Effect of calcium-binding compounds in acid whey on calcium removal during electrodialysis

Nielsen, Emilie N.; Skibsted, Leif H.; Yazdi, Saeed R.; Merkel, Arthur; Ahné, Lilia M.

Published in:
Food and Bioproducts Processing

DOI:
10.1016/j.fbp.2021.11.008

Publication date:
2022

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
Effect of calcium-binding compounds in acid whey on calcium removal during electrodialysis

Emilie N. Nielsen\textsuperscript{a}, Leif H. Skibsted\textsuperscript{a}, Saeed R. Yazdi\textsuperscript{b}, Arthur Merkel\textsuperscript{c,d}, Lilia M. Ahrné\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a} Department of Food Science, University of Copenhagen, Rolighedvej 26, 1958 Frederiksberg, Denmark
\textsuperscript{b} Arla Foods Amba, Agro Food Park 19, 8200 Aarhus N, Denmark
\textsuperscript{c} MemBrain s.r.o. (Membrane Innovation Centre), Pod Viníci 87, 471 27 Stráž pod Ralskem, Czech Republic
\textsuperscript{d} Institute for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Studentská 2, 461 17 Liberec, Czech Republic

\section*{Article Info}
Article history:
Received 18 August 2021
Received in revised form 13 November 2021
Accepted 25 November 2021
Available online 1 December 2021

Keywords:
Calcium
Electrodialysis
Acid whey
Calcium-binding
Lactate
Glucanate
Citrate
Sugars

\section*{Abstract}
Electrodialysis (ED) is an efficient technology to recover calcium from acid whey (AW). The effects of calcium-binding compounds on demineralization and calcium recovery were studied during the ED process, using model solutions imitating AW. The presence of calcium-binding compounds with a higher association constant significantly decreases the extent of the removal of calcium. Lactate, present in high concentrations in AW, has a larger impact than citrate, on removing calcium during ED, although citrate has a higher affinity for calcium-binding. The largest impact of pH was observed for citrate, and at pH of AW, less calcium is bound. Sugars were found to have minor effects, but an additive effect is observed when several calcium-binding compounds are present. Measurement of bound and ionic calcium showed that calcium dissociates during ED reestablishing chemical equilibria except for citrate, which at pH of AW forms the uncharged complex CaH\textsubscript{2}Cit not being transported during ED.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of Institution of Chemical Engineers. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Acid whey is a side-stream from the production of acidified dairy products such as skyr, cottage cheese, quark, and Greek yoghurt. It contains high concentrations of valuable minerals, lactic acid, and sugars but has a low protein content compared to sweet whey (Talebi et al., 2019; Nielsen et al., 2021). The amount of acid whey produced is massive and their use limited, since acid whey needs to be further processed before it can be used as ingredient in other food products. Acid whey is difficult to convert into powder because it contains high lactic acid concentrations, which hinders lactose crystallisation by lowering its glass-transition temperature (Saffari and Langrish, 2014; Chandrapala et al., 2016). Furthermore, discarding acid whey is expensive for the dairies and causes a loss of valuable compounds such as minerals and organic acids. Fractionation and isolation of minerals from acid whey is therefore an important route for the dairy industry to valorise acid whey.

Electrodialysis (ED) has recently been shown to be an efficient and sustainable technology to isolate charged compounds, such as minerals and lactate, the conjugate base of lactic acid, from acid whey (Chen et al., 2016; Nielsen et al., 2021). ED is an electrochemical filtration process, where an applied electrical potential allows the transportation of ions from one solution to another through ion-selective membranes (Rasmussen et al., 2020). However, the composition of acid whey has been shown to influence the ED process and the recovery of minerals and lactate (Nielsen et al., 2021).

Calcium is an essential mineral in the human diet. It contributes to vital processes such as bone metabolism, and as an intracellular mes-
senger among cells, and regulates enzyme activity (Bromer and Pansu, 1999). In many societies, intake of dairy products is important to ful-
fill the required daily intake of around 1.0 g of calcium, and therefore, calcium is one of the most valuable compounds found in acid whey. It is well known that calcium ions can bind to other compounds such as phosphates, α-lactalbumin, some peptides, conjugated bases of organic acids, like lactate, and lactose and its corresponding monosaccharides (Hiraoka et al., 1980; Vavrusova et al., 2013; Jiang et al., 2021a,b). It is our working hypothesis that binding of calcium ions to lactose, glucose, glucose/galactose, lactate, citrate or gluconate will affect the removal of calcium during ED. Lactose is found in all acid whey streams except in lactose-free productions where lactose is cleaved to glucose and galac-
tose by enzymatic hydrolysis (González-Siso, 1996). Citrate, lactate, and gluconate are also naturally found in or added during the production of acidified dairy products. Lactate forms during milk fermentation, while gluconate is the conjugated base of gluconic acid, an oxidation prod-
uct from glucose, which appears during the chemical acidification of milk using delta-gluconolactone as a slow chemical acidifier (Vavrusova et al., 2013).

The affinity of a compound to calcium may be quantified using the association constant, \( K_a \) (Davies, 1962). Citrate has a very high affinity to calcium and, in excess, at higher pH, binds all calcium (Vavrusova and Skibsted, 2016). Lactose, glucose and galactose have shown to have an affinity to calcium, which is approx. 1000 times lower than citrate (Vavrusova et al., 2018; Jiang et al., 2021b). These differences in calcium-binding are expected to affect the recovery of calcium during ED since free calcium ions will be recovered faster than calcium ions bound to other compounds in complexes. Knowledge of the effect of calcium-binding compounds on the recovery of calcium ions during ED is therefore important to design pretreatments of the acid whey that increase the effectiveness of the ED process and, accordingly, the recovery of the valuable minerals like calcium.

This study aims to understand the effects of selected calcium-
binding compounds typically found in acid whey on calcium recovery into the concentrate by ED. Thus, we have investigated the percentage demineralization and recovery of calcium ions from model solutions imitating acid whey composition in terms of lactose, glucose, and equimolar glucose/galactose mixture, lactate, gluconate and citrate during the ED process. Furthermore, the effect of pH and concentration was also evaluated for selected calcium-binding compounds.

2. Materials and methods

2.1. Model solutions

This work was conducted using diluate aqueous solutions as models for acid whey. The composition of all model solutions shown in Table 1. Lactose, galactose, ≥99.5% D-(+)-glucose, ≥99% tri-sodium-citrate-dihydrate, 97% sodium-glucanate and 85% lactic acid were from Sigma-Aldrich (St. Louis, Mis-
souri, USA) and used for creating the model solutions. Besides the calcium-binding compound, all solutions also contained 37 mM calcium (≥97% CaCl₂, Honeywell, Denmark). Solutions with 37 mM calcium (≥97% CaCl₂, Honeywell, Denmark) were used as references. The chemicals were dissolved in MilliQ water. The pH of the solutions was adjusted by 37% HCl (Fisher Scientific, Leics, UK) and 4.0 M NaOH (VWR Chemicals, Fontenay-sous-Bois, France).

2.2. Electrodialysis configuration

The ED experiments were conducted using an EDR-Z/2-10/0.8 ED unit (MemBrain s.r.o., Stráž pod Ralskem, Czech Repub-
ic). The ED unit was equipped with 10 pairs of heterogeneous AM-PES (anionic) and CM-PES (cationic) ion-exchange mem-
brates Ralex® (MEGA a.s., Stráž pod Ralskem, Czech Republic) in CEM-AEM-CEM configuration with a total active surface area of 6.4 × 10⁻² m². A description of the properties of the mem-
brates can be found in Merkel and Ashraf (2019). Fig. 1 shows a schematic representation of the ED configuration. The elec-
trical potential was applied to Pt coated titanium electrodes, and between two Pt wires connected to the end of the stack, the voltage on the membrane stack was measured. The ED unit was operated with a constant voltage of 10 V across the stack. However, the total applied voltage ranged from 11.8 V to 14.6 V; hence the voltage difference corresponds to the actual ED stack resistance. 20 g/L > 99% Na₂SO₄ (Merck KGaA, Darmstadt, Germany) was used as electrolyte solution while deionised water (\( \sigma \leq 10 \mu S/cm \)) was used as the initial concen-
trate. The diluate consisted of the model systems described in Table 1. The diluate, concentrate and electrolyte solutions were recirculated in three separated tanks with constant flows of 55 L/h. The pH, conductivity and temperature of the diluate and concentrate were measured in-line by HQ40d multime-
ters from Hach (Hach, Loveland, Co, USA). The initial mass of diluate was 2.0 kg, while the initial mass of concentrate was 0.75 kg, to increase the current faster in the beginning of the experiments. Each experiment lasted for 120 min Sam-
ple were collected from the diluate every 30 min and frozen at –20°C at the end of the experiments. The membranes were examined after each run and no fouling/scaling was visually observed, however no microscopy observation was done.

2.3. Concentration of calcium and sodium

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) 5100 system including a SPR 4 auto-sampler from Agilent Technologies, was used to determine calcium and sodium concentrations. For detection, wavelengths of 396.85 nm and 588.99 nm were used. For quantification of the two elements, standards obtained from Sigma-Aldrich were used. Before analysis, 1.00 mL samples were digested in 2 mL 37% (w/w) HCl (Merck KGaA, Darmstadt, Germany) and 8 mL 65% (w/w) HNO₃ (Merck KGaA, Darmstadt, Germany) using a Multiwave Gom from Anton Paar (Graz, Austria).

2.4. Ionic calcium

The ionic calcium concentrations were measured by a calcium ion selective electrode ISE25Ca with a reference REF 251 elec-
trode from Radiometer (Copenhagen, Denmark). The electrode was standardised using ≥97% CaCl₂ (Honeywell, Vallen

bæk, Denmark) solutions with concentrations of 6.00 × 10⁻² M, 3.50 × 10⁻² M and 1.00 × 10⁻⁴ M. The ionic strengths of the standard solutions were adjusted with KCl (J.T. Baker, Deventer, Holland) according to the average ionic strength of the samples. The Russell equation was used to determine the average ionic strength (Belessiotis et al., 2016):

\[
I = 1.6 \times 10^{-5} \times \sigma
\]

where \( I \) is the ionic strength (M) and \( \sigma \) is the conductivity (μmho/cm). The samples were measured in triplicates at 25°C.

2.5. Determination of association constants

The association constants based on activity for the binding of Ca²⁺ to a ligand were derived from association constants based on concentration found in the literature. For doing this,
Table 1 – Overview of model solutions used as dilute for electrodialysis. All solutions contain 37 mM calcium in addition to the compounds described in the table.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference solutions</td>
<td>Calcium</td>
<td>Not adjusted (pH 9.5)</td>
</tr>
<tr>
<td>Acid whey model†</td>
<td>Calcium</td>
<td>Adjusted to 4.5</td>
</tr>
<tr>
<td>Conjugated bases</td>
<td>Lactic acid, gluconate, citrate, lactose</td>
<td>Adjusted to 4.5</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>Adjusted to 4.5</td>
</tr>
<tr>
<td></td>
<td>Citrate</td>
<td>Not adjusted (pH 9.0)</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>Adjusted to 4.5</td>
</tr>
<tr>
<td></td>
<td>Glucose/Galactose (equimolar)</td>
<td>Not adjusted (pH 8.3)</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>Adjusted to 4.5</td>
</tr>
</tbody>
</table>

* pH = 4.5 and concentration correspond to acid whey.

![Fig. 1 – Schematic representation of the ED.](image)

the activity coefficients used in the literature were estimated by Davies equation (Davies, 1962):

\[
Ca^{2+} + L^- \rightleftharpoons CaL^+ \quad \text{(2)}
\]

\[
K_a = \frac{a_{CaL^+}}{a_{Ca^{2+}} \times a_{L^-}} \quad \text{(3)}
\]

\[
\log y^{2+} = -A_{DH} \times z^2 \left( \frac{\sqrt{I}}{I + \sqrt{I}} - 0.30 \times I \right) \quad \text{(4)}
\]

where \( y^{2+} \) is the Ca ion activity coefficient, \( A_{DH} \) is 0.51 at 25 °C, and \( z = 2 \) is the charge of calcium. The association constant \( K_a \) was then calculated based on the complex formation:

\[
K_a = K_c \frac{y^{2+}}{y^{2+} + y^-} = \frac{K_c}{y^{2+}} \quad \text{(5)}
\]

where \( K_c \) is the association constant based on concentration (L/mol):

\[
K_c = \frac{[CaL^+]}{[Ca^{2+}] \times [L^-]} \quad \text{(6)}
\]

<table>
<thead>
<tr>
<th>Compound</th>
<th>( K_a )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>10.0</td>
<td>Jiang et al. (2021b)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.3</td>
<td>Jiang et al. (2021b)</td>
</tr>
<tr>
<td>Galactose</td>
<td>7.6</td>
<td>Jiang et al. (2021b)</td>
</tr>
<tr>
<td>Lactate</td>
<td>20.5</td>
<td>Vavrusova et al. (2013)</td>
</tr>
<tr>
<td>Gluconate</td>
<td>35.8</td>
<td>Vavrusova et al. (2013)</td>
</tr>
<tr>
<td>Citrate</td>
<td>1.6 \times 10^4</td>
<td>Vavrusova et al. (2018)</td>
</tr>
<tr>
<td>Hydrogen citrate</td>
<td>2.3 \times 10^3</td>
<td>Vavrusova et al. (2018)</td>
</tr>
<tr>
<td>Dihydrogen citrate</td>
<td>14</td>
<td>Davies and Hoyle (1953)</td>
</tr>
</tbody>
</table>

\( K_a \), association constant based on activity calculated from values found in the literature for concentration-based constants, \( K_c \).

2.6. Statistical analyses

The results in this work are represented as an average ± standard deviation. One way ANOVA was used to determine significant differences (\( P < 0.05 \)).

3. Results and discussion

3.1. Association constants

The calculated association constants show the differences in affinity to calcium of the selected compounds (Table 2). The
compounds with the lowest affinity to calcium are the sugars, where glucose has the lowest association constant of 4.3 (Jiang et al., 2021b). The compound with the highest affinity to calcium is citrate that has an association constant of $1.6 \times 10^6$ (Vavrusova et al., 2018). Hence, citrate has an affinity to calcium which is more than 4000 times higher than the affinity of glucose to calcium. However, the chemical form in which citrate is present depends on the pH (Bates and Pinching, 1949). The pKa values of citrate are $pK_{A1} = 3.13$, $pK_{A2} = 4.76$, $pK_{A3} = 6.4$ and $pK_{A4} = 11.6$ (Bates and Pinching, 1949; Al-Khaldi et al., 2007). The pH of the citrate solutions used in this study are 4.5 and 9.0, and therefore mainly $pK_{A2}$ and $pK_{A3}$ determined the acid/base distribution. The tri-negative citrate ion dominating above pH 6.4 has the highest affinity for metal ions. Lowering the pH decreases the concentration of the tri-negative citrate due to protonation. Below pH 4.76 more than half of the citrate is present as dihydrogen citrate, which has an association constant of 14 (Davies and Hoyle, 1953).

Lactate and gluconate have association constants, which are 2–8 times higher than the association constants of the sugars and dihydrogen citrate (Vavrusova et al., 2013). Binding of calcium is expected for these compounds; however, the amount of bound calcium ions is expected not to be as high as for citrate.

The demineralization and calcium removal from solutions containing each of these compounds at the concentrations and pH commonly found in acid whey are shown in Fig. 2. This figure shows that these calcium-binding compounds reduce the percentage of demineralization and the calcium removal to different extents depending on their association constant for calcium-binding. When they all are present in a multi-component solution, similar to acid whey, their effects are additive. Lactate is the compound that most significantly influences the percentage of demineralization and the extent of calcium removal. In addition to the calcium-binding affinity, the concentration of the compound and pH are confirmed to influence the process as will be discussed in more detail in the following sections.

### 3.2. Calcium recovery by electrodialysis from single-component solutions – effect of concentration and pH

The presence of gluconate affects calcium removal, and the effect increases with increasing gluconate concentration (Fig. 3A). For pH = 4.5, where ~90% of the gluconate is present as the anion, a ratio between calcium and gluconate is around 2 as in the experiment with 37 mM calcium and 18 mM gluconate, the effect of gluconate is only small. After 80 min ED processing, the demineralization of the solution containing 18 mM gluconate starts to differ significantly from the solution only containing calcium. A similar difference is observed after 10 min when the concentration of gluconate is increased to 40 mM and accordingly similar to the calcium concentration with a small excess of gluconate compared to the calcium concentration. The presence of 18 mM gluconate does not significantly affect the time which it takes to reach 70% demineralization when compared to the solution only containing calcium. However, the presence of 40 mM gluconate increases the time for reaching 70% demineralization with 29%. Both 18 mM and 40 mM gluconate affect the time for reaching 90% demineralization (16% and 46%, respectively) (Table 3).

The reduction of calcium concentration in the dilute (Fig. 3C) shows that the calcium solution containing 18 mM gluconate is significantly higher after 90 min than in the solution only containing calcium. However, the standard deviation of the calcium concentration measured after 60 min is high since it was difficult to collect samples for the calcium measurement for one of the duplicates because of calcium-gluconate precipitation. Therefore, it might be possible that the calcium concentrations in the two solutions already start to differ after 60 min. For a solution with 40 mM, a significantly higher concentration in the dilute is observed from the beginning of the process. These observations confirm that the free calcium is the electrochemically active compound at the membrane surface and that the calcium-gluconate complex dissociates when free calcium is removed from the equilibrium mixture.

Lactate, which has an association constant lower than gluconate (Vavrusova et al., 2013), also reduced the removal of calcium during ED. Compared with the calcium solution, the solution containing 18 mM lactate shows only a 2% difference in demineralization after 80 min; however, the difference is significant, while the solution containing 85 mM lactate shows a considerable difference after already 20 min (Fig. 3B). For 18 mM lactate, the free calcium is not reduced dramatically by the complex formation, and the free calcium ions are removed electrochemically at the membrane almost at the same rate. The presence of 85 mM lactate increases the time for reaching 70% demineralization with 56% and only 88% demineralization is reached within 120 min ED processing. The concentrations of calcium in the solutions support that lactate concentration affects the removal of calcium to a smaller degree than gluconate. At 30 min, a small but significant difference in calcium concentration in the dilute is observed when 18 mM lactate is present compared to the solution containing calcium (Fig. 3D). However, there is no difference after 120 min, which means that almost all calcium has been removed. When 85 mM lactate is present, after 30 min, the calcium concentration in the dilute begins to differ significantly compared to the solution containing only calcium. Typically, acid whey contains around 85 mM total lactic acid depending on its origin (Nielsen et al., 2021), and at pH 4.5, around 75% of lactic acid is present as lactate (Chen et al., 2016). Therefore, these results show that lactate in acid whey highly influences the rate of removal of calcium during ED because the total concentration of lactic acid and lactate is high in acid whey.

It should be noted that sodium-gluconate was used as the gluconate source, and sodium was also found in the lactic acid solution due to the neutralisation of lactic acid with sodium hydroxide. Therefore, the effects of sodium on the percentage of demineralization by ED should be considered, as sodium is a monovalent cation, and as known from previous studies, monovalent ions are removed before divalent ions (Rasmussen et al., 2020; Nielsen et al., 2021; Merkel et al., 2021). However, the solutions containing 18 mM gluconate and 18 mM lactate have the same initial sodium concentration (Fig. 3E). Nevertheless, the removal of calcium is significantly slower in the solution containing 18 mM gluconate compared to the solution containing 18 mM lactate; thus, this difference must be a result of gluconates higher association constant and stronger binding of calcium. The presence of sodium might slightly decrease the removal of calcium in the gluconate and lactate solutions, which previously have been observed by Firdaous et al. (2007). However, the compound’s high affinity to calcium has the major impact on removing calcium as it is most clearly seen for the model solutions with an excess of gluconate and lactate.
In Fig. 4A and B, it can be seen that citrate prolong the demineralization process during ED, but the extension depends on pH and the concentration of citrate. From these data, 8 mM citrate at pH 4.5 seems to have the largest impact on removing ions compared with pH 9, which means that calcium demineralization will be limited by the non-transport of calcium across the cation exchange membrane. The transport of citrates across the anion exchange membrane can become a limiting factor. The citrate has a higher affinity to calcium at pH 9.0 than 4.5, and thus, it was expected that citrate at pH 9 would have the highest impact on the calcium removal. However, these results show that the limited transport of the uncharged calcium complex across the cation exchange membrane might have a greater impact than the effect of the pH of the citrate solution on the complex formation. The non-ionic complex CaHCitr is the dominating calcium species at pH below 4.5, while at pH around 9 the negatively charged CaCitr dominates. The neutral molecule CaHCitr is apparently less active during the ED process than the ionic complexes, due to slower diffusion in the electrical field, or form a colloidal solution (Liu et al., 2021). An alternative mechanism could involve a change in citrate protonation during transport through the membranes also resulting in the observed pH effect as seen for phosphate (Rybkina et al., 2019). However, the calcium concentrations measured in the dilute every 30 min show that the calcium is removed fastest in the solution having a pH of 4.5 (Fig. 4D) compared with pH 9.0 (Fig. 4C), where mainly hydrogen citrate and dihydrogen citrate will be present. The effect of pH is substantial after 120 min ED processing, as 2.5 ± 0.24 mM calcium is measured at pH 4.5 compared with 3.8 ± 0.05 mM at pH 9.0. At pH 4.5, a large part of the citrate is still present as hydrogen citrate with an association constant of 2.3 × 10³, which is 7 times lower than the association constant of citrate (Vavrusova et al., 2018). However, it seems that the effect of an increasing association constant from 2.3 × 10³ to 1.6 × 10⁴ is minor when changing the pH since both the affinity of hydrogen citrate and citrate to calcium are very high. Decreasing the pH to lower than 4.5 will increase the amount of dihydrogen citrate, with an association constant of 14. Therefore, the binding of calcium will be lower and may reduce the effect of having citrate present in calcium-containing solution during ED. As a result of the ED process, the pH of the 8 mM citrate solution dropped from 9.0 to 6.4, which reduced the amount of the negatively charged CaCitr⁻ and thereby the citrate’s effect compared to if the pH was maintained at 9.0. The same pH drop was not observed.

Table 3 - Time for reaching 70% and 90% demineralization for all single-component solutions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>Conc. (mM)</th>
<th>D70</th>
<th>D90</th>
<th>Ca (mM) 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference solutions</td>
<td>Ca</td>
<td>9.5</td>
<td>37</td>
<td>54 ± 0</td>
<td>75.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5</td>
<td>37</td>
<td>56 ± 0</td>
<td>78.5 ± 0.7</td>
</tr>
<tr>
<td>Acid whey model</td>
<td>Lactate, gluconate, citrate, lactose</td>
<td>4.5</td>
<td>85, 18, 8, 117</td>
<td>106.5 ± 7.8*</td>
<td>&gt;120</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>4.5</td>
<td>18</td>
<td>57 ± 0</td>
<td>82.5 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85</td>
<td>18</td>
<td>87.5 ± 3.5*</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Conjugated bases</td>
<td>Gluconate</td>
<td>4.5</td>
<td>40</td>
<td>54 ± 2.8</td>
<td>91 ± 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.0</td>
<td>8</td>
<td>72.5 ± 2.1*</td>
<td>115 ± 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5</td>
<td>40</td>
<td>66.5 ± 6.4</td>
<td>81.5 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>Citrate</td>
<td>4.5</td>
<td>8</td>
<td>101.5 ± 4.9*</td>
<td>&gt;120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.0</td>
<td>8</td>
<td>50.5 ± 6.4</td>
<td>81.5 ± 3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.3</td>
<td>222</td>
<td>48.5 ± 2.6</td>
<td>77 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>8.5</td>
<td>222</td>
<td>52.5 ± 0.7</td>
<td>77 ± 0</td>
</tr>
<tr>
<td></td>
<td>Glucose/Galactose</td>
<td>6.1</td>
<td>117</td>
<td>56.5 ± 3.5</td>
<td>80.5 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>4.5</td>
<td>117</td>
<td>59 ± 1.4</td>
<td>85.5 ± 2.1*</td>
</tr>
</tbody>
</table>

* Significant different (<0.05) from reference solution with corresponding pH. Data are missing for those solutions where it was not possible to reach 90% demineralization within 120 min ED processing.
Fig. 3 – Percentage demineralization of solutions at pH 4.5 containing: only 37 mM calcium; 37 mM calcium + 18 mM; or 37 mM calcium + 40 mM gluconate (A); in comparison with 37 mM calcium + 18 mM or 37 mM calcium + 85 mM lactate (B); and their respective calcium concentrations in the diluate for gluconate (C) and lactate (D) solutions during ED processing. Sodium concentration in solutions containing gluconate and lactate (E).

for the 8 mM and 40 mM citrate solutions at pH 4.5, where the pH remained the same during the whole ED process (data not shown). Increasing the concentration of citrate to 40 mM significantly decreases the demineralization rate and also the rate of calcium removal and for these conditions, almost all calcium will be present as complexes in contrast to solutions with an excess of calcium compared to citrate, when free calcium ions still will be active in diffusion in the electrical field. The time for reaching 70% demineralization increases by 81% when 40 mM citrate is present compared to the solution only containing 37 mM calcium. 90% demineralization could not be reached within the 120 min of ED processing. The differences in the demineralization between the calcium solution containing 40 mM citrate and the solution only containing calcium are only observed after 20 min. When 8 mM citrate was present, the differences were only significant after 110 min. The calcium concentrations in the diluate show that more calcium is present in the 40 mM citrate solution after 60 min (27.8 ± 0.55 mM) than in the solution only containing calcium (10.6 ± 0.35 mM). The effect of having citrate present in a calcium solution is, therefore, both pH and concentration-dependent and seems to be related to the distribution between neutral and charged compounds. However, since acid whey contains around 8 mM citrate (Menchik et al., 2019) and the pH is 4.5
or lower, the effect of having citrate present in acid whey is minor. Furthermore, as is evident from Fig. 4E, the citrate solutions also contained sodium, which can delay the removal of calcium during ED. The sodium concentration is especially high in the 40 mM citrate solution and will affect the removal rate of calcium in this solution. The effect of having 40 mM citrate present will not be as pronounced as the present results show; however, citrate still has a large impact on the removal of calcium.

The demineralization of the calcium solutions containing sugars is shown in Figs. 5A and B. No significant differences in demineralization between the calcium solution containing glucose and the solution only containing calcium were observed. This indicates that the low affinity to calcium for glucose does not affect the removal of calcium during ED. This is supported by the calcium concentration measured in samples collected every 30 min during the ED processing (Fig. 5C), in which no significant differences in calcium demineralization in pure calcium solutions or in the presence of glucose were observed. The demineralization of the calcium solution containing a mixture of glucose and galactose was not found to differ from the demineralization of the solution with only calcium. However, a significant difference was found after 120 min ED processing in the calcium concen-
Fig. 5 – Percentage demineralization of solutions at pH 6.1–8.5 (not adjusted) containing only 37 mM calcium; 37 mM calcium + 117 mM lactose, 37 mM calcium + 222 mM glucose or 37 mM calcium + 222 mM glucose/galactose (equimolar) (A); in comparison with solution containing only 37 mM calcium and 37 mM calcium + 117 mM lactose at pH 4.5 (adjusted) (B); and the respective calcium concentration in the dilute solutions (C,D).

Comparison between these two solutions (0.20 ± 0.01 mM and 0 mM, respectively). This indicates that a mixture of glucose and galactose binds calcium stronger than when glucose is present alone, which agrees with the higher association constant of galactose. However, since it is first after 120 min of processing such a difference is observed, the effect on demineralization from a mixture of glucose and galactose is conducted to be minor. The presence of lactose in the calcium solution induces significant differences in the demineralization after 80 min for solutions with pH adjusted to 4.5 (Fig. 5D). Lactose exists in equilibrium between α-lactose and β-lactose and have an equilibrium constant of 0.653, which corresponds to a mixture of approximately 40% α-lactose and 60% β-lactose (Zunft and Schulze, 1990). Jiang et al. (2021b) found that the β-anomer of lactose binds calcium stronger than the α-anomer. However, pH does not affect the equilibrium proportion of α-lactose and β-lactose (Portnoy and Barbano, 2021). The effect of low pH on the removal of calcium from the lactose solution might be found in the rate of lactose mutarotation, which is pH dependent. The rate of mutarotation of lactose is slowest around pH 5 (Portnoy and Barbano, 2021), and is minimal in the lactose solution at pH 4.5. Thus, calcium might have better access to β-lactose in the lactose solution at pH 4.5 than in the lactose solution with pH 6.1. Lactose has an affinity to calcium, which is higher than both glucose and galactose. Therefore, it is expected that the presence of lactose would influence the removal of calcium more than was observed for the two other sugars. The time for reaching 90% demineralization also significantly increases by 9% when lactose is present at pH 4.5. The calcium concentrations in the solution containing lactose and with a pH of 4.5 also show that the removal of calcium is significantly slower after 90 min ED processing. The pH of acid whey is often around 4.5 or lower (Nielsen et al., 2021), and lactose will accordingly reduce the removal of calcium from acid whey during ED.

3.3. Calcium recovery by electrodialysis from an acid whey model solution

The acid whey model solution containing 37 mM calcium, 8 mM citrate, 18 mM gluconate, 117 mM lactose and 85 mM lactate and adjusted to pH 4.5 has a much slower demineralization rate compared to the corresponding single-component solutions (Fig. 6A). After 80 min 56.03 ± 4.47% demineralization is reached, whereas 85.5 ± 1.4% is reached in the 18 mM gluconate solution, and 64.7 ± 2.6% is reached in the 85 mM lactate solution. The slower removal of minerals is also supported by the concentration of calcium determined in the collected samples (Fig. 6B). The percentage demineralization and calcium concentration in the acid whey model solution after ED differed significantly from the solutions containing only a single compound (Fig. 2). The presence of many calcium-binding compounds in one solution strongly decreases the removal of calcium during ED. However, the
significant difference in demineralization is first observed between the acid whey model solution and the solution containing 85 mM lactate after 80 min. Amongst the acid whey model solution components, lactate is the calcium-binding compound that had the largest effect on preventing calcium from being removed during ED, because of its high concentration. Even though lactate has a smaller association constant than both gluconate and citrate, lactate still has the predominant effect. Thus, the concentration of the calcium-binding compound is very important for how it affects the removal of calcium during ED. For model solutions or acid whey where the complex binder is in excess compared to calcium, the low concentration of free calcium hampers the diffusion process to the membranes for separation.

3.4. Distribution of ionic and bound calcium in the solutions before and after 120 min ED

The ionic calcium is determined for all the solutions used before and after 120 min ED processing to understand the distribution of calcium as a free ion or complexed with the calcium-binding compounds. The complexation of calcium has been shown to influence the bioavailability of calcium positively (Wang et al., 2020), so bounded calcium would be more favorable on calcium absorption in humans (Hartley et al., 2020). Large differences were found in the amounts of ionic and complexed calcium in solutions containing higher concentrations of lactate, citrate and gluconate (Fig. 7A), while all the calcium was measured to be ionic in the solutions containing sugars and 18 mM lactate (data not shown). Increasing the concentrations of lactate and gluconate decrease the concentration of ionic calcium. More calcium is bound in the 85 mM lactate solution than in the 40 mM gluconate solution, even though gluconate is known to have the highest association constant (Vavrusova et al., 2013). This supports that the concentrations of the compounds are of high importance for the binding of calcium ions as a mass law effect. More calcium is bound in the 8 mM citrate solution when the pH is 9.0 compared to 4.5 as expected since hydrogen citrate and dihydrogen citrate are dominating at pH 4.5 with lower association constants than citrate (Davies and Hoyle, 1953; Vavrusova et al., 2018). Furthermore, the larger amount of bound calcium in the 8 mM citrate solution at pH 9.0 is also reflected in the demineralization and calcium removal results. Increasing the concentration of citrate to 40 mM increases the concentration of bound calcium. Only a small fraction of the total calcium is found in ionic form. The effect of the very high association constants of citrate and hydrogen citrate can be seen when the amount of bound calcium in the 40 mM citrate solution is compared with the 40 mM gluconate solution, where only a smaller amount of calcium is bound. The acid whey model solution containing 37 mM calcium, 8 mM citrate, 18 mM gluconate, 117 mM lactose and 85 mM lactate has a lower ionic calcium concentration than the corresponding single-component solutions. This supports the previous conclusion that having more than one calcium-binding compound present in a calcium solution increases calcium binding, making it more difficult to remove calcium during ED processing.

The ionic and bound calcium distribution in the solutions, which initially had bound calcium, changes after 120 min ED (Fig. 7B). Bound calcium is only found in the 8 mM citrate solution at pH 9.0, 40 mM citrate solution at pH = 4.5 and the acid whey model solution, but in reduced concentrations, and these solutions have the highest final total calcium concentration. The stoichiometry of the calcium complexes will remain the same in the demineralized solution, but the complex constant will increase due to a decreasing ionic strength and increasing values for the activity coefficients. Increasing the ED processing time had further decreased the concentration of bound calcium. The other solutions, which initially contained bound calcium, only contain ionic calcium after 120 min ED processing. These results show that after the removal of free ions, bound calcium is dissociated during ED processing. An exception seems to be the 40 mM citrate solution at pH 4.5, where the uncharged CaH2Citr dominates. These citrate complexes may dissociate slowly or not at all, as is evidenced by the high concentration of bound calcium. The non-additive effects of the compounds in the acid whey model solution may be related to some unique properties of CaH2Cit, which prevents dissociation and equilibration.

Fig. 8 shows that after 120 min of ED processing, most calcium has been removed in the solutions where all the calcium was present in the ionic form. This means that the initial concentration of bound calcium affects the removal of calcium during ED. However, Fig. 8 also shows that the initial bound

**Fig. 6** - Percentage demineralization of solutions containing only 37 mM calcium and model acid whey solution at concentrations and pH (4.5) typically found in acid whey (calcium 37 mM, lactate 85 mM, gluconate 18 mM, citrate 8 mM, lactose 117 mM) (A); and the respective calcium and sodium concentrations in the dilute solution (B).
calcium concentration does not affect the calcium removal after only 30 min of ED processing. This indicates that at the beginning of the ED process, mainly ionic calcium is removed from the diluate solution. The effect of the initial bound calcium concentration is observed after 60 min of ED processing, and thus, the bound calcium starts to have an effect on the ED process after 30–60 min. After this time, a similar slope is observed between the percentage of calcium removal in the function of the initial bound calcium, which may be related to the dissociation binding into free calcium during the ED processes.

4. Conclusions

This study shows that the presence of calcium-binding compounds in calcium-containing solutions can decrease the removal rate of calcium during ED. Compounds with low association constants such as glucose do not affect the removal of calcium significantly. However, compounds with higher association constant significantly decrease the removal of calcium. In addition, the concentration of the calcium-binding compound is of high importance. Lactate, which is present in high concentrations in acid whey, has a larger impact than citrate on the removal of calcium during ED, even though citrate has an association constant, which is much higher than lactate.

The pH of the solution is also important as it affects the ionisation of the molecules. A large decrease in the removal rate of calcium between pH 4.5, and 6.1 was observed for lactose. However, the largest impact of pH was observed in the citrate-containing solutions since the form of citrate is very pH-dependent. At pH 4.5, a significantly lower amount of calcium was bound to citrate compared to the citrate solution, where the pH was 9.0. Consequently, citrate at pH of acid whey (4.5) and concentration has a minor effect on calcium removal rate. After 120 min of ED processing, it was only possible to detect bound calcium in the solutions containing 8 mM citrate at pH 9.0, 40 mM citrate, and in the acid whey model solution. This shows that ED can be used to both demineralize solutions but also dissociate calcium, which is bound to other compounds.

Funding sources

This study is a part of the Platform for Novel Gentle Processing supported by the Dairy Rationalisation Fund (DDRF), Copenhagen University and Arla Foods. The work performed by MemBrain s.r.o. (Membrane Innovation Centre) was performed within the Institutional support project (Decision No. 6/2018) by the Ministry of Industry and Trade of the Czech Republic.

Declaration of Competing Interest

The authors report no declarations of interest.

References


