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Integrating selective precipitation of α-lactalbumin into a whey valorisation cascade

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*Tjaša Prevc[1], Blaž Lokar[1], Marko Kete[1], Polona Zabukovec[1], Bojana Bogovič Matijašić[2], Maja Zupančič Justin[1], Jernej Oberčkal[1,2]**

[1]Arhel Ltd., Pod Lipami 35, 1218 Komenda, Slovenia
[2]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, 1230 Domžale, Slovenia

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*Corresponding author: jernej.oberckal@bf.uni-lj.si

Abstract

In the Balkans, and probably elsewhere, whey from small dairies, especially acid whey, still poses an environmental challenge. Despite recent advances in whey processing technology, whey is still largely treated as waste or ends up in biogas plants. Due to its high lactic acid content, acid whey cannot be concentrated by conventional drying or used directly in food. However, it contains several valuable components that can be converted into products with high added value. For this purpose, a valorisation cascade was developed, part of which is the extraction of lactoferrin and lactoperoxidase from acid whey or sweet whey. The remaining whey was concentrated and used for the acidic precipitation of α-lactalbumin. In small-scale laboratory experiments, a purity of α-lactalbumin of 72.9 % and 24.8 % was achieved, when isolated from acid whey and sweet whey, respectively. The isolation procedure was then demonstrated on a pilot scale, using 70 L of acid whey, which yielded 220 g of wet isolate of α-lactalbumin with a purity of 78.1 % and a high lysine (11.1 %) and leucine (10.0 %) content. The remaining whey fraction could be used in various fermentation processes or other applications.

Keywords: α-lactalbumin; ß-lactoglobulin; whey; selective precipitation, citrate

Introduction

Whey, the liquid part of milk that is separated from the curd during cheese production, is the most important side stream of the dairy industry. As it is rich in organic substances (biochemical oxygen demand 27-60 g/L, chemical oxygen demand 50-102 g/L), it is not suitable for direct release into the environment (Carvalho et al., 2013; Paskaš et al., 2023). Optimising the processing of whey is therefore crucial for minimising its environmental impact. Acid whey, which is mainly produced in the manufacture of fresh curd cheese or Greek-style yoghurt, poses greater processing challenges than sweet whey due to its higher lactic acid content (0.37 % to 0.65 %) and is therefore less suitable for drying or direct use in food (Menchik et al., 2019). In small dairies, investments in whey valorisation equipment are not profitable and therefore their whey is treated as waste or even improperly disposed of in the environment, even though technological solutions exist. However, the valuable biologically active compounds in whey, such as proteins, should be used in foods or food supplements that benefit human and animal health.

Besides ß-lactoglobulin (ß-LG), α-lactalbumin (α-LA) is the second most abundant protein in bovine whey. It has a favourable amino acid composition as it contains all essential amino acids and is particularly rich in cysteine, tryptophan, lysine and the branched-chain amino acids valine, leucine and isoleucine. α-LA has several uses. Due to its favourable amino acid profile and to improve its similarity to breast milk, it is added to infant formula. It can be used as a dietary supplement to promote gastrointestinal health or neurological function, and it could be used as a therapeutic agent against various diseases (Layman et al., 2018). Proteolysis of α-lactalbumin in the gastrointestinal tract produces bioactive peptides with antimicrobial, antihypertensive, immunostimulant and analgesic properties (Wada and Lönnerdal, 2014; Auestad and Layman, 2021).

In our previous work, procedures for separating individual bioactive proteins from whey were developed. Monolithic chromatography was used to isolate less abundant whey proteins such as lactoferrin, lactoperoxidase and osteopontin (Kete et al., 2019; Matijašić et al., 2020; Justin et al., 2021; Oberčkal et al., 2024). However, isolation of more abundant proteins such as ß-LG and α-LA is more efficient when using non-adsorption techniques (e.g., membrane filtration, two-phase partitioning, high-pressure processing or precipitation) that avoid the limitations due to a limited number of protein binding sites (Jiang et al., 2021; Romo et al., 2023). Building on the work of Pearce (1983), Bramaud et al. (1995), and Alomirah and Alli (2004), Haller and Kulozik (2020) used reversible precipitation at low pH with citrate as Ca^{2+} chelator and heating followed by continuous centrifugation to separate α-LA and ß-LG from reconstituted whey protein concentrate.

Our first aim was to demonstrate the application of citrate-assisted precipitation and separation of ß-LG and α-LA in concentrated sweet and acid whey from a local dairy. A second aim was to separate these two proteins from the main whey fraction by ultrafiltration, so that the remaining whey fraction is relatively unchanged and can be further utilised. In this way, we have integrated the precipitation process into a pilot-scale whey valorisation cascade that enables the reuse of the entire whey and is in line with the zero-waste approach.

Materials and methods

Materials

The acid whey and sweet whey were obtained from the local dairy Mlekarna Celeia (Petrovče, Slovenia). The whey came from the production of fresh curd cheese or other cheeses. Pasteurised milk (76 °C for 15 – 20 s) was either fermented to produce fresh curd cheese (acid whey) or coagulated with rennet to produce different types of cheese and then pooled (sweet whey). Both types of whey were skimmed on site to remove fat and particles and were not additionally pasteurised or filtered before use. Both types of whey were collected on the day of production, transported at 4 °C and processed immediately (see 2.2). Trisodium citrate dihydrate was purchased from ACEF (Fiorenzuola d'Arda, Italy). Citric acid, hydrochloric acid, Tris and Coomassie Brilliant Blue R250 dye were purchased from Merck (Darmstadt, Germany). Molecular weight markers were obtained from ThermoFisher Scientific (MA, USA) and New England Biolabs Inc. (Ma, USA). All other chemicals were of analytical grade or higher. The sequence of mature bovine α-LA was obtained by removing the 19 amino acid signal peptide from the N-terminus of the sequence from the UniProt database (ID P00711).

Whey fraction preparation

Acid whey and sweet whey were processed as previously described (Matijašić et al., 2020). In brief, after microfiltration, lactoferrin was removed by cation-exchange chromatography. The unbound or flow-through fraction (FT) of whey was concentrated 10-fold by ultrafiltration (referred to as concentrated FT, CFT) using the WaterSep ReUse mini-Bio Producer24 m-PES hollow fibre membrane module with 10 kDa cut-off (Sartorius AG, Göttingen, Germany) to concentrate the remaining whey proteins, reduce volume and reagent consumption, and make the process feasible at laboratory and small production scale. All whey variants and fractions were kept in a liquid state and refrigerated from production to analysis, except during the precipitation process.

Preliminary precipitation of proteins from concentrated acid whey FT

The selective precipitation procedure was carried out according to the method of Haller and Kulozik (2020). The proteins were precipitated in two 400 mL aliquots of acid whey CFT by adding 23.1 g citric acid monohydrate and 13.2 g trisodium citrate dihydrate. The pH value after dissolution

of the reagents was 3.50–3.53. The reaction mixtures were stirred by hand in a thermostatic bath and incubated for 30 and 90 minutes after reaching a temperature of 50.0 °C. The reaction was slowed down by immersing the samples in an ice bath. The precipitate was removed from the suspension by centrifugation at 4000 RPM for 30 minutes and the pellet samples were used for the dissolution study.

Dissolution of the precipitate from the preliminary experiment

To determine the conditions for the dissolution of the precipitated proteins, aliquots of 0.5 g of the precipitate were shaken for 1 hour in 40 mL of the following buffers. The pH was then readjusted to the indicated values and the samples were shaken further for a few minutes until no further dissolution was observed: 85 mM glycine pH 1.7, 25 mM sodium phosphate pH 4.6, 25 mM sodium phosphate pH 5.85, 25 mM sodium phosphate pH 8.0, 25 mM sodium phosphate and 400 mM NaCl pH 8.0, 75 mM Tris pH 8.6. After shaking, the samples were refrigerated overnight and then photographed using a smart phone. From the thoroughly homogenised suspensions, we took 1 mL aliquots and centrifuged them at 14000 g for 1 min. We separated the supernatants from the pellets and analysed both under reducing and non-reducing conditions using SDS-PAGE.

Precipitation of proteins from concentrated sweet and acid whey

Ultrafiltration-concentrated FT (CFT) fractions of acid whey and sweet whey were used as starting material. To avoid samples being affected by the removal of aliquots, precipitation was carried out in several samples of 25.0 mL, each serving as a single time point. 0.825 g trisodium citrate dihydrate and 1.445 g citric acid were added to each sample to achieve a pH of 3.50. These quantities were determined in preliminary experiments to adjust the citrate concentration and pH to the appropriate value according to the method of Haller and Kulozik (2020). The solutions were stirred for 15 minutes on a magnetic stirrer and heated to T= 50.0 °C in a thermostatic bath. When the temperature in the sample suspension reached 50.0 °C, individual 25.0 mL aliquots were withdrawn at specific time intervals and cooled in an ice bath to slow down precipitation. The samples were centrifuged at 3350 RCF and 4.0 °C for 30 minutes (FHC-16F, FAITHFUL Instruments, Huanghua, China). The supernatants were kept for analysis. The pellets were weighed and dissolved in 75 mM Tris buffer (pH 8.6).

After incubation at 50.0 °C for 30 min, 0.500 g aliquots of crude wet isolate from acid whey CFT were weighed into centrifuge tubes, rinsed with 2.5 mL dH2O and centrifuged as described. The pellet and the supernatant fractions were collected for analysis. The rinsing procedure was repeated three times. The concentrations of α-LA and β-LG in pellets (resuspended in 75 mM Tris buffer pH 8.6) and supernatants were determined by RP-HPLC.

Precipitation at the pilot scale

70 L of acid whey fraction FT was concentrated to 7.00 L (CFT), and selective precipitation was performed at pH 3.49 by adding 231 g sodium citrate and 404.6 g citric acid for 30 min at 50.0 °C. The solution was quickly cooled in an ice bath and centrifuged by membrane centrifugation in a tubular centrifuge (GQ 75, Nanjing FiveMan Machine CO. Ltd., Nanjing City, China). When the solution flowing through the membrane was clear after visual inspection, the sediment on the membrane was harvested, and the weight and volume of the supernatant were measured. The sediment samples were dissolved in 75 mM Tris (pH 8.6). Precipitate and permeate fractions were collected for further analysis.

SDS-PAGE

SDS-PAGE was performed as previously described by Oberčkal et al. (2023) using a discontinuous Tris-glycine system with a 4 % stacking gel and a 12.5 % separating gel under reducing (in the presence of ß-mercaptoethanol) or non-reducing conditions (Laemmli, 1970). The gels were stained with Coomassie Brilliant Blue R250.

RP-HPLC determination of the whey protein concentration

RP-HPLC analysis of α-LA and β-LG content was performed by Nika Kržišnik at the Faculty of Pharmacy, University of Ljubljana, as described (Osel et al., 2021). The separation was performed on a BioZen™ Intact XB-C8 column (150 × 4.6 mm, 3.6 µm; Phenomenex, Torrance, CA, USA) at 30 °C by gradient elution with 0.1 % trifluoroacetic acid in water (mobile phase A) and 0.1 % trifluoroacetic acid in acetonitrile (mobile phase B).

Chemical analyses

The chemical analyses were carried out according to the specified standards: Protein (ISO 8968-3/IDF 20-3:2004), lactose (ISO 22662/IDF 198:2007), fat (ISO1736/IDF 9:2008) and dry matter content (IDF 26A:1993).

Amino acid composition of the isolate

The total amino acid composition of the crude precipitate from the pilot test was determined by HPLC in the laboratory of ALS Life Sciences (Prague, Czech Republic). The sample was treated with 6M hydrochloric acid in sealed bottles at 115±5 °C for 7 hours to hydrolyse the protein chains. For the analysis of cystine and methionine, the sample was oxidised with performic acid at 2-8 °C for 16 hours prior to acid hydrolysis. The resulting hydrolysate was diluted and filtered. An aliquot was then adjusted to a pH of 2.4, and a known quantity of norleucine was added as an internal

standard prior to dilution. The extracted amino acids were then derivatised with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Fluor) prior to determination by gradient HPLC with fluorescence detection. This procedure is suitable for the quantification of amino acid content in a wide range of samples, including feeds and feed ingredients as well as other protein-containing samples. The method is accredited for total amino acids according to the UK Accreditation Service (UKAS) and is suitable for the determination of 17 of the 20 common amino acids, Asp, Ser, Glu, Gly, His, Arg, Thr, Ala, Pro, Cys, Tyr, Val, Met, Lys, Ile, Leu and Phe. Gln and Asn are converted to Glu and Asp, respectively, during the analysis, and therefore cannot be determined separately from Glu and Asp. The method is not suitable for the determination of Trp. The assay was performed in one replicate.

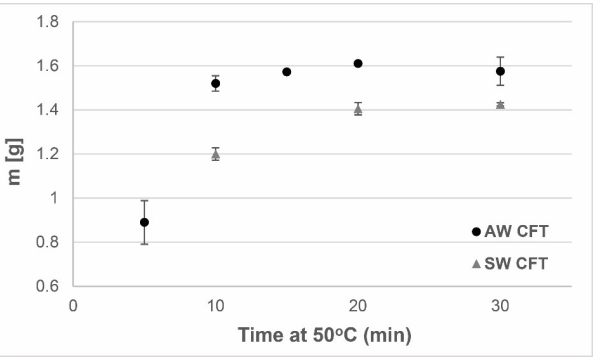

Figure 1. Mass of the crude wet precipitate from 25.0 mL of acid whey and sweet whey CFT after different incubation times at 50 °C. The error bars represent the standard deviation of three replicates.

Results and discussion

Most data on the precipitation of α-LA in the literature refer to reconstituted WPC or WPI as starting material (Pearce, 1983, Bramaud et al., 1995; Lucena et al., 2006; Toro-Sierra et al., 2013; Haller and Kulozik, 2020) and not to the relatively unchanged liquid whey that is produced after milk coagulation. A comparison of the precipitation of α-LA in liquid whey, WPC and WPI was performed by Alomirah and Alli (2004). They used a commercial whey sample after the production of mozzarella cheese (sweet whey). Muller et al. (2003) purified α-LA from acid whey produced by precipitation of caseins with hydrochloric acid, but we could not find an example of purification of α-LA from fermented acid whey in the literature.

Concentration of the whey served several purposes: it facilitated precipitation due to the higher protein concentration and separated the proteins from the bulk of the whey, which could then be further utilised. In addition, by concentrating the proteins, the volume for processing on the pilot scale was reduced. A 10 kDa cut-off membrane retained most of the α-LA (Figure S1).

When comparing sweet whey CFT with acid whey CFT, the mass of crude wet precipitate was lower in sweet whey (Figure 1) although the pH values during precipitation were similar. Acid whey CFT initially contained 6 times the amount of α-LA and 1.7 times the amount of ß-LG of sweet whey CFT (Table 1). The total protein content in acid whey CFT was 2.94 % and in sweet whey CFT 2.35 %. These differences in protein content can be attributed either to the different whey production technology or the sample preparation for analytical HPLC. Acidification during milk fermentation can release some whey proteins associated with casein micelles into the whey phase of acid whey but can also lead to aggregation and precipitation of proteins or co-precipitation of whey proteins with caseins, which could be removed either with the curds or during subsequent skimming of the whey. Different temperatures can influence the distribution of caseins between the micellar and serum phases of the milk (Schiffer et al., 2021; Møller et al., 2023). As the whey came from the local dairy, we had no control over the technology of its production. Another reason for the observed differences could be the sample preparation for RP-HPLC analysis. The samples were exposed to an acidic environment by the addition of trifluoroacetic acid, so that some of the α-LA could further precipitate or not dissolve and be removed by the subsequent microfiltration of the samples before analysis. Especially in sweet whey that was not previously exposed to an acidic environment, this could lead to precipitation of proteins such as α-LA, BSA, IgG and others with an acidic isoelectric point. It is known that the solubility of whey protein is lowest at a pH of about 4.5 (Pelegrine and Gasparetto,

Table 1. The amounts of α-LA and β-LG in the fractions after selective precipitation of acid whey and sweet whey CFT

	Initial mass in 25 mL of concentrated whey	Time [min]	Precipitate		Supernatant		Purity (RP-HPLC) of α-LA in isolate
			m(ß-LG) [% of initial]†	m(α-LA) [% of initial]†	m(ß-LG) [% of initial]†	m(α-LA) [% of initial]†	
Concentrated acid whey FT	m(α-LA) = 119.0 mg m(ß-LG) = 421.8 mg	10	3.9±0.3	76.5±0.1	96.0±0.0	8.7±0.0	67.9 %
		20	3.8±0.2	71.8±3.1	95.2±0.0	6.9±0.0	71.2 %
		30	3.7±0.3	73.6±2.4	95.2±0.2	5.1±0.0	72.9 %
Concentrated sweet whey FT	m(α-LA) = 19.3 mg m(ß-LG) = 250.3 mg	10	4.6±0.3	97.2±5.8	101.2±0.2	15.3±0.6	24.3 %
		20	5.2±0.1	105.9±2.2	101.4±0.1	11.0±0.0	24.8 %
		30	4.9±0.4	110.2±2.3	102.6±0.1	12.6±0.1	24.7 %

†*The values are the average of two experiments ± relative standard deviation.*

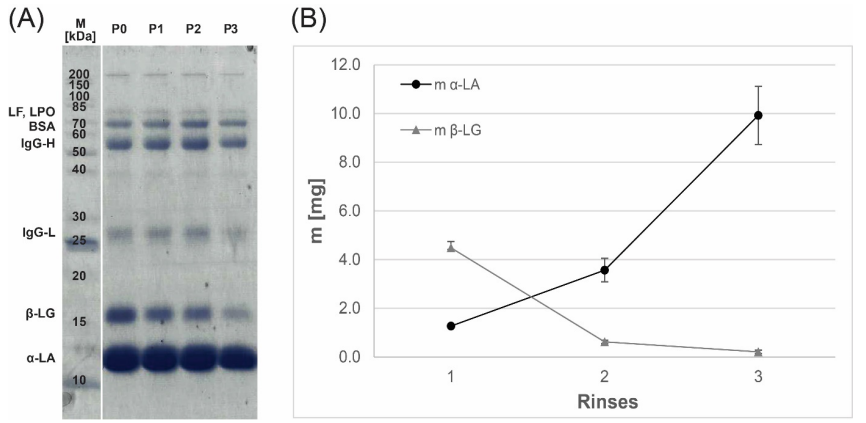

Figure 2. (A) SDS-PAGE of the crude precipitate from acid whey CFT (P0) and the effect of rinsing three times with demineralised water (P1, P2 and P3). The amount of β-LG is decreasing. The spliced lanes are marked by the white dividing line. (B) The amounts of α-LA and β-LG in the supernatant fractions after rinsing 0.500 g acid whey CFT isolate with demineralised water. The error bars represent the standard deviation of three replicates

2005) and that acid and sweet whey differ in protein amount and mineral composition (Carvalho et al., 2013, Lievore et al., 2015; Menchik et al., 2019), both of which could affect the solubility of α-LA and other proteins.

Both the measured extent of precipitation and the amount of residual α-LA in the supernatant were higher in sweet whey CFT (Table 1). This observation, as well as the discrepancies in recovery, can again be attributed to the sample preparation for RP-HPLC as described above. Normally, a relatively lower concentration of proteins, as in sweet whey CFT, would result in a lower level of precipitation. Pearce (1983) observed an order of magnitude lower extent of precipitation when the α-LA concentration was lowered from 2 mg/mL to 1 mg/mL.

As for the yield of α-LA in the precipitate (Table 1), precipitation was successful for both whey variants. However, the α-LA content of the precipitate was four times higher for the acid whey CFT (Table 1), and the chromatographic purity was 71 % compared to only 25 % for the sweet whey isolate. Taken together, these results (at least in the case of this dairy) make acid whey a more favourable starting material for scaling up to the pilot level. After a 20-minute incubation at 50.0 °C, no significant differences were observed in the pellet mass of acid whey CFT with additional incubation time. Even after a 10-minute incubation at 50.0 °C, no significant differences were found in the α-LA content in the precipitation product from acid whey CFT. With sweet whey CFT, however, differences in the amount of α-LA in the precipitate were still detected after 30 minutes. As precipitation was faster in acid whey CFT and therefore a shorter incubation time could be used on a production scale, this type of whey is again the better candidate for scaling up. Both isolates contained neither fat nor lactose.

Compared to the precipitation with citrate in the study of Alomirah and Alli (2004), a higher yield was obtained, which is likely due to the higher temperature in our experiment. They achieved a higher yield in reconstituted WPI and WPC (which contained 5 to 6 times higher protein concentration) than in liquid whey.

Haller and Kulozik (2020) observed that most of the α-LA precipitated already during the heating phase and that subsequent incubation at 50 °C had little effect on the extent of precipitation. However, they used a much shallower temperature gradient of 0.5 °C/min than in our study (about 5 °C/min at laboratory level). Their results suggest that precipitation could be carried out for much longer to increase the yield of precipitated α-LA, especially for the WPC sample, which is closer to native whey.

Figure 2 shows the effect of rinsing the acid whey precipitate (30 min incubation) with water. After the first rinse, the chromatographic purity of α-LA increased from 72 % to 82 %. The protein-to-dry matter ratio increased from 46.5 % in the crude isolate to 89.1 % in the acid whey isolate and from 53.5 % to 85.9 % in the sweet whey isolate. These differences after rinsing are probably due to citrate and soluble minerals, most of which would be removed in the first rinsing step. Although further rinsing steps mainly removed ß-LG, they also resulted in the removal of some α-LA from the isolate (Figure 2b). By the third rinsing step, a total of 19.5 % of α-LA was removed from the pellets, which can be attributed to the higher pH of the demineralised rinse water compared to the reaction mixture. Our dissolution experiments at different pH values and the results of Haller et al. (2021) indicate that the degree of α-LA resolubilisation is pH-dependent (Figure S2). The first rinsing step removed 84 % of the ß-LG and only 5 % of the α-LA, suggesting that a single rinsing step is the preferred method to achieve high process yield.

The purity of α-LA in the precipitate is affected by the presence of bovine serum albumin (BSA), IgG, residual lactoferrin, and lactoperoxidase (Figure 2a). These larger proteins could be largely removed by a prior step of ultrafiltration using a 50 kDa membrane, as previously described by us (Justin et al., 2021), or a 30 kDa membrane, as described by Bhattacharjee et al. (2006), but this was not tested in this study. Alternatively, the amount of BSA (and possibly other large proteins) in the precipitate could be reduced by performing the precipitation at less than 40 °C instead of 50 °C (Bramaud et al., 1997; Alomirah and Alli, 2004).

The pilot experiment served to evaluate the scalability of the isolation process. 220.0 g of crude wet isolate was obtained (Figure 3), containing 27.75 % protein and 37.54 % dry matter. 32.8 g (81.3 %) of the original 40.32 g α-LA in the acid whey CFT was in the pellet, and 1.7 g (4.2 %) remained in the solution. The supernatant contained highly pure ß-LG,

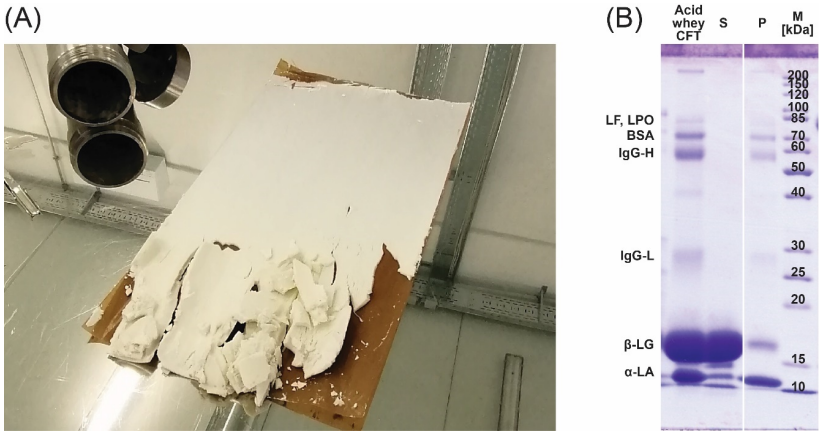

Figure 3. (A) Wet precipitate from concentrated acid whey FT in the pilot test. (B) SDS-PAGE of the fractions from the pilot test. Acid whey CFT - concentrated acid whey FT (starting material), S - supernatant after precipitation, P - precipitate. The spliced lanes are marked by the white dividing line.

and the amounts of larger proteins were below the detection limit of SDS-PAGE (Figure 3b).

The chromatographic purity of α-LA in the isolate was 78.1 %, slightly exceeding the results of the small-scale experiment.

The precipitate dissolved well in Tris pH 8.6, which proved to be an efficient buffer for dissolution (Figures S2 and S3). The different proteins in the precipitate showed a different dissolution profile at different pH values. SDS-PAGE analyses (Figure S3) in the presence or absence of a reducing agent indicate the presence of some aggregates that are sensitive to the reducing agent. Comparison of the two analyses show a decrease in bands >200 kDa (aggregates) with a simultaneous increase in bands at 50 and 25 kDa, suggesting that the aggregates are mainly due to immunoglobulins (heavy chains at 50 kDa and light chains at 25 kDa). However, these aggregates could be an artefact of sample preparation for SDS-PAGE. We have previously observed that heating bovine whey IgG samples in the absence (but not presence) of a reducing agent produces aggregates that are presumably disulfide-linked. Haller et al. (2021) found that resolubilised thermally precipitated α-LA has a very similar (but not the same) structure as native α-LA and can therefore be considered restructured rather than renatured. However, as

Table 2. Amino acid content of α-LA produced in a pilot test and comparison with the theoretical composition of pure α-LA (Uniprot ID P00711).

	Isolate from concentrated acid whey FT		Bovine α-LA mature protein (UniProt ID P00711)		
AA residue	**Content (% w/w)†**	**Content (%)‡**	**Number of residues**	**M (g/mol)**	**Content (% w/w)‡**
Asp	4.67	16.5	13	115.09	10.5
Ser	1.67	5.9	7	87.08	4.3
Glu	3.73	13.2	7	129.11	6.4
Gly	0.89	3.1	6	57.05	2.4
His	0.82	2.9	3	137.14	2.9
Arg	0.63	2.2	1	156.19	1.1
Thr	1.68	5.9	7	101.10	5.0
Ala	0.75	2.6	3	71.08	1.5
Pro	0.98	3.5	2	97.12	1.4
Cys	1.13	4.0	8	103.14	5.8
Tyr	1.4	4.9	4	163.17	4.6
Val	1.24	4.4	6	99.13	4.2
Met	0.22	0.8	1	131.20	0.9
Lys	3.16	11.1	12	128.17	10.8
Ile	1.34	4.7	8	113.16	6.4
Leu	2.83	10.0	13	113.16	10.4
Phe	1.22	4.3	4	147.17	4.1
Asn	/	/	8	114.10	6.4
Gln	/	/	6	128.13	5.4
Trp	/	/	4	186.21	5.3

†Content of the individual amino acids in the wet isolate. ‡ Content of individual amino acids per total mass of amino acids. The assay was performed in one replicate.

α-LA is mainly used in food, the most important properties are its amino acid composition and bioavailability. The amino acid analysis (Table 2) showed that the sum of the individual amino acids accounted for 28.36 % of the total mass of the wet isolate, which was 0.61 % higher than the total protein content, determined by the Kjeldahl method. The isolate was particularly rich in essential amino acids, especially lysine (11.1 %) and leucine (10.0 %). Asp, Asn, Glu and Gln could not be accurately determined by this assay because Asn and Gln are converted into Asp and Glu, respectively, during the analysis. This conversion prevents their independent measurement. Additionally, Trp was not determined, as its quantification requires a separate assay. Table 2 compares the amino acid composition of our isolate with the theoretical composition of pure mature bovine α-LA protein (Uniprot ID P00711). The composition of our isolate correlates well with the composition of mature bovine α-LA. Notable differences exist in the content of Gln, Arg, Ala and Pro, which are 1.8 to 2.5 times more abundant in our isolate. As our product had a favourable amino acid composition and could be completely dissolved at high pH, it should be well suited as a nutrient for use in food.

Conclusions

The conditions for the selective precipitation of α-LA from partially deproteinised acid and sweet whey were investigated. Ultrafiltration, precipitation at low pH and rinsing of the crude precipitate were used to separate ß-LG and α-LA from both the main whey fraction and from each other. A 78.1 % pure isolate of α-LA with a favourable amino acid composition was obtained from concentrated acid whey FT. The isolate could be completely dissolved with the presence of a small amount of aggregates. The precipitation of α-LA was thus successfully integrated into the whey valorisation cascade and the feasibility of the process was demonstrated on a pilot scale.

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Appendix

The following data are available as supporting information: Figure S1: SDS-PAGE analysis of acid whey fractions obtained by ultrafiltration (cut-off 10 kDa); Figure S2: Dissolution of the precipitate in buffers with different pH; Figure S3: SDS-PAGE analyses of the resuspended precipitate in different buffers.

Integriranje selektivnog taloženja α-laktalbumina u višestupanjski proces obrade sirutke

Sažetak

Na Balkanu, a vjerojatno i drugdje, sirutka iz malih mljekara, posebno kisela sirutka, još uvijek predstavlja ekološki izazov. Unatoč nedavnom napretku u tehnologiji prerade sirutke, sirutka se još uvijek uglavnom tretira kao otpad ili završava u bioplinskim postrojenjima. Zbog velikog udjela mliječne kiseline, kisela sirutka se ne može koncentrirati konvencionalnim sušenjem niti izravno koristiti u hrani. Ipak, ona sadrži vrijedne komponente koje se mogu pretvoriti u proizvode s visokom dodanom vrijednošću. U tu svrhu razvijen je višestupanjski proces obrade sirutke, čiji je dio ekstrakcija laktoferina i laktoperoksidaze iz kisele ili slatke sirutke. Preostala sirutka je koncentrirana i korištena za kiselo taloženje α-laktalbumina. U laboratorijskom mjerilu postignuta je čistoća od 72,9 % α-laktalbumina kada je izoliran iz kisele sirutke, odnosno 24,8 % iz slatke sirutke. Postupak izolacije je zatim prenesen na pilot postrojenje, koristeći 70 L kisele sirutke, što je dalo 220 g vlažnog izolata α-laktalbumina čistoće 78,1 % i visokog sadržaja lizina (11,1 %) i leucina (10,0 %). Preostala frakcija sirutke mogla bi se koristiti u raznim procesima fermentacije ili drugim primjenama.

Ključne riječi: α-laktalbumin; ß-laktoglobulin; sirutka; selektivna taloženja, citrat

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