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Influence of processing conditions on the formation of whey protein-citrus pectin conjugates in extrusion

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ABSTRACT

To investigate the formation of whey protein-citrus pectin conjugates during extrusion processing, extrusion trials were performed by a co-rotating twin screw extruder. The processing conditions such as mechanical stress, reaction time and temperature, are varied by varying screw configuration (with/without reverse elements), and barrel temperature (80 °C–180 °C). Biopolymer reactions, such as protein and pectin degradation, protein aggregation as well as conjugation were examined by SDS-PAGE and fluorescence spectroscopy. The highest formation of conjugates was observed in the samples treated with reverse elements and the barrel temperature of 140 °C. As a measure for the processing conditions, material temperature at the die T_m, specific mechanical energy SME, and residence time distribution RTD were measured. The analysis of these parameters showed that T_m and SME are of significant influence on conjugate formation and degradation. Nevertheless, further analyses at defined conditions (time, temperature, shear stress) are necessary to get a better insight on the reactions taking place in extrusion.
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1. Introduction

The increased consumer awareness of nutrition leads to ever-expanding demands for food products based on natural ingredients. Proteins are natural emulsifiers and already applied to stabilize food emulsions. However, the sensitivity of proteins to e.g. changes in pH-value, temperature and concentrations of ions limits their industrial application (Damodaran, 2005; Liu et al., 2012). Previous studies have reported that proteins being covalently linked to carbohydrates, exhibit improved functional properties such as emulsifying activity, gelling, foaming and stabilizing properties (Akhtar and Dickinson, 2007; Al-Hakkak and Al-Hakkak, 2010; Liu et al., 2012; Neirynck et al., 2004; Zhang et al., 2014). Improved emulsifying activity has mainly been observed for high-molecular polysaccharides (e.g. pectin) reacted with proteins (e.g. whey protein and lysozyme) (Dunlap and Côté, 2005; Schmidt et al., 2016; Shu et al., 1996). These covalently linked molecules are often referred to as conjugates.

Conjugation is one of the first reaction steps of the Maillard reaction, whereby a free amino group of the protein reacts with the carbonyl group of the reducing end of a carbohydrate (Hodge, 1953; Maillard, 1912). The reaction is influenced by temperature, pH-value, moisture, reaction time, mass ratio of the amine group and the carbonyl group, and intrinsic properties of reagents such as molecular weight and composition (Baisier and Labuza, 1992; van Boekel, 2001; Wolfrom et al., 1974). The most commonly used methods for conjugation are incubation of lyophilized protein-carbohydrate powders (referred as dry-heating), and of protein-carbohydrate aqueous solutions (referred as wet-heating) under controlled conditions. Both methods are batch processes and exhibit reaction times of several hours to a few days (Akhtar and Dickinson, 2007; de Oliveira et al., 2014; Zhu et al., 2008, 2010; Zhuo et al., 2013). Furthermore, the yield of conjugation achieved by these methods is limited to max. 60% (Zhuo et al., 2013).

Extruders are continuous reactors running at throughputs of up to several hundred tons per hour and short processing times of a few minutes (Riaz, 2000). In extruders, the viscous material is subjected to effective mixing under elevated temperature and pressure. Furthermore, extruded materials are exposed to high mechanical stresses generated by the rotation of screws. Guerrero et al. (2012) have already shown that soy protein reacts with lactose and sucrose to conjugates using extrusion processing. However, very little is known about the influence of extrusion parameters such as mechanical stress, reaction time and temperature on conjugation. This information, however, is essential to control this reaction and to improve its yield during extrusion.

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The scope of this study is to investigate the influence of various processing conditions on the formation of whey protein-pectin conjugates during extrusion. Whey protein concentrate and citrus pectin are used as model ingredients, as they can form conjugates with an improved emulsifying activity (Einhorn-Stoll et al., 2005; Mishra et al., 2001; Neirynck et al., 2004). Processing conditions which are expected to influence reaction conditions during extrusion processing are, residence time, material temperature and the specific mechanical energy as measure for shear stresses. These processing conditions are studied for their effect on conjugate formation.

2. Material and methods

2.1. Materials

Whey protein concentrate (WPC), Textrion PROGEL 800, with typically 83% protein on dry matter basis was provided by FrieslandCampina, Amersfoort, the Netherlands. WPC contains about 6.6% lactose, 4.8% fat, 4.2% ash and 4.8% moisture. Highly methylated citrus pectin (HMCP), Classic CU-L 009/13, was given by Herbstreith & Fox, Neuenbuerg/Enz, Germany. HMCP contains 3% protein, 7.5% moisture and has approximately 70% degree of esterification.

2.2. Extrusion experiments

Extrusion experiments were carried out using a co-rotating twin-screw extruder ZSK 26 Mc (Coperion, Stuttgart, Germany) with screw diameter of 25.5 mm, length to diameter ratio (L/D) of 29 and a die diameter of 3 mm. The barrel consists of 7 sections. Each section, except the first one, can be heated separately. Barrel temperature and screw configuration were varied as shown in Table 1 and Fig. 1, respectively. Compared to screw configuration B, screw configuration A has no reverse transport elements. Screw configuration B was chosen in order to increase mixing of the reactants, shear, and residence time. Screw speed, feed rate and water content were set constant at 180 rpm, 5 kg/h and 35%, respectively. In preliminary experiments these process parameters showed the longest residence times with stable processing conditions.

WPC and HMCP were mixed 1:1 wt ratio in a plowshare mixer FM 50 (Lödige, Warburg, Germany) and were fed to the first barrel section of the extruder by a gravimetrically controlled feeder DDW-DDSR40 (Brabender, Duisburg, Germany). Water was added to the second barrel section of the extruder and was mixed with the material. Material temperature and pressure were measured at the die using a thermocouple type J (Ahlborn, Holzkirchen, Germany) and a pressure sensor type M3 (Gefran, Provaglio d'Iseo, Italy), respectively. Even if the thermocouple is not giving the exact temperature profile, these data were chosen as measure for the maximum product temperature. Samples were taken and dried at 40 °C over night and milled with a rotor beater mill SR3 (Retsch, Haan, Germany). The extrusion experiments were performed in triplicate. For the convenience of the readers, samples are named to the screw configuration (A or B) and the temperature of the last barrel section (80–180). For example "A150" for the sample treated with screw configuration A and the last barrel section temperature of 150 °C.

Table 1
Barrel temperature T of section 1–7.

Section 1	Section 2	Section 3	Section 4–7
–	40 °C	60 °C	80 °C
–	40 °C	60 °C	100 °C
–	40 °C	60 °C	120 °C
–	40 °C	60 °C	140 °C
–	40 °C	60 °C	150 °C
–	40 °C	60 °C	160 °C
–	40 °C	60 °C	170 °C
–	40 °C	60 °C	180 °C

2.3. Residence time distribution measurements

Residence time distributions (RTD) were determined according to the online digital image processing method described by Lee et al. (2009). Once steady state extrusion conditions were achieved, 0.2 g of a red dye (CC-5000-WS-P Cochineal Carmine, CHR. Hansen, Hoersholm, Denmark) was added as a pulse to the first section of the extruder. The color change of the extrudates was captured by a digital camera (Lumix GH2, Panasonic, Kadoma, Japan), and the images were evaluated using an image processing software (MatLAB R2012B, The MathWorks Inc., Natick, USA). The RTD are represented by exit age distribution E(t), and the 10th and 90th percentile of the RTD, referred as t_{10} and t_{90}, respectively, were used as characteristic values to present the shortest and longest residence time of the material in the extruder, as proposed by Leeb et al. (2008).

2.4. Calculation of specific mechanical energy

Specific mechanical energy (SME) is a characteristic value describing the amount of work transferred from the motor into the extruded raw material per unit mass (van Lengerich, 1984). SME was calculated according the following equation (van Lengerich, 1984):

$$SME\ (Wh/kg) = \frac{\frac{n}{n_{max}} \times \frac{M_d - M_{d,unload}}{100}}{\dot{m}} \times P_{max}$$

where n and n_{max} are the actual and maximum screw speed (1800 min^{-1}), and M_d and $M_{d,unload}$ are the actual and empty torque (%), respectively. $\dot{m}$ is the total mass flow (kg/h) and P_{max} the maximum engine power (40 kW).

2.5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were analyzed according to the method described by Laemmli (1970). The first step of this method was to solve 0.1% untreated WPC, HMCP (only used for glycoprotein staining), WPC-HMCP mixture (1:1 wt ratio) or 0.1% of the extrudates for 7 days in deionized water. Afterwards, 40 µl of each solution were mixed with 20 µl phosphate buffer (pH 6.6–7.2) containing 8% w/v SDS, 20% v/v β-mercaptoethanol and 0.015% w/v bromophenol blue (Roti®-Load 1, Carl Roth, Karlsruhe, Germany) and heated at approximately 95 °C for 5 min in a water bath. After incubation the solutions were cooled down to room temperature and the slots of 12% polyacrylamide gels (12% Mini-PROTEAN® TGX™, Bio-Rad, Hercules, California, USA) were loaded with 10 µl of solution, and 5 µl of protein standard (Precision Plus Protein™ Dual Color Standards, Bio-Rad, Hercules, California, USA), respectively. The electrophoresis was carried out at 90 V for 90 min using a tris-glycine buffer (pH 8.3) containing 0.1 w/w SDS. Thereafter, the gel was stained with Coomassie Brilliant Blue R-250 Staining Solution (Bio-Rad, Hercules, California, USA) for protein analysis. For glycoprotein analysis, the gel was stained by the Pierce Glycoprotein Staining Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

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