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Interactions between fava bean protein and dextrans produced by *Leuconostoc pseudomesenteroides* DSM 20193 and *Weissella cibaria* Sj 1b

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ABSTRACT

The aim of this study was to study the interactions between dextran and fava bean protein. Two dextrans produced by *Leuconostoc pseudomesenteroides* DSM 20193 and *Weissella cibaria* Sj 1b were purified and mixed with fava bean protein isolate (FPI) in water or in different buffers. The two isolated dextrans presented a typical dextran structure, mainly α-(1 → 6) linkages (above 95%) and few α-(1 → 3) branches, but they differed in molar mass and conformation. Dry-heating incubation of FPI and dextran mixture facilitated the conjugation of dextran to FPI through the Maillard reaction. Both mixed and conjugated systems were further heat-treated, and different influences of the formed covalent bonds on rheological properties were observed. The *W. cibaria* Sj 1b dextran had a much higher gel-strengthening ability than the *Ln. pseudomesenteroides* DSM 20193 dextran. The intermolecular FPI-dextran interactions played an important role in stabilizing the mixed systems at different pH.

1. Introduction

Fava bean (*Vicia faba* L.) is a widely grown crop utilized for food and animal feed in many countries (Duc, 1997). The seeds contain protein (29%) and starch (39%), with the remainder comprising vitamins, minerals, and dietary fibers (Jezierny, Mosenthin, & Bauer, 2010). The functionality of fava bean protein for food uses, especially as a protein isolate, has been studied at the laboratory scale, where it has shown good solubility, emulsifying, foaming, and gelling properties (Boye, Zare, & Pletch, 2010; Cai, Klamczynska, & Baik, 2001). However, the utilization of fava bean protein isolate (FPI) in the food industry is still minor, despite of its high nutritional value and the increasing global interest in plant-based proteins (Boye et al., 2010).

Dextrans are α-glucan polymers that contain consecutive α-(1 → 6) linkages in the main chain and α-(1 → 2), α-(1 → 3), or α-(1 → 4) in the branch (Bounaix et al., 2009). They have been approved for food use in Europe since 2001 (European Commission, 2001). Dextrans produced *in situ* by lactic acid bacteria (LAB) are drawing increasing attention in the food industry due to their good performance in increasing bread volume, improving the texture, and retarding the staling of wheat sourdough bread (Katina et al., 2009; Korakli, Rossmann, Gänzle, & Vogel, 2001; Tieking, Korakli, Ehrmann, Gänzle, & Vogel, 2003). Due to their long history of safe use, LAB are preferable for producing dextrans *in situ* during fermentation, which is a potential approach for replacing hydrocolloid additives in food products (Katina et al., 2009; Wolter, Hager, Zannini, Czerny, & Arendt, 2014).

The interactions between polysaccharides and proteins are well known in food related systems (de Kruif & Tuinier, 2001; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). Recent investigations into the interactions between whey proteins and dextrans have revealed that the formation of covalent bonds between these two polymers through the Maillard reaction changed the rheological properties of whey protein-dextran mixture (Spotti et al., 2014a, 2014b; Spotti et al., 2013). However, little is known regarding the interactions between legume proteins and dextrans, although the texture of legume-based doughs can be considerably modified by dextrans (Xu, Coda et al., 2017; Xu, Wang et al., 2017). The increasing interest in legume proteins suggests that a better understanding of legume protein-dextran interactions under different conditions could increase the application of dextrans in legume-based food products.

The objective of the present study was to investigate the molecular interactions between FPI and dextran under different conditions. *Leuconostoc pseudomesenteroides* DSM 20193 was chosen due to its high dextran-producing ability, and *Weissella cibaria* Sj 1b was chosen because of the high gel-strengthening ability of the dextran it produced, as indicated in our previous study (Xu, Wang et al., 2017). Dextrans from these two strains were purified, and their structures, molar mass distributions, and rheological behaviors were assessed. The intermolecular

Abbreviations: FPI, fava bean protein isolate; LAB, lactic acid bacteria; NMR, nuclear magnetic resonance; SEC, size-exclusion chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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Table 1
Composition of fava bean protein isolate (FPI) solution, dextran solutions from *Leuconostoc pseudomesenteroides* DSM 20193 (DX_LP) and *Weissella cibaria* Sj 1b (DX_WC), and FPI/dextran (FPI/DX) mixtures and conjugates.

Sample code	FPI (g)	Dextran (g)	Dextran producer	Water (ml)	Incubation time (days)
FPI	1.2	0		6	0
DX_LP	0	1.2	*Ln. pseudomesenteroides* DSM 20193	6	0
DX_WC	0	1.2	*W. cibaria* Sj 1b	6	0
FPI/DX mixture					
FPI/LP_M [a]	1.2	1.2	*Ln. pseudomesenteroides* DSM 20193	6	0
FPI/WC_M	1.2	1.2	*W. cibaria* Sj 1b	6	0
FPI/DX conjugate					
FPI/LP_C [b]	1.2	1.2	*Ln. pseudomesenteroides* DSM 20193	6	6
FPI/WC_C	1.2	1.2	*W. cibaria* Sj 1b	6	6

[a] M indicates a mixture.
[b] C indicates a conjugate formed by dry-heating (Maillard reaction).

interactions between dextran and FPI were generated by mixing these two polymers together, and the intramolecular interactions between these two were formed through the Maillard reaction. The formation of FPI/dextran (FPI/DX) conjugates was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In order to study the effect of dextran on protein gelation, the FPI/DX mixtures and conjugates were further heated, and the rheological properties were evaluated. Finally, the effects of pH on rheological properties of FPI/DX mixtures were studied. To the best of our knowledge, this is the first study on legume protein-dextran interactions.

2. Experimental

2.1. Microbial strains and materials

Leuconostoc pseudomesenteroides DSM 20193 was purchased from Leibniz Institute DSMZ (Braunschweig, Germany). *Weissella cibaria* Sj 1b was obtained from the culture collection of the Division of Food Hygiene and Environmental Health, University of Helsinki. Fava bean flour (*Vicia faba* L. var. *major*) was purchased from CerealVeneta (Padova, Italy), and the composition was reported earlier (Xu, Wang et al., 2017).

2.2. Preparation of FPI

FPI was obtained by isoelectric precipitation (Makri, Papalamprou, & Doxastakis, 2006) and freeze-drying. The composition of the FPI was analyzed according to AOAC official methods 925.10 (moisture) and 923.03 (ash). Protein content was measured with the Lowry assay (Lowry, Rosebrough, Farr, & Randall, 1951) using Bio-Rad DC™ Protein Assay Kit I (Bio-Rad, USA). Carbohydrate content was calculated by analyzing free sugars and starch after sulfuric acid hydrolysis (Xu, Wang et al., 2017). Lipid content was measured according to Lampi et al. (2015).

2.3. Dextran purification and structure elucidation

LAB were grown on De Man, Rogosa, and Sharpe (MRS) agar supplemented with 5% sucrose at 30 °C for four days. The produced slimes were removed carefully from the plates and purified according to a previously reported method (Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008). The purity of the isolated dextran was evaluated by hydrolyzing dextran (10 mg) in 1 M sulfuric acid (2 ml) at 100 °C for 2 h, and quantifying the released glucose according to Xu, Wang et al. (2017). Dextran purity was calculated as the percentage ratio between the released glucose amount and the initial dextran amount. The structure of the purified dextrans was analyzed by nuclear magnetic resonance (NMR) spectroscopy on a 600 MHz Bruker Avance III NMR spectrometer (Bruker BioSpin, Germany) using the Bruker 1D NOESY pulse program (noesygppr1d). Samples (10 mg/ml) were exchanged twice with D_2O, filtered and placed in NMR tubes (Wilmad NMR Tubes, Aldrich chemical company, USA). All the measurements were performed at 50 °C, and the chemical shifts were referenced to acetone (^1H = 2.225 ppm and ^{13}C = 31.55 ppm).

2.4. Size-exclusion chromatography

The molar mass distributions of dextrans were analyzed by size-exclusion chromatography (SEC) using a DMSO-based (DMSO + 0.01 M LiBr) eluent according to Maina et al. (2014). Dextran was analyzed at a concentration of 1 mg/ml after four days of dissolution in DMSO. The SEC data were processed with the OmniSEC 4.5 software (Viscotek Corp.), and the dn/dc value of 0.072 ml/g was used (Basedow, Ebert, & Ruland, 1978).

2.5. Preparation of FPI/DX mixtures and conjugates

Composition of the obtained FPI was: protein (92.0 ± 4.1%), water (6.6 ± 0.3%), carbohydrate (1.5 ± 0.0%), lipid (1.3 ± 0.0%), and ash (4.8 ± 0.0%). The purity of dextrans produced by *Ln. pseudomesenteroides* DSM 20193 and *W. cibaria* Sj 1b was 92.8 ± 6.1% and 81.9 ± 3.9%, respectively. FPI and dextrans were used as such in this study. The FPI/DX mixtures and conjugates were prepared with FPI and dextran at a constant concentration of 20% (w/v) according to Spotti et al. (2014a). In brief, dextran powder (1.2 g) was dispersed in 6 ml of Milli-Q water overnight, followed by the addition of FPI powder (1.2 g). After thoroughly mixing, the FPI/DX mixture was further freeze-dried. Then, the obtained powder was heated at 60 °C with 63% relative humidity for 6 days in order to facilitate the formation of FPI/DX conjugate. The powders of FPI/DX mixtures and conjugates were dissolved in 6 ml of Milli-Q water 24 h before further analysis. FPI suspension (20%) without dextran was prepared as a control. The influence of dry matter changes after dextran addition was eliminated by mixing sucrose (1.2 g) with FPI (1.2 g) in 6 ml Milli-Q water as a reference mixture. All samples were prepared in duplicate. Details about sample preparation and evaluation are listed in Table 1 and Fig. 1.

2.6. SDS-PAGE

FPI, FPI/DX mixtures (FPI/LP_M and FPI/WC_M), and conjugates (FPI/LP_C and FPI/WC_C) were analyzed by SDS-PAGE with 12% resolving gel using a Mini Protein II dual slab cell system (Bio-Rad Laboratories, USA) according to Laemmli (1970). FPI/DX mixtures and conjugates (50 mg) were dissolved in 1 ml of 0.1 M Tris-HCl buffer (pH 6.8) with 10% glycerol, 2% SDS, 1% β-mercaptoethanol, and 0.02% bromophenol blue, followed by heating in a boiling water bath for 5 min. The loading volume was 15 µl, and the running voltage was 150 V. After this, different staining techniques were performed. Proteins were stained with Coomassie Brilliant Blue solution (0.1%) and distained with a mixture of methanol (20%) and acetic acid (20%).

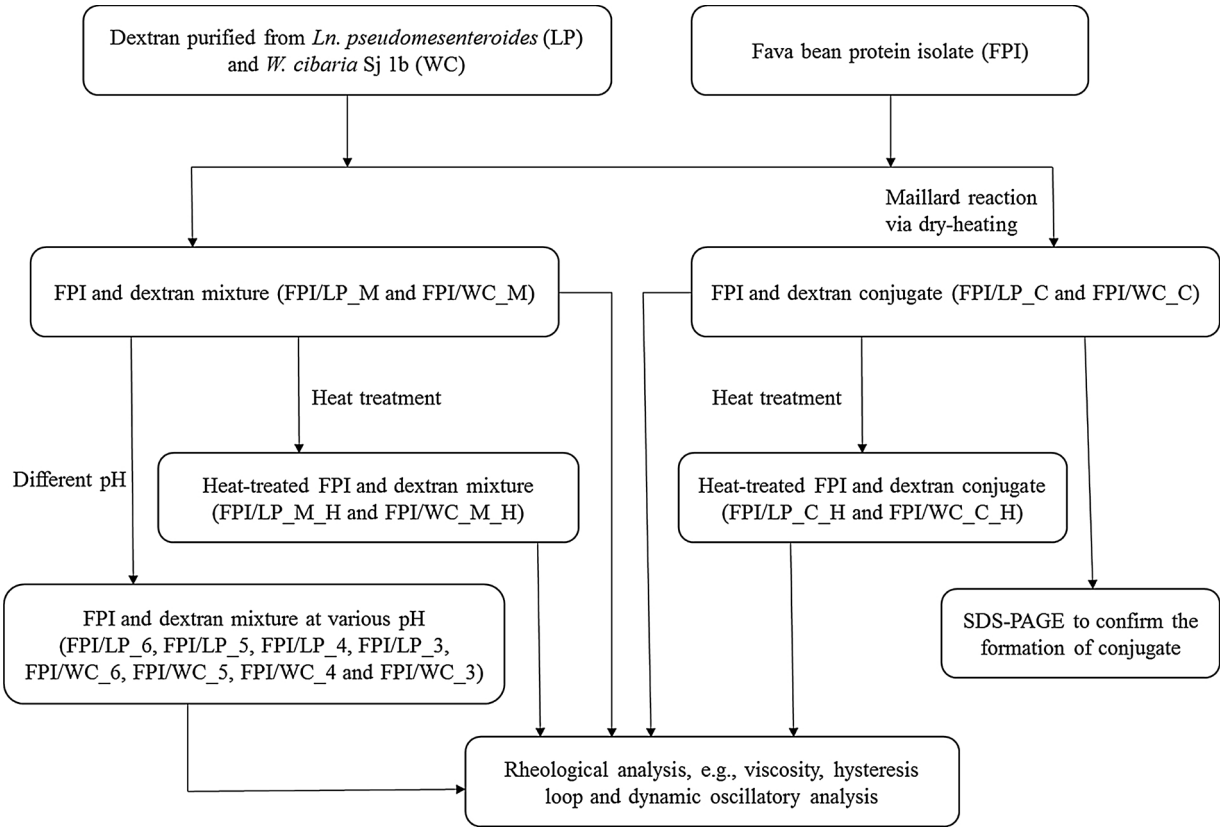

Fig. 1. Schematic summary of sample preparation and evaluation. FPI and dextran mixtures were also studied at a pH range of 3.0–6.0.

Glycoproteins were stained using Periodic acid-Schiff (PAS) staining technique (Zacharius, Zell, Morrison, & Woodlock, 1969).

2.7. Browning intensity and glycosylation degree

The brown color development was evaluated by measuring the absorbance at 420 nm with a UV-1800 spectrophotometer (Shimadzu, Japan). FPI, FPI/DX mixtures, and conjugates were all diluted to a protein concentration of 5 mg/ml with 0.1 M NaOH. Measurements were performed in triplicate.

The modification degree of the primary amino groups was determined indirectly by the specific reaction between O-Phthalaldehyde (OPA, Sigma-Aldrich) and free primary amino groups in proteins, as described by Spotti et al. (2013). FPI/DX mixtures and conjugates were diluted to a protein concentration of 3 mg/ml, and measurements were performed in triplicate. Glycosylation degree (GD) was calculated according to the following equation:

$$GD = (Am - Ac)/Ac \times 100\%$$

where Am is the absorbance of the mixture and Ac the absorbance of the conjugate.

2.8. Viscosity flow curves and hysteresis loops

The viscosity of dextran solutions at different concentrations (up to 22%) were measured under shear rates from 2 to 100 1/s (up and down sweeps) by a HAAKE RheoStress rheometer (RS 50, HAAKE Rheometer, Germany), and only the values at 100 1/s were used. Then, the plots of shear viscosity as a function of dextran concentration were plotted.

The viscosity flow curves of FPI, FPI/DX mixtures, and conjugates were analyzed by the same method used for dextran solutions. The hysteresis loop area between the upward and downward flow curves was calculated using the RheoWin Pro software. Measurements were conducted in duplicate.

2.9. Dynamic oscillatory rheology

The dynamic moduli (G', G") were recorded as a function of frequency from 0.1 to 10 Hz by HAAKE RheoStress rheometer at 20 °C, using a parallel plate system (1 mm gap). Measurements were conducted in duplicate after sample equilibration.

2.10. Heat treatment

FPI, FPI/DX mixtures, and conjugates were incubated in a water bath at 90 °C for 15 min. After cooling to room temperature, the rheological properties were evaluated as described in Sections 2.8 and 2.9.

2.11. Effect of pH on FPI/DX mixtures

FPI and FPI/DX mixtures were dispersed thoroughly to their original concentration in 0.1 M sodium citrate buffer at different pH (6.0, 5.0, 4.0, and 3.0). Then, the rheological properties were evaluated as described in Section 2.8 and 2.9.

2.12. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using Origin 8.6 (OriginLab Inc., USA). Means were compared using Tukey's test ($P < 0.05$).

3. Results and discussion

3.1. Dextran properties

3.1.1. Structure

The structures of the isolated dextrans were analyzed by NMR spectroscopy. As shown in Fig. 2A, dextrans from *Ln. pseudomesenteroides* DSM

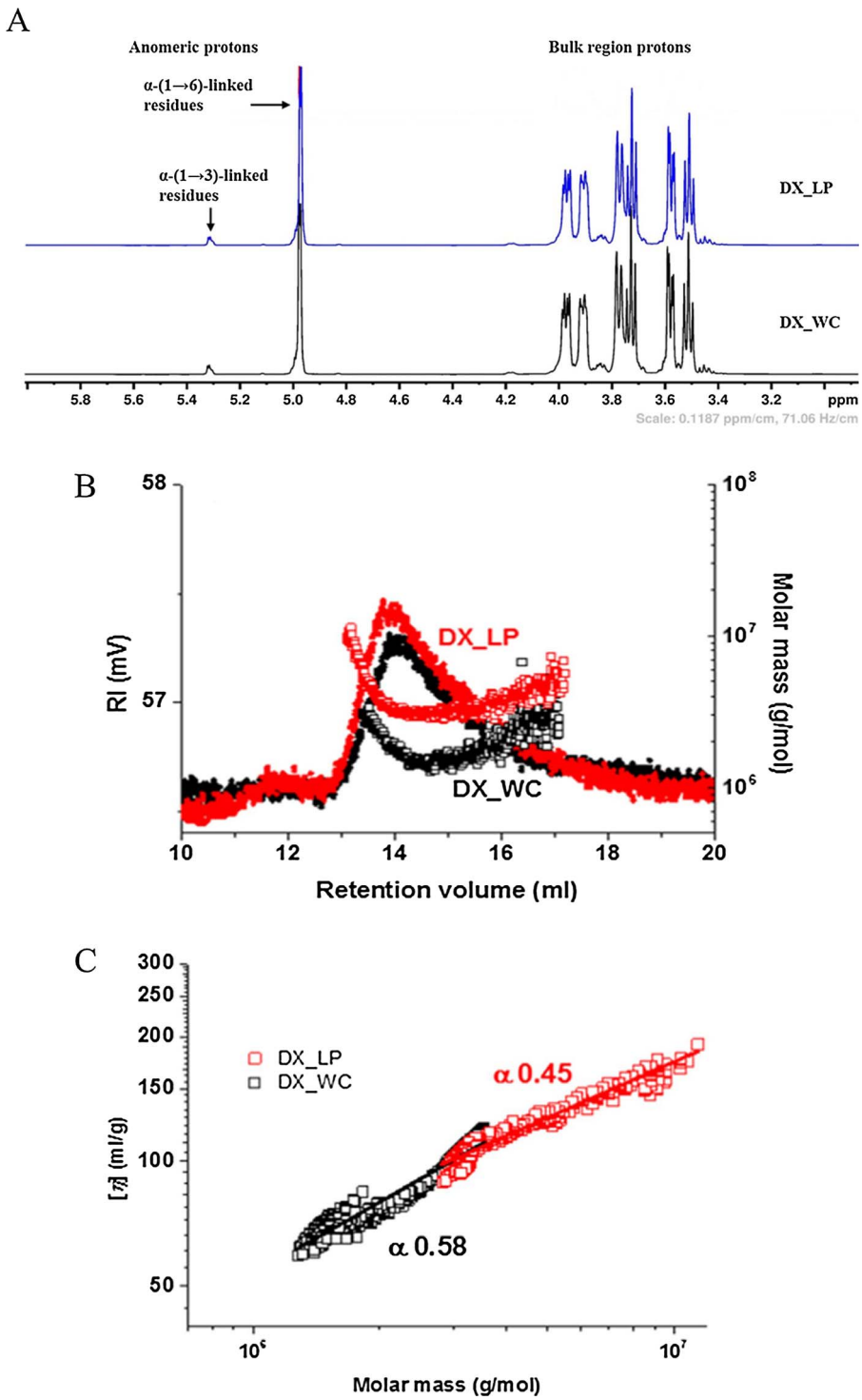

Fig. 2. The 1D ^{1}H spectra (A) and molar mass distributions (B) of dextrans from *Ln. pseudomesenteroides* DSM 20193 (DX_LP) and *W. cibaria* Sj 1b (DX_WC) and the Mark-Houwink plots of the two dextrans (C). Squares represent molar mass and lines represent detector signals (B).

20193 (DX_LP) and *W. cibaria* Sj 1b (DX_WC) both presented a similar structure to the commercial dextran produced by *Ln. mesenteroides* B512F (Maina et al., 2008). The peak around 4.98 ppm in ^{1}H spectra revealed a typical α-(1 → 6) chain-extending anomeric signal, and the anomeric signal around 5.32 ppm indicated the α-(1 → 3) linked branches in dextran (Maina et al., 2008). The degree of branching determined from the relative intensities of the ^{1}H anomeric signals was about 5.8% for DX_LP and 4.1% for DX_WC, which were similar values to those found for dextrans produced by *Leuconostoc* spp. and *Weissella* spp. (Maina et al., 2008; Shukla et al., 2014).

3.1.2. Macromolecular properties

The chromatograms of DX_LP and DX_WC are overlapped (Fig. 2B), suggesting a similar hydrodynamic size for both samples (SEC is a size-based separation technique). A small peak before the main peak was found in the chromatogram of DX_LP. This pre-peak was clearly visible in the light-scattering and viscosity signals and might indicate the presence of aggregates in the solution (Fig. S1, Supplementary data). The viscometric radius (R_η) across the peaks for both samples are also very similar, as suggested by the similar elution volumes of the two samples (Fig. S1). However, despite the similar elution volumes, the

Table 2
Molar mass averages (*Mw, Mn*), polydispersity indices (*Mw/Mn*), average intrinsic viscosity [η], average viscometric radius (Rη), Mark-Houwink α values, and critical overlap concentrations (c*) of the dextrans from *Ln. pseudomesenteroides* DSM 20193 (DX_LP) and *W. cibaria* Sj 1b (DX_WC).

Sample code	*Mw* [a] (10^3 g/mol)	*Mn* [b] (10^3 g/mol)	*Mw/Mn*	[η] (ml/g)	R_η (nm)	α	c* (%)
DX_LP	4379	3801	1.15	109	41.00	0.45	8
DX_WC	2452	1993	1.23	81	29.86	0.58	9

[a] Weight-average molar mass.
[b] Number-average molar mass.

molar masses across the peaks differed significantly between the two samples, with DX_LP possessing a higher molar mass than DX_WC (Fig. 2B). This difference in the relationship between size and molar mass of the samples reflects differences in molecular density, and DX_LP was denser than DX_WC. The density difference can also be seen in Mark-Houwink plots (Fig. 2C), in which intrinsic viscosity ([η]) was plotted against molar mass. The plot slope is lower for DX_LP than for DX_WC, indicating a difference in solution conformation between the two dextrans (Pitkänen, Virkki, Tenkanen, & Tuomainen, 2009). This difference might be due to the number and the length of branches on dextran chains. DX_LP contains more branches than DX_WC, as confirmed by NMR analysis. The branches in DX_LP might be also longer, as suggested by its higher molecular density.

The average values for molar mass, intrinsic viscosity, and viscometric radius of the two dextrans also differed (Table 2). When compared with DX_LP, the weight-average molar mass of DX_WC was approximately two times lower, but in the same order of magnitude. The two dextrans showed similar polydispersity indices (*Mw/Mn*). In addition, the intrinsic viscosity values, as well as the Mark-Houwink α values, indicated that both dextrans adopted a compact conformation in solution (Maina et al., 2014). The sample recovery rates in the SEC analysis were both below 50%, since dextrans with high molar mass are not completely soluble in DMSO-based eluent. Therefore, the SEC analysis only reveals the differences between the soluble parts of the two dextrans.

3.1.3. Rheological behavior

Consistent with previous studies (McCurdy, Goff, Stanley, & Stone, 1994; Tirtaatmadja, Dunstan, & Boger, 2001), the two dextran solutions (up to 22%) both showed a Newtonian behavior (Fig. S2). The plots of shear viscosity as a function of concentration for the purified dextrans both presented two distinct regions separated by a critical overlap concentration (c*) (Fig. S2), indicating a typical behavior of random coil polymers in aqueous solution (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). In the dilute region below c*, the viscosity-concentration plot showed a lower slope when compared with the slope above c*, pronouncing the lesser dependence of viscosity on concentration in this region. The c* was approximately 8% for DX_LP and was 9% for DX_WC (Table 2). The slightly higher c* of DX_WC might be because of its lower molar mass, since higher c* values are normally found in dextran solutions with lower molar mass (Pinder, Swanson, Hebraud, & Hemar, 2006). The dynamic rheological behavior of dextran solutions (22%) was also studied, with the two solutions showing a liquid-like behavior (Fig. S3). This agrees with the conclusion that dextrans could not form gels due to their flexible structures in aqueous solution (McCurdy et al., 1994).

3.2. Conjugation of dextran to FPI

3.2.1. SDS-PAGE

As reported by Spotti et al. (2014a), smaller polysaccharides have easier access to protein amino acid groups, resulting in a higher extent of the Maillard reaction. Therefore, a high content of dextran (20%) was used in order to facilitate the Maillard reaction, since the dextrans used in this study possess a high molar mass (10^6 Da). The conjugation of dextran to FPI was confirmed by SDS-PAGE. Proteins were identified by Coomassie brilliant blue staining (Fig. 3A) and glycoproteins by PAS staining (Fig. 3B). The characteristic bands of the proteins in FPI changed after incubation. In detail, lanes 1, 2 and 4, corresponding to FPI, FPI/LP_M and FPI/WC_M, respectively, showed the same bands, whereas these characteristic bands were diminished in lanes 3 and 5, which correspond to FPI/LP_C and FPI/WC_C, respectively. Furthermore, a broad band was observed near the top of the separating gel in lanes 3 and 5, indicating the formation of compounds with high molar

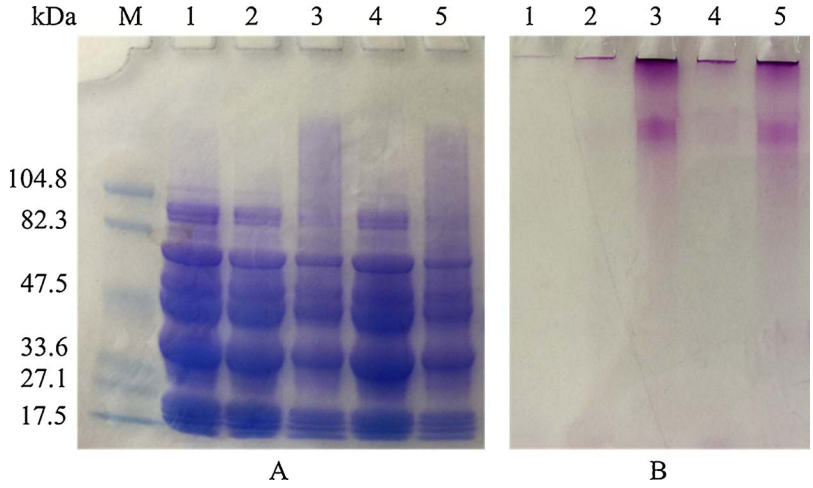

Fig. 3. SDS-PAGE of protein marker (M), FPI (1), FPI/DX mixtures: FPI/LP_M (2) and FPI/WC_M (4), and FPI/DX conjugates: FPI/LP_C (3) and FPI/WC_C (5). A: Coomassie Brilliant Blue stain; B: Periodic Acid-Schiff (PAS) stain.

Table 3
The browning intensity (A_{420}), Glycosylation degree (GD), viscosity, hysteresis loop area, G' and tan δ of FPI solution, dextran solutions from *Ln. pseudomesenteroides* DSM 20193 (DX_LP) and *W. cibaria* Sj 1b (DX_WC), FPI/DX mixtures, and conjugates with or without heat treatment.

Sample code [A]	Heat	A_{420}	GD (%)	Viscosity [B](Pa s)	Loop area (10^4 Pa/s)	G' [C](Pa)	tan δ
FPI	− [D]	0.26 ± 0.02 [a]	0	0.11 ± 0.01 [a]	ns [E]	0.82 ± 0.51 [a]	2.96 ± 1.99 [a]
FPI_H	+ [F]	ns	0	1.18 ± 0.14 [a]	0.75 ± 0.09 [a]	133.15 ± 31.33 [bc]	0.44 ± 0.06 [a]
DX_LP	−	ns	0	2.34 ± 0.03 [a]	ns	11.09 ± 0.26 [a]	1.74 ± 0.06 [a]
FPI/LP_M	−	0.42 ± 0.01 [bc]	0	11.10 ± 0.56 [b]	6.79 ± 0.41 [b]	105.61 ± 4.56 [bd]	1.40 ± 0.03 [a]
FPI/LP_M_H [G]	+	ns	0	16.17 ± 1.43 [c]	9.96 ± 0.85 [c]	199.43 ± 2.24 [ce]	1.14 ± 0.01 [a]
FPI/LP_C	−	0.48 ± 0.01 [b]	7.24	11.07 ± 1.32 [b]	6.78 ± 0.89 [b]	130.88 ± 8.99 [bc]	1.11 ± 0.02 [a]
FPI/LP_C_H	+	ns	7.24	13.83 ± 0.14 [bc]	8.27 ± 0.04 [bc]	205.65 ± 32.90 [ce]	0.84 ± 0.11 [a]
DX_WC	−	ns	0	2.26 ± 0.12 [a]	ns	28.37 ± 1.87 [ad]	1.48 ± 0.01 [a]
FPI/WC_M	−	0.40 ± 0.03 [c]	0	10.34 ± 0.01 [b]	6.62 ± 0.01 [b]	238.10 ± 5.3 [e]	1.03 ± 0.06 [a]
FPI/WC_M_H	+	ns	0	16.81 ± 0.23 [c]	10.66 ± 0.20 [c]	1045.74 ± 11.03 [f]	0.59 ± 0.07 [a]
FPI/WC_C	−	0.43 ± 0.02 [bc]	6.62	10.94 ± 0.81 [b]	6.97 ± 0.54 [b]	177.24 ± 6.07 [bce]	0.96 ± 0.02 [a]
FPI/WC_C_H	+	ns	6.62	16.57 ± 0.93 [c]	10.46 ± 0.61 [c]	700.51 ± 21.8 [g]	0.46 ± 0.02 [a]

[a–g] Values in the same column with different letters are significantly different ($p < 0.05$).
[A] Details about sample code can be found in Table 1.
[B] Values were taken at the shear rate of 100 1/s.
[C] Values were taken at the frequency of 1.0 Hz.
[D] Without heat treatment.
[E] ns = not shown.
[F] With heat treatment (90 °C, 15 min).
[G] H stands for heat treatment.

mass in the conjugates (Liu, Zhao, Zhao, Ren, & Yang, 2012). However, molar mass determination of the FPI/DX conjugates was not possible by SDS-PAGE.

PAS staining revealed the presence of glycoproteins only in lanes 3 and 5, where the featured pink band appeared at the top of the stacking and separating gel (Fig. 3B). Therefore, the conclusion can be drawn that FPI/DX conjugates were formed in FPI/LP_C and FPI/WC_C through the Maillard reaction. Similar electrophoretic patterns have also been observed in other studies using different protein/polysaccharide mixtures (Liu et al., 2012; Spotti et al., 2014a, 2014b).

3.2.2. Browning intensity and glycosylation degree

The browning intensity was measured as an index of the Maillard reaction process. Among the measured samples, FPI showed the lowest absorbance at 420 nm (Table 3). With dextran mixed, the absorbance of FPI/LP_M and FPI/WC_M both increased due to the brown color of the dextran solution itself. The absorbance was higher for the conjugated samples (FPI/LP_C and FPI/WC_C) than for the mixed samples (FPI/LP_M and FPI/WC_M), indicating the appearance of browning compounds in the conjugates. However, no significant difference was found between the absorbance of mixed and conjugated samples with the same dextran, indicating a limited occurrence of the Maillard reaction between FPI and dextran. A limited extent of the Maillard reaction was also observed previously between a peanut protein isolate and commercial dextrans (Liu et al., 2012).

The glycosylation degree, which was measured indirectly by a specific reaction between amino acids and OPA, was used to describe the percentage of amino acids involved in the Maillard reaction. According to Table 3, the glycosylation degree was slightly higher for FPI/LP_C (7.24%) than for FPI/WC_C (6.62%), corresponding to its higher absorbance value at 420 nm. Compared with the glycosylation degree reported by other researchers using whey protein and commercial dextrans (Spotti et al., 2013; Sun et al., 2011), the glycosylation degree of FPI in this study is lower. Possible reasons could include structural differences between whey and fava bean proteins and molar mass differences among the dextrans used, since smaller polysaccharides have an easier access to amino acid groups, resulting in a higher extent of the Maillard reaction (Spotti et al., 2014a). The weight-average molar mass of the dextrans used in this study are much higher than those used by Spotti et al. (2013) and Sun et al. (2011), which could explain the lower glycosylation degree.

3.3. Interactions between FPI and dextran

3.3.1. Viscosity

The addition of dextran considerably increased the viscosity of FPI, as shown by the viscosity values of FPI/DX mixtures (Table 3), confirming the viscosity-improving ability of dextrans reported in our previous studies (Xu, Coda et al., 2017; Xu, Wang et al., 2017). The addition of the same amount of sucrose, as a reference, did not change the viscosity of FPI (results not shown). The viscosity was slightly higher for FPI/LP_M than for FPI/WC_M, pointing to the effect of molar mass on viscosity. Very similar viscosity values were found between the mixed and conjugated systems with the same dextran, indicating that the Maillard reaction between FPI and dextran has no obvious influence on viscosity.

The influence of heat treatment on FPI/DX mixtures and conjugates was studied as heat treatment is an important method in food processing, and there is no study on the effect of dextran on protein gelation during heat treatment. Varying viscosity increases were observed after heat treatment (Table 3). In detail, the viscosity of the FPI solution increased from 0.11 Pa s to 1.18 Pa s after heat treatment, indicating a thickening effect caused by heat-induced gelation of FPI. This thickening effect was more obvious in FPI/DX mixtures and conjugates. Generally, the viscosity increases were higher in mixed systems than in conjugated systems, suggesting that the intermolecular interactions between FPI and dextran play a major role in viscosity improvements. The covalent bonds formed through the Maillard reaction had a greater effect on the viscosity increase of FPI/LP_C, since the viscosity of FPI/LP_C did not significantly increased after heating, unlike FPI/WC_C, which showed a significantly higher viscosity value after heating. This difference might be attributable to the differences in molar mass and conformation between the two dextrans. However, more work is still needed to study the behavior and function of dextrans in legume protein-based systems during heat treatment.

3.3.2. Hysteresis loop

Hysteresis loops are observed in viscoelastic materials during the shear rate sweep, and materials with larger hysteresis loop showed better structural reversibility (Purwandari, Shah, & Vasiljevic, 2007). In the present study, hysteresis loops were observed in all FPI/DX mixtures and conjugates (Fig. S4), and the loop areas were used to evaluate the effects of dextran addition on structural reversibility. The loop areas

of FPI and dextran solutions are not shown as they remained as Newtonian fluids (Table 3). After heat treatment, the FPI solution showed a thixotropic behavior and had the lowest loop area compared with dextran-added samples, revealing a positive effect of dextran on structural reversibility. Similar to the observations on viscosity, the Maillard reaction had no obvious influence on hysteresis loop areas of systems before heat treatment. However, heat treatment promoted the influence, since FPI/WC_C showed a significantly higher loop area after heat treatment, while FPI/LP_C did not. This suggested the susceptibility of the system with dextran from *Ln. pseudomesenteroides* DSM 20193 to interaction changes during heating process, similar to the phenomenon observed on viscosity increases in this system.

3.3.3. Dynamic oscillatory rheology

The dynamic rheological properties of samples with or without heat treatment were all evaluated, as these properties are associated with the functions of food proteins with gelling properties. Heat-induced gelation is frequently observed in globular protein solutions and was also observed in our study. Without heat treatment, FPI solution showed a liquid-like behavior (Fig. S5A). However, after heat treatment, it showed a solid-like behavior (Fig. S5B), and the G' value, as a measure of gel stiffness, increased considerably from 0.82 Pa to 133.15 Pa (Table 3). Consistent with the result reported by Spotti et al. (2014b), the addition of dextran increased the gel stiffness of both mixed systems (FPI/LP_M and FPI/WC_M), with a higher gel stiffness in FPI/WC_M. After heat treatment, a significantly higher gel stiffness was observed in FPI/WC_M_H (1045.74 Pa), when compared with FPI/LP_M_H (199.43 Pa). Furthermore, FPI/WC_M_H presented a lower dependence of G' on frequency than FPI/LP_M_H, indicating a more stable gel structure (Fig. 4).

The value of tan δ, which is an index of relative viscoelasticity, also indicated different effects of the two dextrans on fava bean protein gelation (Table 3). Compared to the systems with *Ln. pseudomesenteroides* DSM 20193 dextran, a lower tan δ was found in systems with *W. cibaria* Sj 1b dextran after heat treatment, suggesting a more rigid character of the gels formed in these systems (Spotti et al., 2014b). This further confirmed the high gel-strengthening ability of the dextran from *W. cibaria* Sj 1b, as first observed in our previous study (Xu, Wang et al., 2017). The gel-strengthening ability of dextrans in protein solutions might be due to the microphase separation between protein and dextran molecules and the low entropy of the mixing process (Spotti et al., 2014b; Turgeon et al., 2003).

In contrast to the findings of Spotti et al. (2014a) and Sun et al. (2011), the formation of covalent bonds in FPI/LP_C did not reduce the gel stability or gel stiffness (Fig. 4 and Table 3). However, the covalent bonds formed in FPI/WC_C reduced the gel stiffness, especially after heat treatment, but had no obvious effect on gel stability. This could be partially explained by the difficulties in forming disulfide bonds by the conjugates, as these bonds are responsible for the formation of protein networks. Moreover, in conjugated systems, the steric hindrance generated by the dextran-protein conjugation may also suppress the intermolecular interactions (mostly hydrophobic interactions) between neighboring proteins in aqueous solution (Spotti et al., 2014a). The differences in gel stiffness of the mixed and conjugated systems with different dextrans suggested a possible effect of the molar mass and conformation of the dextran on gelation of FPI. One hypothesis is that *W. cibaria* Sj 1b dextran was a good filler in the protein network, contributing to protein network consolidation. However, further work is still needed.

3.4. Effects of pH on FPI-dextran interactions

3.4.1. Viscosity and hysteresis loop

As dextrans are produced by LAB, together with a lowered pH, the effects of pH on rheology of FPI/DX mixtures were studied, in order to understand the role of dextran in the maintenance of fermented food

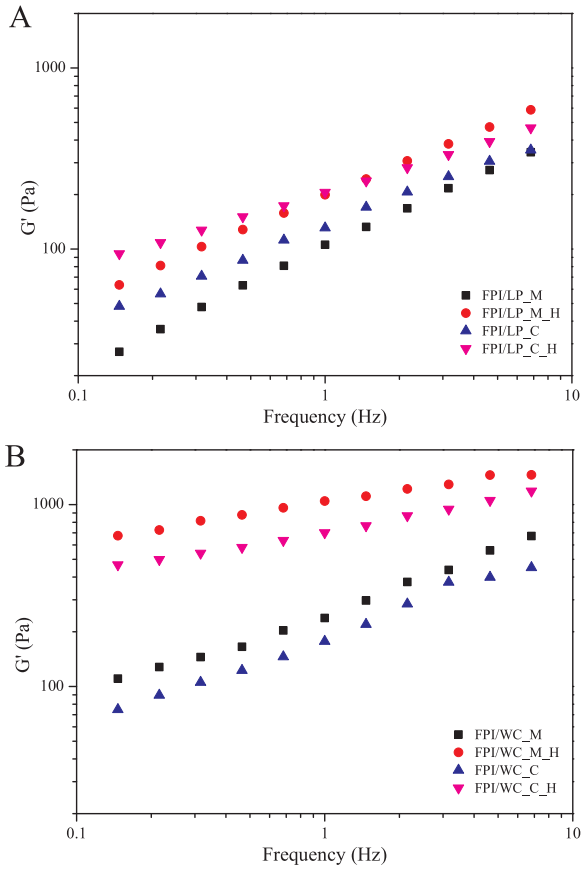

Fig. 4. Frequency sweeps of FPI/LP mixtures and conjugates without (FPI/LP_M, FPI/LP_C) or with heat treatment (FPI/LP_M_H, FPI/LP_C_H) (A) and FPI/WC mixtures and conjugates without (FPI/WC_M, FPI/WC_C) or with heat treatment (FPI/WC_M_H, FPI/WC_C_H) (B).

structure. The viscosity of FPI solutions was affected by pH, and in very acidic buffer, fava bean proteins started to form precipitates, resulting in inhomogeneous solutions. For this reason, the viscosity, hysteresis loop areas, G', and tan δ values of FPI solutions at pH of 4.0 and 3.0 are not shown (Table 4). The addition of dextran considerably increased the viscosity of the FPI at different pH, with all systems showing a typical shear-thinning behavior (Fig. S6). In samples with *Ln. pseudomesenteroides* DSM 20193 dextran (FPI/LP), the viscosity was the highest at pH 6.0 and lowest at pH 4.0, which is close to the isoelectric point of fava bean protein (Sosulski & McCurdy, 1987). Interestingly, in samples with *W. cibaria* Sj 1b dextran (FPI/WC), the viscosity did not change significantly at different pH (Table 4). Previous reports have indicated that dextrans remain unaffected by changes in pH during acidification (McCurdy et al., 1994), and the ionic strength and pH only influence protein self-association in systems with proteins and nonionic polysaccharides, e.g., dextran (Syrbe, Bauer, & Klostermeyer, 1998). This could explain the low viscosity value of FPI/LP_4, since fava bean proteins are aggregated at its isoelectric point. However, the minor viscosity changes in FPI/WC system at different pH indicated a stabilizing ability of the dextran against protein aggregation. The effects of pH on the hysteresis loop areas of the two systems with different dextrans were similar to those observed for viscosity, with *W. cibaria* Sj 1b dextran showing a stabilizing function (Table 4).

3.4.2. Dynamic oscillatory rheology

The G' values of FPI solutions at pH 6.0 and pH 5.0 were significantly lower when compared with other samples mixed with dextrans (Table 4). In FPI/LP system, the highest gel stiffness was found for FPI/LP_6 and the lowest for FPI/LP_3. Generally, the G' value was

Table 4
Viscosity, hysteresis loop area, G', and tan δ of FPI and FPI/DX mixtures at different pH values (6.0, 5.0, 4.0, 3.0).

Sample code [A]	Viscosity [B](Pa s)	Loop area (10^4 Pa/s)	G' [C](Pa)	tan δ
FPI_6	0.12 ± 0.00 [a]	–	13.65 ± 1.16 [a]	–
FPI_5	0.11 ± 0.02 [a]	–	22.35 ± 4.16 [a]	–
FPI_4	–	–	–	–
FPI_3	–	–	–	–
FPI/LP_6	12.78 ± 0.07 [b]	7.92 ± 0.18 [a]	151.04 ± 17.11 [bd]	1.19 ± 0.01 [abc]
FPI/LP_5	8.94 ± 0.72 [c]	5.45 ± 0.36 [b]	109.47 ± 8.84 [bc]	1.32 ± 0.05 [ab]
FPI/LP_4	6.23 ± 0.10 [d]	3.93 ± 0.02 [c]	111.35 ± 8.07 [bc]	1.19 ± 0.00 [ac]
FPI/LP_3	6.65 ± 0.97 [d]	3.92 ± 0.57 [c]	90.31 ± 3.62 [c]	1.35 ± 0.05 [b]
FPI/WC_6	8.88 ± 0.08 [c]	5.66 ± 0.06 [b]	163.32 ± 2.36 [d]	1.11 ± 0.02 [c]
FPI/WC_5	8.19 ± 0.25 [cd]	5.02 ± 0.25 [bc]	128.59 ± 4.12 [bcd]	1.24 ± 0.01 [abc]
FPI/WC_4	8.80 ± 0.14 [c]	5.48 ± 0.12 [b]	148.53 ± 11.33 [bd]	1.18 ± 0.00 [ac]
FPI/WC_3	6.97 ± 0.02 [cd]	4.29 ± 0.03 [bc]	134.65 ± 7.12 [bcd]	1.18 ± 0.02 [ac]

[a–d] Values in the same column with different letters are significantly different ($p < 0.05$).
[A] FPI/LP and FPI/WC stand for mixtures of FPI and dextran from *Ln. pseudomesenteroides* DSM 20193 and *W. cibaria* Sj 1b, respectively; numbers at the end indicate the pH value.
[B] Values were taken at the shear rate of 100 1/s.
[C] Values were taken at the frequency of 1.0 Hz.

higher for the FPI/WC system than for the FPI/LP system at the same pH. Changes in pH did not considerably affect the gel stiffness, especially in the FPI/WC system, indicating a stronger stabilizing capacity of *W. cibaria* Sj 1b dextran. Furthermore, no significant difference was found among the tan δ values of the FPI/WC system, confirming the stronger stabilizing ability of *W. cibaria* Sj 1b dextran.

During acidification, several physicochemical changes occurred in FPI solution. The acidification progressively destabilized the initial structure of fava bean proteins, leading to protein aggregation. However, the addition of dextran induced various interactions between dextran and fava bean proteins that prevented the proteins from aggregating. This further stabilized the protein network and resulted in a relatively stable gel stiffness in the pH range of 3.0–6.0. The better stabilizing ability of *W. cibaria* Sj 1b dextran might be attributed to its lower molar mass, making it a better filler in the protein network, but further evidence is needed. In this study, the addition of dextran significantly affected the gel network of FPI solution, differing from earlier reports that indicated a small influence of exopolysaccharides on rheological properties of fermented milk (Gentès, St-Gelais, & Turgeon, 2011; Hassan, Ipsen, Janzen, & Qvist, 2003).

4. Conclusions

Dextrans produced by *Ln. pseudomesenteroides* DSM 20193 and *W. cibaria* Sj 1b possessed a similar structure, but differed in molar mass, conformation, and functional interactions with the FPI. The Maillard reaction between FPI and dextran showed different influences on rheological properties of the two conjugated systems, especially after heat treatment, revealing the effect of molar mass and conformation of dextran on fava bean protein network. Dextran stabilized the FPI/DX mixtures at different pH by intermolecular interactions with the FPI. The texture-modifying effect of dextran on fava bean protein illustrates the great potential of dextran using in legume-based foods. Dextrans with various properties may further meet some specific requirements for different foods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.carbpol.2018.02.082.

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