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Short communication

Evaluation of casein as a binding ligand protein for purification of alpha-lactalbumin from beta-lactoglobulin under high hydrostatic pressure

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ABSTRACT

Fractionation of β-lactoglubulin (β-lg) and α-lactalbumin (α-la) using conventional separation technologies remains challenging mainly due to similar molecular weight. Herein, casein (CN) was used as ligand protein to specifically aggregate β-lg under high hydrostatic pressure (HHP) in order to separate α-la after acidification to pH 4.6. Specifically, we studied the effect of different concentration of CN on α-la purity and recovery. Model solutions of α-la, β-lg and CN (from 0 to 5 mg/mL) were pressurized (600 MPa–5 min). After acidification and centrifugation of pressure-treated solutions, purity of α-la was increased up to 78% with a recovery of 88% for solution without CN. In contrast with our initial hypothesis, the presence of CN decreased β-lg pressure-induced aggregation and co-precipitation upon acidification and significantly reduced purity (~71%). Therefore, our results suggest a chaperone-like activity of CN on β-lg pressure-induced aggregation which needs further investigation.

1. Introduction

Alpha-lactalbumin (α-la) and beta-lactoglobulin (β-lg) are the two major whey proteins (WP) of bovine milk that represent respectively, 4 and 10% of the total proteins. These WP are of industrial importance due to their nutritional and functional properties as well as in a context of valorization of whey from dairy processing (Jouan, 2002). In addition, these proteins represent an interesting source of bioactive peptides with various activities such as antimicrobial, opioid, antihypertensive, etc. (O'Mahony & Fox, 2013). Moreover, WP, mainly for α-la, are part of key biological functions such as in milk lactose biosynthesis, or source of key amino acids involved in the synthesis of the neurotransmitters (e.g. tryptophan and serotonin) (Kamau, Cheison, Chen, Liu, & Lu, 2010; O'Mahony & Fox, 2013; Toro-Sierra, Tolkach, & Kulozik, 2013). Although, WP present many interesting features, bovine β-lg, which is absent in human milk, is not desirable in infant formula due to it's allergenicity (Bansal & Bhandari, 2016; Kamau et al., 2010; O'Mahony & Fox, 2013). Therefore, their separation is of growing interest for infant formula application. Nevertheless, due to their similar molecular weight (14.2 and 18.6 kDa for α-la and β-lg, respectively), they are hardly fractionable using conventional industrial technologies such as membrane filtration (Bansal & Bhandari, 2016; El-Sayed & Chase, 2011; Smithers, 2015). However, their differences in terms of structure make them more or less sensitive to various treatment such as high hydrostatic pressure (HHP). Particularly, HHP is known to maintain the protein covalent linkages associated to disulfide bonds. Thus, α-la which contains 4 disulfide bonds (in comparison to 2 for β-lg), is more compact and thus more resistant to HHP treatment. Additionally, β-lg also contains one free sulfhydryl group which is also available for covalent interactions and disulfide interchanges with various milk proteins upon denaturation. Recently, we proposed a novel and eco-friendly technology for the fractionation of α-la and β-lg using HHP and casein (CN) as a ligand to modulate interactions between CN and β-lg followed by acidification to pH 4.6, corresponding to isoelectric point of CN (Marciniak, Suwal, Britten, Pouliot, & Doyen, 2018). In the same work, we obtained an α-la enriched-fraction with 86% purity and a recovery of 77%. However, for the industrial implementation of this process, the concentration of the ligand (CN) needs to be optimized. Thus, the aim of this study was to determine the α-la purification degree and recovery rate using HHP as a pre-treatment at varying CN concentration.

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2. Material and methods

The experimental design and materials and method used in the present study were similar to our previous study (Marciniak et al., 2018). All experiments and analyses were made in triplicate and statistical analyses were executed using the SAS University Edition (SAS Institue Inc, Cary, NC). Tukey test ($\alpha = 0.05$) was performed as a comparison test. Briefly, model milk protein solutions composed of α-la, β-lg (Davisco Food international Inc. Le Sueur, Minnesota, USA) and acidic casein (CN – Sigma Aldrich St Louis, Missouri, USA – lot # SLBM5975 V) were prepared by solubilizing proteins in 0.2 M Tris-HCl buffer (pH 6.6). Concentrations of α-la and β-lg were fixed at 2.5 $mg.mL^{-1}$ whereas concentration of CN was varied from 0 to 5 $mg.mL^{-1}$ corresponding to final protein ratio (α-la: β-lg: CN) of 1:1:0 to 1:1:2. The WP were submitted to HHP treatment (300 s 600 MPa) followed by an acidification at pH 4.6 to precipitate CN and CN-β-lg complexes (aggregates) and centrifugation at 11000*g* for 25 min. Mono-dimensional PAGE under native condition was performed to analyze aggregation profile. The protein concentrations were determined using HPLC, as described previously (Marciniak et al., 2018). The recovery rates, defined as the protein concentration in the supernatant relative to its initial concentration, were calculated using Eqs. (1) and (2) for α-la and β-lg, respectively. The purification degrees, defined as the concentration of protein relative to the total concentration of proteins in the supernatant (soluble fraction) and pellet (insoluble fraction) for α-la and β-lg, respectively, were calculated using the Eqs. (3) and (4).

In supernatants:

$$\alpha\text{–}la\ protein\ recovery\ rate(\%) = \frac{[sup]_{\alpha-la}}{[initial]_{\alpha-la}} \cdot 100 \quad (1)$$

$$\beta\text{–lg}\ protein\ recovery\ rate(\%) = \frac{[sup]_{\beta-lg}}{[initial]_{\beta-lg}} \cdot 100 \quad (2)$$

$$\alpha\text{–}la\ purification\ degree(\%) = \frac{[sup]_{\alpha-la}}{[sup]_{total\ proteins}} \cdot 100 \quad (3)$$

In pellets:

$$\beta\text{–lg}\ purification\ degree(\%) = \frac{[pellet]_{\beta-lg}}{[pellet]_{total\ proteins}} \cdot 100 \quad (4)$$

where [sup], [pellet] and [initial] correspond to the concentrations ($mg.mL^{-1}$) of proteins in supernatant, pellet, and initial solutions.

3. Results and discussion

3.1. Relationship between CN concentration and protein denaturation/aggregation

Fig. 1A and B show the native PAGE profiles of pressure-treated milk protein solutions and supernatants recovered after acidification and centrifugation. While the intensity of bands corresponding to CN increase with CN concentration (Fig. 1A), intensity of bands corresponding to α-la remain constant. However, a slight increase in the band intensity of the bands related to β-lg was observed as the CN concentration increases. The sample with a CN concentration of 5 $mg.mL^{-1}$ showed the highest band intensity indicating that β-lg was less associated with CN. Even though pressure-induced aggregates are not clearly visible within the gel, these aggregates could have simply been washed from the top of the wells by the migration buffer. As expected, after acidification at pH 4.6 and centrifugation of the samples (Fig. 1B), no band corresponding to CN were detected in the gel whereas α-la bands displayed a higher intensity as compared to other proteins. Although the band intensities related to β-lg were very light, they seemed to increase as the concentration of CN increased. These observations indicate that β-lg interacts less with itself and/or with CN, when more CN are used.

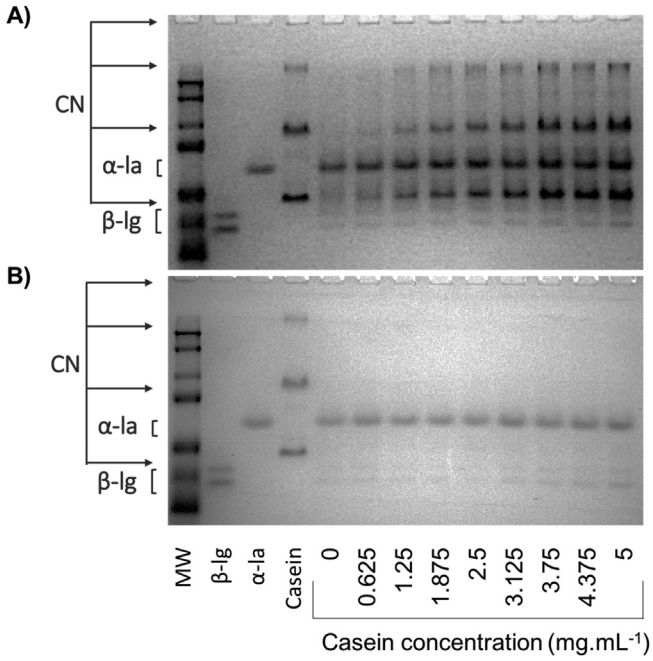

Fig. 1. PAGE profile under native condition of pressure-treated (600 MPa, 300 s) milk protein solutions (A) and pH 4.6-soluble fractions (supernatants) (B) at varying concentration of casein (CN) used as ligand. Concentrations of β-lg and α-la was maintained constant at 2.5 $mg.mL^{-1}$ each.

In strong agreement with our previous results, a higher sensitivity of β-lg to HHP treatment was observed in contrast to α-la (Bravo, Felipe, Lopez-Fandino, & Molina, 2013; Huppertz, Fox, & Kelly, 2004a,b; Marciniak et al., 2018; Patel, Singh, Anema, & Creamer, 2006). Surprisingly, the native PAGE profiles of protein solution (Fig. 1A) and the supernatant (Fig. 1B) recovered after acidification at pH 4.6 where no CN were present (0 $mg.mL^{-1}$) were similar to other samples containing CN (from 0.625 to 5 $mg.mL^{-1}$) in terms of bands corresponding to α-la and β-lg. These observations are in agreement with those of Huppertz et al. (2004b) and Pizzano, Manzo, Adalgisa Nicolai, and Addeo (2012), respectively upon pressurization (600 MPa–30 and 60 min) and heat-treatment (UHT) of whey suggesting that HHP and UHT treatment of solution containing only the WP can induce self-aggregation of β-lg which can be precipitated upon acidification to 4.6 (Huppertz et al., 2004b; Pizzano et al., 2012).

3.2. Protein recoveries and purification degrees

To confirm our results obtained by native-PAGE analysis, the amount of α-la and β-lg recovered in supernatants after acidification at pH 4.6 were determined by HPLC (Fig. 2A). Concerning α-la, no significant difference was observed whatever the concentration of CN ($p = 0.1512$). Indeed, the recovery rate of α-la (concentration in the supernatant after acidification at pH 4.6 and centrifugation) remained constant with an average value of 85 ± 6.3%. Regardless the concentration in CN, and in contrast to results obtained for α-la, the recovery rate of β-lg was drastically reduced by the HHP treatment decreasing its rate by 77% as compared to its initial value. In addition, the concentration of CN had a significant impact ($p = 0.0005$) on β-lg recovery. For samples containing higher concentration of CN (i.e. from 3.75 $mg.mL^{-1}$) the recovery rate of β-lg increased significantly suggesting that a lesser amount of β-lg were being precipitated during acidification. Indeed, β-lg recovery varied from an average value of 23 ± 1.7 (from 0 to 3.125 $mg.mL^{-1}$) to 33 ± 1.5% (from 3.75 to 5 $mg.mL^{-1}$). These results are in accordance with what we observed previously with PAGE analyses (Fig. 1).

Our data on protein recovery rates, indicate that up to protein ratio

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