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Influence of the Maillard reaction on the properties of cold-set whey protein and maltodextrin binary gels

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ABSTRACT

The Maillard conjugation of proteins and reducing saccharides is used to modify the technological functionality of whey proteins. In this study, whey protein isolate (WPI) was conjugated with maltodextrin (at 1:1 ratio and two total solid contents of 100 and 200 mg mL^{-1}) through the Maillard reaction and used to form cold-set gels. The glycation reaction increased the strength of hydrogen bonding of whey proteins and preferentially modified α-lactalbumin, in comparison with β-lactoglobulin. It also increased the reducing power of binary protein-saccharide solution and allowed formation of self-standing cold-set WPI gel at a low protein content (i.e., ≈50 mg mL^{-1}). Microscopic imaging showed micro-phase separated maltodextrin domains, interrupting the protein network, in gels made of protein-maltodextrin physical mixtures, whereas Maillard conjugation resulted in more homogenous microstructures at both total solid contents. The Maillard reaction increased gel firmness and water-holding capacity and caused a reduction in the extent of gel swelling.

1. Introduction

Whey proteins are highly nutritious and show various technologically important functionalities such as emulsification (Singh & Dalgleish, 1998), foam formation (Zhu & Damodaran, 1994), and heat-induced and cold-set gelation (Cavallieri & Da Cunha, 2008; Sarkar et al., 2016; Torres, Murray, & Sarkar, 2017) properties. Nonetheless, modification of whey proteins by diverse methods, e.g., heating (Foegeding, Davis, Doucet, & McGuffey, 2002), high pressure and ultrasonic treatments (Jambrak, Mason, Lelas, Herceg, & Herceg, 2008), partial hydrolysis by proteolytic enzymes (Chobert, Bertrand-Harb, & Nicolas, 1988), as well as crosslinking by the enzyme transglutaminase (Aboumahmoud & Savello, 1990), glutaraldehyde and citric acid (Bagheri, Yarmand, Madadlou, & Mousavi, 2014) has been implemented to modulate whey proteins functionality and confer tuned properties.

Chemical modification of proteins is easy to perform, imposes low labour cost and allows a wide variety of functionalisation possibilities by means of versatile chemicals and reagent combinations. However, most chemically modified proteins are questioned by consumers with regards hazardous traces of non-food-grade ingredients, such as carcinogens (Nagasawa, Ohgata, Takahashi, & Hattori, 1996). The Maillard reaction has long been applied in the conventional food industry for miscellaneous purposes ranging from bakery to coffee roasting (Moreira, Nunes, Domingues, & Coimbra, 2012; Zanoni, Pierucci, & Peri, 1994). It is a non-enzymatic browning reaction and includes addition of reducing carbohydrates to proteins, which may improve protein functionality such as heat stability (Aoki et al., 1999), emulsification (Zhu, Damodaran, & Lucey, 2010), foaming properties (Corzo-Martínez, Sánchez, Moreno, Patino, & Villamiel, 2012), and solubility (Shepherd, Robertson, & Ofman, 2000). However, control over the reaction is needed to prevent formation of advanced polymerised glycation end-products, which have been found associated with chronic diseases such as diabetes, arteriosclerosis, kidney failure, early ageing, and Alzheimer's (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016). In contrast, a well-controlled Maillard reaction can increase the antioxidant activity of proteins (Liu & Zhong, 2013).

The Maillard reaction has been utilised to modify the textural properties of heat-set whey protein hydrogels. Spotti et al. (2013) showed that, unlike WPI-dextran mixed gels, heat-set gels fabricated from the Maillard reaction conjugates of whey protein isolate (WPI) and dextran did not fracture under uniaxial compression test (80% deformation), which was related to the texture alteration from

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brittle to rubbery due to the formation of new covalent bonds in effect of the Maillard reaction. Gels made from the protein-dextran conjugates had a significantly lower hardness than mixed gels. Likewise, Sun et al. (2011) reported that glycosylation of WPI with dextran caused a10-fold reduction in the storage modulus (as an indication of stiffness) of the subsequently formed heat-set gel. In contrast to the heat-induced procedure, cold-set gelation of whey proteins allows addition of heat-labile bioactive compounds into the protein gel (Maltais, Remondetto,& Subirade, 2009). A two-step process is carried out to form cold-set whey protein gels: first, proteins are heat-denatured at non-gelling conditions (i.e., at low ionic strength, low protein content and at pHs far from the proteins' isoelectric point), which leads to formation of whey protein soluble aggregates; this is followed by cooling the protein solution to room temperature and gelation induction by adding salts and/or adjusting pH toward the isoelectric point (Ju & Kilara, 1998). Cold set WPI gels usually have a more homogenous microstructure (Nicolai, Britten, & Schmitt, 2011) and a higher water-holding capacity than heat-induced gels (Veerman, Baptist, Sagis, & van der Linden, 2003). There is no report in the literature, to the best of the authors' knowledge, about modification of the texture and functionality of cold-set whey protein gels by glycation of protein building blocks through the Maillard reaction.

Maltodextrin is a hydrolysis product of starch consisting of α-(1,4) and α-(1,6) linked D-glucose polymers and/or oligomers with a dextrose equivalent less than 20. Neutral and reducing nature of maltodextrin causes proteins glycation, without complication resulting from the formation of electrostatic complexes (Dickinson & Semenova, 1992; O'Regan & Mulvihill, 2009). In addition, saccharides that are substituted at the C-4 hydroxyl (like maltodextrin) might be less energetically prone to post-Amadori Maillard reactions (Shepherd et al., 2000), decreasing the formation extent of advanced polymerised products. In the current study, whey proteins were conjugated with maltodextrin through the Maillard reaction and the resulting salt-induced cold-set hydrogels were characterised to investigate the effect of glycation on the gel properties and to produce protein gels with potential bioactive characteristics.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) with >90% protein content was donated by Arla food ingredients (Viby J, Denmark). Maltodextrin powder with dextrose equivalent of 18–20 was purchased from Ingredion Germany GmbH (Hamburg, Germany). Other chemicals used were of analytical grade and supplied from Merck (Darmstadt, Germany).

2.2. Preparation of WPI-maltodextrin solutions

WPI powder was dissolved in distilled water (100 mg mL^{-1}) and sodium azide was added (50 ppm) as bactericide. Afterwards, the

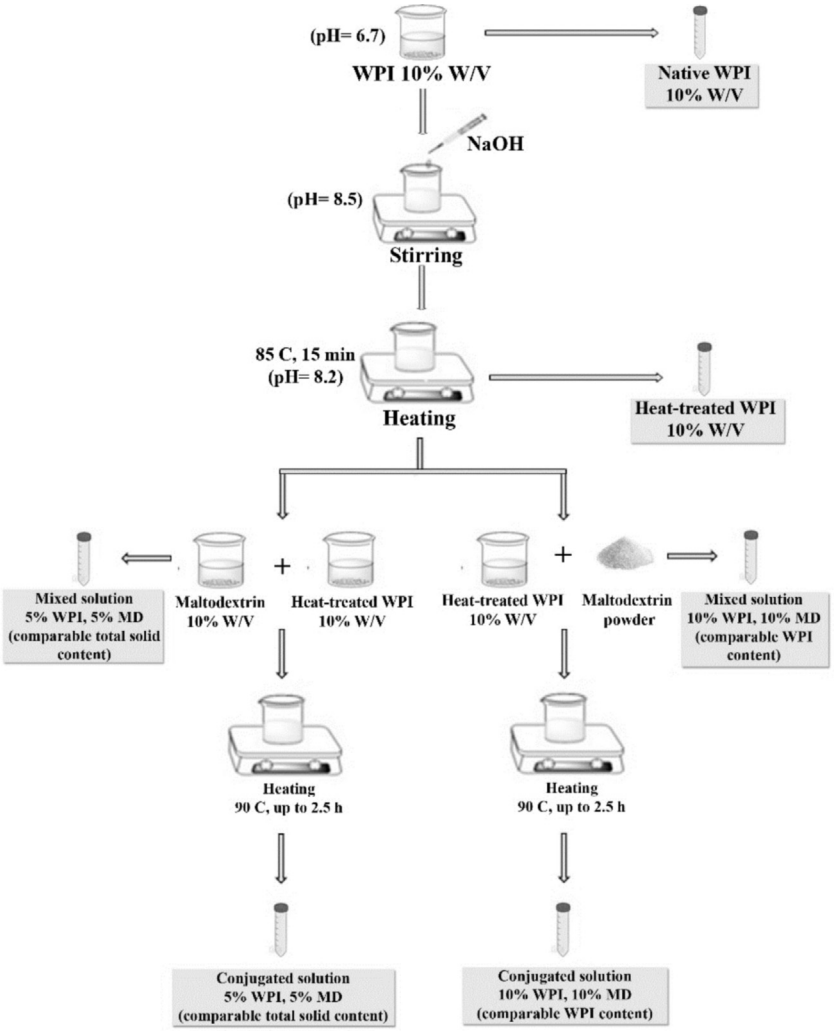

Fig. 1. Schematic description of samples.

solution (50 mL) was stirred for 2 h and left for 12 h at 4 °C to hydrate completely. Then, the pH was adjusted to 8.50 ± 0.05 by stepwise addition of 5 M NaOH and heated in a shaking water bath for 15 min at 85 °C, to form WPI soluble aggregate solution (pre-heated WPI sample). After heat treatment, sample were rapidly cooled to room temperature using ice water and used for preparation of control cold-set gel.

WPI-maltodextrin mixed and conjugated gel samples were made at either comparable total solid content (100 mg mL^{-1}) or comparable WPI content (100 mg mL^{-1}) to control cold-set WPI gel (Fig. 1). WPI-maltodextrin mixed samples with comparable total solid content were prepared by mixing a pre-heated WPI solution (100 mg mL^{-1}) and a maltodextrin solution (100 mg mL^{-1}) at a 1:1 ratio, yielding a binary protein-polysaccharide solution of 100 mg mL^{-1} total solid content. WPI-maltodextrin mixed samples with comparable WPI content were prepared by adding dry maltodextrin (100 mg mL^{-1}) into pre-heated WPI solution (100 mg mL^{-1}), resulting in a binary solution with twofold total solid content (i.e., 200 mg mL^{-1}) to control cold-set gel. Furthermore, WPI-maltodextrin conjugated solutions at either comparable total solid content (100 mg mL^{-1}) or comparable WPI content (100 mg mL^{-1}) were prepared by heating at 90 °C for 2.5 h in closed falcons. Fig. 1 illustrates schematically how the samples were prepared.

2.3. Measurement of the Maillard reaction advanced products

Formation of the final stages products of the Maillard reaction in WPI-maltodextrin conjugated samples was surveyed by measuring absorbance over the reaction period (0, 0.5, 1, 1.5, 2 and 2.5 h) at 420 nm (Kim & Lee, 2009) using a UV–visible spectrophotometer (CecilCE2502, Cecil Ins., Cambridge, UK).

2.4. Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of WPI (native), heat-treated (pre-heated) WPI, maltodextrin, WPI-maltodextrin mixed samples and WPI-maltodextrin conjugates were acquired using a FTIR spectrometer (Spectrum one, Perkin Elmer, Torrence, MA, USA). Samples were freeze-dried prior to being mixed with potassium bromide powder as the reference material and then spectra were procured at transmission mode from wavenumber range of 2000 to 600 cm^{-1} at 4 cm^{-1} resolution.

2.5. SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was performed using a 5% (w/v) stacking gel and a 15% (w/v) separating gel according to the method of Laemmli (1970). Solution and suspension samples having equal protein concentration of 4 mg mL^{-1} were dissolved in a sample buffer containing β-mercaptoethanol, followed by heating at 95 °C for 10 min before loading. The electrophoresis process was carried out at a constant voltage of 150 V for 4 h. The gel was stained using Coomassie Brilliant Blue and then it was scanned after bleaching in 20% methanol solution and 10% acetic acid.

2.6. Reducing power

Reducing power of samples was measured following the method described by Peng, Kong, Xia, and Liu (2010) with some modifications. Equal portions of each sample (0.5 mL) was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%), incubated at 50 °C for 20 min then charged with 2.5 mL trichloroacetic acid (10%). Afterwards, the mixtures were centrifuged at 4000× *g* for 10 min. The supernatant (2.5 mL) was blended with 2.5 mL distilled water and 0.5 mL of ferric chloride solution (0.1%). Thereafter, mixtures were kept for 10 min, and the absorbance was read at 700 nm using a UV–visible spectrophotometer (CecilCE2502, Cecil Ins., Cambridge, UK).

2.7. Cold set gelation

The pH of WPI and WPI-maltodextrin samples was adjusted to 7.5 with 1.0 M HCl and 5 mL of samples was transferred into plastic cylindrical tubes (50 mm height and 15 mm inner diameter). Gelation was induced by adding an appropriate volume of $CaCl_2$ solution to samples at a final concentration of 200 mM Ca^{2+}. Then, samples were left for 24 h at 4 °C. Gel samples were equilibrated at 25 °C for 2 h prior to characterisation.

2.8. Hydrogel characterisation

2.8.1. Microstructure

Microstructure of gel samples was imaged using a Zeiss-DSM 960A scanning electron microscope (Carl Zeiss, Jena, Germany) at 30,000× magnification. Gel samples were freeze-dried and cut into small pieces which were mounted on aluminium stubs and then sputter-coated with gold prior to imaging.

2.8.2. Textural analysis

The firmness of gel samples was assessed by penetration test which was carried out using a texture analyser machine (M350-10CT, Testometric, Lancashire, UK) equipped with a stainless steel cylindrical probe (8 mm diameter). Cylindrical (35 mm height × 15 mm diameter) samples were penetrated to a depth of 10 mm at a constant speed of 1 mm s^{-1}. Firmness was the maximum force (N) needed to penetrate samples.

2.8.3. Water holding capacity

Water holding capacity (WHC) was calculated based on the method of Zhang, Hsieh, and Vardhanabhuti (2014). Gel samples were prepared within 5 mL plastic cylindrical tubes and centrifuged at 4000× *g* for 15 min. Then, WHC was determined using the following equation:

$$\mathrm{WHC}(\%) = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

where W_i and W_f are the weight of water in gel before centrifugation (g) and the weight of water loss during centrifugation (g), respectively.

2.8.4. Swelling experiment

The swelling capacity of hydrogels was determined based on the method described by Maltais, Remondetto, and Subirade (2009). Gels made of WPI-maltodextrin binary solutions (mixed or conjugated) at 100 mg mL^{-1} total solid content (50 mg mL^{-1} WPI content) could not hold their structure and degraded during the swelling experiments. Therefore, only the gels made of heat-treated WPI without maltodextrin (100 mg mL^{-1}), and WPI-maltodextrin binary solutions at 200 mg mL^{-1} total solid content (100 mg mL^{-1} WPI content) were analysed for swelling study. Gel samples were weighed and immersed in an enzyme-free simulated gastric fluid at 37 °C for 2 h. The acidic fluid was decanted and excess water on the surface was removed with a piece of paper towel. Afterwards, samples were accurately

weighed and swelling percentage of gels was determined by the following equation:

$$\text{Swelling}(\%) = \frac{m_t - m_0}{m_0} \times 100 \quad (2)$$

where m_t and m_0 are the gel mass at time t and the initial gel mass, respectively.

2.9. Statistical analysis

The results were analysed by one-way ANOVA with SPSS software version 16 (IBM software, NY, USA) using Duncan's test at significance level of $p < 0.05$. Each experiment was replicated three times.

3. Results and discussion

3.1. Glycation of whey proteins

Heat treatment of the WPI solution without added maltodextrin decreased pH by a value of ≈0.2 unit. This minor reduction indicates a limited extent of the Maillard reaction that typically happens between whey proteins and small amounts of lactose that is ordinarily present in WPI powder (Mulcahy, Fargier-Lagrange, Mulvihill, & O'Mahon, 2017). Reduction of pH due to heat treatment was much more intensive in WPI solutions with added maltodextrin (Fig. 2) and pH of the binary solution at 200 mg mL^{-1} total solid content dropped to approximately 7.5. Consumption of free amino groups of proteins as a result of the Maillard reaction and formation of organic acids such as acetic acid and formic acid explain the pH reduction (Mulcahy, Park, Drake, Mulvihill, & O'Mahony, 2016). Formation of coloured products during the Maillard reaction shows degradation of Amadori compounds (intermediate phase) and a reaction cascade which develops high-molecular weight advanced products (Hemmler et al., 2017). As expected, protein content had a remarkable effect on the formation extent of coloured products (Fig. 2). A higher total solid content caused a higher extent of coloured products formation. Fig. 2 shows progressive development of coloured products over the reaction time. However, pH reduction, particularly at the total solid content of 100 mg mL^{-1} slowed down after 90 min. This reaction time (i.e., 90 min) was therefore chosen to form conjugated products for the subsequent analyses and cold-set gel formation.

3.2. FTIR analyses

The main spectral features of proteins are the intense amide I, amide II and amide III bands, placed approximately between 1700 and 1600 cm^{-1} (C═O stretching), 1550–1500 cm^{-1} (N–H deformation) and 1300–1200 cm^{-1} (C–N stretching and N–H deformation), respectively (Chang & Tanaka, 2002). These bands could be modified by the Maillard reaction (Farhat, Orset, Moreau, & Blanshard, 1998). Amide I band, at 1638 cm^{-1} in the spectrum of WPI-maltodextrin mixture (200 mg mL^{-1}), shifted to 1634 cm^{-1} as a consequence of conjugation (Fig. 3), which indicates stronger hydrogen bonding (Barth, 2007) of whey proteins in the conjugates. Moreover, the intensity of amide II and amide III bands in glycated system at 200 mg mL^{-1} total solid content was lower compared with non-glycated sample. The reduced intensity of amide II band can be ascribed to protein aggregation due to the heat treatment (Madadlou, Floury, & Dupont, 2018) during the Maillard reaction. In agreement with our observation, Su, Huang, Yuan, Wang, and Li (2010) reported that conjugation of carboxymethyl cellulose with soy protein isolate resulted in reduction of intensity of amid II and amid III bands, associated with the consumption of –OH group of carboxymethyl cellulose and amino groups of soy proteins.

Peaks at the region of 1180–953 cm^{-1}, which are caused by C–C and C–O stretching vibrations and C–H bending, are often associated to "saccharide" bands (Zhang et al., 2013). The intensity of the band at about 1077 cm^{-1} in the spectrum of WPI-maltodextrin conjugate was lower than that in the spectrum of WPI-maltodextrin mixture at a comparable total solid content of 200 mg mL^{-1}. This band is attributed to the glycosidic linkage of di- or oligosaccharides (Luo et al., 2013; Yaylayan, 1997). Although the glycosidic bonds of di- or oligosaccharides remain generally unchanged during conjugation, a small fraction of glycosidic linkage can be cleaved (Wang, Qian, & Yao, 2011). The band at 850 cm^{-1} in maltodextrin spectrum standing for anomeric region (Sivakesava, & Irudayaraj, 2001), disappeared at WPI-maltodextrin conjugates (200 mg mL^{-1}), suggesting that the anomeric region of maltodextrin attached to whey proteins.

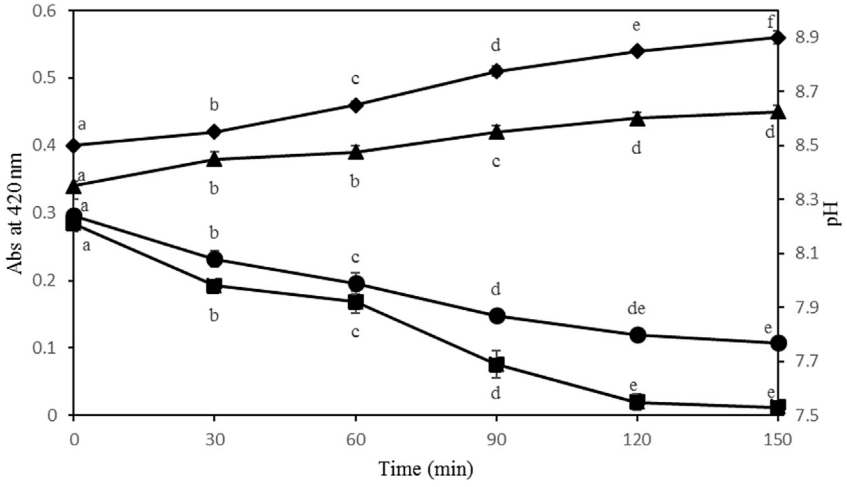

Fig. 2. Absorbance (at 420 nm; ▲, ◆) and pH value (●, ■) of WPI solutions with added maltodextrin at two levels of total solid content: 100 mg mL^{-1} (▲, ●) and 200 mg mL^{-1} (◆, ■). The same letters in each curve show statistically indifferent values ($p > 0.05$).

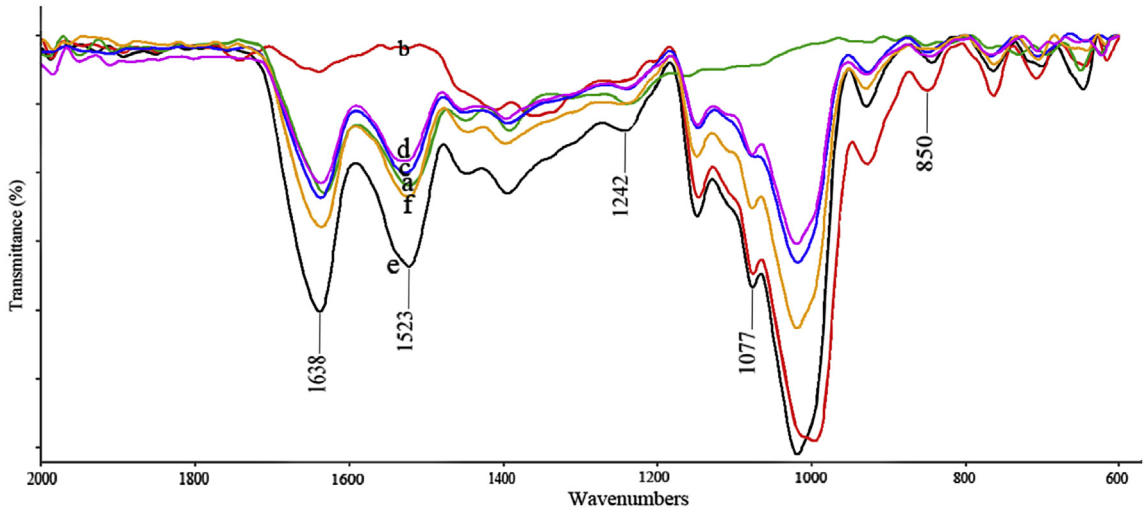

Fig. 3. Fourier transform infrared spectra of (a) heat-treated WPI solution, (b) maltodextrin, (c) WPI-maltodextrin mixture at 100 mg mL^{-1} total solid content, (d) WPI- maltodextrin conjugate at 100 mg mL^{-1} total solid content, (e) WPI- maltodextrin mixture at 200 mg mL^{-1} total solid content, (f) WPI-maltodextrin conjugate at 200 mg mL^{-1} total solid content.

3.3. SDS-PAGE

Fig. 4 shows the SDS-PAGE patterns of different samples. Heat treatment of whey proteins caused formation of high-molecular mass compounds (lane 2), which could not migrate into separating gel, resulting in intense drags at the loading end of the gel. These drags may be high-molecular mass conjugates of WPI-lactose and are associated with the presence of lactose in commercial WPI powder. However, lactose impurity even at a high level, could not inhibit the Maillard reaction between maltodextrin and WPI (Ding, Valicka, Akhtar, & Ettelaie, 2017). In glycated samples (lanes 5 and 6), the intensities of bands at about 66 kDa, 18 kDa and 14 kDa that are attributed to bovine serum albumin, β-lactoglobulin and α-lactalbumin, respectively (Mulcahy et al., 2017), were significantly lower than WPI-maltodextrin mixtures (lanes 3 and 4). The Maillard reaction (lanes 5 and 6) caused a higher reduction in the intensity of the band corresponding to α-lactalbumin (14 kDa) than that representing β-lactoglobulin (18 kDa). It was recently reported that α-lactalbumin is more prone to the Maillard reaction than β-lactoglobulin (Cardoso, Wierenga, Gruppen, & Schols, 2018).

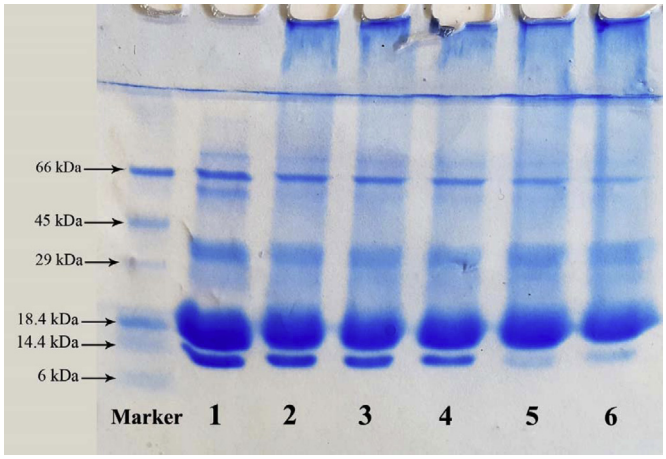

Fig. 4. SDS-PAGE patterns of native WPI (lane 1), heat-treated WPI (lane 2), WPI-maltodextrin mixture at 100 mg mL^{-1} (lane 3) and 200 mg mL^{-1} (lane 4) total solid content, WPI- maltodextrin conjugates at 100 mg mL^{-1} (lane 5) and 200 mg mL^{-1} (lane 6) total solid content sample series, under reducing condition.

Likewise, the heat-treated WPI (lane 2), large Maillard reaction products caused drags at the loading end of lanes 5 and 6.

3.4. Reducing power

Heat treatment of WPI solution caused an increase in the reducing power (Fig. 5), which means a potential increase of antioxidant activity (Elias, Kellerby, & Decker, 2008). As can be observed in Fig. 5, the reducing power of WPI-maltodextrin mixture at 100 mg mL^{-1} total solid content (50 mg mL^{-1} WPI content) was significantly less than WPI sample without added maltodextrin (100 mg mL^{-1} WPI content) ($p < 0.05$), which is ascribed to the lower protein content of the WPI-maltodextrin mixture. In agreement to our findings, Liu, Kong, Han, Sun, and Li (2014) observed that increasing protein concentration in WPI-glucose system resulted in a significant increase in the reducing power of solution. Conjugation of WPI and maltodextrin increased the reducing power of samples at both 100 and 200 mg mL^{-1} total solid content series ($p < 0.05$). The Maillard reaction produces a broad range of products with reducing activity, such as high molecular weight compounds, components with reducing hydroxyl groups and the hydrogen donating intermediate reductones (Daglia et al., 2008; Rao, Chawla, Chander, & Sharma, 2011; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997). In addition, structural changes that occur in proteins due to the Maillard reaction have been found highly consistent with augmentation of antioxidant activity (Liu et al., 2014). WPI-maltodextrin conjugates at 200 mg mL^{-1} total solid content exhibited the highest extent of reducing power ($p < 0.05$), which shows the effect of higher protein content on the extent of formation of Maillard reaction products, in agreement with browning results (Section 3.1).

3.5. Characterisation of WPI gels

3.5.1. Scanning electron microscopy (SEM)

Fig. 6 shows the scanning electron microscopy (SEM) micrographs of cold-set gels formed by $CaCl_2$ addition at both sample series of 100 and 200 mg mL^{-1} total solid content. The cold-set gel from heat-treated WPI sample without added maltodextrin had a relatively homogenous microstructure with serum pools embedded throughout the matrix (Fig. 6a). Addition of maltodextrin into WPI solution without subsequent Maillard conjugation

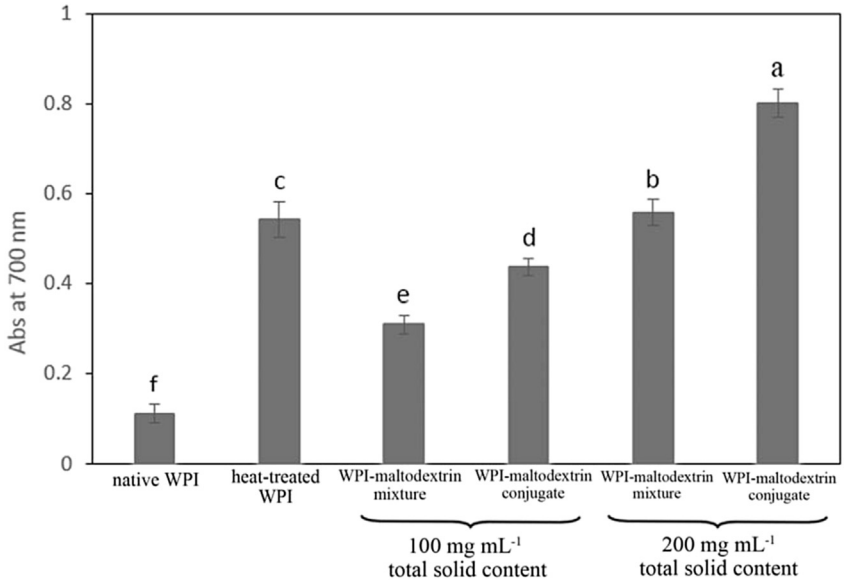

Fig. 5. Reducing power of samples at 100 mg mL^{-1} and 200 mg mL^{-1} total solid content.

resulted in less homogenous networks (Fig. 6b,d). We postulate that maltodextrin and whey proteins underwent segregative micro-phase separation due to thermodynamic incompatibility (Grinberg & Tolstoguzov, 1997). The phase separation concentrates incompatible polymers in separated domains, causing higher effective concentrations than corresponding nominal concentrations (Beaulieu, Turgeon, & Doublier, 2001). Hence, at the WPI content of 50 mg mL^{-1}, a self-standing gel was formed as a consequence of maltodextrin addition; whereas, pre-heated WPI at 50 mg mL^{-1} concentration without added maltodextrin did not form a self-standing gel. Domains of a continuous protein network lacking serum pools is observed at Fig. 6d, which is ascribed to the efficient water immobilisation at high total solid content of biopolymers (200 mg mL^{-1}) that decreased free water.

The Maillard reaction between whey proteins and maltodextrin led to formation of more homogenous gel networks (Fig. 6c,e) than physical mixtures of WPI and maltodextrin (Fig. 6b,d) at comparable total solid contents. Covalent attachment of maltodextrin to proteins could enhance the biopolymers compatibility. The size of serum pools in conjugate gels was smaller at 200 mg mL^{-1} total solid content (Fig. 6e) than 100 mg mL^{-1} total solid content (Fig. 6c), which is associated with the extensive binding of water by the biopolymers.

3.5.2. Textural properties

Table 1 summarises the results of textural analysis of the gels made using pre-heated WPI, WPI-maltodextrin mixture and conjugates at 100 and 200 mg mL^{-1} total solid contents, induced by addition of $CaCl_2$ at room temperature. Cold-set WPI gel without added maltodextrin (100 mg mL^{-1} total solid content) had a significantly higher firmness value than mixed maltodextrin-WPI gels at both 100 and 200 mg mL^{-1} total solid contents ($p < 0.05$). Maltodextrin could interfere with protein gel formation, as evidenced by SEM images. A less homogenous gel network with interrupted protein lattice argues the lower firmness values of the mixed WPI-maltodextrin gels.

The Maillard conjugation of whey proteins and maltodextrin increased the firmness of binary gels, so that the firmness of WPI-maltodextrin conjugate gel at 200 mg mL^{-1} total solid content was comparable with that of control WPI gel (Table 1) ($p > 0.05$). Therefore, formation of high molecular mass protein-polysaccharide conjugates, compensated network heterogeneity. Furthermore, consumption of free amino groups of protein in the Maillard reaction could result in decreased electrostatic repulsion between proteins, leading to a more inter-connected protein network compared with the mixed samples. Sun et al. (2011) and Spotti et al. (2014) observed that the elastic modulus and stiffness of WPI-dextran conjugated heat-set gels were lower than those of the control WPI gel. This was mainly associated to the weakened hydrophobic interactions due to the inhibitory effect of bulky hydrophilic part and steric hindrance of dextran molecules, as well as lowered accessibility of thiol groups and reduction in formation of disulphide bonds. Unlike heat-induced gelation where protein denaturation, aggregation and gelation occur simultaneously, in cold-set gelation technique, denaturation and aggregation phenomena are carried out separately (Bryant & McClements, 1998). Therefore, inter-molecular disulphide bonds and hydrophobic interactions could be formed extensively at the first stage of cold-set gelation (Hoffmann & van Mil, 1997; Roefs & Kruif, 1994), due to the absence of maltodextrin during the heat treatment. However, additional disulphide bonds are formed during the gelation step of cold-set approach (Alting, Hamer, de Kruif, Paques, & Visschers, 2003); therefore, presence of maltodextrin could somewhat prevent the formation of these covalent bonds.

3.5.3. Water holding capacity

The ability of whey protein gels to hold water under centrifugal force depends on the feature of building molecules, their structure, and pore size of network (Kuhn, Cavallieri, & Da Cunha, 2010; Vardhanabhuti, Foegeding, McGuffey, Daubert, & Swaisgood, 2001). As reported in Table 1, WPI-maltodextrin mixed gels at both 100 and 200 mg mL^{-1} total solid content series, had lower WHC values than the WPI gel without added maltodextrin ($p < 0.05$). This could be attributed to the reduction of gel network homogeneity and larger pore size of the mixed samples. The Maillard conjugation of whey proteins and maltodextrin increased the WHC of binary gels at both total solid content series, so that the WHC of WPI-maltodextrin conjugate gel at 200 mg mL^{-1} total solid content was comparable to that of control WPI gel ($p > 0.05$). The increased WHC of binary gels due to the Maillard reaction is correlated with the less porous microstructure of corresponding gels (Fig. 6).

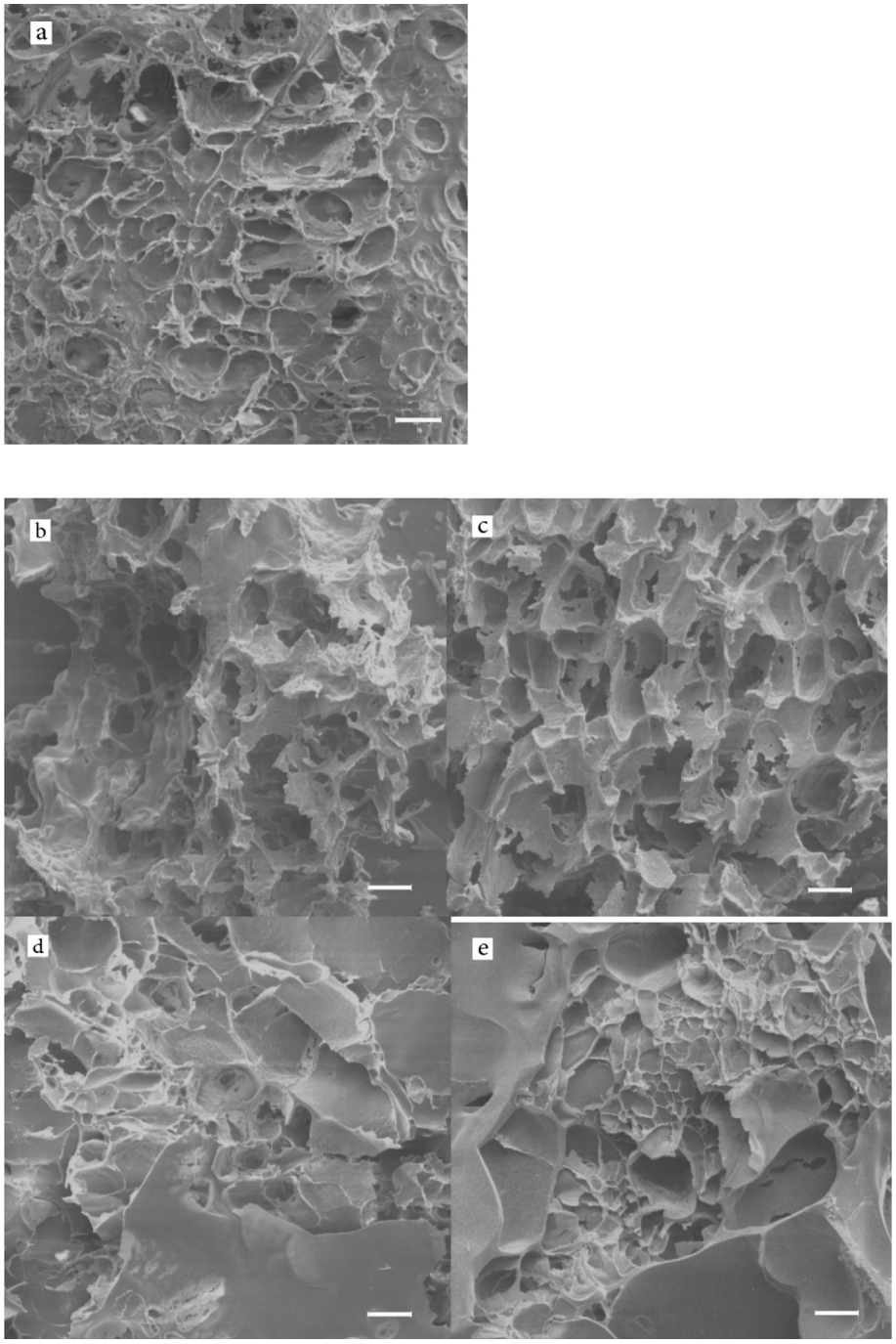

Fig. 6. SEM micrographs of salt-induced cold-set gels prepared from heat-treated WPI (a; 100 mg mL^{-1} total solid content), WPI-maltodextrin mixture (b; 100 mg mL^{-1} total solid content; d, 200 mg mL^{-1} total solid content) and conjugate (c; 100 mg mL^{-1} total solid content; e, 200 mg mL^{-1} total solid content). Scale bar corresponds to 100 μm.

Table 1
Water holding capacity and textural properties of gel samples.[a]

Parameter	Heat-treated WPI gel TS 100 mg mL^{-1}	WPI-maltodextrin mixed gel TS		WPI-maltodextrin conjugated gel TS	
		100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}
Firmness (N)	3.48 ± 0.14^{a}	0.69 ± 0.05^{c}	3.07 ± 0.07^{b}	1.05 ± 0.09^{b}	3.46 ± 0.05^{a}
WHC (%)	98.35 ± 1.11^{a}	62.43 ± 3.63^{c}	75.90 ± 4.21^{b}	71.99 ± 6.47^{b}	99.00 ± 0.3^{a}

[a] Abbreviations are: WPI, whey protein isolate; TS, total solid content. Values are means; means within a row with different superscript letters differ significantly ($p < 0.05$).

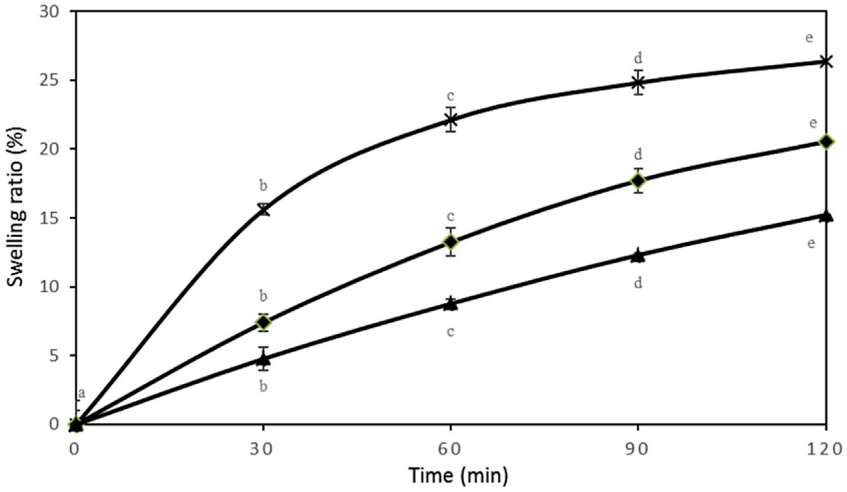

Fig. 7. Swelling ratio of salt-induced cold-set gels prepared from heat-treated WPI (×), WPI-maltodextrin mixture (◆) and conjugate (▲) at 200 mg mL^{-1} total solid content. Different letters in each curve show statistically different values for swelling ratio ($p > 0.05$).

3.5.4. Swelling

The ability of hydrogels to swell in aqueous solutions is considered as a determinant factor to control the release rate of entrapped bioactive components from the gel matrix (Begam, Nagpal, & Singhal, 2003). WPI-maltodextrin mixed and conjugated gels at 100 mg mL^{-1} total solid content disintegrated during the swelling experiments, so that they were removed from the swelling analysis. As could be observed in SEM micrographs (Fig. 6), these gels had a highly discontinuous microstructure, which resulted in weaker gels and subsequent disintegration during water diffusion into gel network. As shown in Fig. 7, WPI cold-set gel (control sample) had the highest extent of swelling, which could be related to its homogenous microstructure that brought about a higher water imbibition capacity. Furthermore, WPI-maltodextrin conjugated at comparable protein concentration swelled to a lesser degree than the mixed sample series. As it has been already reported by Peters, Luyten, Alting, Boom, and van der Goot (2015), reduced solubilisation of biopolymers in effect of higher degree of crosslinking (as it occurs during the Maillard reaction) could result in reduction of swelling ratio. On the other hand, free amino groups of protein were consumed and involved in conjugation, which reduced the electrostatic repulsion among the ionised positively charged groups of gel network under acidic aqueous media of swelling analysis (Ozel, Cikrikci, Aydin, & Oztop, 2017).

4. Conclusion

The Maillard reaction of whey proteins and maltodextrin allowed formation of self-standing cold-set gels at a low WPI content (i.e., 50 mg mL^{-1}). It is possible to increase the firmness and WHC of WPI-maltodextrin binary cold-set gels to those of saccharide-free WPI gels, by the Maillard reaction. Furthermore, the reaction increased the reducing power of the binary solution, which shows potential to produce cold-set WPI gels of higher antioxidant activity. On the other hand, gel swelling in enzyme-free simulated gastric fluid decreased as a consequence of the Maillard conjugation. A lower swelling extent may cause a lower gel digestibility (this assumption requires experimental verification), which can be exploited at fabrication of niche foods intended for obesity treatment by reducing stomach emptying rate and energy uptake.

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