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Short communication

Colloidal stabilisation of β-casein enriched whey protein concentrate

Yonas Hailu [a], James A. O'Mahony [b], Mark A. Fenelon [a], Noel A. McCarthy [a, *]

[a] Food Chemistry & Technology Department, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland
[b] School of Food and Nutritional Sciences, University College Cork, Cork, Ireland

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ABSTRACT

The aim of this study was to produce a colloidally stable whey protein concentrate enriched in β-casein. Microfiltration (MF) of skim milk was completed at ~7 °C (pore size 0.1 μm) followed by ultrafiltration of the MF permeate at ~9 °C to concentrate the β-casein-whey protein fraction (BWPC). The casein:whey protein ratio of the BWPC system was ~25:75. Skim milk was then added to ultrafiltration retentate at a level supplying 0, 0.1, 0.3 and 0.5% (w/w) of the total protein, followed by evaporation and spray drying. During evaporation of the BWPC stream without skim milk addition, there was significant precipitation of β-casein, resulting in an increase in particle size due to uncontrolled aggregation. Addition of low amounts of skim milk was effective at reducing the precipitation of β-casein during evaporation at 50 °C, indicating that the addition of micellar casein to the BWPC stream was able to prevent uncontrolled β-casein aggregation.

1. Introduction

Bovine milk protein is used extensively within the infant milk formula (IMF) industry in the form of blending skim milk and whey protein concentrates (WPCs). The casein to whey protein ratio of human milk is ~40:60 (Liao et al., 2017) and is comprised of ~64% β-casein, ~24% κ-casein and ~12% α_S-casein (Malacarne, Martuzzi, Summer, & Mariani, 2002). Bovine milk contains a casein to whey ratio of ~80:20 the casein of which is comprised of ~40% β-casein, 10% κ-casein and 50% α_S-casein (McCarthy, Wijayanti, Crowley, O'Mahony, & Fenelon, 2017).

Increasing the proportion of β-casein and α-lactalbumin is an important objective in efforts to improve the nutritional properties of IMF and can be achieved through the addition of enriched or purified protein ingredients (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015). The need to use multiple dairy ingredients to achieve a humanised protein profile places additional challenges on IMF manufacturers in terms of ingredient sourcing/transit and prediction of ingredient functionality/stability during reconstitution, thermal processing and drying. McCarthy et al. (2017) showed, through the use of cold microfiltration (MF) (<10 °C), β-casein could permeate the membrane along with whey proteins creating a unique combined casein-whey protein ingredient. However, the colloidal stability of this β-casein enriched ingredient was relatively low.

β-Casein is an amphiphilic protein, consisting of a hydrophilic N-terminal domain and a hydrophobic C-terminal domain (Rollema, 1992) and, at low concentrations and temperatures, β-casein exists as a monomer in solution (Dauphas, Amestoy, Llamas, Anton, & Riaublanc, 2008). However, increasing either β-casein concentration or temperature leads to an increase in hydrophobic interactions, resulting in protein aggregation and micelle formation. This has been shown to be an issue during heat treatment or during water removal via evaporation and spray drying, with McCarthy et al. (2017) suggesting that future work be performed on improving the heat stability of β-casein enriched whey. Crowley, Kelly, O'Mahony, and Lucey (2019) also highlighted that the colloidal stability of β-casein concentrate ingredients was significantly affected by the presence of minerals commonly found in nutritional product formulations. Therefore, the aim of this work was to investigate the potential stabilisation effect of skim milk addition to β-casein enriched whey protein ultrafiltration (UF) retentate.

2. Materials and methods

2.1. Production of β-casein enriched whey protein concentrate

Skim milk (1000 L) was obtained from a local dairy company and added to 1000 L of reverse osmosis water and chilled overnight at 5 °C at Moorepark Technology Limited, Moorepark, Teagasc, Fermoy, Co. Cork. Subsequently, MF was performed at ~7 °C using 19 Tami isoflux ceramic membranes (TAMI industrials, France)

* Corresponding author.
E-mail address: noel.mccarthy@teagasc.ie (N.A. McCarthy).

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situated in parallel (pore size 0.14 μm; surface area 6.65 m^2). The MF retentate was continuously chilled using a heat exchanger at 4 ± 1 °C during retentate recirculation (i.e., retentate returned to the feed). The feed recirculation rate was adjusted to 825 L h^{-1} at a feed pressure of 4.6 bar. The transmembrane pressure was maintained at 1 ± 0.25 bar. The MF permeate (1400 L) was then ultrafiltered using 10 kDa spiral wound membranes (Memtech, Israel) at ~9 °C with a feed recirculation rate of 850 L h^{-1} at 1.6 bar pressure and a membrane inlet pressure of 4 bar generated using a booster pump, until a final volume concentration factor (VCF) of 10 was attained. The concentrated UF retentate (~150 L) (BWPC) was divided into four batches (35 L each) prior to addition of skim milk (i.e., BWPC-0 = no skim milk addition; BWPC-0.1 = 0.1% of total protein sourced from skim milk; BWPC-0.3 = 0.3% of total protein sourced from skim milk; BWPC-0.5 = 0.5% of total protein sourced from skim milk). Subsequently, all batches were evaporated using a single-effect falling-film evaporator (Anhydro F1 Lab, Copenhagen, Denmark) at 50 ± 2 °C under re-circulation mode until a dry matter content of 18% (w/w) was reached. All concentrates were dried using a pilot-scale Anhydro-Lab 1 spray dryer (Anhydro F1 Lab Dryer; Copenhagen, Denmark), equipped with a two-fluid nozzle atomiser in counter-flow operation. Inlet and outlet temperatures were set at 178 and 88 °C, respectively.

2.2. Protein profiling and analysis

Total nitrogen and protein content was determined using the Kjeldahl method (ISO, 2001) and a nitrogen to protein conversion factor of 6.38. The protein profile of all samples was analysed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and performed according to the method of Laemmli (1970) with a slight modification as described by McCarthy et al. (2017). Bands were stained using Instant Blue stain-destain solution (Expedeon Instant blue™) overnight and destained with distilled water for 2 h.

Reverse phase-high performance liquid chromatography (RP-HPLC) was performed for protein quantification using a Poroshell 300SB-C18 (2.1 mm diameter × 75 mm length, 5 μm; Agilent Technologies, Ireland) column, equipped with a Zorbax poroshell guard column (1.0 mm diameter × 17 mm length, 5 μm; Agilent Technologies). BWPC ingredient powders were rehydrated at 10%, w/w, in distilled water, and a 200 μL aliquot was diluted in 7 M urea buffer containing 20 mM bis-Tris propane and 71.5 mM 2-mercaptoethanol (pH 7.5) at a 1:20 ratio (v/v). Diluted samples were left for 1 h at 23 °C prior to filtration using a 0.2 μm syringe filter (Agilent Technologies, Econofltr, PES 25 mm). Protein analysis was carried out in triplicate to quantify total and individual casein (β-, α- and κ-casein) and whey protein (β-lactoglobulin and α-lactalbumin) fractions.

2.3. Particle size analysis

Particle size of BWPC samples was measured using a Mastersizer 3000 laser light diffraction unit (Malvern Instruments, Malvern, UK), with a refractive index set at 1.46 and 1.33 for protein and dispersant (i.e., water), respectively. The absorption index of the disperse phase was set at 0.01 and the obscuration maintained at 6–12%. All measurements were performed in triplicate.

2.4. Colloidal stability analysis

A LUMISizer (LUM GmbH, Berlin, and Germany) analytical centrifuge was used to evaluate the sedimentation properties of 8% (w/w) protein solutions. Aliquots (~0.4 mL) of sample were transferred using a syringe and wide-bore needle into polycarbonate cells (PC 110-131XX; Lum GMBH). Continuous light transmission was measured over a 60 min period under centrifugation at 3500 RPM at 37 or 50 °C. Sedimentation to the bottom of the cuvette was measured using SEPVIEW v.4.1 software at a laser wavelength of 865 nm (Lerche, 2002). Stability of protein solutions was presented as instability index values, where 0 implies high stability and 1 indicates complete separation.

2.5. Statistical analysis

The data obtained were analysed using ANOVA and the level of significance set at $P < 0.05$ using SAS version 9.4. The mean comparison was made using Fisher's pair comparisons. Production of all protein samples was performed in duplicate, with analysis conducted in triplicate.

3. Results and discussion

3.1. Protein profile

SDS-PAGE and HPLC protein profiles of all BWPC ingredients are shown in Fig. 1. SDS-PAGE analysis showed distinct protein bands for β-casein, β-lactoglobulin and α-lactalbumin in BWPC-0 (Fig. 1A; lane 1), similar to the HPLC chromatogram (Fig. 1B; profile 1). The protein content of BWPC-0 was 37.6% (w/w, dry basis) with a casein:whey protein ratio 25:75 and ~100% of the casein composed of β-casein (Table 1). Previously, McCarthy et al. (2017) showed a similar protein content for a β-casein enriched whey protein ingredient (34.4%, w/w, protein) but showed a higher casein:whey protein ratio of 34.5:64.5. The addition of skim milk to the BWPC-0 sample led to an increase in the casein:whey protein ratio (Table 1). This resulted in a proportional decrease in β-casein (100–81.2%) and increase in α-casein (0–17.7%), as measured by HPLC (Table 1; Fig. 1B). Previously, Malacarne et al. (2002) and Potočnik, Gantner, Kuterovac, and Cividini (2011) reported a total α-casein concentration of ~11.8 and 11.1–12.5% in human milk, respectively. The α-casein in the current study at the higher skim milk protein addition (BWPC-0.5) was 17.7% (Table 1), which may further complement its use in infant formula. The level of β-lactoglobulin (~83%) and α-lactalbumin (~16%) as a percentage of total whey protein did not change with increasing levels of skim milk addition (Table 1).

3.2. Particle size and stability of protein systems

Particle size measurements of BWPC samples measured after evaporation are shown in Fig. 2. BWPC-0 showed a bimodal size distribution with a major peak from 10 to 624 μm (D_{90} = 211 μm), indicating significant protein aggregation/flocculation. A number of previous studies (Dauphas et al., 2005; Riaublanc et al., 2004) have shown that increasing either β-casein concentration or temperature led to an increase in hydrophobic interactions, resulting in β-casein self-association/aggregation and micelle formation. However, a significantly lower particle size was observed in BWPC samples with increasing skim milk, with D_{90} values of 34.6, 17.8 and 2.4 μm for BWPC-0.1, 0.3 and 0.5, respectively. Although it is important to note that all BWPC samples exhibited bimodal profiles, there was a clear shift towards a larger volume of particles in the size range 0.1–1.0 μm with increasing skim milk addition (Fig. 2).

Instability index values of rehydrated BWPC powders measured at 37 and 50 °C are shown in Table 2. Similar to particle size data, suspension stability was significantly ($P < 0.001$) effected by both skim milk addition level and measurement temperature (37 and 50 °C). At 37 °C, no difference in instability index was observed between BWPC-0 and BWPC-0.1 but it significantly decreased thereafter at higher skim milk addition levels for BWPC-0.3 and BWPC-0.5. This may be a result of the significant influence that

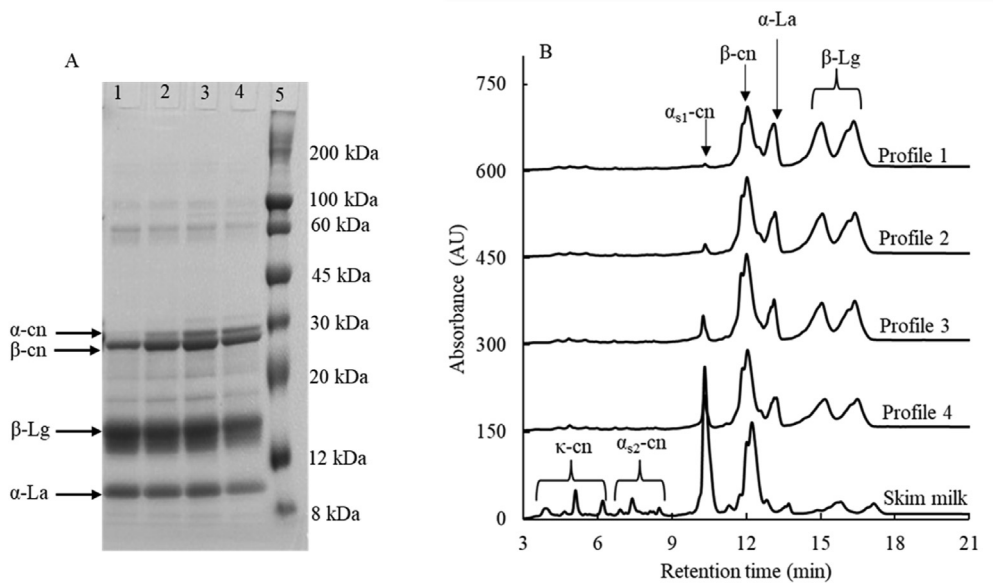

Fig. 1. Sodium dodecylsulphate-polyacrylamide gel electrophoresis (A) and high performance liquid chromatography (B) of β-casein enriched whey protein concentrate (BWPC) ingredients containing 0 (lane 1; profile 1), 0.1 (lane 2; profile 2), 0.3 (lane 3; profile 3), 0.5% (lane 4; profile 4) protein addition from skim milk and molecular mass marker (lane 5).

Table 1
Total protein content, casein:whey protein ratio and percentage of casein and whey protein fractions of β-casein enriched whey protein concentrate (BWPC) ingredients containing 0, 0.1, 0.3 or 0.5% (w/w) protein addition from skim milk.[a]

Component	BWPC-0	BWPC-0.1	BWPC-0.3	BWPC-0.5	Skim milk
Protein (g 100 g^{-1})*	37.6 ± 0.1	39.9 ± 0.4	40.1 ± 0.0	40.3 ± 0.3	41.3 ± 0.2
Casein:whey protein ratio	25.0:75.0	35.7:64.3	43.3:56.7	48.0:52.0	85.0:15.0
% β-CN of TCN	100 ± 0.0^{a}	94.0 ± 0.3^{b}	85.6 ± 0.9^{c}	81.2 ± 2.7^{d}	39.5 ± 1.6^{e}
% α-CN of TCN	0 ± 0.0^{e}	5.7 ± 0.5^{d}	13.1 ± 1.1^{c}	17.7 ± 1.7^{b}	48.3 ± 1.1^{a}
% κ-CN of TCN	0 ± 0.0^{b}	0.3 ± 0.3^{b}	1.3 ± 0.2^{b}	1.2 ± 1.1^{b}	12.2 ± 2.6^{a}
% β-Lg of TW	83.5 ± 0.8	83.6 ± 0.1	83.9 ± 0.2	83.5 ± 0.2	86.9 ± 4.1
% α-La of TW	16.5 ± 0.5	16.4 ± 0.1	16.1 ± 0.2	16.5 ± 0.2	13.1 ± 4.1

[a] Abbreviations are: TCN, total casein; TW, total whey protein. *Protein is given on a dry matter basis. Values presented are the means ± standard deviations of n = 3 measurements; means not sharing a common superscript in a row differ significantly ($P < 0.05$).

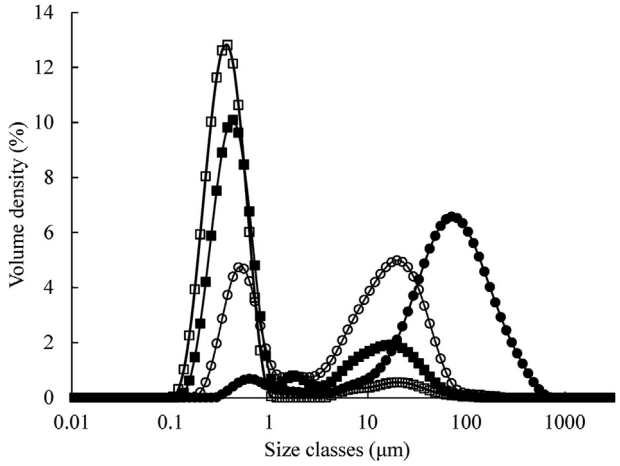

Fig. 2. Particle size distribution profiles of β-casein enriched whey protein concentrate (BWPC) ingredients containing 0 (●), 0.1 (○), 0.3 (■) or 0.5% (□) protein addition from skim milk measured after evaporation. Particle size values (in μm; average of triplicate analysis) of BWPC-0, BWPC-0.1, BWPC-0.3, and BWPC-0.5 were 211, 34.6, 17.8, and 2.4, respectively, for D_{90}, and 94.6, 14, 5, and 4.4, respectively, for $D_{4,3}$.

temperature plays on the hydrophobic interactions of β-casein, whereby increasing the temperature causes β-casein to move from the serum to the colloidal phase, forming a β-casein enriched micelle. Once equilibration of β-casein in the micelle has occurred, the κ-casein from the added skim milk (Table 1) provides colloidal stabilisation of the β-casein against calcium-induced precipitation. However, samples measured at 50 °C were less stable than their counterparts at 37 °C and it was only at a skim milk protein addition level of 0.5% that the instability values were significantly lower. The higher instability at 50 °C may be explained by the greater affinity of β-casein to calcium at this temperature, as was previously shown by Li et al. (2019). Horne and Lucey (2014) also described an increase in the calcium-binding affinity of casein with increasing temperature, and described the relationship between Arrhenius activation energy, electrostatic repulsion and hydrophobic attraction. The authors showed that the Arrhenius activation energy

Table 2
Instability index values of reconstituted β-casein enriched whey protein concentrate (BWPC) powders (8%, w/w, protein) measured at 37 or 50 °C containing 0, 0.1, 0.3 or 0.5% protein addition from skim milk.[a]

Sample	Separation temperature	
	37 °C	50 °C
BWPC-0	0.82 ± 0.02^{a}	0.88 ± 0.03^{a}
BWPC-0.1	0.83 ± 0.03^{a}	0.91 ± 0.01^{a}
BWPC-0.3	0.41 ± 0.05^{b}	0.86 ± 0.01^{a}
BWPC-0.5	0.33 ± 0.05^{c}	0.48 ± 0.04^{b}

[a] Values presented are the means ± standard deviations of n = 3 measurements; means not sharing a common superscript in a column differ significantly ($P < 0.05$).

decreased in a non-linear manner with increasing temperature and was greatly affected by the calcium ion concentration.

Overall, the addition of skim milk improved BWPC stability and while micellar casein was added in this study, other potential options may be feasible. Previously, Crowley et al. (2015) found that using polyvinylidene-difluoride based membranes (pore size 0.45 μm) allowed for the partial permeation of micellar casein into the permeate stream, which may actually avoid the need to add skim milk. A number of other studies over the last decade have also examined the effect of membrane type and pore size on the fractionation of casein and whey proteins. Jørgensen et al. (2016) used ceramic membranes (0.20 μm pore size), and although this study used relatively high filtration temperatures (i.e., 50 and 60 °C), a significant amount of casein permeated, giving a casein distribution similar to that in the original skim milk and therefore without the enrichment of β-casein. However, Coppola, Molitor, Rankin, and Lucey (2014) examined the effect of casein transmission during MF (0.08 μm pore size polymeric membranes) of skim milk at 7 and 23 °C and found that at low temperature casein transmission occurred and represented ~25% of the total protein in the permeate stream, with the authors stating that this casein fraction was almost entirely composed of β-casein.

4. Conclusion

The production of β-casein enriched whey protein streams was possible under cold MF conditions, but was highly susceptible to aggregation and precipitation once the temperature or protein concentration was increased. Through the re-addition of low amounts of micellar casein in the form of skim milk a significant increase in protein stability was achieved. This method could be optimised by refining the membrane pore size to allow the permeation of whey proteins, β-casein and low amounts of micellar casein, negating the need for re-addition of skim milk.

Declaration of competing interest

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.

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