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Structural changes and functional properties of highly concentrated whey protein isolate-citrus pectin blends after defined, high temperature treatments

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ABSTRACT

Extrusion processing can be used for conjugation of whey proteins with citrus pectins. However, many reactions can take place simultaneously, and so far less is known about the influence of the extrusion parameters on the structural and functional properties of the reaction products. This study focuses on the influence of elevated temperatures on the structural and functional properties of highly concentrated whey protein isolate-citrus pectin blends. Defined temperature treatments were performed by using a closed-cavity rheometer. Structural changes due to non-disulfide, covalent cross-links, and the formation of fluorescent compounds were analyzed. Functional properties (e.g. viscosity, emulsifying capacity) were determined of selected samples. The results showed that the emulsifying capacity can be improved by defined temperature treatments at 120 °C and 140 °C. Samples with an improved emulsifying capacity also exhibited higher maximum fluorescence intensity indicating the formation of Maillard reaction products (e.g. conjugates).

1. Introduction

With an ever-expanding consumer awareness of health and nutrition, the use of natural food ingredients has gained increasing importance. Biopolymers such as whey proteins are often used as natural emulsifiers in bakery, meat, dairy and other food products (Morr & Ha, 1993). However, the industrial application is limited due to the sensitivity of whey proteins against changes in temperature, concentration of ions and pH (Damodaran, 2007, p. 217). Therefore, the functionalization of whey proteins has been the focus of many researchers during the last years. It is well-known that mixing and heating of whey proteins with high molecular polysaccharides result in covalently linked molecules with improved functional properties e.g. emulsifying capacity and stabilizing properties (Akhtar & Dickinson, 2003; Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Neirynck, Van der Meeren, P, Bayarri Gorbe, Dierckx, & Dewettinck, 2004; Schmidt et al., 2016). These molecules are referred to as protein-polysaccharide-conjugates and are formed during the initial stage of the Maillard reaction (Hodge, 1953; Maillard, 1912).

The most commonly used methods for conjugation are incubation of lyophilized protein-polysaccharide powders (referred to as dry heating) and of protein-polysaccharide aqueous solutions (referred to as wet heating) under controlled conditions. Both processes can only be run batch wise and exhibit reaction times of several hours up to a few days. Further methods for conjugation with shorter reactions times (seconds to a few minutes) are pulsed electric field (Sun, Yu, Zeng, Yang, & Jia, 2011) and ultrasonic treatments (Li, Xue, Chen, Ding, & Wang, 2014), as well as extrusion processing (Guerrero, Beatty, Kerry, & Caba, 2012; Koch, Emin, & Schuchmann, 2017). Extrusion is a continuous process with reaction times of a few minutes. It is well-known that the Maillard reaction can take place during extrusion processing (Ames, Guy, & Kipping, 2001; Camire, 1991; Davidek, Illmann, Rytz, & Blank, 2013). However, up to now only a few studies focused on conjugation during extrusion processing and on the functional properties of the reaction products (Bueno, Pereira, Menegassi, Arêas, & Castro, 2009; Guerrero et al., 2012; Koch et al., 2017).

Besides the Maillard reaction many other reactions can proceed during heat treatments of whey protein with citrus pectin. These reactions can involve denaturation (Areas, 1992; Mulvihill & Donovan, 1987), degradation (Burgess & Stanley, 1976), and

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polymerization of proteins (Areas, 1992; Burgess & Stanley, 1976), as well as degradation of pectin and thus caramelization (Axelos & Branger, 1993; Morris, Foster, & Harding, 2002; Schols & Voragen, 2002). Up to now, very little is known about the influence of extrusion parameters such as mechanical stress, reaction time and temperature on the reactions taking place in highly concentrated systems. This information; however, is essential to control the reactions. During extrusion, the material is exposed to broadly distributed thermal and mechanical stresses simultaneously. Moreover, these stresses are also coupled to each other which make extrusion trials not suitable to consider the effect of these stresses separately. Defined elevated temperatures and mechanical stresses can be applied by using a specific rheometer e.g. closed-cavity rheometer (CCR) (Emin & Schuchmann, 2017; Habeych, van der Goot, & Boom, 2009; Madeka & Kokini, 1994, 1996; Pommet, Morel, Redl, & Guilbert, 2004). This provides the opportunity to consider elevated temperatures and mechanical stresses in highly concentrated systems independently. Moreover, water evaporation can be avoided.

This study aims at gaining a better understanding on the impact of elevated temperatures on structural changes (i.e. non-disulfide, covalent links) and functional properties (i.e. water solubility, viscosity and emulsifying activity) of highly concentrated whey protein isolate-citrus pectin blends.

2. Material and methods

2.1. Material

Whey protein isolate (WPI), German Prot 9000, with 90 g/100g protein on dry matter was obtained from Sachsenmilch Leppersdorf GmbH (Leppersdorf, Germany). According to the supplier, the WPI contains typically $\leq$ 3 g/100g lactose, $\leq$ 1 g/100g fat and an ash content of 4 g/100g. The moisture content is stated to be $\leq$ 5 g/100g. Highly methylated citrus pectin (HMCP), Classic CU-L 009/13, was provided by Herbstreith & Fox (Neuenbuerg/Enz, Germany). According to the manufacturer, the HMCP exhibits a protein content of 3 g/100g, a moisture content of 7.5 g/100g and an average molecular weight of about 79.8 kDa determined by intrinsic viscometry. For the preparation of the emulsions, pure rapeseed oil (Bernhard Schell, Lichtenau, Germany) was used.

2.2. Sample preparation and temperature treatments

A blend of WPI and HMCP (1:1 g:g) was adjusted to a moisture content of 28 g/100g, and mixed in a Thermomix (Vorwerk, Wuppertal, Germany) for 3 min. To achieve a homogeneous water distribution, the samples were stored for 3 days at 8 °C. Subsequently, temperature treatments were performed by using a closed-cavity rheometer (RPA flex, TA Instruments, New Castle, Delaware, USA). Fig. 1 shows a schematic illustration of the device. The biconical test chamber consists of two cones in opposite. The grooved cones, to prevent slippage, are thermoregulated by direct heating and forced air cooling. To prevent water evaporation the test cavity is sealed, and can be pressurized up to 8000 kPa. During the experiments the lower cone is driven by a motor and oscillates with a defined frequency and amplitude. At a constant strain and frequency of 1% and 1 Hz (linear viscoelastic region), respectively, samples were heated at 80 °C, 100 °C, 120 °C and 140 °C for 0.75 min, 2 min, 6 min and 10 min. Two samples were prepared for each condition. Subsequently, samples were dried in a vacuum dryer (Heraeus, Hanau, Germany) at 40 °C and 8 kPa and milled (POLYMIX® PX-MFC 90 D, Kinematica, Luzern, Switzerland) to a particle size < 500 μm.

2.3. Size-exclusion chromatography

Size-exclusion chromatography (SEC) experiments were performed to analyze changes in the molecular size distribution of non-disulfide, covalent cross-links by HPLC (Shimadzu, Kyoto, Japan). Therefore, samples (1 mg/ml) were solved in a 0.2 mol/L phosphate buffer (pH 7) consisting of 0.033 mol/L SDS, 0.54 mol/L NaCl, 8 mol/L Urea, and 0.002 mol/L dithiothreitol (DTT). Experiments showed a maximum in solubility when solving for 7 days. Afterwards, samples were centrifuged at 4637 × g for 30 min ensuring that no insoluble fractions contaminate the analysis. For the same purpose, the mobile phase, a 0.2 mol/L phosphate buffer with 2 mol/L Urea (pH 7), was filtered through 0.2 μm cellulose acetate filters (Sartorius, Goettingen, Germany) and degassed. Finally, 100 μl of the soluble sample fraction was injected by an auto sampler to the TSKgel G3000SW$_{XL}$ column (Tosoh Bioscience, King of Prussia, USA). The measurements were conducted with a flow rate of 0.5 ml/min at 25 °C and the absorbance of the samples was recorded by an UV/VIS detector at 280 nm. The analyses were performed in triplicate for each sample.

2.4. Fluorescence spectroscopy

Fluorescence spectroscopy was performed to detect fluorescent compounds that are formed during the Maillard reaction (Leclère & Birlouez-Aragon, 2001; Matiacevich & Pilar Buera, 2006). An Infinite 200 Pro microplate reader (Tecan, Crailsheim, Germany) was used to determine the fluorescence spectra of the samples. The sample solutions were prepared by solving 25 mg in 10 ml of 0.2 mol/L phosphate buffer containing 0.033 mol/L SDS, 0.54 mol/L NaCl, 8 mol/L Urea and 0.002 mol/L dithiothreitol (DTT) for 7 days. The excitation spectra from 300 to 400 nm were scanned at an emission wavelength of 420 nm. For the emission spectra, the maximum in excitation at 368 nm was set constant, and emission spectra were recorded from 400 to 580 nm. Excitation and emission slits were set to 2 nm. The spectrum of each sample was recorded in triplicate. Maximum fluorescence intensity of the emission spectra at 488 ± 5 nm was used as a measure for the amount of fluorescent

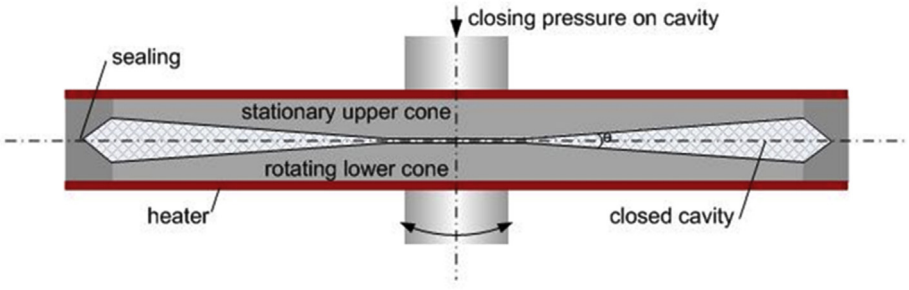

Fig. 1. Schematic illustration of the closed-cavity rheometer.