride was comparable to the Merck standard across all egg types.
- The maximum cheese yield (12.69%) was achieved using unpasteurized milk with Merck calcium chloride; the lowest yield (11.02%) was observed with pasteurized milk without it.
- Calcium chloride influenced lipolysis and proteolysis during cheese-making, although there were no significant pH differences among cheeses produced with different sources of $CaCl_2$.
- Moisture content and syneresis showed an inverse relationship, indicating the impact of eggshell-derived $CaCl_2$ on cheese texture and quality.
- The textural properties of cheese made with poultry eggshell-derived $CaCl_2$ were similar to those made with the Merck sample, suggesting its viability as a functional ingredient.

While this study demonstrates the potential of using poultry eggshells for $CaCl_2$ extraction, there are limitations, including the variability in eggshell composition and the need for further optimization of the extraction process. Future research should focus on:

- Investigating the long-term stability and sensory properties of cheese produced with eggshell-derived $CaCl_2$.
- Exploring additional drying methods and their effects on the quality of extracted calcium chloride.
- Conducting cost-benefit analyses to evaluate the economic feasibility of scaling up this process for industrial applications.

Acknowledgments

The authors would like to acknowledge everyone who has helped us in the accomplishment of research work, for their scientific and valuable help and cooperation with this project.

Received: Oct.29, 2024; Accepted: Dec. 30, 2024

REFERENCES

[1] Vakilian K., Nateghi L., Javadi A., Anarjan N., Extraction and Characterization of Pectins from Ripe Grape Pomace Using Both Ultrasound Assisted and Conventional Techniques, *Food Anal. Methods*, (2024).

[2] Nateghi L., Zarei F., Zarei M., Optimize the Extraction Conditions of Pectin Extracted from Saveh Pomegranate Peels, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **41(11):** 3835-3850. (2022).

[3] Kashani A., Hasani M., Nateghi L., Asadollahzadeh M.J., Kashani P., Optimization the Conditions of Process of Production of Pectin Extracted from the Waste of Potato Peel, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **41(4):** 1288-1304 (2021).

[4] Vakilian, K., Nateghi, L., Javadi, A., Anarjan, N. Optimization of Conventional and Ultrasound-Assisted Extraction of Pectin from Unripe Grape pomace: Extraction Yield, Degree of Esterification, and Galacturonic Acid Content, *J. Food Meas. Charact*., **17:** 5777–5793 (2023).

[5] Ravindran R., Jaiswal A.K., Exploitation of Food Industry Waste for High-Value Products, *Trends Biotechnol*, **34(1):** 58-69 (2016).

[6] Waheed M., Yousaf M., Shehzad A., Inam-Ur-Raheem M., Khan M.K.I., Khan M.R., Ahmad N., Aadil R.M., Channelling Eggshell Waste to Valuable and Utilizable Products: A Comprehensive Review, *Trends Food Sci. Technol.*, **106:** 78-90 (2020).

[7] Wee R., Top Egg Producing Countries in the World, In, 2020. World Atlas, (2019).

[8] Abdulrahman I., Tijani H.I., Mohammed B.A., Saidu H., Yusuf H., Ndejiko Jibrin M., Mohammed S., From Garbage to Biomaterials: An Overview on Eggshell Based Hydroxyapatite, *J. Mater*., **2014:** 1-7 (2014).

[9] Perez-Huerta A., Dauphin Y., Comparison of the Structure, Crystallography and Composition of Eggshells of the Guinea Fowl and Graylag Goose, *Zoology*, **119(1):** 52-63 (2016).

[10] Pliya P., Cree D., Limestone Derived Eggshell Powder as a Replacement in Portland Cement Mortar, *Constr. Build. Mater.*, **95:** 1-9(2015).

[11] Vandeginste V., Food Waste Eggshell Valorization through Development of New Composites: A Review, *Sustainable Mater. Technol*., **29:** e00317 (2021).

[12] Polat S., Sayan P., Ultrasonic-Assisted Eggshell Extract-Mediated Polymorphic Transformation of Calcium Carbonate, Ultrason. Sonochem., **66:** 1-11 (2020).

[13] Waheed M., Butt M.S., Shehzad A., Adzahan N.M., Shabbir M.A., Suleria H.A. R., Aadil R.M., Eggshell Calcium: A Cheap Alternative to Expensive Supplements, *Trends Food Sci. Technol*., **91:** 219-230 (2019).

[14] Ajala E., Eletta O., Ajala M., Oyeniyi S., Characterization and Evaluation of Chicken Eggshell for Use as a Bio-Resource, *Arid Zone J. Eng. Technol. Environ*., **14:** 26-40 (2018).

[15] Younas K., Afzaal M., Saeed F., Shankar A., Kumar Bishoyi A., Khare N., Imran A., Mahmood K., Amhed A., Asghar A., Samar N., A Mini- Review on Egg Waste Valorization, *J. Sci. Food Agric*. (2024).

[16] Ganesan V.S., Paramathevar N., Utilising Calcined Eggshell Waste as a Multifunctional Sustainable Agent as a Nano-Adsorbent, Photocatalyst and Priming Elicitor, *Environ. Sci. Pollut. Res*., **31(8):** 12112-12130 (2024).

[17] Rashid A.A,, Huma N., Zahoor T., Asgher M. Optimization of pH, Temperature and $CaCl_2$ Concentrations for Ricotta Cheese Production from Buffalo Cheese Whey using Response Surface Methodology, *J. Dairy Res.*, **84(1):** 109-116 (2017).

[18] Strelec I., Peranovic K., Ostojcic M., Aladic K., Pavlovic H., Djerdj I., Tatar D., Maravic N., Skoko Z., Budzaki S., Eggshell Waste Transformation to Calcium Chloride Anhydride as Food-Grade Additive and Eggshell Membranes as Enzyme immobilization Carrier, *Green Process. Synth*., **13(1):** 20230254 (2024).

[19] Garnjanagoonchorn W., Changpuak A., Preparation and Partial Characterization of Eggshell Calcium Chloride, *Int. J. Food Prop*., **10:** 497-503 (2007).

[20] ASTM International. Standard designation: E449-96 (Reapproved 2001): "Standard Test Methods for Analysis of Calcium Chloride", West Conshohocken, PA: ASTM Int. (2001).

[21] Zonoubi R., Goli M., The Effect of Complete Replacing Sodium with Potassium, Calcium, and Magnesium Brine on Sodium-Free Ultrafiltration Feta Cheese at the End of the 60-Day Ripening Period: Physicochemical, Proteolysis–Lipolysis Indices, Microbial, Colorimetric, and Sensory Evaluation, *Food Sci. Nutr.*, **9(2):** 866-874 (2021).

[22] Association of Official Analytical Chemists, "Official Methods of Analysis of AOAC International" (17th Ed.). AOAC. (2002).

[23] Park Y.W. Proteolysis and Lipolysis of Goat Milk Cheese, *J. Dairy Sci*., **84:** 84-92 (2001).

[24] Farokhian F., Jafarpour M., Goli M., Askari-khorasgani O., Quality Preservation of Air-Dried Sliced Button Mushroom (Agaricus Bisporus) by Lavender (*Lavendula angustifolia* mill.) Essential Oil, *J. Food Process Eng.*, **40(3):** 1-10 (2016).

[25] Ayyash M.M., Sherkat F., Francis P., Williams R.P.W., Shah N.P., The Effect of Sodium Chloride Substitution with Potassium Chloride on Texture Profile and Microstructure of Halloumi Cheese, *J. Dairy Sci.*, **94(1):** 37-42 (2011).

[26] Pokorski P., Hoffmann M., Valorization of Bio-Waste Eggshell as a Viable Source of Dietary Calcium for Confectionery Products, *J. Sci. Food Agric*., **102(8):** 3193-3203 (2022).

[27] Platon N., Arus V.A., Ursu A., Georgescu A.M., Nistor I.D., Silion M. Valorization of Eggshells Waste for Bread Production, *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Ind.*, **23 (1):** 49-61 (2022).

[28] Fox P.F., Guinee T.P., Cogan,T.M., Sweeney P.L.H., Fundamentals of Cheese Science, *Springer*, 1-803 (2020).

[29] Fox P.F., Sweeney P.L.H., Cheese: Chemistry, Physics and Microbiology, Academic *Press. Burlington*, **1:** 1-640 (2004).

[30] Jeantet R., Croguennec T., Schuck P., Brule G., Handbook of Food Science and Technology 3: Food Biochemistry and Technology, *John Wiley & Sons*, 1-63 (2016).

[31] Ong L., Dagastine R.R., Kentish S.E., Gras S.L., The Effect of Calcium Chloride Addition on the Microstructure and Composition of Cheddar Cheese, *Int. Dairy J*., **33:** 135-141 (2013).

[32] Abu-Alruz K.H.A.L.E.D., Quality of White-Brined Nabulsi Cheese Made with Different Proportions of Sheep's and Cows' Milk, *Curr. Res. Nutr. Food Sci*., **11(1):** 88-110 (2023).

[33] Bansal N., Fox P.F., Sweeney P.L., Factors That Affect the Aggregation of Rennet- Altered Casein Micelles at Low Temperatures, *Int. J. Dairy Technol*., **61(1):** 56-61 (2008).