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Effects of conventional processing methods on whey proteins in production of native whey powder

Klaus Muuronen ^{a,b*}, Riitta Partanen ^b, Hans-Jürgen Heidebrecht ^{c,d}, Ulrich Kulozik ^{c,d}

^a *Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, P.O. Box 16300, FI-00076 Espoo, Finland*

^b *Valio Ltd., R&D Center, PO Box 30, 00039 Valio, Finland*

^c *Chair of Food and Bioprocess Engineering, Technical University of Munich, Weihenstephaner Berg 1, 85354 Freising, Germany*

^d *ZIEL Institute for Food & Health, Technical University of Munich, Germany*

* Corresponding author. Tel.: +358 50 398 73 81

E-mail address: klaus.muuronen@valio.fi (K. Muuronen)

26

27 ABSTRACT

28

29 Native whey, produced by microfiltration of skim milk, has great potential in infant nutrition and as
30 a functional food ingredient; however, processes to produce whey powder using high temperatures
31 can adversely affect protein quality. The individual effects of three critical processing steps were
32 investigated: standard thermal pasteurisation, membrane concentration and spray drying on protein
33 quality when performed under the mildest conditions considered feasible for industrial operation.
34 HPLC-analysis was used to measure the degree of denaturation of the six most abundant whey
35 proteins (β -lactoglobulin, α -lactalbumin, bovine serum albumin, immunoglobulins, lactoferrin and
36 lactoperoxidase) before and after each processing step. Overall, denaturation was minimal
37 throughout our tests, although the tendency to denature was unequal between the individual proteins
38 and also varied between tested processing types and medium compositions. In the production of
39 native whey with microfiltration, retentions varied greatly between the proteins but were not
40 affected by pasteurisation of milk.

41

1. Introduction

The biological functions of milk during the neonate's growth are numerous and mostly mediated by proteins, or peptides released upon their digestion (Fox, 2008; O'Mahony & Fox, 2013) with established roles in being the source of amino acids, transferring nutritionally important ingredients, protecting against microorganisms, modulating the immune system and acting as releasing signal agents that affect many physiological systems (Korhonen, 2009; Pellegrino, Masotti, Cattaneo, Hogenboom, & de Noni, 2013; Sanghoon & Hae-Soo, 2009). More recently they have also been linked with growing number of health benefits (Korhonen, Pihlanto-Leppälä, Rantamäki, & Tupasela, 1998; Madureira, Pereira, Gomes, Pintado, & Malcata, 2007), especially for infants whose protein digestion is less efficient than adults due to higher gastric pH and lower protease activity (Perdijk, van Splunter, Savelkoul, Brugman, & van Neerven, 2018). Native whey proteins have also been proposed as a possible reason why consumption of raw milk correlates with lower risk of developing allergies, asthma and respiratory infections in children (Abbring, Hols, Garssen, & van Esch, 2019; Perdijk et al., 2018). Although health benefits may be less accessible for the adult digestive system, undenaturated whey proteins have proven their value in improving functional properties of many dairy products (Mulvihill & Donovan, 1987).

To perform their biological functions, proteins fold into their specific, three-dimensional form, which can be easily disturbed by changes in their environment leading to their denaturation and loss of the original biological activity (Denature, 2020, Haque & Adhikari, 2015). For the functionalities to reach a consumer, the proteins have to tolerate certain amount of processing, such as agitation and elevated temperatures; this poses a challenge for industrial applicability as several of these proteins are prone to heat denaturation at temperatures typically used in pasteurisation, evaporation and drying of dairy products. As an example, in their recent study, Brick et al. (2017)

calculated that a typical pasteurisation of at 72 °C for 20 s was enough to reduce the number of identifiable proteins by approximately 20%.

As there are obvious health risks related to consumption of unpasteurised milk, an alternative approach on how to process and deliver bioactive proteins to consumers would be favourable. Milk microfiltrate, or native whey (also referred to as milk serum, serum protein and ideal whey), is a promising ingredient as it contains great number of native whey proteins with small quantity of caseins and has some unique properties compared to typical cheese whey in both nutritional and technological functionality (Gésan-Guiziou, 2015; Korhonen, 2009). In the production of native whey, the denaturation of the native proteins can be avoided using low temperatures, i.e., below 20 °C, which has the additional benefit of increased release of β -casein from the casein micelle, allowing it to permeate the microfiltration membrane together with whey proteins (Hekken & Holsinger, 2000). Therefore, native whey could be used to simultaneously supplement a dairy product with bioactive proteins and to adjust its casein composition. A prime example would be an infant formula with desired bioactivities from whey proteins and more human-like protein composition from increased β -casein content and near absence of bovine milks α_{s2} -caseins (Woychik, 1991), which has also been linked to better digestibility in infant nutrition (Nakai & Li-Chan, 1987). Whether or not the demands for digestibility and product safety could be met with such infant formula is open for debate, but perhaps nutritional benefits could be applied in products aimed at slightly older children.

The denaturation rate of whey proteins is known to be influenced by their surrounding media, especially pH, ionic strength and mineral composition, dry matter content and casein content (Anema 2006; Kessler & Beyer, 1991). Under the processing conditions typical for milk or whey, the corresponding proteins easily denature and aggregate (Anema, 2009; Kessler & Beyer, 1991). Denaturation of whey proteins during heat treatment has been researched on many occasions, using different analytical methods and varying feeds, most often milk or sweet whey (Mulvihill &

Donovan, 1987; Wijayanti, Bansal, & Deeth, 2014), but far more rarely with native whey. The aim of this work was to investigate whether it would be possible to produce a whey protein product in powder form with a truly native protein content using only processing steps commonly used within the dairy industry and to determine the optimal process.

2. Material and methods

A flowchart of experimental procedures is presented in Fig. 1. Raw milk was fat separated (RM) and pasteurised (PM). To obtain native whey, skimmed milk was microfiltrated (NW). Following microfiltration, native whey was concentrated (NWC). Unpasteurised concentrate was dried into a powder to make it storable (NWP). Three different drying methods; spray-, freeze- and vacuum-drying were compared. Loss of nativity during heat treatments was calculated by comparing against a non-treated feed sample, i.e., pasteurised native whey (PNW) against unpasteurised native whey (NW).

Reversed-phase high-performance liquid chromatography (RP-HPLC) was chosen to measure the degree of denaturation in this study because it can measure multiple proteins simultaneously, quickly and accurately (Anema, 2009; Kessler & Beyer, 1991).

2.1. Pre-treatment of milk

To obtain the best possible quality of the native whey, it was produced on site from freshly produced unpasteurised milk, retrieved separately for each batch. Three types of milk samples were obtained from a local dairy (Molkerei Weihestephani GmbH & Co. KG, Freising, Germany): untreated raw milk, skimmed raw milk and skimmed milk pasteurised at 72 °C for 15 s. Both skimmed milk samples, raw and pasteurised, underwent the same industrial cream separation

process in the dairy. To ensure that the raw milk samples (RM) had the highest possible amount of native proteins, separation conditions used at local dairy were compared against raw milk samples skimmed at 10 °C in a centrifuge ($6000 \times g$ for 10 min). Pasteurised samples (PM) were heat treated at the dairy with indirect high temperature short time (HTST) method (72 °C, 15 s), used commonly in dairy industry. To avoid microbiological growth all samples were immediately cooled down to a storage temperature of 5 °C and further processed within four days from collecting. Raw milk samples were collected between April and May of 2018.

2.2. Protein separation and concentration

To separate native whey from milk, 220 L of unpasteurised skimmed milk was concentrated batchwise with a cross flow microfiltration system, using a 6.3” polymeric (PVDF) spiral wound (SW) element with a nominal pore size of 0.1 μm and 9.9 m^2 of active membrane area (Synder Filtration, Vacaville, CA, USA). Filtration conditions were kept constant throughout all tests: temperature between 10 and 12 °C, average transmembrane pressure (Δp_{TM}) at 0.6 bar and pressure difference across the element (ΔP) at 0.8 bar. To ensure equal permeate quality between trials and to allow the system to reach a steady state, each run was started with an equal circulation step (30 min), during which both permeate and concentrate were returned to the feed tank.

Following the circulation step, microfiltration permeate for further trials was separated by concentrating the milk until a volumetric concentration factor of 3.3 was reached. Only raw milk (RM) was used to produce permeate for further tests, but the initial circulation phase was repeated also for pasteurised milk (PM). The feed tank of the system had a maximum volume of around 100 L, which was kept full until all 220 L of milk had been fed into the plant. Concentration time varied between 60 and 70 min during which approximately 150 L of permeate was collected into 40 L

cans, constantly weighed to monitor the progress of concentration. Upon being filled, the cans were quickly cooled in an ice bath and taken to cold storage (+5 °C).

The 150 L of native whey was then concentrated in a reverse osmosis step to produce appx. 38 L of native whey concentrate, (NWC). The crossflow filtration unit used for concentration was equipped with two 4" polymeric (PA) spiral wound reverse osmosis elements (Dow, US), installed in series with a combined membrane area of 5.6 m² and salt rejection of 99.4 %. Filtration temperature was maintained at 10 °C, Δp_{TM} at 50 bar and pressure difference between inlet and outlet (ΔP) at 0.8 bar. Concentration was started directly by collecting the permeate into a 40 L canister and continued until target Brix value (28°) was reached. Permeate was weighed and average concentration factor was calculated to be 4.4. Total duration of RO-concentration varied between 120 and 150 min.

2.2.1. *Pasteurisation of native whey samples*

Native whey and native whey concentrates were pasteurised at 72 °C for 15 s (HTST) and 63 °C and 30 min (LTLT), according to Fox, Uniacke-Lowe, McSweeney, and O'Mahony (2015). For indirect HTST pasteurisation, (PNW) and (PNWC) in Fig. 1, a tubular heat treatment pilot plant was used (GEA, UHT pilot plant, Type L). For the longer (LTLT) treatments, 30 mL stainless steel tubes (17 mm inner diameter) were filled with milk and immersed in a circulating water bath at 64 °C. A reference tube was equipped with a thermometer. Pasteurisation time of 30 min was defined from the time when the sample temperature exceeded 62 °C.

2.2.2. *Drying of native whey samples*

To test the effect of drying method on protein denaturation, three different drying methods, spray-, freeze- and vacuum drying were compared. Before drying, pH was standardised to 6.80 with 1 M NaOH solution. Each of the three unpasteurised native whey concentrate samples, (NWC in

Fig. 1), was divided to be used for all three drying methods. Dried samples (NWP) were sealed into airtight glass jars with small as possible air space.

2.2.3. *Spray drying*

For spray drying tests, a mini spray drying unit, equipped with a two-fluid atomiser, was used (B-290, Büchi Labortechnik AG, Switzerland). During spray drying feed material was kept in an ice bath and no pre-heating was used. The feed mass flow rate varied, depending on drying temperature, between 2.7 kg h⁻¹ for the higher and 10.9 kg h⁻¹ for the lower temperature. Air flow was kept at the maximum setting for each test. Two different air inlet and outlet temperature combinations, 160/95 °C (SD-H) and 115/50 °C (SD-L), were selected based on literature to cover the typical range used in industrial spray drying of whey products (Bansal & Bhandari, 2016; Filková & Mujumdar, 1995) and the temperature range reported to cause denaturation of studied proteins (65–83 °C) (Boscolo, Leal, Ghibaudi, & Gomes, 2007; Rüegg, Moor, & Blanc, 1977). Feed samples were continuously cooled during spray-drying, and no preheating was applied to avoid denaturation prior to actual drying, as it has been shown earlier to be a major contributor to spray-drying related denaturation (Oldfield, Taylor, & Singh, 2005).

2.2.4. *Freeze- and vacuum drying*

A laboratory freeze dryer (Christ Delta 1-24 LSC) was used to produce both vacuum (VD) and freeze-dried (FD) native whey powder samples. The same samples were used in both procedures and their handling differed only in that the freeze-dried samples were pre-frozen to -80 °C before placing them into the dryer. All samples were freeze dried within five weeks from their production. For vacuum drying, the pressure was lowered to 15 mPa, and sample temperature set to +20 °C. Freeze-drying temperature was -15 °C, while using the same pressure as with vacuum drying. Both freeze- and vacuum-drying experiments were completed in 48 h. Dried samples were

pulverised with a mortar and sealed into a glass jar with small as possible air space to wait for analysis.

2.2.5. Protein analysis

Overall protein level was analysed based on the total nitrogen concentration according to Dumas' method using Nitrogen analyser Vario Max cube (Elementar Analysensysteme GmbH, Langenseibold, Germany). Nitrogen to protein conversion factor used for milk samples was 6.38. For native whey samples, consisting of approximately 80% whey and 20% caseins proteins, the conversion was adjusted to 6.40, according to protein conversion factors for milk proteins (Dupont, Croguennac, Brodkorb, & Kouaouci, 2013; Maubois & Lorient, 2015).

Non-aggregated, acid soluble, whey proteins, ALA, BLG, BSA, IG, LF were quantified using reverse phase high performance liquid chromatography (RP-HPLC). Samples were analysed within 2–3 days and stored at 5 °C before that. Powder samples (NWP) were weighed and dissolved in distilled water at room temperature to match total solids concentration of native whey samples (NW), approximately 6%, and allowed to hydrate under continuous stirring with a magnetic stirrer until no powder particles were visible and the solution was visually clear, typically this was completed in 30 to 60 min. Prior to inserting samples into the RP-HPLC-column, caseins were removed by acid precipitation at pH 4.6 and filtering the supernatants through a 0.45 µm filter (Chromafil, Xtra RC45/25). Analyses were carried out according to the method originally described by Voswinkel and Kulozik (2011), and later improved by Heidebrecht et al. (2018), using a PLRP-S 300 Å/8µm column, 150 × 4.6 mm (Latek, Eppelheim, Germany) with an adapted elution gradient to increase the peak sharpness. Eluent A contained 0.1% (v/v) trifluoroacetic acid (TFA) dissolved in water and eluent B was a mixture of 0.0555% (v/v) TFA, 80% (v/v) acetonitrile and 20% (v/v) water. The flow rate was 1 mL min⁻¹ and the temperature 40 °C.

Loss of individual proteins was determined by comparing treated sample to the untreated ones, e.g., pasteurised samples against unpasteurised one (Kessler & Beyer, 1991). Genetic variants of BLG were calculated as one in the results, as well as all classes of immunoglobulins. To quantify the aggregated whey proteins, BLG and ALA, and different casein proteins (α -, β - and κ -caseins), a method using guanidine buffer solution to dissolve proteins, originally described by Bobe, Beitz, Freeman, and Lindberg. (1998), and further developed by Dimpler, Wohlschläger, and Kulozik (2016), was used. In accordance with the latter, Agilent Zorbax 300SB-C18, 150×4.6 mm, (Santa Clara, CA, USA) column was used. Eluents A contained 0.1% TFA in 90% water and 10% acetonitrile solution, while eluent B consisted of 0.07% TFA in 10% water and 90% acetonitrile solution. Flow rate was kept at 1.2 mL min^{-1} and temperature at 40°C .

2.2.6. Powder composition

Volumetric Karl Fischer titration was used to determine the water content of all powder samples. To determine the basic composition of native whey three parallel spray-dried samples were analysed applying ISO-standardised methods for minerals (ISO, 2007) and lactose (ISO, 2002).

2.2.7. Data handling and analysis

All experiments were repeated at least three times, unless stated otherwise. The analysis results presented in tables and figures represent the mean values with error values or bars according to their standard deviation. To standardise the results between batches of variable protein content, native protein concentrations were compared against their total nitrogen content according to Eq. 1.

$$\text{Ratio of native protein} = \frac{[\text{Native protein}]}{[\text{Total Nitrogen}] \times \text{Protein conversion factor}} \quad (1)$$

3. Results and discussion

3.1. *Effect of separation and pasteurisation on protein denaturation*

While the composition of milk samples remained constant when taken from the same batch, milk as a biological raw material has a varying composition, which was noticed to cause deviation between batches. Raw milk samples, skimmed at 10 °C, had the lowest possible thermal history and thus the highest possible amount of native whey proteins. Separating of milk fat at more typical temperature of 50 °C was not found to cause any loss of nativity on any of the quantified proteins and was thus selected to be used for following experiments (Table 1). A similar conclusion was reported earlier for IG's (Li-Chan, Kummer, Losso, Kitts, & Nakai, 1995). The overall whey protein denaturation degree during industrial HTST- pasteurisation (72 °C, 15 s) of milk was calculated based on the combined HPLC results to be $9 \pm 6\%$ which, given the deviation, is in line with the 4% reported earlier for full fat milk by Guinee et al. (1995), but high compared with the 1% calculated by Dannenberg and Kessler (1988) for denaturation of BLG and ALA in skim milk. A relatively high value of 11% was recently reported by Svanborg, Johansen, Abrahamsen, and Skeie (2014), who used similar pasteurisation and protein analysis methods as was used in this work.

While the major whey proteins were not as strongly affected by HTST-pasteurisation, having denaturation degrees below 10%, greater average losses of 17 to 29% were observed for IGs, BSA, LF and LPO, as shown in Table 1. The average loss of IG's was $29 \pm 7\%$, which is in line with the 24–38% reported earlier by Li-Chan et al. (1995) and 24% by Lorenzen et al. (2011) for commercial pasteurised skim milk samples after homogenisation and pasteurisation. Our results for industrially pasteurised milk samples suffered from far greater deviation between results than samples produced in laboratory scale, and while the reason behind it was left unclear to us, it points

out that special attention should be given to the increased complexity when transferring our findings to industrial scale.

3.2. *Microfiltration of skim milk – effect of milk pasteurisation on protein composition in native whey*

Microfiltration was used to separate native whey from caseins and other larger particles in milk. During the first 20 to 25 min of the initial 30-min circulation step, a gradual change in permeate, from turbid to clear was visible. Initial change in turbidity of permeate is typical for polymeric SW-membranes and believed to be caused by casein, which initially permeates the membrane and then, after formation of a deposited casein layer on membrane surface, cause changes to the separation characteristics acting as a secondary filtration layer (Le Berre & Daufin (1996). Given the complexity of fluid dynamics involved in formation of a deposit layer, the timing for this phenomenon was surprisingly consistent throughout the experiments, but is most likely linked to the specific conditions of each system as, e.g., Zulewska, Newbold, and Barbano (2009) reported far shorter times of 2 to 3 min needed for the above-mentioned effect to take place when using a membrane with larger pore size and higher filtration temperature.

Compared with earlier work, our native whey samples had a similar composition of major whey proteins (ALA and BLG), but information on minor whey proteins concentrations, or their nativity, was scarce. Average protein contents in MF-permeates (6.0 g L^{-1} for raw milk and 6.2 L^{-1} for pasteurised milk) were comparable to results by Zulewska et al. (2009) (5.8 g L^{-1}) and Beckman, Zulewska, Newbold, and Barbano (2010) (5.1 g L^{-1}), who also used polymeric SW-membranes, but a temperature of 50°C . Slightly lower total protein content in their permeates was likely due to the higher filtration temperature causing the β -casein equilibrium to shift towards micellar form. As a result, the permeates in previously mentioned studies had significantly lower

casein concentrations of 0.01 or 0.02% respectively, compared with the 0.15% in our samples. The majority of the casein was made of β -casein (58 %), followed by α -CN and κ -CN (Table 3).

Arranging proteins by their retention rates from low to high in Fig. 2 (ALA, BLG, IG, LF, LPO, BSA) shows that some proteins with interesting biological activities, such as IG, LF and LPO, were not favoured by microfiltration as a method to separate caseins from whey proteins. While it was known that not all proteins permeate the microfiltration membrane at an equal rate (Gosch, Apprich, Kneifel, & Novalin, 2013), our assumption was that the difference in permeation of the individual proteins would result from aggregation of heat labile whey proteins to caseins, namely κ -casein, during pasteurisation of milk. Native whey, obtained from both pasteurised and unpasteurised milk, however, had nearly equal composition of the individual native proteins measured (Table 1). This indicates that denaturation occurring during pasteurisation is not the predominant factor that could be used to explain why certain proteins, such as BSA, are less prone to permeate the microfiltration membrane. Explanation of what does cause this difference in permeability, is possibly linked with the different proteins interact with the surface of the microfiltration membrane but was unfortunately beyond our understanding.

Protein retention did not seem to correlate perfectly with either increasing molecule size (ALA, BLG, BSA, LF, LPO, IGs) or isoelectric point (ALA, BSA, BLG, IGs, LF, LPO) (Hahn, Schulz, Schaupp, & Jungbauer, 1998; Zydney, 1998). Low retentions for ALA and BLG were likely due their small size and low and negative surface charge at used pH. Correspondingly, being both large and having strong positive charge, LF and LPO can be expected to have higher retention, but neither can be used to explain why the highest retention was observed for BSA while IGs were able to permeate at much higher rate. Possibly, overall salinity or glycosylation of some of the biologically active and steric characteristics might be aspects to be looked in future work to better understand these differences in permeation. Overall retention of native whey proteins in

unconcentrated milk was calculated to be 48% for unpasteurised and 42% for pasteurised milk, which agrees with the removal rate of 49%, reported by Zulewska et al. (2009).

As a similar decrease in retention was observed for the individual proteins (Fig. 2), native proteins were found in nearly equal amounts in both permeates, despite their lower initial content in pasteurised milk (Table 1). The reason for this difference in retention is unclear, especially as they are in contradiction with the results reported earlier by Svanborg et al. (2014), who reported lower retention for both native BLG and ALA when unpasteurised milk was used. Although raw material handling was similar in the experiments, the cause for the contradictory outcome might arise from differences in filtration experiments, as they used ceramic membrane, above 50 °C temperature and collected samples after concentration instead of circulation at the initial concentration. Recently Heidebrecht and Kulozik (2019), using the same analytical method, reported similar retention for measured proteins using ceramic MF-membrane to separate whey proteins from unpasteurised skim milk at 50 °C. Permeation of LF and IG's in microfiltration of raw milk was 43% and 50%, while Gosch et al. (2013) reached 27 % for LF and nearly 100% for IGs, possibly due to differences in using a ceramic or polymeric membrane or analytical method used. Crowley et al. (2015) reported a noticeable retention for LF during microfiltration of milk at ≤ 4 °C, when using polymeric membrane cassettes with nominal pore size of 0.1 μm , but far less with 0.45 μm . The decline was also observed in our results for 0.1 μm membrane, but to a lesser extent.

RO-concentration did not cause denaturation of target proteins, as their relative proportions to total protein and other constituents (Table 4), remained unchanged during RO-concentration. The permeate collected during RO-concentration was colourless and had total protein concentration of < 0.1%, affirming that the loss of protein was minimal, and that the protein composition remained consistent.

336

337 3.3. *Effect of native whey pasteurisation on protein denaturation*

338

339 Concentration of each of the native proteins identified in milk samples were also within
340 calibration range in all pasteurised whey samples, although results for the dilute MF-permeates
341 admittedly at the lower end of it. Our results with native whey showed no significant difference
342 between LTLT and HTST pasteurisation as almost no denaturation of any of the measured proteins
343 was detected in either sample (Table 2), which was in contrast with the results for similarly
344 pasteurised skim milk. Possibly due to lack of micellar casein, which was demonstrated earlier by
345 Kessler and Beyer (1991) to induce denaturation of whey proteins. Similar results were recently
346 reported for HTST pasteurised native whey by Bogahawaththa, Chandrapala, and Vasiljevic (2017).

347 However, contrary to other studies, our findings also cover some of the minor whey proteins
348 whose denaturation might be overlooked when focusing on the overall denaturation degree. Baro et
349 al. (2011) used combination of mass spectrometry, HPLC and SDS-PAGE to compare the impact of
350 HTST and LTLT pasteurisation on native protein profile on human milk, whose composition native
351 whey closely mimics. Their findings indicated that, while neither method caused significant
352 denaturation, HTST was better at preserving the original protein profile, including lactoferrin, lipase
353 and immunoglobulins with known biological activities. Surprisingly, according to our results, there
354 was no significant difference in protein nativity between samples, which were HTST-pasteurised
355 before or after concentration, even though the possibility of interactions was higher in the more
356 concentrated solution. This was possibly due to high lactose to protein ratio (7.6) in native whey,
357 shown earlier to inhibit the denaturation of whey proteins (Anema, 2006; Arakawa & Timasheff,
358 1982). Although the past work on this subject is quite extensive, it is not easy to draft up a universal
359 conclusion from it. Overall concentration of a solution has been shown to have lesser effect on
360 denaturation rate of BLG and ALA, than protein to solids- or casein to whey protein ratios (Anema,

2006; Kessler & Beyer, 1991). Recently Marx, Bernauer, and Kulozik (2018) reported that overall concentration had no effect on the denaturation degree in cheese whey concentrates, when pasteurised at temperatures below 80 °C for 30 s.

3.4. *Effect of drying method on denaturation of whey proteins*

In all powder samples, disregarding the drying method or temperatures used, the concentration of native was nearly identical between all drying methods (Table 5). Comparable concentrations of native and non-native (Table 3) BLG and ALA confirms their low denaturation degree in powder samples. To compare powder samples to their feed material and to exclude the effect of varying moisture content in powder samples, native protein concentrations were standardized against their respective total protein concentrations, as presented in Table 4. The only major difference was found with LPO, which lost more than 40% of its nativity in all drying processes, irrespective of temperatures used, leading to the conclusion that the dehydration caused LPO to aggregate, either directly or during the short storage time as powder. All powder samples dissolved visibly in distilled water and after 1 h of continuous stirring, no flakes or powder particles could be seen in the transparent whey solution.

It should be noted though, that due to uneven scaling of surface area and volume of the drying chamber, some drying conditions, such as residence time inside the chamber, cannot be replicated across the vastly different sized laboratory- and industrial units. A visible result of which is the lesser agglomeration of powder particles when produced in laboratory scale (Nijdam & Langrish, 2007). While freeze drying is known to be a gentle drying method, widely used to produce bioactive protein products (Maltesen & van de Weert, 2008), our results show that laboratory scale spray drying caused as little, or no, denaturation for the proteins in native whey. Of the variables affecting denaturation of proteins during spray drying, outlet temperature was found to

be the most critical one and was thus chosen as the primary variable in this work (Haque & Adhikari, 2015). Although outlet temperatures exceeded those needed for denaturation of whey proteins, the temperature inside a droplet during spray-drying should remain considerably lower, not exceeding 70 °C, due to the cooling effect of evaporation continuing until moisture is completely evaporated (Bernard, Regnault, Gendreau, Charbonneau, & Relkin, 2011; Kelly & Fox, 2016).

The high lactose content, combined with low concentration and decreased molecular mobility of proteins in the dry particle could explain their ability to retain native state during drying irrespective of the outlet temperature (Anema, 2006; Mensink, Frijilink, van der Voort Maarschalk, & Hinrichs, 2017). Lactose and protein concentrations in our spray dried powder (SD-H) were $72.5 \pm 1.7\%$, $9.8 \pm 0.2\%$, respectively. Combined with the short time needed for all the water in a droplet to evaporate, temperature seemed to stay low enough to prevent denaturation of whey proteins, even at the highest outlet temperature used (95 °C). It is likely that further increases in drying temperature would lead to protein denaturation but with limited gains, as the residual water content reached here was a satisfactory $5.1 \pm 0.1\%$ (SD-H in Table 5). On the other hand, lower drying temperatures could lead to higher moisture content in powders (e.g., $8.3 \pm 0.7\%$ in SD-L) adversely affecting their storage stability, while providing no real benefits in terms of protein quality. Proteins at the surface of a droplet during spray-drying are known to be more prone to unfolding, making controlling of the droplet size crucial in minimizing the overall denaturation level in a dried product (Haque & Adhikari, 2015; Maltesen & van de Weert, 2008). Although droplet size was likely inconsistent in our trials due to varying feed flow rates, it was left without further attention at this time, as no significant difference was seen between samples dried with different inlet- and outlet temperatures.

Only three earlier reports on spray-drying induced denaturation on native whey were found. Bernard et al. (2011), using a pilot scale unit, spray-dried native whey protein concentrate in 170–

260 °C inlet temperatures and reported losses of 35% for native BLG and 29% for ALA at temperature combination of 170 °C (inlet) and 85 °C (outlet). Further increases in temperature resulted in even higher losses. Anandharamakrishnan, Rielly, and Stapley (2007, 2008) studied on two occasions the effect of outlet temperatures between 60 and 120 °C on denaturation of native whey protein isolate, rehydrated into 20, 30 and 40% solutions, in pilot scale, while maintaining a steady temperature drop between inlet and outlet of 100 °C. Measuring the enthalpy of denaturation, Anandharamakrishnan et al. (2007) found that the lowest loss of nativity, 30%, occurred at 80 °C, while temperature 120 °C resulted in 95% loss. Loss of soluble protein was, however, considerably lower and was confirmed by RP-HPLC results of soluble ALA and BLG (Anandharamakrishnan et al., 2008). Anandharamakrishnan et al. (2008) also reported almost no loss of soluble ALA and BLG at outlet temperatures of 60 and 80 °C, while increasing the temperature to 100 °C resulted in approximately 8 and 13% loss of ALA and BLG, respectively. On both occasions, the highest denaturation degrees were seen when the highest feed concentration (40 %) was used. Similarly, low losses of native IgG and BSA during pilot scale spray drying of skimmed milk at temperature between 160-200 °C (inlet) and 87-101 °C (outlet) were also reported by Oldfield et al. (2005). Wang, Timilsena, Blanch, and Adhikari (2017) compared the effect of spray- and freeze drying on denaturation enthalpy of purified lactoferrin solution in laboratory scale and found no significant difference.

4. Conclusions

Overall, separating native whey from milk and processing it into a pasteurised powder was accomplished with minimal effect on protein nativity, even if not without changes to protein composition as permeation through microfiltration membrane was highly uneven between measured proteins. Whether raw or pasteurised milk was used as a raw material in microfiltration, had

surprisingly little effect on protein composition of native whey, and the differences observed with less heat tolerant proteins were in line with those observed during pasteurisation of milk. In other words, protein denaturation prior to microfiltration did not explain why certain proteins, e.g., BSA, were underrepresented in native whey.

Processing native whey further into a pasteurised powder caused no further changes in protein distribution and only minimal decline in overall protein nativity. Standard HTST or LTLT - pasteurisation of native whey, caused negligible denaturation of any of the measured proteins, as did both spray- and freeze-drying. As spray-drying and HTST-pasteurisation are both widely used and cost efficient, these results could be of great economic importance when establishing a production process for a whey powder with improved biological functionality. Changing the dry matter content, e.g., to produce native whey protein concentrate, could lead to a different outcome and should be studied independently. For future work and industrial scale applicability, further attention should also be given to processing steps that were excluded from our work but would add to the overall heat load of the product, such as evaporation and pre-heating of concentrate prior to drying. Careful selection of microfiltration membrane and operating conditions to counter the uneven transmission of proteins could have great impact on product composition. Considering the similarities in dry matter composition of native whey and human milk, our results indicate that majority of biological functionalities originally present in milk proteins could be incorporated in powder products aimed at young children.

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Table 1Average native whey protein composition of milk and native whey samples. ^a

Protein	RM10	RM50	PM	NWRM	NWPM
Total protein	35.5 ± 1.16	35.9 ± 1.09	36.0 ± 0.50	6.02 ± 0.13	6.31 ± 0.42
Native BLG	4.27 ± 0.05	4.24 ± 0.07	3.99 ± 0.34	2.08 ± 0.10	2.29 ± 0.07
Native ALA	1.11 ± 0.01	1.12 ± 0.02	1.05 ± 0.08	0.75 ± 0.02	0.77 ± 0.02
Native IG	0.45 ± 0.00	0.47 ± 0.02	0.32 ± 0.03	0.20 ± 0.02	0.15 ± 0.02
Native BSA	0.21 ± 0.00	0.22 ± 0.01	0.17 ± 0.01	0.04 ± 0.01	0.04 ± 0.00
Native LF	0.20 ± 0.00	0.21 ± 0.01	0.16 ± 0.01	0.08 ± 0.00	0.07 ± 0.00
Native LPO	0.19 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.05 ± 0.01	0.06 ± 0.00

^a Abbreviations are: RM10, raw milk skimmed at 10 °C; RM50, raw milk skimmed at 50 °C; PM, pasteurised milk; NWRM, microfiltration permeate from raw milk; NWPM, microfiltration permeate from pasteurised milk. Values are in g L⁻¹; total protein based on nitrogen concentration.

Table 2

Average concentration of native whey proteins detected in native whey and native whey concentrate after pasteurisation. ^a

Protein	NWC	PNWC (LTLT)	PNWC (HTST)	NW	PNW (HTST)
BLG	12.13 ± 1.49	11.68 ± 1.76	11.66 ± 1.69	2.69 ± 0.65	2.63 ± 0.45
ALA	4.15 ± 0.35	3.82 ± 0.58	3.97 ± 0.43	0.92 ± 0.18	0.96 ± 0.12
IG	1.15 ± 0.14	1.10 ± 0.15	1.09 ± 0.10	0.26 ± 0.07	0.25 ± 0.04
BSA	0.25 ± 0.03	0.23 ± 0.04	0.24 ± 0.03	0.06 ± 0.02	0.05 ± 0.01
LF	0.43 ± 0.03	0.35 ± 0.08	0.41 ± 0.03	0.09 ± 0.02	0.10 ± 0.01
LPO	0.39 ± 0.06	0.32 ± 0.10	0.35 ± 0.09	0.07 ± 0.02	0.08 ± 0.01

^a Abbreviations are: NWC, native whey concentrate; PNWC (LTLT), LTLT pasteurised native whey concentrate; PNWC (HTST), HTST pasteurised native whey concentrate, NW, native whey; PNW (HTST), HTST pasteurised native whey.

Table 3

Average protein compositions of milk, native whey, native whey concentrates (g L⁻¹) and native whey powders (g kg⁻¹). ^a

Protein	RM10	NWRM	NWPM	NW	NWC	NWP
Total protein	35.5 ± 1.2	6.1 ± 0.1	6.3 ± 0.4	7.4 ± 0.6	28.5 ± 1.9	95.9 ± 3.4
Casein proteins	29.6 ± 0.6	1.4 ± 0.3	1.5 ± 0.2	1.5 ± 0.2	7.8 ± 1.1	23.3 ± 4.4
α-CN	14.3 ± 0.3	0.4 ± 0.2	0.1 ± 0.1	0.5 ± 0.2	2.6 ± 0.3	6.7 ± 2.8
κ-CN	3.8 ± <0.1	0.1 ± 0.1	0.0 ± <0.1	0.1 ± 0.1	0.6 ± 0.1	2.5 ± 0.5
β-CN	11.5 ± 0.4	1.0 ± 0.1	1.3 ± 0.2	0.9 ± 0.1	4.7 ± 0.2	14.1 ± 1.8
Whey proteins	5.2 ± 0.2	2.7 ± <0.1	2.9 ± 0.1	3.3 ± 0.7	14.9 ± 3.1	57.1 ± 5.8
BLG	3.9 ± 0.1	1.9 ± < 0.1	2.1 ± <0.1	2.4 ± 0.6	10.6 ± 2.3	44.5 ± 2.2
ALA	1.1 ± <0.1	0.8 ± < 0.1	0.8 ± <0.1	0.9 ± 0.2	4.0 ± 0.6	14.2 ± 1.7

^a Denatured proteins are included in the results. Abbreviations are: RM10, raw milk skimmed at 10 °C; NWRM, native whey from unpasteurised milk (CF = 1); NWPM, native whey from pasteurised milk (CF = 1); NW, native whey (MF-permeate from concentration phase); NWC, native whey concentrate; NWP, native whey powder. Number of samples: RM10 = 6, NWP = 17, all others = 3. Total protein based on nitrogen concentration.

Table 1

Ratio (%) of native proteins to total protein in native whey, native whey concentrate and native whey powder. ^a

Protein	Milk pasteurisation pre MF		NWC pasteurisation		Drying	
	NWRM	NWPM	NWC	PNWC (HTST)	NWP (SD-H)	NWP (FD)
BLG	39.9 ± 3.3	36.2 ± 0.0	41.3 ± 3.2	39.8 ± 0.7	39.8 ± 1.6	40.4 ± 4.8
ALA	13.7 ± 0.4	12.1 ± 0.0	14.2 ± 0.5	13.6 ± 0.3	14.5 ± 0.1	14.7 ± 0.7
IG	3.8 ± 0.4	2.4 ± 0.1	3.9 ± 0.3	3.7 ± 0.1	4.0 ± 0.2	3.8 ± 0.3
BSA	0.9 ± 0.1	0.6 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.2
LF	1.4 ± 0.0	1.2 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.5 ± 0.1
LPO	1.1 ± 0.2	0.9 ± 0.0	1.3 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1

^a Abbreviations are: NWRM, microfiltration permeate from raw milk; NWPM, microfiltration permeate from pasteurised milk; NWC, native whey concentrate; PNWC (HTST), HTST pasteurised native whey concentrate; NWP (SD-H), spray dried (160/95 °C) native whey concentrate; NWP (FD), freeze dried native whey concentrate. Total protein based on total nitrogen concentration.

Table 5

Average concentrations of residual water, total protein and native whey proteins in powder samples. ^a

Component	FD	VD	SD-L	SD-H
Water	107 ± 1	64 ± 0	83 ± 7	51 ± 1
Total protein	92 ± 4	98 ± 2	94 ± 4	98 ± 2
Native BLG	39.0 ± 4.2	43.4 ± 2.2	44 ± 1.8	43.0 ± 1.9
Native ALA	15.3 ± 0.9	15.0 ± 0.4	15.5 ± 0.1	15.0 ± 0.4
Native IG	3.7 ± 0.2	4.4 ± 0.3	4.3 ± 0.2	4.2 ± 0.3
Native BSA	0.9 ± 0.2	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.1
Native LF	1.4 ± 0.1	1.2 ± 0.4	1.5 ± 0.1	1.5 ± 0.1
Native LPO	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.1

^a Abbreviations are: NWP, native whey proteins; FD, freeze-dried powder; VD, vacuum-dried powder; SD-L, spray-dried powder (110/55 °C); SD-H, spray-dried powder (160/95 °C).

Figure legends

Fig. 1. Manufacturing process for all milk, native whey, native whey conc. and -powder samples

Fig. 2. Average retention of native whey proteins after 30 min of microfiltration of unpasteurised (blue) and pasteurised (red) milk at steady conditions; the dotted line represents the average retention for the cumulative proteins.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: