Eggshell-Waste-Derived Calcium Acetate, Calcium Hydrogen Phosphate and Corresponding Eggshell Membranes

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Featured Application: Calcium acetate monohydrate and calcium hydrogen phosphate anhydrous of food-grade additive purity, as well as corresponding eggshell membranes as a valuable by-product, has been produced from eggshell waste by commonly used industrial processes which might be easily transferable on the industrial scale level.

Abstract: The development of innovative transformation techniques for various wastes generated by the agri-food industry is one of the goals of sustainable waste management oriented toward “zero-waste” approach. This includes the production of bulk and fine chemicals, bioactive compounds, enzymes and functional materials from various waste. The present research shows the possibility of the production of value-added products from eggshell waste using chemical transformation by acetic and o-phosphoric acid at a laboratory scale level. Eggshell-derived calcium acetate monohydrate and calcium hydrogen phosphate, both of high purity, as well as corresponding eggshell membranes as valuable by-products, were produced. Chemical transformation of 100 g of eggshell waste with 10% (w/v) acetic acid resulted in 111.41 ± 2.13 g of calcium acetate monohydrate and 2.90 ± 0.14 g of eggshell membranes, with a chemical yield in calcium acetate monohydrate of 79.16 ± 1.98%. On the other hand, the transformation of 100 g of eggshell waste in 15% (w/v) o-phosphoric acid yielded 77.06 ± 6.21 g of calcium hydrogen phosphate and 2.94 ± 0.08 g of eggshell membranes, with a chemical yield in calcium hydrogen phosphate of 71.36 ± 5.58%. Eggshell-derived calcium hydrogen phosphate met all prescribed criteria for food-grade additive, as determined by physicochemical analysis. On the other hand, calcium acetate monohydrate met the majority, except water insoluble and formic acid and oxidizable impurities content. Based on the obtained results, it can be concluded that the proposed eggshell waste transformation process by two different acids might be useful for the production of calcium acetate and calcium hydrogen phosphate as food-grade additives, as well as eggshell membranes as valuable by-products.

Keywords: eggshell waste utilization; acid treatment; calcium acetate; calcium hydrogen phosphate; eggshell membranes

1. Introduction

Huge amounts of eggshell waste (ESW) have been produced worldwide annually, but the majority of it unfortunately ends up in landfills, presenting a significant ecological burden, as well as a potential biohazard [1–4]. This is quite surprising since numerous scientific reports and patents [3,5–16] clearly show the multiple usage possibilities of ESW. Regardless of the fact that ESW may be used to produce low-value added products, such as fertilizers or food for pets and animals, or to produce single value-added products, there is still a multitude of possibilities for ESW utilization, especially those oriented toward the production of high value-added products approaching the “zero-waste” model.
ESW originating from fresh hen egg breaking in households, restaurants, as well as egg breaking plants, could be roughly divided into three structurally different parts each containing unique set of high value molecules of potential use (Figure 1). Adherent egg white layer comprising the inner part of ESW, present in the amount of about 3% of ESW on dry weight basis, is a rich source of valuable egg white proteins, such as ovalbumin, ovotransferrin and lysozyme [1,4,5,17]. This sticky egg white layer is adhered to the eggshell membranes (ESM) that comprise about 3% of ESW on dry weight basis, and presents a rich source of collagen fibers, hyaluronic acid and other valuable glycosaminoglycans [1,18–21]. Finally, the outer part of ESW is a calcified matrix mainly composed of calcium carbonate comprising between 92 and 95% of ESW on dry weight basis, and some highly valuable antimicrobial eggshell proteins present in low amounts [1,4,16,22,23]. As we have just stated, each part of ESW could be used for the production of high value-added products, instead of being used partially, or in the worst-case scenario discarded on landfills.

In this respect, our research group has been oriented towards the possibility of the complete utilization of eggshell waste, using the most common industrial processes, such as mixing, filtration, evaporation and drying, but with the aim of producing high-value-added products. Our most recent publication [1] has proven the possibility of ESW utilization for the production of egg white protein solution, calcium chloride dihydrate as a food-grade additive, and eggshell membranes, using ESW transformation with 5% (w/v) hydrochloric acid. In continuation of our efforts oriented toward the complete utilization of ESW, in our present research, we have examined the possibility of chemical transformation of ESW with acetic and -phosphoric acid to the corresponding calcium salts and eggshell membranes. Although the use of -phosphoric and acetic acid for the production of corresponding Ca-salts from eggshell waste, such as calcium hydroxyapatite [2,8,10,13,27–39], calcium triphosphate [13,28,30–32,34,38,40–42], calcium diphosphate [42,43] and much less studied calcium monophosphate [42] or calcium acetate [44,45], have been reported, the novelty of our research lies in the use of eggshell halves and pieces instead of milled ones, the chemical transformation of eggshell waste calcium carbonate with diluted acids instead of those with

<table>
<thead>
<tr>
<th>Adherent Egg White</th>
<th>Eggshell Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins ~ 10–12%</td>
<td>Proteins ~ 60–90%</td>
</tr>
<tr>
<td>Ovalbumin ~ 54%</td>
<td>Collagen I-VX ~ 10–15%</td>
</tr>
<tr>
<td>Ovotransferrin ~ 12%</td>
<td>Proteoglycans ~ 30–50%</td>
</tr>
<tr>
<td>Ovomucoid ~ 1.5%</td>
<td>Glycosaminoglycans ~ 25%</td>
</tr>
<tr>
<td>Lysozyme ~ 3.4%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Structural parts of eggshell waste and their most valuable components. Contribution of each structurally different part of eggshell waste in total eggshell waste amount is approximated on the basis of reports by Strelec et al. [1], while the most valuable components of each part of eggshell waste comes from references [1,2,4,5,17–26].
higher percentages, the use of room temperature during the ESW chemical transformation into salt solutions and ESMs instead of higher ones, as well as the production of calcium salts as the major product and corresponding eggshell membranes as valuable by-products. Moreover, ESW-derived calcium acetate monohydrate and calcium hydrogen phosphate obtained in this research were fully examined in the prescribed demands regarding their suitability as food-grade additives.

2. Materials and Methods

2.1. Chemicals and Reagents

Transformation process of eggshell waste halves and pieces collected from households and local restaurant from city of Osijek (Croatia) into calcium acetate and calcium hydrogen phosphate was performed using 10% (w/v) acetic acid and 15% (w/v) o-phosphoric acid prepared from acetic acid (99.9%) purchased from VWR Chemicals (Leicestershire, UK), and 85% o-phosphoric acid from Carlo Erba (Emmendingen, Germany). Precipitation of calcium salts from a concentrated solutions of eggshell-derived calcium acetate and calcium hydrogen phosphate was performed using acetone obtained from Grammol (Zagreb, Croatia). Complexometric determination of calcium content in eggshell-derived calcium salts was performed using calconcarboxylic acid purchased from Acros Organics (Geel, Belgium) and disodium salt of ethylenediaminetetraacetic acid from Fischer Scientific (Leicestershire, UK). All other chemicals used in this research were of pro analysis purity.

2.2. Chemical Transformation of Eggshell Waste by Acetic and o-Phosphoric Acid

Chemical transformation of eggshell waste using acetic and o-phosphoric acid (Figure 2) was based on designed ESW transformation process using 5% (w/v) hydrochloric acid reported by our research group [1] but with some modifications. These included the use of higher acid (w/v) percentage, the lack of neutralization of the excess of acids and organic matter flocculation and drying of obtained Ca-salts at lower temperatures.

The starting point in designing ESW transformation process using acetic and o-phosphoric acid was the selection of right percentage (w/v) of acid used. This was based on the report by Torres-Mansilla and Delgado-Mejía [46], in which the authors used a four times greater stoichiometric amount of acetic and o-phosphoric acid than necessary to completely dissolve ESW calcium carbonate and release the eggshell membranes completely devoid of ESW calcium carbonate. Based on the amount of calcium carbonate present in ESW collected from households and restaurants reported in our previous research [1], the desired concentration of acetic and o-phosphoric acids necessary for complete removal of ESW calcium carbonate was calculated, and preliminary study for complete dissolution of ESW calcium carbonate, using a 7.5, 10 and 12.5% (w/v) solution of acetic acid (corresponding to ESW calcium carbonate and acetic acid molar ratio of: 1:2.4; 1:3.2; and 1:4) and 10, 12.5, and 15% (w/v) solution of o-phosphoric acid (corresponding to ESW calcium carbonate and o-phosphoric acid molar ratio of 1:2.3; 1:2.9; and 1:3.4), was performed by monitoring time-dependent solvation of ESW calcium carbonate every 15 min for 4 h. Based on the preliminary results, 10% (w/v) acetic or 15% (w/v) o-phosphoric acid chemical transformation process of ESW for 3 hours was selected as most adequate, and transformation process of ESW was performed in future examinations.

Chemical transformation of eggshell waste (ESW) started with washing 90 g of ESW (halves and pieces) with 900 mL of distilled water 3 × 30 min on orbital shaker IKA KS 260 basic (IKA, Staufen, Germany) set at 250 rpm min⁻¹ in order to remove possible impurities, as well as adherent egg white proteins [1]. Washed eggshells were mixed with 1350 mL of 10% (w/v) acetic or 15% (w/v) o-phosphoric acid, and the process of ESW chemical transformation was performed by mixing on a magnetic stirrer (LLG, Meckenheim, Germany) set at 500 rpm min⁻¹ for 3 h at room temperature. After complete conversion of eggshells calcium carbonate to corresponding calcium salts, separation of ESM from salt solutions was performed by filtration through a plastic mesh screen of 1 mm pore size. Obtained calcium salt solutions were subjected to clarification by two successive
steps of vacuum filtration through filter paper Whatman 114 followed by Whatman 1, and subsequent calcium acetate solution evaporation at 100–130 °C to the 1/10 of volume, or calcium hydrogen phosphate solution evaporation to the 1/2 of volume. Precipitation of crystals from saturated salt solutions cooled down to room temperature was performed by addition of 3 volumes of acetone. Precipitated crystals of calcium acetate and calcium hydrogen phosphate were separated from the rest of liquid by vacuum filtration through filter paper Whatman 1 and dried at 60 °C for 24 h. Afterwards, dried salts were ground in a mortar with a pestle and kept in sealed plastic containers until analysis.

**Figure 2.** Schematic representation of the proposed process of eggshell waste utilization using 10% (w/v) acetic and (15% w/v) o-phosphoric acid.
Eggshell membranes (ESMs), separated from calcium salt solutions, were washed, dried and milled using a procedure previously reported by Strelec et al. [1]. In brief, obtained ESMs were washed with distilled water and acetone, followed by separation via filtration, and drying of ESM between 18 and 24 h at 60 °C. Afterwards, dried ESMs were milled to a size lesser than 0.5 mm and used for analysis.

Suitability of the proposed ESW transformation process using 10% (w/v) acetic or 15% (w/v) o-phosphoric acid (Figure 2) was estimated by calculating mass and/or chemical yield.

Yield of ESW-derived Ca-salts was calculated via mass and chemical yield as shown in Equations (1) and (2):

\[
\text{Mass Yield} \left[ \frac{g}{100 \, g \, \text{ESW}} \right] = \frac{m(\text{Calcium salt})}{m(\text{Eggshell Waste})} \times 100 \tag{1}
\]

\[
\text{Chemical Yield} \% = \frac{m(\text{Calcium salt})}{m(\text{Calcium salt})_{\text{(theoretical)}}} \times 100 \tag{2}
\]

Yield of ESW-derived eggshell membranes (ESM) was calculated via mass yield as shown in Equation (3):

\[
\text{Mass Yield} \left[ \frac{g}{100 \, g \, \text{ESW}} \right] = \frac{m(\text{Eggshell Membranes})}{m(\text{Eggshell Waste})} \times 100 \tag{3}
\]

2.3. Characterization of Eggshell-Derived Calcium Salts

ESW-derived calcium acetate and calcium hydrogen phosphate powders were analyzed on dry matter content via drying at 105 °C until constant mass, calcium content via complexometric titration with 25 mM EDTA-2Na as titrant and calconcarboxylic acid as indicator, soluble protein content via Bradford method [47], as well as on pH of 10% solution, all performed according to Strelec et al. [1].

Fluoride content in the produced Ca-salts was determined using ion-selective electrode method, while As, Pb, Cd, Al and Hg content using ICP-MS technique, all performed by an external accredited laboratory. In addition, calcium and phosphorus content in ESW-derived calcium hydrogen phosphate powders were determined using an X-ray analysis performed by an external accredited laboratory.

Loss on drying at 155 °C, water insoluble content, formic acid and oxidizable impurities in ESW-derived calcium acetate, as well as loss on drying at 200 °C and loss on incineration at 800 °C in ESW-derived calcium hydrogen phosphate were determined according to FAO and EU regulative [48–52].

FTIR-ATR analysis of commercially available calcium acetate monohydrate and calcium hydrogen phosphate, and ESW-derived calcium acetate and calcium hydrogen phosphate was performed on Carry 630 FTIR ATR spectrometer (Agilent, Santa Clara, CA, USA) in the range of 650–4000 cm⁻¹.

2.4. Characterization of Eggshell Derived Eggshell Membranes

Chemical analysis of produced ESMs included determination of dry matter, total protein content using Kjeldahl method, and lipid content using a procedure previously reported by Strelec et al. [1]. FTIR-ATR analysis of acid-derived ESM was performed on Carry 630 FTIR ATR spectrometer (Agilent, Santa Clara, CA, USA) in the range of 650–4000 cm⁻¹.

2.5. Statistical Analysis

Statistical analysis of obtained data included determination of average values and standard deviations of at least three independent determinations performed in triplicate via Microsoft Excel 2019 MSO.
3. Results and Discussion

3.1. Eggshell Waste Transformation using 10% Acetic and 15% o-Phosphoric Acid

The present study investigated the possibility of chemical transformation of ESW using acetic and o-phosphoric acid into value-added products, including corresponding calcium salts and eggshell membranes completely devoid of remnants of ESW calcium carbonate. In this respect, it was necessary to design a transformation process that fulfils both demands. Therefore, the starting point in designing the ESW transformation process using acetic and o-phosphoric acid was the report by Torres-Mansilla and Delgado-Mejía [46], in which the authors used four times greater stoichiometric amount of acids than necessary to completely dissolve the ESW calcium carbonate. Regardless of the proposed molar ratio of ESW calcium carbonate and molar ratio of acids used by Torres-Mansilla and Delgado-Mejía [46] for the complete dissolution of ESW calcium carbonate, we used 7.5, 10 and 12.5% (w/v) solution of acetic acid and 10, 12.5, and 15% (w/v) solution of o-phosphoric acid, and monitored the complete removal of ESW calcium carbonate from ESMs every 15 min for 4 h. The obtained results (Figure 3) show that the use of 10% and 12.5% (w/v) acetic acid was suitable for the complete dissolution of the ESW calcium carbonate in 3 h, while in the case of o-phosphoric acid, the complete dissolution of ESW calcium carbonate was achieved in 3 h only when using 15% (w/v) o-phosphoric acid. Therefore, the complete solubilization of ESW calcium carbonate using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid in 3 h was used for the simultaneous production of calcium salt solutions and corresponding eggshell membranes.

![Figure 3](image-url)

**Figure 3.** Time dependent solubilization of eggshell waste calcium carbonate using 7.5, 10.0 and 12.5% (w/v) acetic acid (a,c) and 10.0, 12.5 and 15.0% (w/v) o-phosphoric acid (b,d). Results are expressed as average values of three independent determinations each performed in triplicate.
Following the aforementioned defined parameters for the simultaneous production of calcium salts and corresponding ESMs using acetic and \(\alpha\)-phosphoric acid, the ESW transformation process was performed using the procedure shown in Figure 2, and results of the three independent acid transformation processes are shown in Table 1.

### Table 1. The Yield of Eggshell Membranes, Calcium Acetate and Calcium Hydrogen Phosphate Produced from Eggshell Waste.

<table>
<thead>
<tr>
<th>Eggshell-Waste-Derived Product</th>
<th>Mass Yield [g/100 g ESW]</th>
<th>Chemical Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% (w/v) acetic-acid-derived ESM</td>
<td>2.90 ± 0.14</td>
<td>n. a. (^3)</td>
</tr>
<tr>
<td>15% (w/v) (\alpha)-phosphoric-acid-derived ESM</td>
<td>2.94 ± 0.08</td>
<td>n. a. (^3)</td>
</tr>
<tr>
<td>ESW-derived Calcium Acetate Monohydrate</td>
<td>111.41 ± 2.13</td>
<td>79.16 ± 1.18 (^4)</td>
</tr>
<tr>
<td>ESW-derived Calcium Hydrogen Phosphate</td>
<td>77.06 ± 6.21</td>
<td>71.36 ± 5.58 (^5)</td>
</tr>
</tbody>
</table>

\(^{12}\) mass yield calculated as shown in Equations (1) and (3). \(^3\) not applicable. \(^4\) chemical yield calculated as shown in Equation (2) but on the basis of calcium acetate monohydrate. \(^5\) chemical yield calculated as shown in Equation (2) but on the basis of calcium hydrogen phosphate.

The designed ESW transformation process (Figure 2) has been proven to be suitable for the simultaneous production of calcium salts and corresponding eggshell membranes (Table 1). The eggshell’s calcium carbonate was completely dissolved with the use of 10% (w/v) acetic and 15% (w/v) \(\alpha\)-phosphoric acid (Figure 3b,d) into the corresponding Ca-salts and ESMs completely devoid of the calcium carbonate remnants, at ESW conversion rate of 22.22 g/L h. The chemical transformation of 100 g of ESW containing approximately 85% of dry matter using 10% (w/v) acetic acid resulted in 111.41 ± 2.13 g of calcium acetate monohydrate and 2.90 ± 0.14 g of ESM, and a chemical yield in calcium acetate monohydrate of 79.16 ± 1.18%. On the other hand, the transformation of 100 g of ESW using 15% (w/v) \(\alpha\)-phosphoric acid yielded 77.06 ± 6.21 g of calcium hydrogen phosphate and 2.94 ± 0.08 g of ESM, with a chemical yield in calcium hydrogen phosphate of 71.36 ± 5.58%.

Chemical reactions between ESW calcium carbonate and 10% (w/v) acetic and 15% (w/v) \(\alpha\)-phosphoric acid are shown by Equations (4) and (5):

\[
\text{CaCO}_3 + 2\text{CH}_3\text{COOH} \rightleftharpoons \text{Ca}\{(\text{CH}_3\text{COO})_2\} + \text{CO}_2 + \text{H}_2\text{O} \quad (4)
\]

\[
\text{CaCO}_3 + \text{H}_3\text{PO}_4 \rightleftharpoons \text{CaHPO}_4 + \text{CO}_2 + \text{H}_2\text{O} \quad (5)
\]

The possibility of the production of calcium acetate using ESW have been reported by two patents [53,54] and two scientific publications [44,45]. The reported mass yield of calcium acetate using ESW was 70% [53], 88% [45], 95.3% [54], and 95.5% [44] which was quite lower than mass yield (111.41 ± 2.13 g/100 g of ESW) achieved in this research. Moreover, it should be pointed out that none of the aforementioned authors perform the simultaneous production of Ca-acetate and corresponding ESMs from eggshell waste.

Numerous reports deal with the transformation of ESW using \(\alpha\)-phosphoric acid [2,8,10,13,27–43,55], but the possibility of the production of calcium hydrogen phosphate from ESW has been reported by only one scientific report [42] and one patent [56]. Laohavisuti et al. [42] prepared calcium hydrogen phosphate of high purity/yield of 99.63% using ESW, which is much higher than the mass yield observed in our research (77.06 ± 6.21%), but authors used an ESW calcination at 500 °C for two hours where all organic matter was incinerated, and a much greater \(\alpha\)-phosphoric concentration (50%) for the solvation of ESW calcium carbonate. Ash and Scheideler [56], in their patent, used 85% \(\alpha\)-phosphoric acid diluted with water in a ratio of 1:2, where ESW-derived foam obtained during the transformation process was dried in order to obtain calcium hydrogen phosphate with an approximate yield of 94% per dry matter. While both aforementioned reports clearly achieved a greater yield in calcium hydrogen phosphate than that observed in our research (~77%), it should be pointed out that the authors did not perform the simultaneous production of calcium hydrogen phosphate and corresponding ESM. Moreover, authors...
used a much higher o-phosphoric acid percentage for ESW transformation than that used in our research (50% or ~30%, contrary to 15% used in our research), and in the case of Laohavisutti et al. [42] an energetically demanding process of ESW calcination at 500 °C was used.

The production of ESMs from ESW using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid transformation process (Figure 2) resulted in a similar mass yield of 2.90 ± 0.14 and 2.94 ± 0.08 g per 100 g of ESM (Table 1). The obtained results were in accordance with our previous report [1], where 2.84 ± 0.16 g of ESM per 100 g of originally used ESW was obtained using a 5% (w/v) hydrochloric acid treatment of ESW, but were lower than those reported in the patent of Thoroski [57], where an ESM yield of 6 to 10% per ESW was obtained by ESW mechanical treatment, including milling and sieving.

Since data on the possibility of ESW utilization using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid (Table 1) clearly showed that the proposed process of ESW utilization (Figure 2) has a great potential to be used for the production of calcium salts and ESMs, it was necessary to characterize the obtained ESMs and derived calcium salts.

3.2. Characterization of Eggshell-Waste-Derived Eggshell Membranes

ESW-derived ESMs obtained through the transformation of ESW halves and pieces using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid are shown in Figure 4a,c, and milled ones in Figure 4b,d. ESW-derived ESMs differentiated in their color regarding the acid used. Those obtained using 15% (w/v) o-phosphoric acid treatment of ESW showed a somewhat whiter intensity than those obtained using 10% (w/v) acetic acid treatment (Figure 4b,d), indicating possible differences in the ESMs chemical composition.

![Figure 4. Eggshell Waste Derived Eggshell Membranes after Drying (a,c) and Milling (b,d). Legend: (a,b)—10% (w/v) acetic-acid-derived ESM; (c,d)—15% (w/v) o-phosphoric-acid-derived ESM.](image)

The basic chemical composition of acid-derived ESW eggshell membranes and the raw ones is shown in Table 2. ESM obtained through ESW transformation using 10% (w/v) acetic acid had a somewhat higher amount of total proteins (96.48 ± 1.75 g per 100 g of ESW per dry weight basis) than those produced using 15% (w/v) o-phosphoric acid (93.36 ± 1.69 g per 100 g of ESW per dry weight basis), but both had a much higher protein content than the raw one (84.26 ± 0.77 g per 100 g of ESW per dry weight basis). On the other hand, the lipid content in ESMs obtained via acid transformation were slightly lower than those observed in raw EMS (Table 2). However, it should be noted that lipids were present in an amount lesser that 1%.

<table>
<thead>
<tr>
<th>Table 2. Chemical Composition of Obtained Eggshell Membranes 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Dry matter [g/100 g]</td>
</tr>
<tr>
<td>Proteins [g/100 g d.w.b.] 2</td>
</tr>
<tr>
<td>Lipids [g/100 g d.w.b.] 2</td>
</tr>
</tbody>
</table>

1 result present mean value ± standard deviation of five independent determinations. 2 d.w.b.—dry weight basis.
Since the basic chemical analysis of the amount of total proteins and lipids of obtained ESMs (Table 2) indicated some differences in chemical composition, it was necessary to elucidate which components of raw ESM has been extracted using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid. This was performed via FTIR-ATR analysis as shown in Figure 5.

FTIR-ATR analysis (Figure 5) revealed that raw eggshell membranes exhibited a higher intensity for all detected absorbance peaks than ESMs obtained using a 10% (w/v) acetic and 15% (w/v) o-phosphoric acid ESW treatment. The observed differences in the amount of lipids between raw and 10% (w/v) acetic and 15% (w/v) o-phosphoric-acid-derived ESMs (Table 2) were proven via decreased peak intensity with a wavenumber of 2919 cm$^{-1}$ (Figure 5) attributable to the C-H stretching vibration of endogenous lipids [58]. Strikingly, much higher amounts of total proteins were detected in EMS obtained using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid treatment than those in raw ESM (Table 2), which was apparently opposite to the observed FTIR-ATR absorbance intensity of peaks attributable to the protein presence in examined ESM samples (Figure 5). While the absorbance intensity peaks in FTIR-ATR spectra (Figure 5) detected at 3306, 1640 and 1152 cm$^{-1}$ could be attributed to the N-H and C-N stretching vibrations of amide bonds present in proteins including collagen fibers (3360 and 1640 cm$^{-1}$), and N-H deformation and C-N stretching vibration of amide bonds present in proteins (1152 cm$^{-1}$), N-H and O-H stretching vibrations detected at 3306 cm$^{-1}$ could be also attributed to the presence of polysaccharides, as well as C=O and C-N stretching vibrations of amide bonds present in glycosaminoglycans containing N-acetyl-glucosamine and N-acetyl-galactosamine including hyaluronic acid (1640 cm$^{-1}$), and C-O stretching vibration in carbohydrates (1152 cm$^{-1}$). Moreover, the absorbance intensity of peaks detected in the region between 995 and 1077 cm$^{-1}$ (Figure 5) could be assigned to the C-O stretching vibration of constitutive polysaccharides [1,58–61]. Thus, the observed differences in the estimated total protein content determined via the Kjeldahl method (Table 2) and FTIR-ATR spectra of examined ESMs (Figure 5) might be explained by imperfection of both of methods used. While the Kjeldahl method for the determination of total protein content detects not only the amount of proteins but also all amino-containing compounds, including glycosaminoglycans present in ESMs, FTIR-ATR analysis detects intensity of stretching and deformation vibrations.
of N-H and C-N of amide bonds in proteins or amino-containing polysaccharides, C=O and C-N stretching vibrations of amide bonds present in glycosaminoglycans, as well as C-O stretching vibration of constitutive polysaccharides. Based on the aforementioned results, it seems quite possible that constitutive oligo- and polysaccharides, as well as some of constitutive glycosaminoglycans of raw ESMs, have been extracted during the ESW transformation using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid, leading to the observed increase in the amount of total proteins (Table 2) and the decreased intensity of peaks in FTIR-ATR spectra (Figure 5).

3.3. Characterization of Eggshell-Waste-Derived Calcium Acetate and Calcium Hydrogen Phosphate

The transformation process of ESW using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid (Figure 2) ended with the production of calcium acetate monohydrate and calcium hydrogen phosphate powders shown in Figure 6.

![Figure 6. Eggshell-Waste-Derived Calcium Acetate Monohydrate (a) and Calcium Hydrogen Phosphate (b).](image)

Both obtained ESW-derived Ca-salts were quite white (Figure 6), indicating that produced salts might be of high purity. Therefore, food-grade additive criteria analysis of calcium acetate and calcium hydrogen phosphate prescribed by the FAO [50,51] and EU [48,49] were applied for the analysis of ESW-derived calcium acetate and calcium hydrogen phosphate. Physicochemical analysis of ESW-derived calcium acetate monohydrate in comparison with the demands for food-grade additive is shown in Table 3, and ESW-derived calcium hydrogen phosphate in Table 4.

### Table 3. Physicochemical Analysis of Eggshell-Derived Calcium Acetate Monohydrate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Food Additive Criteria ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter [%]</td>
<td>98.78 ± 0.94</td>
<td>n. c. ²</td>
</tr>
<tr>
<td>Calcium [% d.w.b.] ³</td>
<td>22.46 ± 0.43</td>
<td>n. c.</td>
</tr>
<tr>
<td>pH of 10% solution</td>
<td>7.01 ± 0.26</td>
<td>6–9</td>
</tr>
<tr>
<td>Soluble proteins [g/100 g d.w.b.]</td>
<td>0.15 ± 0.09</td>
<td>n. c.</td>
</tr>
<tr>
<td>Loss on Drying [%]</td>
<td>2.60 ± 1.39</td>
<td>&lt;11</td>
</tr>
<tr>
<td>Water insoluble [%]</td>
<td>0.6 ± 0.08</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Formic acid and oxidizable impurities [mg/kg]</td>
<td>4392.42 ± 337.86</td>
<td>&lt;1000</td>
</tr>
<tr>
<td>Fluoride [mg/kg]</td>
<td>&lt;10</td>
<td>n. c.</td>
</tr>
<tr>
<td>As [mg/kg]</td>
<td>&lt;1</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Pb [mg/kg]</td>
<td>&lt;1</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Hg [mg/kg]</td>
<td>&lt;0.015</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

¹ Food Additive Criteria of FAO [50] and EU [49]. ² n. c.—not included in criteria for additive. ³ d.w.b.—dry weight basis.
Table 4. Physicochemical Analysis of Eggshell-Derived Calcium Hydrogen Phosphate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Food Additive Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter [%]</td>
<td>99.47 ± 0.19</td>
<td>n. c. ²</td>
</tr>
<tr>
<td>Calcium [% d.w.b.] ³</td>
<td>25.64 ± 0.68</td>
<td>n. c.</td>
</tr>
<tr>
<td>Calcium [% d.w.b.] ⁴</td>
<td>23.57 ± 0.07</td>
<td>n. c.</td>
</tr>
<tr>
<td>Phosphorus [% d.w.b.] ⁴</td>
<td>17.25 ± 0.20</td>
<td>n. c.</td>
</tr>
<tr>
<td>pH of 10% solution</td>
<td>4.13 ± 0.15</td>
<td>n. c.</td>
</tr>
<tr>
<td>Soluble proteins [g/100 g d.w.b.]</td>
<td>not detected</td>
<td>n. c.</td>
</tr>
<tr>
<td>Loss on Drying [%]</td>
<td>1.40 ± 0.16</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Fluoride [mg/kg]</td>
<td>&lt;10</td>
<td>&lt;50</td>
</tr>
<tr>
<td>As [mg/kg]</td>
<td>&lt;0.36</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cd [mg/kg]</td>
<td>&lt;0.04</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Pb [mg/kg]</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Hg [mg/kg]</td>
<td>&lt;0.01</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Al [mg/kg]</td>
<td>&lt;26.99</td>
<td>&lt;80</td>
</tr>
</tbody>
</table>

¹ Food Additive Criteria of FAO [51] and EU [49]. ² n. c.—not included in criteria for additive. ³ determined by complexometric titration; d.w.b.—dry weight basis. ⁴ determined by X-ray analysis.

ESW-derived Calcium Acetate Monohydrate contained 22.46 ± 0.43% of calcium per dry weight basis. This was quite close to the theoretical amount of calcium in Ca(CH₃COO)₂ × H₂O of 22.75%. While loss on drying at 155 °C, pH of 10% solution and amount of As, Pb and Hg completely fulfilled the criteria prescribed by the FAO [50] and EU [49], the amount of water insoluble, as well as formic acid and oxidizable impurities content were out of the prescribed criteria, indicating that some additional purification steps are necessary to obtain calcium acetate of food-grade additive purity. In addition to the prescribed criteria for additive purity, the amount of soluble proteins present in the produced calcium acetate monohydrate was determined, and based on the obtained results (Table 3), it can be concluded that soluble proteins were present in the minor amount of 0.15 ± 0.09 g per 100 g of Ca(CH₃COO)₂ × H₂O.

ESW-derived calcium hydrogen phosphate contained 25.64 ± 0.68% of calcium determined via complexometric titration and 23.57 ± 0.07% of Ca and 17.25 ± 0.20% of phosphorus determined by X-ray analysis (Table 4), indicating that the obtained CaHPO₄ is probably present in anhydrous form. This was additionally proven by the loss on drying and loss on incineration (Table 4), where ESW-derived CaHPO₄ fulfilled the criteria for calcium hydrogen phosphate anhydrous prescribed by the FAO [51] and EU [49]. Moreover, based on the analysis of fluoride, As, Cd, Pb, Hg and Al content, the obtained ESW-derived CaHPO₄ meet all criteria to be used as a food-grade additive (Table 4).

FTIR-ATR analysis of commercial (Figure 7a) and ESW-derived calcium acetate monohydrate of three production batches (Figure 7b) revealed an identical peak position and similar absorbance intensities attributable to the presence of symmetric and asymmetric vibration stretching of C-O bonds in acetate detected at 1602, 1528, 1446, 1416, 1028 and 670 cm⁻¹ [62]. This additionally proved that ESW-derived Ca(CH₃COO)₂ × H₂O is of high purity, and dominantly present in monohydrate form.

FTIR-ATR spectra of three independent production batches of ESW-derived calcium hydrogen phosphate (Figure 7d) and those of commercial CaHPO₄ anhydrous (Figure 7c), have shown identical peak positions and similar absorbance intensities of major peaks centered at 883, 983, 1058 and 1129 cm⁻¹ attributable to the symmetric stretching of P=O bonds in phosphate ion (983, 1058 cm⁻¹), asymmetric stretching of P=O bonds in phosphate ion (1129 cm⁻¹) and deformation vibrations of P-O-H group in hydrogen phosphate ion (883 cm⁻¹) [63–65]. This additionally proved that ESW-derived CaHPO₄ is of high purity and dominantly present in anhydrous form.
While the yield of the produced calcium acetate monohydrate and calcium hydrogen phosphate (Table 1) was quite satisfactory, question remains whether omitting the step of addressing in the future research.

The obtained Ca(CH₃COO)₂ × H₂O and CaHPO₄ salts were white (Figure 6) and their FTIR-ATR spectra (Figure 7) were identical to the commercial calcium acetate monohydrate and calcium hydrogen phosphate anhydride which indicated their high purity.

Based on all that was aforementioned, it should be clearly stated that the proposed ESW transformation process using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid shows a great potential to be used for the subsequent production of corresponding calcium salts and eggshell membranes. The ESW chemical transformation using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid yielded 111.41 ± 2.13 g of calcium acetate monohydrate and 2.90 ± 0.14 g of eggshell membranes per 100 g of ESW containing ~85% dry matter, and 77.06 ± 6.21 g of calcium hydrogen phosphate and 2.94 ± 0.08 g of ESM (Table 1). While the yield of the produced calcium acetate monohydrate and calcium hydrogen phosphate (Table 1) was quite satisfactory, question remains whether omitting the step of neutralization of the excess of 10% (w/v) acetic and 15% (w/v) o-phosphoric acid, previously reported by our research group for the production of ESW-derived calcium chloride using 5% (w/v) hydrochloric acid [1], was the right choice, but this is a question which should be addressed in the future research.

The obtained Ca(CH₃COO)₂ × H₂O and CaHPO₄ salts were white (Figure 6) and their FTIR-ATR spectra (Figure 7) were identical to the commercial calcium acetate monohydrate and calcium hydrogen phosphate anhydride which indicated their high purity.
This was confirmed via physicochemical analysis (Tables 3 and 4), where the obtained Ca(CH$_3$COO)$_2 \times$ H$_2$O fulfilled almost all the prescribed criteria for food-grade additive purity [49,50], while CaHPO$_4$ fulfilled all [49,51]. ESW-derived calcium acetate monohydrate had a higher amount of insoluble matter and formic acid and oxidizable impurities (Table 3) than prescribed for food-grade additives [49,50], attributable to the presence of degradation products of unreacted acetic acid formed during the salt solution evaporation. Therefore, it seems quite possible that the neutralization of the excess of acetic acid using calcium hydroxide reported in our previous research [1], dealing with the neutralization of excess of hydrochloric acid, might be quite useful for achieving Ca(CH$_3$COO)$_2 \times$ H$_2$O of additive purity and even much higher yield. Nevertheless, the ESW-derived calcium acetate monohydrate in our research was produced in much higher yield than ESW calcium acetate reported by Yao et al. [44], Lizhong [53], Zhang et al. [54] and Nobre et al. [45] and with concomitant production of eggshell membranes as valuable by-products. On the contrary, the ESW-derived CaHPO$_4$ (Table 4) fulfilled all criteria for additive purity [49,51], but its yield (Table 1) was lower than those reported by Laohavisuti et al. [42] and Ash and Scheideler [56]. However, CaHPO$_4$ purity of food-grade additive (Table 4), as well as subsequent production of ESM during ESW transformation process using 15% o-phosphoric acid (Figure 2) might justify its production.

The transformation of ESW calcium carbonate to calcium acetate monohydrate and calcium hydrogen phosphate (Figure 2) yielded a small but valuable amount of ESMs (Table 1) enriched in the protein content (Table 2) and depleted of constitutive polysaccharides and at least some glycosaminoglycans (Figure 5). Although the amount of produced eggshell membranes were ~2.9%, the significant value of ESMs is reflected by their potential use as food supplement for the alleviation of joint pains [66], as well as suitable material for enzyme immobilization [7], justifying their concomitant production.

4. Conclusions

The present study shows the possibility of eggshell waste utilization via chemical transformation with 10% (w/v) acetic and 15% (w/v) o-phosphoric acid performed at a laboratory scale level but using the most common industrial operations. The proposed eggshell waste acid transformation process shows great potential for the production of calcium acetate monohydrate and calcium hydrogen phosphate of additive purity and corresponding eggshell membranes as valuable by-products intended as food supplements and/or enzyme immobilization carriers. However, its possible application on the industrial scale level clearly needs additional “fine-tuning” research. Therefore, future perspectives of the proposed ESW transformation process using 10% (w/v) acetic and 15% (w/v) o-phosphoric acid should include the possibility of introduction of neutralization of the excess of acid used, scaling-up of the transformation process, the use on industrial eggshell waste, as well as a potential replacement of Ca-salts acetone precipitation via spray-drying. Based on everything aforementioned, this should contribute to the development of the process of complete eggshell waste utilization approaching a “zero waste” model.

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