Food Hydrocolloids 81 (2018) 60–70

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Modification of the interfacial properties of sodium caseinate using a commercial peptidase preparation from *Geobacillus stearothermophilus*

Jacob Ewert [a], Claudia Glück [a], Benjamin Zeeb [b], Jochen Weiss [b], Timo Stressler [a, *], Lutz Fischer [a]

[a] University of Hohenheim, Institute of Food Science and Biotechnology, Department of Biotechnology and Enzyme Science, Garbenstr. 25, 70599 Stuttgart, Germany
[b] University of Hohenheim, Institute of Food Science and Biotechnology, Department of Food Physics and Meat Science, Garbenstr. 25, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 16 October 2017
Received in revised form
29 January 2018
Accepted 19 February 2018
Available online 23 February 2018

Keywords:
Peptide
Surfactant
Milk protein hydrolysis
Technofunctionality
Foam
Emulsion

ABSTRACT

Sodium caseinate was hydrolyzed with the commercial enzyme preparation Sternzym BP 25201, containing a thermolysin-like peptidase from *Geobacillus stearothermophilus* as the only peptidase. The hydrolysis was carried out at 65 °C with an enzyme activity of 1 nkat mL^{-1} or 15 nkat mL^{-1}, leading to various degrees of hydrolysis (DH) ranging between 0.1 and 8.5%. The hydrolysates obtained were analyzed in a multi-scale approach, covering the hydrolysate properties (viscosity, hydrophobicity, peptide composition) and their interaction at oil–water (emulsion) and air–water (foam) interphases. The viscosity and surface hydrophobicity generally decreased with an increasing DH. Longer, more hydrophobic peptides, which self-assembled into network-like supramolecular particles, were detected up to a DH of 2.2%. Compared to untreated sodium caseinate, these structures could increase the half-life of emulsions (+400%) and foams (+31%). This was most probably caused by an increase in particle size (45.2-fold). By contrast, a higher DH led to a less hydrophobic product and smaller, spherical-shaped supramolecular structures. Foams and emulsions prepared with those hydrolysates were not stable and phase separated within minutes (for example, emulsion half-life = 5 min; foam half-life = 4.6 min at DH of 8.5%).

1. Introduction

Milk proteins are increasingly being used in the food industry due to their technofunctionality (Kilara & Panyam, 2003). The technofunctionality of proteins includes the water-holding capacity, oil- and fat-binding properties, gelling properties and the ability to stabilize oil–water (emulsion) or gas–water (foam) interphases (Zayas, 1997). The strong amphiphilic nature of milk proteins enables them particularly to adsorb onto the interphase (Caessens, Gruppen, Visser, Aken, & Voragen, 1997). The proteins have to diffuse from the bulk liquid phase to the close proximity of the interphase and, subsequently, adsorb, based on flip-flop mechanism, for the stabilization of an interphase (Germain & Aguilera, 2014). The proteins undergo conformational changes here during the adsorption at the interphase and expose their hidden hydrophobic groups to the non-aqueous phase (oil or gas), while hydrophilic groups orient to the continuous liquid phase – a fact that is also known as surface denaturation (McClements, 2004). In addition, the proteins might also aggregate and form a network, called the lamella (Ewert et al., 2016). The oil or gas phase is stabilized according to the size (steric hindrance) or charge distribution (electrostatic repulsion) of the proteins in the lamella (Germain & Aguilera, 2014).

In addition to the native food proteins used commonly, modified (chemically or enzymatically) proteins can also be applied (Celus, Brijs, & Delcour, 2007). These modifications can improve the technofunctional properties, such as the attachment onto the interphase (Kilara & Panyam, 2003). An example of these

* Corresponding author.
E-mail addresses: j.ewert@uni-hohenheim.de (J. Ewert), Claudia.Baur@uni-hohenheim.de (C. Glück), zeeb@uni-hohenheim.de (B. Zeeb), j.weiss@uni-hohenheim.de (J. Weiss), t.stressler@uni-hohenheim.de (T. Stressler), lutz.fischer@uni-hohenheim.de (L. Fischer).

https://doi.org/10.1016/j.foodhyd.2018.02.036
0268-005X/

modifications is the enzymatic protein hydrolysis. During the hydrolysis, proteins are broken down into peptides of different sizes and free amino acids, as a result of cleavage of the peptide bonds (Sinha, Radha, Prakash, & Kaul, 2007). Possible consequences of a hydrolysis are: a decreased molecular weight, an increased number of ionizable groups, an exposure of hydrophobic groups from the inner protein molecule, an increased solubility, a decreased viscosity and, consequently, changes regarding the environmental interactions at the interphase (Panyam & Kilara, 2004; Tavano, 2013). In contrast to the chemical hydrolyses (conditions for acid hydrolysis: 2 M HCl, 110 °C, 24 h incubation), the enzymatic process usually avoids side reactions which might lead to toxic substances such as lysino-alanine and a decrease of the nutritional value of the protein source (Merz et al., 2015a; Sinha et al., 2007; Tavano, 2013). A further benefit of the enzymatic hydrolysis is that it provides a more uniform product, can be performed under milder conditions and, thus, avoid the extreme environments required for chemical treatments (Sun, 2011; Tavano, 2013). For the enzymatic hydrolysis peptidases can be applied. Consistent with the International Union of Biochemistry and Molecular Biology (IUBMB), all enzymes, which act on a peptide bond are called peptidases (Ewert, Glück, Strasdeit, Fischer, & Stressler, 2018). The specificity of the peptidase used for hydrolysis and the resulting degree of hydrolysis (DH) of the protein hydrolysate are the major parameters for the technofunctional behavior obtained (Althouse, Dinakar, & Kilara, 1995; Kunst, 2002; van der Ven, Gruppen, de Bont, & Voragen, 2002). The hydrolysis product has to be investigated in a multi-scale approach for evaluation, taking into account the molecular structure of the product, the microscopic effects (e.g. droplet size) at the interphase and the macroscopic effects (e.g. overrun, stability) of the emulsion or foam (Germain & Aguilera, 2014). Regarding the enzyme used to produce technofunctional protein hydrolysates, our group recently suggested the application of thermolysin (EC 3.4.24.27) (Ewert et al., 2016), due to its near neutral optimum pH (~7.0), high optimum temperature (70–80 °C) and stability (stable ≥ 50 °C), as well as specificity to hydrophobic amino acids (preferred at P1': Leu, Phe, Ile, Val) (Rawlings & Salvesen, 2013). Compared to thermolysin from *Bacillus thermoproteolyticus*, the thermolysin-like peptidase (TLP) from *Geobacillus stearothermophillus* ssp. (also called stearolysin, boilysin) offers an improved temperature stability. Takii, Taguchi, Shimoto, and Suzuki (1987), for example, showed that TLP from *Geobacillus stearothermophillus* KP1236 was stable for 18 h at 60 °C and pH 6.0–8.6, while this treatment inactivated *Bacillus thermoproteolyticus* thermolysin greatly (residual activity: 6, 34 and 7% at pH 7.0, 7.5 and 8.0, respectively). Our own investigations prior to this study using the commercial TLP preparation Sternzym BP 25201 (Stern-Wywiol Gruppe, Ahrensburg, Germany) confirmed the characteristics described in the literature (see supplementary data, Table S1).

To the best of our knowledge, no studies are available so far describing the effect of a TLP hydrolysis on the interfacial properties of sodium caseinate. As already suggested in the literature (Althouse et al., 1995; Kunst, 2002; van der Ven et al., 2002), we hypothesized that a low DH might lead to an improved emulsifying and foaming behavior of the hydrolysate, while a high DH might diminish these properties. Therefore, the objective of the current study was to investigate the foaming and emulsifying properties of caseinate, hydrolyzed with the technical TLP preparation Sternzym BP 25201, in a multi-scale approach and to further investigate the mechanism behind the changes in the interfacial behavior due to the applied hydrolysis.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade and obtained from Sigma-Aldrich GmbH (Schnelldorf, Germany), Carl Roth (Karlsruhe, Germany), AppliChem GmbH (Darmstadt, Germany) and Merck AG (Darmstadt, Germany). Sodium caseinate powder was obtained from FrieslandCampina (Amersfoort, Netherlands). Its specifications were: 90.6% (w/w) protein, < 1% lactose (w/w), <1% fat (w/w) and 4.1% (w/w) minerals. Sternzym BP 25201 was a friendly gift from SternEnzym (Stern-Wywiol Gruppe, Ahrensburg, Germany). The medium chain triacylglyceride mixture Mygliol 812 N® was purchased from Sasol Germany GmbH (Brunsbüttel, Germany).

2.2. Sodium caseinate hydrolysis using Sternzym BP 25201

The initial proteolytic activity of Sternzym BP 25201 was determined with the *ortho*-phthalaldehyde (OPA) assay described by Stressler, Eisele, Schlayer, Lutz-Wahl, and Fischer (2013), using 10% (w/v) sodium caseinate powder in tap water as the substrate.

The main hydrolysis for this study was performed in two separate batch processes. Therefore, 10% (w/v) sodium caseinate powder (1 L each) was dissolved in tap water and preincubated for 30 min at 65 °C (hydrolysis temperature). The hydrolysis was started by adding an initial activity of either 1 nkat mL^{-1} or 15 nkat mL^{-1} Sternzym BP 25201 to the substrate. The pH of the caseinate solution (pH = 6.75) was measured and adjusted automatically with 2 M NaOH. Samples (80 mL) were taken over 24 h and subsequently heated at 95 °C for 20 min to inactivate the enzyme. After the heat treatment, no enzyme activity (checked with the OPA-assay and azocasein-assay) or further hydrolysis were detectable and the samples were stored at −20 °C until further analysis. An unheated sample (5 mL) was taken at the beginning and end of the hydrolysis and its microbial load was tested on plate count agar plates (0.5% (w/v) tryptone, 0.25% (w/v) yeast extract, 0.1% (w/v) glucose, 1.5% (w/v) agar; incubated for 3 d at 30 °C). In addition, the initial and remaining enzyme activity was determined using the azocasein-assay described by Ewert et al. (2016). The samples were applied to a PD-10 desalting column (GE Healthcare, Little Chalfont; UK) prior to the remaining activity determination, to remove small peptides because they can influence the azocasein-assay.

2.3. Analysis of the hydrolysis product

2.3.1. Degree of hydrolysis

Firstly, each heated sample (450 μL) was mixed with 10% (w/v) SDS (50 μL) to solubilize the proteins. Afterwards, the number of free α-amino groups of each sample was measured using a derivatization protocol with OPA described by Stressler et al. (2013). Subsequently, the DH was calculated, according to Merz et al. (2015b) as follows:

$$DH\ [\%] = \frac{h}{\frac{c_{Protein}}{M^* - M_{H_2O}}} * 100\% \quad (1)$$

where h is the concentration of free amino groups (mol L^{-1}) determined by OPA, $c_{protein}$ is the concentration of protein (g L^{-1}), M_{H2O} is the molecular weight of water (18 g mol^{-1}) and M^* is the