



Effect of Maltodextrin Dextrose Equivalent on Electrospinnability and Glycation Reaction of Blends with Pea Protein Isolate

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Abstract

Compared to commonly applied wet and dry heating procedures, a combination of electrospinning and heat treatment can facilitate glycation of proteins with reducing polysaccharides. This study investigates how the amount of reducing carbonyl groups (i.e. dextrose equivalent, DE) of different maltodextrins influences electrospinnability and subsequent glycation in blends with pea protein isolate (PPI). In the first step of the study, maltodextrin-PPI dispersions were electrospun. The concentrations of PPI and maltodextrin DE 2 were kept constant in the aqueous spinning dispersion. The addition of 0.05 or 0.1 g/mL maltodextrin DE 12 or 21 slightly affected the electrical conductivity and dynamic viscosity of the spinning dispersions, however, fiber production rate and morphology were dominated by the presence of maltodextrin DE 2 (0.8 g/mL). In the second step of the study, fibers were heated (60 °C, 75% rel. Humidity, 0–24 h). SDS-PAGE analysis and the measurement of free amino groups confirmed the covalent attachment of maltodextrin carbonyl groups to free amino groups of PPI. The fastest glycation and the lowest relative amount of free amino groups ($49.70 \pm 6.54\%$) after 24 h heating was measured for the fibers with the highest amount of reducing carbonyl groups. The fibers with the lowest amount of reducing carbonyl groups showed no significant ($p < 0.05$) decrease of free amino groups after heat treatment. The results suggest that within the boundaries of electrospinnability, the degree of glycation can be adjusted by varying the amount of reducing carbonyl groups in the fibers.

Keywords Maltodextrin · Pea protein isolate · Conjugation · Maillard reaction · Needleless electrospinning · Microfibers

Introduction

Plant-based proteins have experienced a rise in demand in the food industry due to an increased interest in plant-based food [1]. These proteins are not merely employed in the production of meat substitutes but also serve a role as technofunctional ingredients such as emulsifiers, foam stabilizers, and gelling agents [2]. Proteins derived from legumes such as soy, chickpea, or pea are one of the most important groups of plant proteins. Pea (*Pisum sativum* L.) proteins in particular are of interest to the food industry due to their high nutritive value,

low allergenic potential, and high availability [3, 4]. However, the low solubility and the hydrophobic surface of these proteins negatively impact their technofunctionality [5]. A way to overcome these problems is the glycation of proteins with a reducing polysaccharide via the first stage of the Maillard reaction [6, 7]. The covalent linkage occurs mainly between ϵ -amino groups of lysine residues and carbonyl groups of reducing carbohydrates [8]. Numerous studies using various proteins and polysaccharides have demonstrated increased protein solubility and protection of the technofunctionality of proteins against extrinsic factors such as pH and heat treatment upon its connection to a hydrophilic polysaccharide [7, 9, 10]. Protein-polysaccharide conjugates have a high potential to be used in transparent protein beverage formulations which have a low pH value or require heat treatment [11]. Influencing parameters on the reaction include temperature, time, type of reducing carbohydrate and protein, and the ratio of the free amino to reducing carbonyl groups [9]. Commonly, the reactants are either dissolved, freeze-dried and subsequently heated (dry state method) or dissolved and heated (wet state method). These methods have disadvantages such as the

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generation of undesired Maillard by-products due to high temperatures and/or prolonged reaction times as well as rather low reaction yields [12, 13]. A new approach to enable the efficient production of glycated proteins is to physically structure the reactants by electrospinning prior to heat treatment. The close molecular contact of the proteins and polysaccharides in the electrospun fibers and their high surface-to-volume ratio is supposed to facilitate the glycation compared to dry and wet state methods [14]. The technique was successfully used for conjugate formation of whey protein isolate with dextran [15] and maltodextrin [16]. Electrospinning is a fiber production technique where fine fibers are produced from electrically charged polymer solutions in a strong electric field [17]. In needleless electrospinning, multiple solution jets are generated simultaneously from the surface of a slowly rotating, horizontal metal cylinder covered with the charged polymer solution. These jets eject as soon as the electrical forces overcome the surface tension of the polymer solution and travel towards an electrically grounded collector above the rotating cylinder. On their way, the solvent rapidly evaporates and the jets stretch and bend into fine fibers [18]. In this approach, high molecular weight polysaccharides do not only deliver the reducing carbonyl group for the glycation but also enable the formation of stable fibers in which the proteins are embedded [14]. The most influential parameters on the electrospinning process are the type of polymer used, its molecular weight and structure, polymer concentration, the spinning dispersion's viscosity, surface tension, and electrical conductivity, as well as ambient parameters such as temperature and relative humidity [19].

In this study, the aim was to explore the potential of using the electrospinning method for the production of pea protein isolate (PPI)-maltodextrin conjugates. Maltodextrins with different dextrose equivalents (DE) differing in molecular weight and amount of reducing groups were used. Their ratio was varied in order to achieve high fiber production rates during electrospinning as well as a high degree of glycation after heating the fibers. It was hypothesized that a higher amount of high DE maltodextrin with a low molecular weight and a high amount of reducing groups would decrease the quality of electrospun fibers while at the same time promoting the glycation reaction with PPI. This study is expected to contribute to the knowledge necessary to successfully apply electrospinning in the production of glycated proteins with improved technofunctionality.

Materials and Methods

Materials

Pea protein isolate (Pisane® C9, lot number 2016407102, protein content 79.76% w/w, determined by Dumas flash

combustion method with a protein conversion factor of 6.25) was provided by Cosucra Groupe Warcoing S.A. (Pecq, Belgium). Maltodextrins (Eliane™ MD 2, GLUCIDEX® 12 D, and GLUCIDEX® 21 D) were kindly provided by Avebe U.A. (Veendam, Netherlands) and Roquette Frères (Lestrem, France). Other reagents and chemicals were acquired from Carl Roth GmbH & Co. KG (Karlsruhe, Germany) and Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and were of analytical grade, unless otherwise stated.

Determination of Maltodextrin Molecular Weight with High-Performance Size-Exclusion Chromatography (HP-SEC)

Maltodextrins were dissolved ($c = 10$ mg/mL) in the mobile phase (5 mM acetic acid, containing 0.25 M NaCl) and stirred overnight at room temperature before they were filtered (pore size 0.45 μ m, regenerated cellulose filters Chromafil RC-20/25, Machery-Nagel GmbH & Co. KG, Düren, Germany). Analysis was carried out on an Agilent HP Series 1100 with a tandem of the silica packed columns TSKgel G4000SW_{XL} and TSKgel G2000SW_{XL} (TOSOH Bioscience, Tokyo, Japan) protected by a SW_{XL} guard column with an injection volume of 20 μ L at a flow rate of 0.6 mL/min. The molecular weight distribution was calculated from the signal of a refractive index detector (RID) with the help of a six-point calibration curve using dextran standards ranging from 12 to 270 kDa (Fluka Analytical, Buchs, Switzerland). Number average molecular weight (M_n) and weight average molecular weight (M_w) were calculated according to Eq. 1 and 2 with M_i being the molecular weight of fraction i and c_i being the concentration of fraction i . Two samples of each maltodextrin were analyzed.

$$M_n = \frac{\sum c_i}{\sum \frac{c_i}{M_i}} \quad (1)$$

$$M_w = \frac{\sum M_i \cdot c_i}{\sum c_i} \quad (2)$$

Dextrose Equivalent of Maltodextrins

The dextrose equivalent value (DE) was determined by measuring the amount of reducing sugars following the Nelson-Somogyi assay [20]. Calibration was done with glucose and the absorbance was read at 540 nm with a spectrometer (Lamda 750S, PerkinElmer, Beaconsfield, UK). The DE was calculated from the percentage of glucose in the maltodextrin dry matter. The analysis was performed on two independently prepared samples.

Preparation and Characterization of Electrospinning Dispersions

Spinning dispersions from various maltodextrins and pea protein isolate were prepared with the concentrations given in Table 1 by dispersing the components in demineralized water and stirring them overnight at ambient temperature to ensure complete hydration. Concentrations were derived from preliminary experiments where the maximum spinnable PPI concentration was 0.063 g/mL (corresponds to a pea protein content of 0.05 g/mL) when mixed with maltodextrin DE 2 at its critical spinning concentration of 0.8 g/mL [21]. Samples were labeled according to the ratios of the individual components. 80-DE2:5-PPI serves as a control sample without any other added second maltodextrin.

Electrical Conductivity

The electrical conductivity of the biopolymer spinning dispersions was measured with a microprocessor conductivity meter LF357 (WTW GmbH, Weilheim, Germany). Three independently prepared samples were measured.

Dynamic Viscosity

Dynamic viscosity of the spinning dispersions at 25 °C was measured with a rotational rheometer (MCR 502, Anton Paar GmbH, Ostfildern, Germany) equipped with a plate-plate measurement system (P-PTD200 and PP50, Anton Paar GmbH, Ostfildern, Germany) at a gap width of 0.5 mm. Shear rate was increased linearly from 0.1 to 1000 s⁻¹. The analysis was performed in duplicate on two independently prepared samples.

Needleless Electrospinning and Fiber Characterization

Electrospinning was performed with a needleless upward roller electrospinning setup where the spinning dispersion was charged (63.8 kV) with a high voltage power supply (SL 60, Spellman, Hauppauge, NY, USA) in the sample container and loaded onto a stainless steel cylinder

spinneret by rotation (55 rpm). Fibers were collected on an electrically grounded rotating stainless steel cylinder (30 rpm) above the spinneret. The distance between spinneret and collector was 15.5 cm. Electrospinning was performed at 21–25 °C and a relative humidity of 10–15%. The fiber production rate was determined by weighing the fibers after a spinning time of 1 h. Three independently prepared samples were used.

Scanning Electron Microscopy (SEM)

Fiber morphology was studied using a scanning electron microscope (JSM-IT100, JEOL USA, Inc., Peabody, USA) at a high vacuum and an acceleration voltage of 3 kV. Fibers for electron microscopy were collected during electrospinning for 120 s on a conductive tab attached to the collector of the electrospinning apparatus. The average fiber diameter was obtained by image analysis (Digimizer, MedCalc Software bvba, Ostend, Belgium) and calculated as an average of 50 fibers for each sample.

Fiber Protein Content

Fiber protein content was measured using the Dumas flash combustion method (AOAC 46–30.01) using a Dumatherm® DT N Pro (C. Gerhardt GmbH & Co. KG, Königswinter, Germany). Protein content was calculated from the total nitrogen content using a protein conversion factor of 6.25. Three independently prepared samples were analyzed.

Preparation of Maltodextrin-PPI Conjugates

Electrospun fibers (35 ± 0.02 mg) were placed inside a climate chamber (Mettmert HCP50, Mettmert GmbH & Co. KG, Schwabach, Germany) at 60 °C and 75 ± 0.5% relative humidity (RH) for 6, 12, 18, and 24 h, respectively.

Measurement of Free Amino Groups

In order to determine the degree of glycation, the free amino group content in unheated and heated fibers was

Table 1 Preparation scheme for the electrospinning dispersions made with maltodextrin with different dextrose equivalents (DE) and pea protein isolate (PPI) at different concentration ratios

	Maltodextrin DE 2 (g/mL)	Maltodextrin DE 12 (g/mL)	Maltodextrin DE 21 (g/mL)	Pea protein (g/mL)
80-DE2:5-PPI	0.8	–	–	0.05
80-DE2:5-DE12:5-PPI	0.8	0.05	–	0.05
80-DE2:10-DE12:5-PPI	0.8	0.1	–	0.05
80-DE2:5-DE21:5-PPI	0.8	–	0.05	0.05
80-DE2:10-DE21:5-PPI	0.8	–	0.1	0.05

DE: dextrose equivalent; PPI: pea protein isolate

analyzed with the *ortho*-phthaldialdehyde (OPA) method according to the slightly modified microtiter plate assay of Barba, Carbonell-Capella, Esteve and Frigola [22]. The OPA reagent was prepared fresh daily by dissolving 80 mg of OPA in 2 mL of ethanol and mixing with 50 mL of 0.1 M sodium tetraborate buffer (pH 9.7–10), 5 mL of 20% w/v SDS solution, and 0.2 mL of β -mercaptoethanol. Unheated and heated fibers were diluted to a biopolymer concentration of 10 mg/mL with ultrapure water. 25 μ L of the sample were mixed with 475 μ L of ultrapure water and 500 μ L 12% w/v SDS solution and stored overnight at 4 °C. Samples/blanks (8 μ L), 0.1 M sodium tetraborate buffer (pH 9) (8 μ L) and 250 μ L of OPA reagent were pipetted into 96-well black plates (Brand GmbH & Co. KG, Wertheim, Germany) and measured in a Synergy HT microtiter plate reader (BioTek Instruments GmbH, Bad Friedrichshall, Germany) at $\lambda_{\text{ex}} = 340$ nm and $\lambda_{\text{em}} = 455$ nm after 2 min. A six-point L-lysine calibration curve ($c = 0.1$ – 1.0 mg/mL) was used for quantification. Three independently prepared samples were analyzed.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Changes in the molecular weight were analyzed by SDS-PAGE under reducing conditions following the method of Laemmli [23] on a Mini-PROTEAN Tetra Cell (Bio-Rad Laboratories, Hercules CA, USA). Samples of unheated and heated fibers were diluted 1:1 with sample buffer to obtain a final protein concentration of 2 mg/mL and denatured at 90 °C for 5 min. Gels (4–20% Mini-PROTEAN TGX precast gel, Bio-Rad Laboratories, Hercules CA, USA) were loaded with 10 μ L of sample. Electrophoresis was carried out in 0.025 M Tris-HCL buffer solution (pH 8.3, including 0.192 M glycine and 0.1% w/w SDS) at ambient temperature for 35 min at 200 V. Afterwards, the gel was stained for 30 min with Coomassie Brilliant Blue R-250. The molecular weight of the samples was determined according to a molecular

weight standard (Roti®-Mark PRESTAINED, Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

Statistical Analysis

Means and standard deviations were using Excel (Microsoft, 98,052 Redmond, WA, USA). Statistically significant ($p < 0.05$) differences between samples regarding electrical conductivity, fiber production rate, fiber protein content, fiber diameter, and free amino groups after heating were tested with a one-way analysis of variance (ANOVA) and a Tukey post-hoc test under assumed equal variances and normal distribution of the results (SPSS Statistics 25, IBM, Armonk, NY, USA). Different letters represent significant differences.

Results and Discussion

Characterization of Maltodextrins

Maltodextrins with stated dextrose equivalent (DE) values were compared for their measured DE values, their molecular weight, and their polydispersity (Table 2). The determined DE values for the maltodextrin DE 2 (2.70 ± 0.36), DE 12 (11.51 ± 0.34), and DE 21 (22.01 ± 2.05) were in good alignment with the values stated by the manufacturers. The maltodextrin DE 2 had the highest weight-average molecular weight M_w (131.93 ± 0.47 kDa) and number-average molecular weight M_n (6.62 ± 3.03 kDa). For the maltodextrins DE 12 ($M_w = 23.16 \pm 1.68$ kDa; $M_n = 1.27 \pm 0.45$ kDa) and DE 21 ($M_w = 22.01 \pm 2.05$ kDa; $M_n = 1.03 \pm 0.34$ kDa) lower molecular weights were observed. The DE value of a maltodextrin represents the percentage of the reducing sugar ends and is inversely related to the molecular weight of the starch-derived oligosaccharides [24]. A higher DE, hence a higher concentration of reducing sugars, indicates longer hydrolysis times resulting in a decreased molecular mass of the molecules [25]. The polydispersity indices (PDI) ranged between 4.91 ± 1.85 (maltodextrin DE 21)

Table 2 Dextrose equivalent (DE), weight-average molecular weight M_w , number-average molecular weight M_n and polydispersity index (PDI) for tested maltodextrins

Maltodextrin	DE value	M_w (kDa)	M_n (kDa)	PDI
DE 2	2.70 ± 0.36	131.93 ± 0.47	6.62 ± 3.03	22.22 ± 10.08
DE 12	11.51 ± 0.34	23.16 ± 1.68	1.27 ± 0.45	19.27 ± 5.50
DE 21	22.01 ± 2.05	4.73 ± 0.21	1.03 ± 0.34	4.91 ± 1.85

Results are expressed as the mean \pm standard deviation of two independent experiments

DE: dextrose equivalent; M_w : weight-average molecular weight; M_n : number-average molecular weight; PDI: polydispersity index

and 22.22 ± 10.08 (maltodextrin DE 2), reflecting a broader molecular weight distribution of the saccharides in the maltodextrins with decreasing DE.

Characterization of Spinning Dispersions

Electrical Conductivity

Electrical conductivity is an essential parameter for the electrospinning process due to the fact that a higher conductivity favors elongated jets and consequently fiber formation [26]. The solvent, the polymer, and additives such as salts contribute to the overall electrical conductivity of spinning dispersions [27]. The significantly highest ($p < 0.05$) electrical conductivity was observed for the maltodextrin-PPI spinning dispersion with only maltodextrin DE 2 and pea protein isolate (80-DE2:5-PPI) (Table 3). Generally, a higher added percentage of a second maltodextrin led to a decrease in conductivity. Since it had previously been shown that the protein is the main contributor to the electrical conductivity of maltodextrin-protein spinning dispersions [21, 28], this finding was expected because the addition of a second maltodextrin decreased the overall percentage of the pea protein in the dispersion. Responsible for the electrical conductivity of proteins in solution is their polyelectrolyte character [29, 30].

Dynamic Viscosity of Spinning Dispersions

Rheological properties of spinning dispersions are one of the most important parameters for the electrospinning process [31, 32]. An optimal dynamic viscosity is required since at too low viscosities no continuous fiber formation is possible, whereas at too high viscosities it is difficult to eject jets from the polymer solution [27]. All maltodextrin-PPI spinning dispersions were shear

thinning and showed a decrease in dynamic viscosity upon increasing the shear rate at the shear rate analyzed from 0.1 to 1000 s^{-1} (Fig. 1). Shear thinning behavior is linked to an entangled polymer network that is disturbed at sufficiently high shear rates [33, 34]. This entanglement of polymer chains in the spinning dispersion is one prerequisite for successful electrospinning since it prevents the solution jet from breaking up into droplets during stretching in the electric field [35]. Based on the results of Stijnman, Bodnar and Hans Tromp [36] where a weak tendency of shear thinning below shear rates of 1000 s^{-1} was a necessary but insufficient condition for fiber formation, it was presumed that all studied maltodextrin-PPI samples would be electrospinnable. The dynamic viscosity of all samples was in the narrow range between 7.3 ± 0.9 (80-DE2:5-DE21:5-PPI) and $8.3 \pm 0.5 \text{ Pa}\cdot\text{s}$ (80-DE2:5-PPI) at 0.1 s^{-1} . It was expected that the addition of a second maltodextrin to the spinning dispersion would increase the dynamic viscosity due to the overall increase of the polymer concentration in the spinning dispersion. However, both maltodextrins DE 12 and 21 caused a small reduction of the dynamic viscosity compared to the spinning dispersion 80-DE2:5-PPI. The added maltodextrins DE 12 and 21 had very low molecular weights compared to the maltodextrin DE 2 (Table 2). Their addition broadened the overall molecular weight distribution of the saccharides in the spinning dispersion. Generally, the narrower the molecular weight distribution of a polymer, the lower the concentration necessary (i.e., the entanglement concentration c_e) to reach an entangled polymer network [31]. Hence, a possible explanation for the slightly lower dynamic viscosity might be that the polymer chains of maltodextrin DE 12 and 21 were too short to contribute to the entangled network of the long maltodextrin DE 2 chains but long enough to interfere with this entanglement and consequently causing a decrease in dynamic viscosity of the spinning dispersions.

Table 3 Electrical conductivity of spinning dispersions, fiber production rate, mean fiber diameter, and protein content of electrospun fibers prepared from different maltodextrin-pea protein isolate (PPI) ratios

	Electrical conductivity (mS/cm)	Fiber production rate (g/h)	Mean fiber diameter (μm)	Fiber protein content (% w/w)	Ratio spinning dispersion protein content to fiber protein content
80-DE2:5-PPI	0.84 ± 0.02^a	1.27 ± 0.36^a	2.54 ± 1.00^b	4.34 ± 0.03^a	1:0.74
80-DE2:5-DE12:5-PPI	0.75 ± 0.01^b	0.86 ± 0.13^a	2.47 ± 1.13^b	3.91 ± 0.07^c	1:0.70
80-DE2:10-DE12:5-PPI	0.68 ± 0.02^c	0.89 ± 0.26^a	2.89 ± 0.79^{ab}	4.12 ± 0.02^b	1:0.78
80-DE2:5-DE21:5-PPI	0.76 ± 0.01^b	0.87 ± 0.33^a	3.31 ± 1.22^a	4.03 ± 0.04^{bc}	1:0.73
80-DE2:10-DE21:5-PPI	0.70 ± 0.01^c	1.50 ± 0.25^a	2.70 ± 0.99^b	4.04 ± 0.09^{bc}	1:0.77

Results are expressed as the mean \pm standard deviation of three independent experiments. Different letters indicate significant differences ($p < 0.05$) between samples

DE: dextrose equivalent; PPI: pea protein isolate

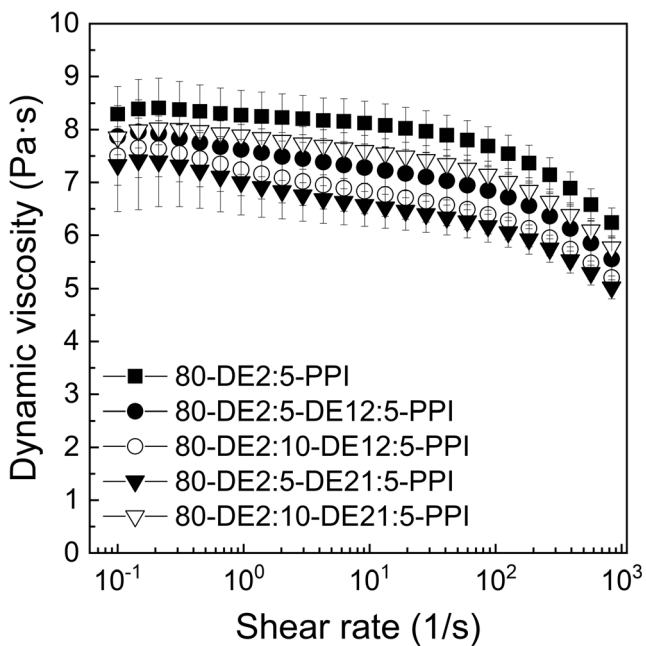


Fig. 1 Dynamic viscosity of maltodextrin-PPI spinning dispersions versus shear rate. Error bars indicate standard deviations

The same observation was made for the addition of maltodextrins to potato starch pastes [37]. However, it has to be pointed out again that the overall influence of either 0.05 or 0.1 g/mL maltodextrin DE 12 and 21 on the dynamic viscosity of the spinning dispersions was small.

Characterization of Electrospun Fibers

Electrospinnability

The ability of a solution to be spun into fibers in an electrostatic field is called electrospinnability [32]. Electrospinnability was assessed by studying the fiber production rates for the maltodextrin-PPI spinning dispersions (Table 3). The fiber production rates ranged between 0.86 ± 0.13 (80-DE2:5-DE12:5-PPI) and 1.50 ± 0.25 g/h (80-DE2:10-DE21:5-PPI) and did not differ significantly ($p < 0.05$). It was concluded that the influence of a second added maltodextrin on the spinning dispersions' relevant properties such as electrical conductivity (Table 3) and dynamic viscosity (Fig. 1) was not big enough to alter the electrospinnability of the system. Electrospinnability was dominated by the presence of maltodextrin DE 2 ($c = 0.8$ g/mL) in the systems. It was previously shown that this concentration was the critical spinning concentration of the maltodextrin DE 2 in a needleless setup and that the system tolerates the addition of further compounds without a loss of electrospinnability [21]. In comparison to maltodextrin-soy protein blends, the electrospinnability of maltodextrin with pea protein was higher [28], making pea protein a more suitable legume protein for the production of electrospun fibers. Pea protein was previously successfully electrospun in blends with

pullulan ($M_w \sim 100$ kDa) by Aguilar-Vázquez, Loarca-Piña, Figueroa-Cárdenas and Mendoza [38].

Fiber Morphology and Diameter

The morphology of the maltodextrin-PPI fibers analyzed by SEM is shown in Fig. 2. In the pictures A1 – E1, unsystematic bead defects between the continuous fibers were visible. Some fibers also showed a ribbon-shaped and fused morphology. The observed fiber defects might be a result of the broad molecular weight distribution of maltodextrin (Table 2) and PPI (Fig. 4) since it is known that largely polydisperse polymers impair electrospinnability [39]. Furthermore, the dynamic viscosity of the maltodextrin-PPI spinning dispersions was much higher (up to 8.3 ± 0.5 Pa·s for 80-DE2:5-PPI at 0.1 s^{-1}) compared to other electrospinning studies using pullulan ($M_w \sim 100$ kDa) as carrier molecule for pea protein (up to 0.6 Pa·s) [38] or for amaranth protein (up to 3.5 Pa·s) [40]. This might have also caused defects and fused fibers due to a limitation in deformability of the polymer jet and a faster solidification of the highly concentrated PPI-maltodextrin dispersions [41]. The pictures A2 – E2 showed continuous fibers with smooth surfaces and only a few areas with accumulated fiber material. It was concluded that the pea protein was successfully incorporated into the maltodextrin fibers. No clear difference between the SEM micrographs of the different maltodextrin-PPI blends was observed. The mean fiber diameters ranged from 2.47 ± 1.13 (80-DE2:5-DE12:5-PPI) to $3.31 \pm 1.22 \text{ }\mu\text{m}$ (80-DE2:5-DE21:5-PPI), which was the significantly largest ($p < 0.05$) fiber diameter (Table 3). The other samples did not differ significantly from each other and no clear trend could be observed between the different spinning dispersions and the fiber diameters. The high standard deviations of the fiber diameters reflect a broad fiber diameter distribution. Generally, needleless electrospinning produces less homogeneous fiber diameters compared to classical needle electrospinning. This broad fiber diameter distribution is caused by the ejection of jets from droplets of various sizes directly from the open surface of the spinning solution [42]. The findings align with the previous assumption that neither the differences in electrical conductivity nor dynamic viscosity of the spinning dispersions were large enough to alter their electrospinnability substantially.

Fiber Protein Content

Since the incorporation of the pea protein from the spinning dispersions into the electrospun fibers is important for the subsequent conjugation reaction between protein and maltodextrin, the protein content of the fibers was measured and compared to the initial protein content of the spinning dispersions (Table 3). The lowest protein content was measured for 80-DE2:5-DE12:5-PPI fibers with $3.91 \pm 0.07\%$ w/w. The

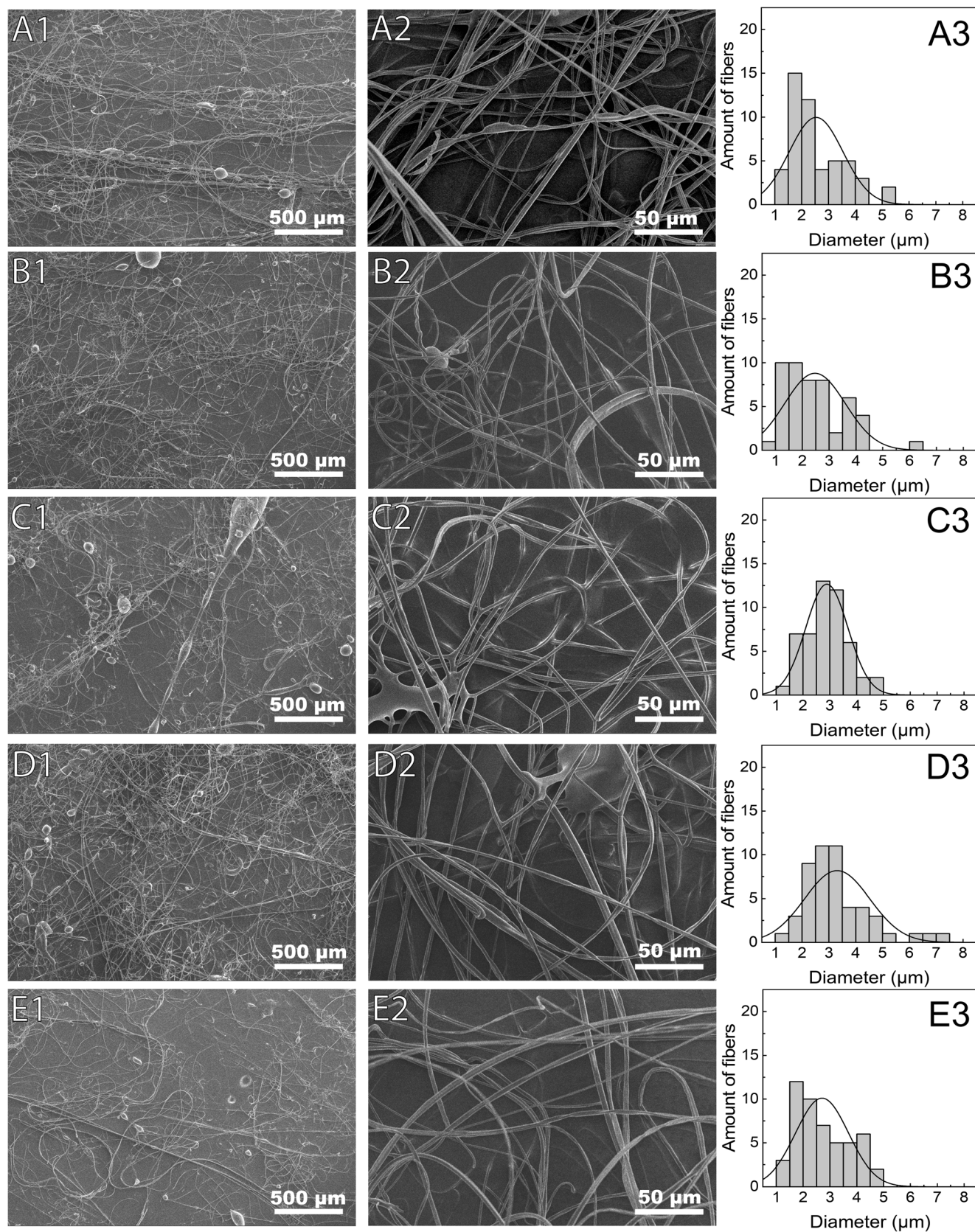


Fig. 2 Scanning electron micrographs (A1 – E1, A2 – E2) and diameter histograms (A3 – E3) of electrospun maltodextrin-PPI fibers from (A) 80-DE2:5-PPI, (B) 80-DE2:5-DE12:5-PPI, (C) 80-DE2:10-DE12:5-PPI, (D)

80-DE2:5-DE21:5-PPI, and (E) 80-DE2:10-DE21:5-PPI. Scale bar represents 500 μm in left (1) images and 50 μm in right (2) images

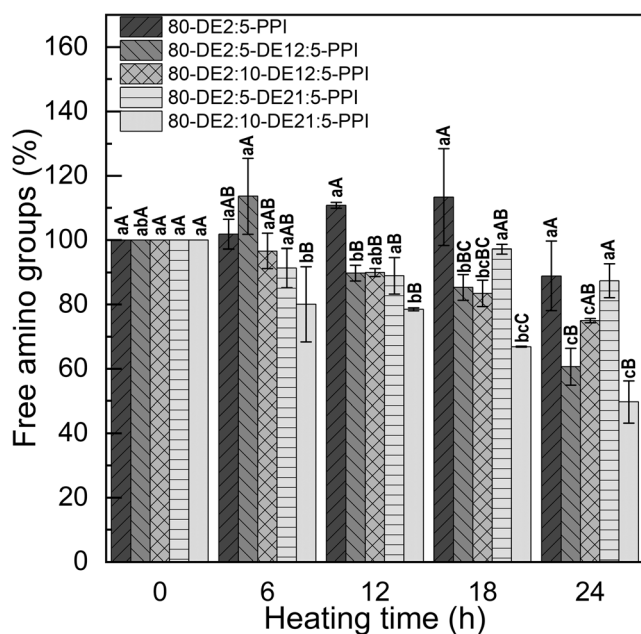


Fig. 3 Changes in free amino groups of maltodextrin-PPI fibers heated at 60 °C and 75% relative humidity for 0–24 h. Error bars indicate standard deviation. Different lowercase letters indicate significant differences between heating times of the same sample ($p < 0.05$), uppercase letters indicate significant differences between samples at the same heating time ($p < 0.05$)

highest protein content was $4.34 \pm 0.03\%$ w/w for 80-DE2:5-PPI fibers. Compared to the initial pea protein amount in the spinning dispersions, the major part of the pea protein (70–78%) could be incorporated into the electrospun fibers (Table 3). Spinning dispersions had a pH ranging from ~6.5 to ~6.8 (data not shown). Considering the protein solubility of the pea protein isolate of ~60% at pH 7 (data not shown), it is suggested that the majority of the insoluble pea protein interfered with the entanglement of the maltodextrin network and was thus not incorporated into the electrospun fibers [28, 43]. This resulted in a lower fiber protein content compared to the protein content of the spinning dispersion. Again, no trend between the ratio of spinning dispersion protein content to

fiber protein content and the composition of the spinning dispersion was observed.

Characterization of Conjugates

Free Amino Groups

The decrease of free amino group content is an indicator of the early Maillard reaction stage wherein the condensation reaction between free amino groups and reducing carbonyl groups of the polysaccharide occurs [44]. In order to study the influence of the maltodextrin composition of the sample and the heating time on the glycation reaction, free amino groups were quantified using the OPA fluorometric assay before and after heating the samples. Figure 3 shows the loss of free amino groups expressed as percentage of the initial content in the unheated fibers. Looking at the development over time (lowercase letters), 80-DE2:10-DE21:5-PPI showed the fastest glycation with a significant decrease ($p < 0.05$) down to $80.10 \pm 11.70\%$ free amino groups after 6 h heating at 60 °C and $75 \pm 0.5\%$. After 12 h, also the free amino groups of 80-DE2:5-DE12:5-PPI started to decrease significantly ($p < 0.05$) down to $89.77 \pm 2.44\%$. The only samples which did not show any significant changes in their free amino group content compared to the unheated fibers over the whole heating period of 24 h were 80-DE2:5-PPI ($88.88 \pm 10.78\%$ after 24 h) and 80-DE2:5-DE21:5-PPI ($87.41 \pm 5.28\%$ after 24 h). Compared to each other (uppercase letters), the sample 80-DE2:10-DE21:5-PPI showed the significantly lowest ($p < 0.05$) relative amount of free amino groups ($66.86 \pm 0.16\%$) after a heating time of 18 h. After 24 h, both the samples 80-DE2:10-DE21:5-PPI ($49.70 \pm 6.54\%$) and 80-DE2:5-DE12:5-PPI ($60.65 \pm 5.71\%$) were the samples with the significantly lowest ($p < 0.05$) relative amount of free amino groups compared to the other samples after the same heat treatment. Considering the mean diameters of the electrospun fibers (Table 3), no correlation between glycation behavior

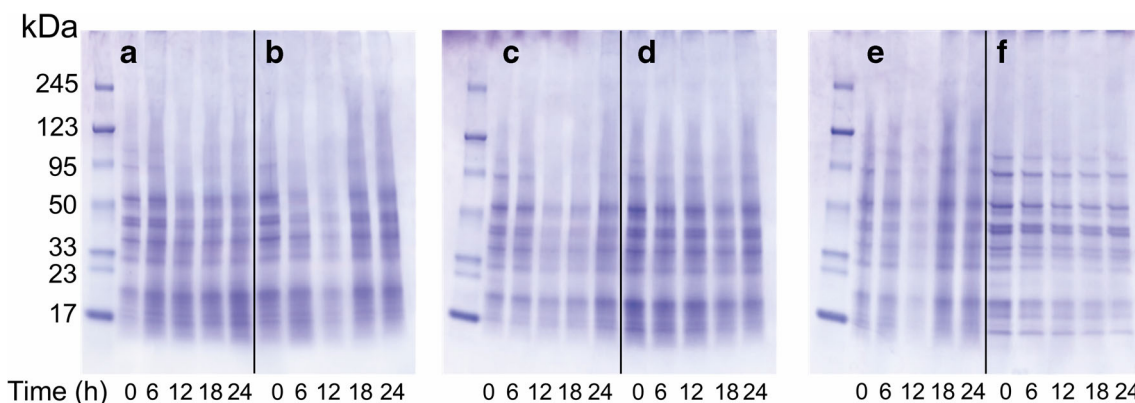


Fig. 4 SDS-PAGE pattern of electrospun maltodextrin-PPI fibers from (a) 80-DE2:5-PPI, (b) 80-DE2:5-DE12:5-PPI, (c) 80-DE2:10-DE12:5-PPI, (d) 80-DE2:5-DE21:5-PPI, (e) 80-DE2:10-DE21:5-PPI, and (f) PPI heated at 60 °C and 75% relative humidity for 0–24 h

and fiber diameter distribution was observed. However, the sample with the highest overall amount of reducing carbonyl groups (80-DE2:10-DE21:5-PPI) showed the fastest and largest decrease of free amino groups, whereas the sample with the lowest amount of reducing carbonyl groups (80-DE2:5-PPI) showed no significant ($p < 0.05$) decrease. Although no direct correlation between the amount of reducing carbonyl groups in the fiber samples and the formation of glycated pea protein could be established, the addition of a second maltodextrin with a higher DE (12 or 21) to the blends of maltodextrin DE 2 and pea protein isolate generally promoted the Maillard reaction. This observation has previously been made for different proteins and sugars glycated under commonly used dry and wet heating conditions by Mulcahy, Mulvihill and O'Mahony [45], Wooster and Augustin [46], and Chevalier, Chobert, Popineau, Nicolas and Haertlé [47]. All reported a decrease in reactivity with increasing carbohydrate chain length. The chain length is reversely related to the amount of reducing carbonyl groups. The higher the amount of reactive groups, the faster the Maillard reaction can occur [47]. Furthermore, high molecular weight polysaccharides only have a low mobility due to steric hindrance further slowing down the glycation reaction [46].

Firstly, these results demonstrate that highly sought-after glycated pea proteins can be produced by electrospinning PPI with maltodextrins and subsequently heating the fibers. This makes the electrospinning approach a viable production method for novel plant protein ingredients. Secondly, the findings show that the maltodextrin composition in the fibers is crucial for successful glycation. The findings contribute to the knowledge of improving plant protein technofunctionality and can help to create plant protein ingredients that can substitute animal-based proteins in new food product formulations.

SDS-PAGE Analysis

In order to investigate the covalent coupling between the maltodextrin and the pea protein after heating the fibers, SDS-PAGE was performed under reducing conditions. Figure 4 shows the SDS-PAGE patterns of unheated and heated fiber samples with different maltodextrin compositions (A – E) as well as unheated and heated PPI (F) as reference sample. Unheated fibers, as well as the PPI samples, showed the characteristic bands of pea proteins at 19–97 kDa with legumin (α -legumin at 38–60 kDa and β -legumin at 19–22 kDa), vicilin (30–33 kDa), convicilin (70 kDa), and lipoxigenase (97 kDa) [38, 48, 49]. Heating the maltodextrin-PPI fibers led to the appearance of a longitudinal smeared band over the whole range of the gel, indicating an increase in molecular weight of the proteins. This behavior was most distinct for the 80-DE2:10-DE21:5-PPI sample immediately after 6 h and for the 80-DE2:10-DE12:5-PPI sample after 18 and

24 h. This observation matches the results of the OPA-assay (Fig. 3), where these two samples showed the highest decrease of free amino groups upon heating. The SDS-PAGE pattern of PPI without the presence of maltodextrin did not change over the heating period of 24 h. It was concluded that the smeared bands of the heated maltodextrin-PPI fibers were not caused by protein aggregation but by glycation. The wide molecular range of the stained band can be explained by the high polydispersity (Table 2) of the maltodextrins [50, 51]. Similar behavior was previously reported for SDS-PAGE patterns of whey protein isolate-maltodextrin conjugates produced with the electrospinning approach [16].

Conclusion

This study demonstrated the successful production of pea protein isolate glycated with maltodextrin in electrospun fibers. It was shown that the spinnability was predominantly determined by the high amount (0.8 g/mL) of long chain polysaccharides of the maltodextrin with dextrose equivalent 2 in the mixture. The addition of up to 0.1 g/mL of a second maltodextrin (dextrose equivalents 12 or 21) slightly altered the spinning dispersions' electrical conductivity and dynamic viscosity but not its electrospinnability. The addition of a second maltodextrin with a higher dextrose equivalent enhanced the glycation reaction upon heating the electrospun fibers due to the higher amount of carbonyl groups present in the fibers. The sample with the highest amount of carbonyl groups showed the fastest and highest decrease of the free amino groups over the heating period. Overall, the study showed that the amount of reactive carbonyl groups in the electrospun fibers can be adjusted to reach a higher degree of glycation as long as the limits for electrospinnability are not exceeded. Further investigations must be conducted to evaluate the influence of the degree of glycation and the molecular weight of the maltodextrin on the technofunctionality of glycated pea protein isolate.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of PepsiCo Inc.

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