International Dairy Journal 49 (2015) 8–15

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Self-assembly of caseinomacropeptide as a potential key mechanism in the formation of visible storage induced aggregates in acidic whey protein isolate dispersions

Nanna Stengaard Villumsen [a, *], Hanne Bak Jensen [a], Thao Thi Thu Le [a], Hanne Søndergaard Møller [a], Rune Thorbjørn Nordvang [b], Line Ravn Nielsen [c], Søren Bang Nielsen [c], John Sørensen [d], Marianne Hammershøj [a], Lotte Bach Larsen [a]

[a] Department of Food Science, Aarhus University, P.O Box 50, DK-8830 Tjele, Denmark
[b] BIOENG, Department of Chemical and Biochemical Engineering, Technical University of Denmark, Sølvtoft Plads, Building 227, DK-2800 Kgs. Lyngby, Denmark
[c] Arla Foods Ingredients Group P/S, Sønderupvej 26, DK-6920 Videbæk, Denmark
[d] Arla Foods Strategic Innovation Centre, Rørdrumvej 2, DK- 8220 Brabrand, Denmark

ARTICLE INFO

Article history:
Received 24 February 2015
Received in revised form
5 May 2015
Accepted 5 May 2015
Available online 19 May 2015

ABSTRACT

Visible aggregates formed during storage in acidic whey protein isolate (WPI) dispersions represent a challenge to the beverage industry. Batch-to-batch variations are observed that prevents consistent quality and shelf-life prediction. Heat-treatment of WPI dispersions at 120 °C for 20 s instead of conventional heating at 95 °C for 180 s often prevents the aggregate formation, and varying levels of divalent cations were proposed to contribute to the observed batch-to-batch variations. In this study, the composition of the visible aggregates was examined. Caseinomacropeptide (CMP) was enriched in the visible aggregates compared with the surrounding clear liquid. Disruption of electrostatic interactions between glycosylated and non-glycosylated CMPs were studied by addition of calcium, acidification, and enzymatic de-sialidation. The treatment strategies each significantly decreased time-dependent turbidity development in acidic WPI dispersions. This suggests that the storage-induced aggregates may be prevented by disruption of electrostatic interactions between negatively-charged sialic acid residues and positively-charged amino acids.

1. Introduction

Whey protein isolates (WPIs) are produced predominantly by combinations of membrane filtrations and spray drying of cheese whey (Huffman & Harper, 1999). The proteins of commercial WPIs are mainly composed of β-lactoglobulin (β-LG; ~40–50%), α-lactalbumin (α-LA; ~20%), and caseinomacropeptide (CMP; ~20%). Minor whey proteins constitute the remaining part of the protein content (Bonnaillie & Tomasula, 2008). Protein-fortified beverages produced by re-hydration of WPI powders is a rapidly expanding market (Etzel, 2004). Sterile liquid products that are stable for up to six months at ambient temperature are produced by thermal processing. The shelf-stable products are considered a convenient thirst-quenching source of protein, particularly by health conscious consumers (Ha & Zemel, 2003). By formulating the WPI dispersions at acidic rather than at neutral pH, solution-clarity is obtained (Dissanayake, Ramchandran, Piyadasa, & Vasiljevic, 2013). This is desirable, as clear liquid beverages are considered to be more thirst quenching than their opaque counterparts by consumers (Beucler, Drake, & Foegeding, 2005).

However, a continuing challenge in the beverage industry is the formation of visible protein aggregates, which appear during storage of acidic WPI dispersions and lead to decreased consumer acceptability. The level of this storage-induced aggregation (SIA) varies between batches (Villumsen et al., 2015), even though the processing steps and the raw material are seemingly the same. Recently, it was described that changing the heat treatment of acidic WPI dispersions from the conventional hot filling heating method of 95 °C for 180 s to 120 °C for 20 s, and/or lowering the storage temperature from 20 °C to 4 °C could efficiently prevent the

* Corresponding author. Tel.: +45 61710305.
E-mail address: nannavillumsen@hotmail.com (N.S. Villumsen).

http://dx.doi.org/10.1016/j.idairyj.2015.05.003
0958-6946/

formation of SIA in the liquid products (Villumsen et al., 2015). Furthermore, small variations in the contents of divalent cations in different WPI batches may contribute to the observed differing levels of SIA formation in WPI dispersions. To date, protein chemical characterisation of SIA and the molecular mechanisms responsible for its formation remains to be described. Likewise, the preventive effects of the heat treatment at 120 °C for 20 s and the reasons for the observed batch variations remain to be outlined.

The 64 amino acid long caseinomacropeptide (CMP) molecule is the frequently glycosylated and phosphorylated C-terminal fragment of κ-casein, which is released by rennet during cheese production. The two major genetic variants A and B are denoted CMP(A) and CMP(B), respectively. Up to three phosphorylations can appear on a single CMP molecule (Holland, Deeth, & Alewood, 2006). Roughly 50% of CMP is glycosylated by highly heterogeneous carbohydrate chains (Saito, Yamaji, & Itoh, 1991). The glycosylated CMP molecule is denoted gCMP while the non-glycosylated is often called aCMP for a-glycosylated CMP (Neelima, Sharma, Rajput, & Mann, 2013). The distribution of monosaccharide, disaccharide, trisaccharide (straight or branched), and tetrasaccharide chains has been determined by high pressure liquid chromatography (HPLC) to constitute 0.8%, 6.3%, 18.4%, 18.5% and 56.0%, respectively. The highly acidic sialic acid, which is mainly represented by N-acetylneuraminic (NeuNac) and N-glycolylneuraminic acid, is the terminal residue on the majority of the carbohydrate chains (Saito et al., 1991).

Farías, Martinez, and Pilosof (2010) reported that formation of opaque gels and sedimentations can occur with time due to the formation of CMP oligomers at acidic pH at room temperature. The time-frame for the formation of these visible aggregates varied from 2 to 21 days, depending on acidity and CMP concentration of the solution. The proposed CMP self-assembly mechanism in their study is driven by hydrophobic and electrostatic interactions (Farías et al., 2010). At pH ~3, aCMP, which has a pI of ~4.15, carries a net positive charge due to its three lysine residues and the positively-charged N-terminal. The most glycosylated variant of gCMP has a pI of ~3.1. However, due to the highly acidic sialic acid residues on the glycosylated polypeptide, local negative charges appear even at pH 2.2. This facilitates electrostatic interaction with positive charges on aCMP (Farías et al., 2010; Kreuß, Strixner, & Kulozik, 2009). It was recently suggested that CMP self-assembly is a contributing factor to visual changes observed in acidic WPI beverages during storage (Villumsen et al., 2015). Furthermore, it was suggested that divalent cations may bind to the negatively-charged sialic acids and thereby potentially affect the process. Different levels of these cations between batches may therefore be a driver behind the observed visible differences. Likewise, increased acidity could be expected to decrease the level of electrostatic interactions due to increased protonation of sialic acid residues. Hydrolysis by sialidases, a class of enzymes that catalyse the hydrolysis of terminal sialic acids (Kim, Oh, Kang, & Kwon, 2011), would also potentially decrease the level of CMP oligomerisation, due to the loss of the negatively-charged sialic acid residues on gCMP.

The present study aimed to assess the hypothesis that CMP self-assembly is involved in the formation of SIA in acidic WPI dispersions. This was done by comparative protein composition analyses of the SIA the surrounding liquid. Further the effects of the mineral contents and glycosylation patterns on the aggregation were examined.

2. Materials and methods

2.1. Whey protein isolate samples and chemicals

WPI powders from two different batches of the same commercial WPI product were provided by Arla Foods Ingredients P/S, (Videbæk, Denmark). The two batches were known to represent extremes with respect to SIA formation in acidic WPI dispersions produced from the powders. The first batch yielded beverages with a low level of SIA formation (batch-low). The second batch yielded beverages with a high level of SIA formation (batch-high). The calcium contents of batch-low and -high WPI powders were 0.0169% and 0.0018% (w/w), respectively, as determined by inductively coupled argon-plasma emission spectrometry (Villumsen et al., 2015). The WPI powders were rehydrated to 7% (w/v) protein, adjusted to pH 3, and heated at 95 °C for 180 s or at 120 °C for 20 s, as described by Villumsen et al. (2015).

Immobilised pH gradient (IPG) strips and precast SDS-PAGE gels were from BioRad (Hercules, CA, USA). All chemicals and reagents applied were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Storage of whey protein isolate dispersions

Liquid samples from the four combinations of batches and heat treatment (2 WPI batches: low and high × 2 heat treatments: 95 °C for 180 s and 120 °C for 20 s) were stored for six months at 20 °C without light exposure. During this storage period, visible SIAs were formed in three of the four samples. In batch-low WPI dispersion heated at 95 °C for 180 s, SIA appeared as short threads floating in the liquid, while in batch-high WPI dispersion heated at 95 °C for 180 s, SIA appeared as a soft gel-like precipitate at the bottom of the bottle. In the batch-high WPI dispersion heated at 120 °C for 20 s, SIA was seen as a minor haze formation. In batch-low WPI dispersion heated at 120 °C for 20 s, no SIA formation was observed during the 6-month storage period (Villumsen et al., 2015). More described details of the levels of SIA formation were given by Villumsen et al. (2015).

At the end of the 6-month storage, six samples (A–F) from batch low and high were selected for analyses. The six samples were as follows: A, isolated SIA from batch low WPI dispersion heated at 95 °C for 180 s; B, isolated SIA from batch high WPI dispersion heated at 95 °C for 180 s; C, clear liquid from batch low WPI dispersion heated at 95 °C for 180 s; D, clear liquid from batch high WPI dispersion heated at 95 °C for 180 s; E, clear liquid from batch low WPI dispersion heated at 120 °C for 20 s; F, clear liquid from batch high WPI dispersion heated at 120 °C for 20 s. The SIAs were isolated for further analyses by sucking up a sample using a laboratory pipette. For neither of the WPI dispersions was it possible to obtain a sample of SIA without including some of the clear liquid from the solution. The diffuse nature of SIA in batch-low WPI dispersions made it partly dissolve – or disperse into species not visible to the eye – upon merely gentle agitation, which also impaired SIA sampling; therefore, more of the surrounding WPI liquid as “contamination” was present in the SIA sample from the batch-low than from the batch-high dispersion. The isolated SIA samples were manually homogenised using micro-tubes pestles (Vitro, Fredensborg, Denmark) and vortexed before further analyses.

2.3. Effect of calcium, pH and de-sialidation on turbidity in WPI dispersions

Ways to prevent time-dependent turbidity increases in the WPI dispersions were studied by three different treatment strategies: addition of calcium, increased acidity or sialidase treatment. All three treatments were tested in a batch-high WPI dispersion 7% (w/v) protein. The powder was weighed out, re-dispersed and the individual ‘SIA-preventing’ treatments were done prior to the heat treatment.