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Research on the production of high-purity calcium lactate from poultry eggshells and their application in food

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Abstract:

Poultry eggshell waste is an environmental concern. Transforming biowaste into value-added products is one of the promising green solutions. Converting eggshell calcium carbonate into organic calcium is a promising strategy to treat this waste. The aims of this study are to produce high-purity lactate from poultry eggshell waste and to apply this preparation in food. Calcium sourced from poultry eggshells, recovered from food production facilities, has been thoroughly treated to remove membranes. The calcium was then extracted with lactic acid to produce high-purity organic calcium. The extraction process parameters were optimised: the ratio of eggshell to 16% lactic acid solution was 1:12 (w/v), reaction temperature 35°C, crystallisation at 25°Bx, and drying at 80°C for 6 hours. This process yielded a calcium lactate product with a purity exceeding 99%, and a recovery efficiency surpassing 90%, meeting the quality criteria outlined in Vietnamese standard QCVN 4-13:2010/BYT. Additionally, 0.2% (126 mg Ca/330 ml) of this calcium product was successfully incorporated into passion fruit juice, providing a high-quality product with potential to supplement organic calcium in human diets.

Keywords: **calcium carbonate, calcium lactate, eggshell, lactic acid, passion fruit.**

Classification numbers: **3.1, 3.6**

1. Introduction

The domestic poultry industry has seen significant growth over the past decade, a trend that is expected to continue. In 2022, poultry egg production exceeded 18 billion eggs, generating vast quantities of eggshell waste - potentially amounting to hundreds of millions of tonnes annually. This is especially problematic in hatcheries, where tonnes of eggshells are discarded daily, contributing to hazardous environmental waste. Such waste sources release greenhouse gases that exacerbate global warming, presenting a pressing issue. Therefore, developing sustainable techniques for the reduction of eggshell waste is critical to addressing these environmental concerns. Transforming biowaste into value-added products is one of the promising green solutions [1].

Numerous studies worldwide have explored the potential of this abundant material. M. Waheed, et al. (2019) [2] recently proposed a significant new concept: "Eggshell calcium: An inexpensive alternative to costly supplements". Eggshells consist of approximately 2% water and 98% dry matter, with the latter containing 93% calcium minerals and 5% crude protein. Eggshells are a rich source of calcium (98.2%), with 2.7 g of eggshell powder providing roughly 100% of the recommended daily calcium intake for adults. In light of the increasing global prevalence of calcium deficiency, the demand for pure and highly bioavailable calcium sources is rising. While oyster shells are another source of calcium carbonate, they pose the risk of containing toxic elements such as lead, aluminium, mercury, and cadmium - risks not present in eggshell calcium [2].

Calcium lactate, a form of organic calcium, is widely used in the food industry as a food additive (E code E327). It is recognised as safe (GRAS) by the United States Food and Drug Administration and serves as a firming agent, flavour enhancer, leavening agent, calcium fortification supplement, stabiliser, and thickener. In medicine, calcium lactate is used to treat calcium deficiencies and is administered orally to manage conditions such as osteoporosis, osteomalacia, rickets, hypocalcaemia, and myasthenia gravis. It is also recommended for pregnant and lactating women. Additionally, calcium lactate is frequently found in digestive enzymes and functional foods [1, 3].

Recycling eggshells into valuable calcium compounds, particularly calcium lactate, is an environmentally friendly and efficient approach [1, 4]. Converting eggshell calcium carbonate ($CaCO_3$) into organic calcium enhances its solubility and absorption, resulting in higher bioavailability. This process requires selecting safe, commonly used organic acids in food that can displace weak carbonic acid (H_2CO_3) and bind with calcium ions (Ca^{2+}) from eggshells. Optimising the technological parameters of this process is essential to

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achieving the highest purity and recovery efficiency of the calcium product. The acid dissociation constant (Ka) and pKa values indicate the acid's strength, where $pKa=-\log_{10}(Ka)$; lower pKa values correspond to stronger acids. Lactic acid ($CH_3CH(OH)CO_2H$) has a Ka of 1.38×10^{-4} and a pKa of 3.86, while carbonic acid has a Ka of 4.5×10^{-7} and a pKa of 6.3. The reaction equation is as follows:

$$2C_3H_6O_3+CaCO_3\rightarrow Ca(C_3H_5O_3)_2+CO_2\uparrow+H_2O \qquad (*)$$

F. Li, et al. (2010) [4] proposed a direct reaction between eggshells and lactic acid, achieving a calcium lactate yield of 79.23% and a calcium content of 86.93% under optimal conditions. Various methods for producing calcium lactate from eggshells have been reported, including bacterial fermentation, where lactic acid is produced and reacted with eggshells. In this process, powdered eggshells are first converted into a liquid through heating and pressure homogenisation, followed by treatment with papain to hydrolyse protein impurities. After enzymatic hydrolysis, glucose and a combination of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* are added to ferment the enzymatically treated eggshell liquid, yielding organic calcium. The extraction rate reached 86.36%, comprising 93.62% calcium lactate and 5.46% calcium gluconate [5]. However, this method was found to be inefficient and complicated to scale up for high-purity product recovery [3].

Recently, A.H. Prayitno, et al. (2022) [6] demonstrated the production of nano-calcium lactate from chicken eggshells. After membrane separation, the shells were dried, ground, and calcined at 1000°C to convert them into CaO. Nano-calcium lactate was then formed by reacting CaO with lactic acid at 50°C for 30 minutes with stirring, supplemented by ethanol to aid crystallisation. The product was then dried at 105°C for 72 hours, resulting in particles sized 75 nm. This result highlights the potential applications of calcium derived from poultry eggshells.

2. Materials and methods

2.1. Materials

Eggshells were obtained from the HaiHa Kotuboki company, Hanoi, Vietnam. They were pre-processed, washed, and dried. Other materials include: EDTA (Disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2O_8Na_2.2H_2O$), white powder, China; Lactic acid, colourless solution, concentration 85%, 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%; Passion fruit at technical maturity for juice extraction, sugar, carboxymethyl cellulose (CMC), etc.

2.2. Methods

2.2.1. Eggshell preparation

The collected eggshells were hydrolysed using 2% NaOH at 70°C for 45 minutes, followed by filtration and washing to remove the membrane. The material was then hydrolysed with Alcalase at 90°C for 45 minutes, followed by further filtration and washing to eliminate any remaining membrane. The product was dried, and the eggshell membrane was successfully removed. The processed eggshell exhibited a calcium carbonate ($CaCO_3$) purity of 99.69%.

2.2.2. Calcium lactate extraction from poultry eggshells

After the removal of the eggshell membrane, the calcified matrix was solvated. The mixture was then subjected to centrifugation and filtration to separate the pellets. The supernatant was evaporated to concentrate the calcium lactate solution. The concentrated solution was crystallised once or twice to further purify the product, and the crystals were washed with ethanol to remove impurities. Finally, the purified material was dried and ground into calcium lactate powder (Fig. 1).

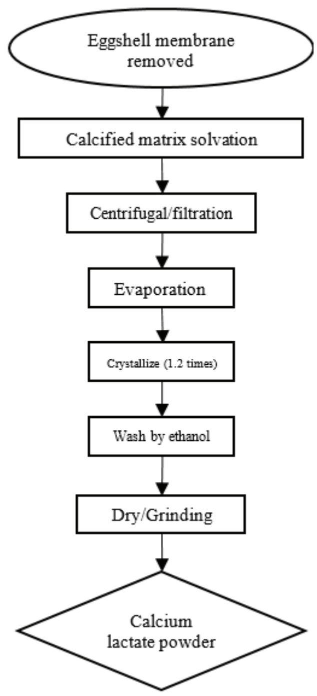

Fig. 1. Flow chart of the calcium lactate extraction process from poultry eggshells [7].

The moisture content of the calcium lactate powder was determined using the TCVN 10788:2015 standard, while the calcium content was analysed via the EDTA titration method (TCVN 6198:1996). The total calcium lactate content (expressed as total lactic acid) was measured using NIFC.02.M.32 (HPLC). Specifically, 0.1-0.5 g of the sample was accurately weighed into a 50 ml volumetric flask and diluted with distilled water. After filtering and dilution, the sample was analysed via HPLC-PDA, using a mobile phase of 0.1% o-phosphoric acid, an anion chromatography column, and a detection wavelength of 210 nm. L-(+)-Lactic acid with a purity of ≥98% (CAS number 79-33-4, SIGMA) was used as a reference.

The recovery efficiency and purity of the calcium lactate preparations were determined based on the (*) reaction. Theoretically, 10 g (corresponding to 0.1 M) of $CaCO_3$ reacts with 18.18 g (corresponding to 0.2 M) of lactic acid to produce 21.82 g (corresponding to 0.1 M) of $Ca(C_3H_5O_3)_2$.

Purity of calcium lactate preparation [7] was determined by the following equation:

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

where $C_{Ca(tt)}$ is the calcium content in the product sample determined by EDTA titration method and $C_{Ca\,(lt)}$ is the theoretical calcium content in calcium lactate (*).

Recovery efficiency of calcium was prepared using the following formula [8]:

$$H = \frac{m}{m_0} \times Purity_{\ ca} \times 100\ (\%)$$

where m is the mass of calcium lactate salt obtained (g) and m_0 is the mass of calcium lactate salt according to the theoretical reaction equation (g) (*).

2.2.3. Passion fruit juice supplemented with calcium lactate derived from poultry eggshell production

Fortified passion fruit juice containing calcium lactate was prepared (Fig. 2). The pH of the juice was measured in accordance with TCVN 4835:2002 (ISO 2917:1999), while acidity was determined via titration with 0.1 N NaOH. The absolute viscosity of the juice was measured using a DV2T (cP) meter (Brookfield Ametek - USA), and the juice's colour intensity was assessed through L, a, and b parameters using a Colorlife SPH860 colourimeter.

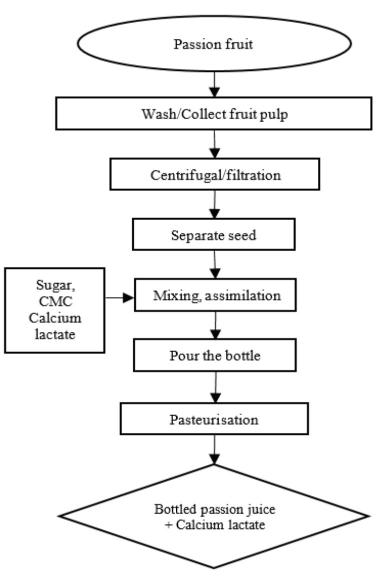

Fig. 2. The process of producing passion fruit juice supplemented with calcium lactate from poultry eggshells [8].

3. Results and discussion

3.1. Production of high-purity calcium lactate products from poultry eggshells

3.1.1. Determination of the lactic acid content during the extraction process

Calcium lactate was produced by dissolving calcium carbonate with lactic acid, (*) reaction. Theoretically, every 10 g of $CaCO_3$ reacts with 18 g of lactic acid if the reaction proceeds completely. This study investigated the extraction of 10 g of membrane-free eggshells using different lactic acid concentrations: 18, 19, 20 and 21 g, corresponding to concentrations of 15.5, 16, 16.5, and 17%, with an eggshell-to-lactic-acid ratio of 1:12 (w/v) and a reaction temperature of 35°C [4] (Fig. 3).

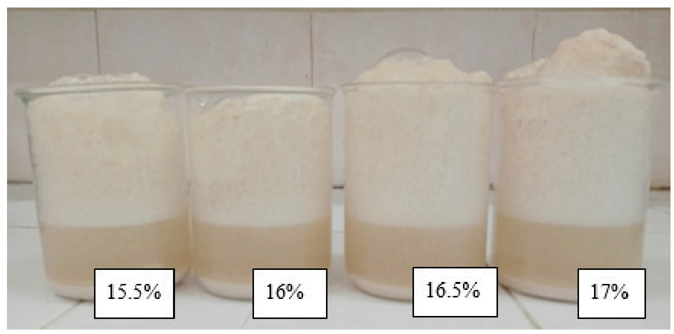

Fig. 3. Calcium lactate preparation samples extracted in lactic acid at different concentrations.

The calcium extraction solutions were concentrated to the same level, crystallised, washed with absolute alcohol, and dried at 70°C for 8 hours. The calcium content of the calcium lactate preparations was measured via EDTA titration method, and the results of the influence of lactic acid concentration on the production of calcium lactate are shown in Table 1.

Table 1. Effect of lactic acid concentration on eggshell calcium extraction process.

Lactic acid content (%)	Calcium content in calcium lactate product obtained (%)	Purity of calcium lactate product obtained (%)	Recovery efficiency (%)
15.5	16.90	92.30	75.93
16	**17.98**	**98.05**	**90.98**
16.5	17.78	97.16	90.48
17	17.41	95.10	90.37

At 17% lactic acid concentration, the amount of calcium lactate recovered was the highest, although the purity did not meet the Vietnamese standard QCVN 4-13:2010/BYT. However, at 16%, the product demonstrated high calcium content and purity, with a recovery efficiency of 90.98%. These findings align with the results of F. Li, et al. (2010) [4], where the optimal lactic acid content for calcium extraction was 18.5 g to extract 10 g of eggshell, diluted in 100 ml of distilled water. Hence, a 16% lactic acid concentration was chosen for further studies.

3.1.2. Investigation of the crystallisation condition

The calcium lactate extract obtained from 10 g of eggshell using 16% lactic acid was centrifuged to separate the residue and concentrated to facilitate crystallisation at 10°C for 24 hours. The product was dried at the same temperature. The effect of solute concentration after evaporation on the crystallisation process and product recovery is shown in Table 2.

Table 2. Effect of calcium lactate concentration after evaporation on crystallisation and product recovery.

Calcium lactate concentration after evaporation (°Bx)	23	25
Volume of calcium lactate salt recovered after drying (g)	18.313	20.229
Calcium content (%)	17.91	**17.96**
Recovery efficiency (%)	82.04	**90.98**
Purity (%)	97.66	**98.05**

At a concentration of 25°Bx, the crystallisation process yielded the best results, with the highest product volume, recovery efficiency, and purity. However, at 27°Bx, the solution solidified into a soft mass, making it difficult to separate crystals and acid. After the first crystallisation, residual calcium lactate remained in the solution, necessitating a second crystallisation to improve recovery efficiency. Therefore, a 25°Bx concentration and two crystallisation cycles were selected for further study (Fig. 4).

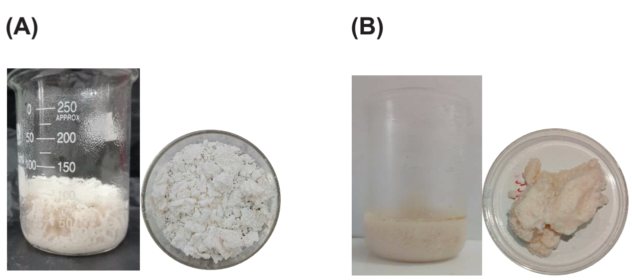

Fig. 4. Calcium lactate after crystallisation at different concentrations (A) 25°Bx, **(B)** 27°Bx.

3.1.3. Investigation of drying temperature and drying time

Eggshell powder (10 g) was extracted using lactic acid under the selected conditions (16% lactic acid concentration, 25°Bx crystallisation), followed by washing with alcohol and drying at different temperatures ranging from 60 to 80°C. The effects of drying temperature and time on product weight and humidity were investigated, as shown in Fig. 5.

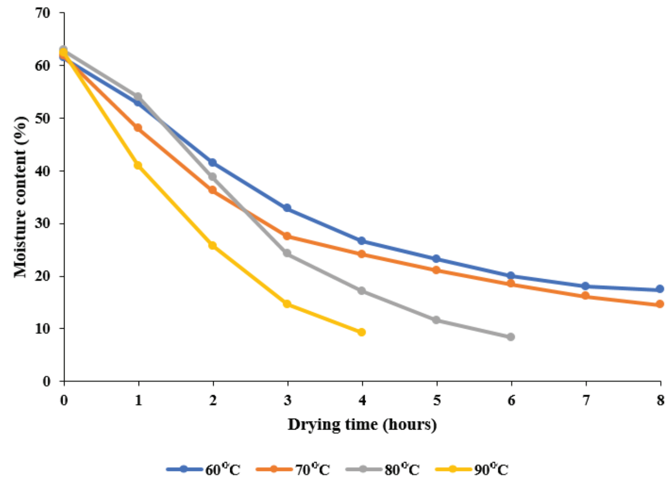

Fig. 5. Effect of drying condition on product weight and humidity of calcium lactate.

Calcium lactate is highly hygroscopic, meaning it readily absorbs moisture during storage, usually existing as pentahydrate $C_6H_{10}CaO_6.5H_2O$ with 30% moisture content, or trihydrate $C_6H_{10}CaO_6.3H_2O$ with 20% moisture. Therefore, the moisture content after drying should be reduced to less than 10%. During the initial drying phase, moisture is quickly released, and higher temperatures accelerate moisture loss. After drying for 3 hours at 90°C, the moisture content remained at 14.63%; however, the product exhibited a yellowish colour at this temperature. As a result, a drying condition of 80°C for 5 hours was chosen, achieving a moisture content of 8.37% (Fig. 6). These results are consistent with those of F. Li, et al. (2010) [4], who recommended that the drying temperature should not exceed 80°C.

Fig. 6. Sample of calcium lactate during drying at 80°C for the first 5 hours.

3.1.4. Evaluation of the quality of calcium lactate preparations from poultry eggshells

The findings demonstrate that the parameters for recovering calcium lactate from eggshells were optimised as follows: a ratio of eggshell to 16% lactic acid solution of 1:12 (w/v), a reaction temperature of 35°C, crystallisation at 25°Bx, and drying at 80°C for 6 hours. This process resulted in a recovery efficiency of 90.98% and a calcium lactate purity of 99.1%. These results compare favourably with those of F. Li, et al. (2010) [4], where the calcium lactate yield was 79.23%, and the calcium content was 86.93%. Q. Huang, et al. (2019) [9] used four strains of lactic acid bacteria

(*E. mundtii*, *Streptococcus thermophilus*, *Lactobacillus casei*, and *L. bulgaricus*) to ferment eggshells in the presence of glucose, yielding 40 g/l of calcium lactate with a purity of 93%. The quality assessment of the obtained calcium lactate (Table 3) indicates that it meets the QCVN 4-13:2010/BYT standards for use as a food additive. As such, this study aims to incorporate this organic calcium source into food products (e.g., fruit juice) to address calcium deficiencies in human diets.

Table 3. Evaluation of calcium lactate preparations from poultry eggshells.

Parameters	According to QCVN 4-13:2010/BYT	Sample of calcium lactate preparation	Method
Sensory	White to cream colour crystalline powder or granules, almost odourless	The granular product was a creamy white colour, and it was white after grinding[b]	Perception with the naked eye
pH	6-8	6.03[b]	TCVN 12348:2018
Acidity	Amount of NaOH 0.1 N used <0.5 ml	Amount of NaOH 0.1 N used: 0.75 ml[b]	According to QCVN 4-13:2010/BYT
Calcium lactate content	>98%	99.1%[a]	NIFC.02.M.32 (HPLC)
Humidity	<30%	16.7%[a]	TCVN 8900-2:2012
Lead content	<1 mg/kg	0.094 mg/kg[a]	NIFC.03.M.45 (ICP-MS)
Solubility	Soluble in water, insoluble in ethanol	Concentration can reach 48 g/l [b]	

a: Evaluation at the National Testing Centre; b: Laboratory assessment.

3.2. Effect of calcium lactate from poultry eggshells as a rich-calcium source in bottled passion fruit juice

Passion fruit juice samples were prepared according to the process outlined in Section 2.2.3. The ingredients included 10% passion fruit juice, 90% sugar syrup, and ascorbic acid and CMC additives, achieving a dry matter concentration of 13°Bx.

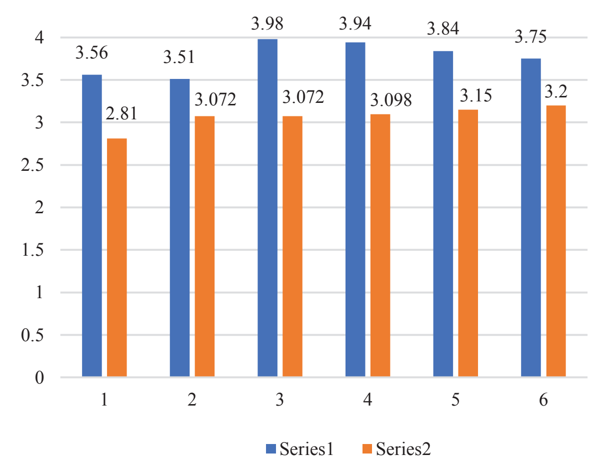

Fig. 7. pH and acidity (ml of 0.1N NaOH/5 ml) of passion fruit juice samples with and without adding calcium lactate.

Calcium lactate extracted from poultry eggshells was added at concentrations ranging from 0.15 to 0.3%, equivalent to a calcium content of 90-182 mg per 330 ml bottle. The juice was then sterilised at 85°C for 10 minutes. Quality criteria, including calcium content, colour, viscosity, pH, acidity, and sensory properties, were evaluated according to the described methods and compared with a control sample (without calcium lactate) and a commercial sample of canned passion fruit juice from the Doveco brand. The results are presented in Figs. 7-10.

The pH of the research samples ranged from 3.51 to 3.98, closely resembling the commercial samples. Fruit juice samples supplemented with calcium showed a slight decrease in pH and a marginal increase in acidity, likely due to the presence of lactate salts, though these changes were not significant.

Colour evaluation was based on L, a, and b values. The L index, which represents the brightness of the juice, ranged from 21.17 to 22.7, with the research samples exhibiting slightly higher brightness than the market samples. The a index, indicating the intensity of the orange-yellow colour typical of passion fruit, was higher in the research samples, though there was no significant difference between samples with and without calcium lactate. The b index, representing yellow intensity, also showed no notable variation, with values ranging from 10.38 to 10.92.

Market samples displayed a lighter yellow hue, while research samples had a more orange-yellow tone. There was no significant difference in colour between the control sample (without calcium lactate) and the supplemented samples. The samples with 0.2 and 0.25% calcium lactate exhibited the most harmonious colours.

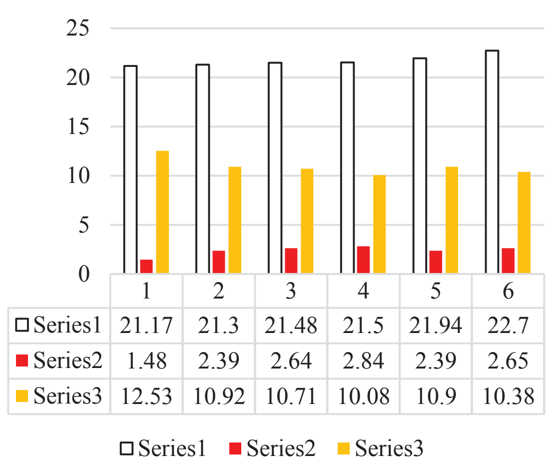

Fig. 8. L, a, b colourimetric values of passion fruit juice samples with and without adding calcium lactate.

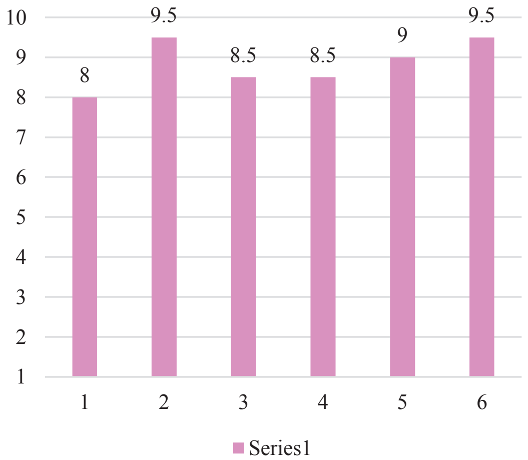

Fig. 9. Viscosity (consistency) values of passion fruit juice samples with and without calcium lactate addition.

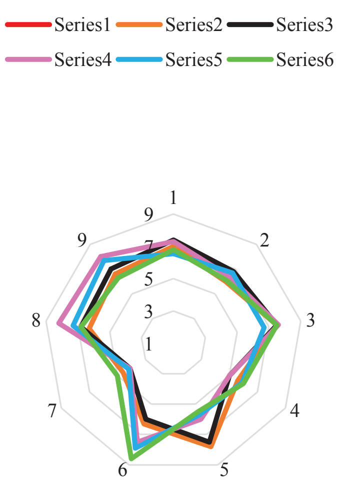

Fig. 10. Sensory evaluation of passion fruit juice samples with and without calcium lactate addition.

The viscosity of the passion fruit juice samples exhibited minimal differences. The addition of calcium lactate resulted in a slight increase in viscosity, with sensory evaluations indicating improved consistency. The samples supplemented with 0.2 and 0.25% calcium lactate were found to have the most balanced viscosity, providing a desirable mouthfeel.

To further confirm the quality of passion fruit juice samples supplemented with calcium lactate derived from poultry eggshells, consumer taste and sensory factors, in addition to physical and chemical indicators, remain critical for determining product acceptability. Particular attention was given to detecting any off-flavours after heating, especially the potential presence of an eggshell odour. The sensory evaluation panel, using descriptive tests, assessed nine typical properties of the product, with the results summarised in Fig. 8. The market sample was found to have a higher sweetness level and an artificial passion fruit flavour. The sample supplemented with 0.2% calcium lactate was considered to have the most natural colour, harmony, and highest overall evaluation score.

To determine if any calcium content was lost during processing, the calcium content in the fruit juice samples was analysed. As shown in Table 4, the calcium content in the samples slightly exceeded the amount added. This suggests that the original calcium in the raw materials contributed to the final calcium content, meaning the added calcium was largely retained during processing.

Table 4. Calcium content in passion fruit juice samples.

Samples	Calcium lactate added content (mg/bottle of 330 ml)	Calcium content in the sample (mg/bottle of 330 ml)
Control sample	-	5.23
Calcium lactate added 0.15%	91.08	96.27
Calcium lactate added 0.2%	121.44	126.59
Calcium lactate added 0.25%	151.80	156.95
Calcium lactate added 0.3%	182.16	187.21

Bottled passion fruit juice products supplemented with calcium lactate from poultry eggshells

According to the nutrition survey by the Vietnam Ministry of Health [10], the rate of stunting malnutrition (height-for-age) among children under five in Vietnam is 19.6%, with mountainous regions experiencing rates as high as 38%. Additionally, a report from the International Osteoporosis Foundation in 2018 revealed that the daily calcium intake in Vietnam is among the lowest globally, at less than 500 mg per day. This places Vietnam among the countries with the most severe calcium deficiency.

Thus, the passion fruit juice sample supplemented with 0.2% calcium lactate from eggshells - equivalent to 126 mg of calcium per 330 ml bottle - provides over one-quarter of the daily calcium requirement for children and more than one-fifth of the daily requirement for adults. It is recommended to consume 1-2 bottles per day to help alleviate calcium deficiency among the Vietnamese population.

This research is expected to be expanded and scaled up for industrial application, leveraging the abundant supply of eggshell waste to create valuable nutritional supplements while addressing environmental pollution.

4. Conclusions

The technological parameters for the extraction of calcium lactate from poultry eggshells were established, producing a high-purity product (99.1%) that meets the quality criteria of Vietnamese standard QCVN 3-4:2010. The optimised process involved a ratio of eggshell to lactic acid solution (16%) of 1:12 (w/v), with crystallisation at 25°Bx, drying at 80°C for 6 hours, and a recovery efficiency of 90.98%.

Incorporating 0.2% calcium lactate (126 mg Ca per 330 ml bottle) from poultry eggshells into passion fruit juice significantly enhanced the product's quality and sensory properties. The supplemented juice offers a valuable source of organic calcium to reduce calcium deficiency. Due to limitations in the current research design, it is recommended that this extraction process be tested in an industrial setting.

The results demonstrate the potential for transforming biowaste, such as eggshells, into value-added products like calcium lactate, playing a crucial role in both waste management and the food and pharmaceutical industries. The conversion of eggshells into useful supplements also contributes to solving environmental issues.

CRediT author statement

Phan Thanh Tam: Methodology, Data analysis, Writing - Reviewing and Editing; Nguyen Hai Van: Methodology, Data analysis, Writing; Nguyen Ngoc Hieu: Methodology, Data analysis.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] S. Seesanong, C. Seangarun, B. Boonchom, et al. (2023), "Efficient, green, and low-cost conversion of bivalve-shell wastes to value-added calcium lactate", *ACS Omega*, **8(30)**, pp.27044-27055, DOI: 10.1021/acsomega.3c02042.

[2] M. Waheed, M.S. Butt, A. Shehzad, et al. (2019), "Eggshell calcium: A cheap alternative to expensive supplements", *Trends Food Sci. Technol.*, **91**, pp.219-230, DOI: 10.1016/j.tifs.2019.07.021.

[3] A.E. Tamer, L. Wu, M. Younes, et al. (2021), "Biotechnological applications of eggshell: Recent advances", *Frontiers in Bioengineering and Biotechnology*, **9**, DOI: 10.3389/fbioe.2021.675364.

[4] F. Li, M. Ma, Y. Li, et al. (2010), "Technology of preparation of calcium lactate from eggshell", *Transactions of The Chinese Society of Agricultural Engineering*, **26(2)**, pp.370-374, DOI: 10.3969/j.issn.1002-6819.2010.02.065.

[5] Y. Shao (2017), *Method for Preparing Organic Calcium with Eggshells*, Patent -CN106307551A.

[6] A.H. Prayitno, T.A. Siswoyo, Y. Erwanto, et al. (2022), "Characterisation of nano-calcium lactate from chicken eggshells synthesized by precipitation method as food supplement", *Jurnal Ilmu Ternak Dan Veteriner*, **26(4),** pp.139-144.

[7] I. Strelec, K. Tomičić, M. Zajec, et al. (2023), "Eggshell-waste-derived calcium acetate, calcium hydrogen phosphate and corresponding eggshell membranes", *Applied Sciences*, **13(13**), DOI: 10.3390/app13137372.

[8] R. Rosnah, N.A. Taslim, A.M. Aman, et al. (2021), "Physicochemical characteristics of chicken eggshell flour produced by hydrochloric acid and acetic acid extraction", *Open Access Macedonian Journal of Medical Sciences*, **9(A)**, pp.428-432, DOI: 10.3889/oamjms.2021.6238.

[9] Q. Huang, X. Huang, R. Yang, et al. (2019), *A Kind of Method that Compound Lactobacillus-Fermencucumber Eggshell Prepares Calcium Lactate*, Patent - CN110373444A.

[10] Ministry of Health (2021), "The Ministry of Health announced the results of the 2019-2020 nutrition census", https://moh.gov.vn/hoat-dong-cua-lanh-dao-bo/-/asset_publisher/TW6LTp1ZtwaN/content/bo-y-te-cong-bo-ket-qua-tong-ieu-tra-dinh-duong-nam-2019-2020, accessed 15 April 2021 (in Vietnamese).