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Effect of Oat Particle Concentration and Size Distribution on the Phase Behaviour of Mixtures with Gelatin

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Abstract The present study examines the effect of oat particle addition on the structural properties of gelatin gels. In doing so, gelatin concentration was 2% (w/w) and a variable amount of oat (0-4%, w/w) was employed. The latter came at three different particle size distributions, i.e. 28.2, 82.9, and 182.2 μm. Mechanical observations were carried out using small deformation dynamic oscillation in shear alongside thermal studies with micro differential scanning calorimetry. Scanning electron microscopy images provided tangible evidence of the changing morphology in the binary mixture with the addition of oat particles. Phase separated matrices are formed where gelatin is the continuous phase supporting the discontinuous inclusions of oat particles. There was an immediate decrease in the mechanical strength of the composite gel with the addition of oat particles, which was accompanied by a parallel drop in the enthalpy values of helical associations in gelatin. Increasing concentrations of oat with the smallest particle-size distribution are capable of disturbing rapidly the gelatin network, as compared to the larger counterparts.

Keywords: *oat particles, dietary fibre, gelatin, particle size distribution, phase separation*

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1. Introduction

Dietary fibre is a class of carbohydrate polymers of plant origin that are not hydrolysed by the endogenous enzymes in the small intestine and have been shown to induce physiological and metabolic changes benefiting health [1]. Examples of dietary fibre include cellulose, hemicellulose, gums, pectin substances, resistant starch and fractions of lignin [2]. Oat is an excellent source of dietary fibre with cholesterol-lowering properties and attenuation of glycaemic and insulin responses. Whole grains from oat are processed prior to being utilised in foods, and examples of unit operations include milling, baking, fermentation, germination, flaking, extruding, puffing and drying. These processes influence the structure of the oat grain and the physicochemical properties of their macronutrients and microconstituents [3].

Particle size and bulk volume, surface area characteristics, hydration, rheological properties, and adsorption or entrapment of minerals and organic molecules are known to have variable influence on the functionality of dietary fibre in processed products [4]. For example, it has been suggested that reduction in the particle size of fibre increases the inhibitory effect against α-amylase and pancreatic lipase in the gastrointestinal tract [5]. Further, Alqahtani et al. [6] showed a positive relationship between size distribution of insoluble oat particles and viscosity of fibre-enriched UHT beverages.

Consumers are becoming increasingly aware of the requirement for good nutrition in the diet, which focuses on food with a high dietary-fibre content to reduce the risk of developing chronic diseases including colon cancer, atherosclerosis, diabetes, hypertension and obesity [7,8]. Inclusion of dietary fibre in formulations might improve their nutritional profile but is often adversely associated with the texture and sensory profile of these preparations [9].

To overcome undesirable changes to the consistency of fibre-enriched foods, manufacturing increasingly recognises the utility of protein-polysaccharide mixtures as functional aids in materials processing [10]. Gelatin is used widely to impart specific structure, flow behaviour, mouthfeel and appearance in food, with specific examples being fruit gums, desserts, yogurt, ice cream, processed meat and sausages [11]. This protein is derived from collagen through alkaline or acidic processes that break up the secondary and higher structures with varying degrees of hydrolysis of the polypeptide backbone [12].

Mixing of two distinct macromolecules often encourages the formation of two microscopic layers, with each containing most of one constituent and little of the other. The phenomenon is known as phase separation and, in the gel state, the phase morphology of the mixture determines overall structure and techno-functionality [13,14,15]. For example, manipulation of biopolymer

concentration and experimental protocol to promote agarose gelation upon cooling or whey protein denaturation upon heating results in phase inversion from polysaccharide to protein continuous matrices [16]. Low cooling rates promote phase separation and structuring of the faster gelling biopolymer, with the slow gelling counterpart existing in the form of liquid inclusions [17].

Incorporation of food-grade particles in a "rubbery" biopolymer matrix may increase the mechanical strength of the mixture. This degree of network enhancement depends on the phase volume and the shape/size, i.e. maximum packing fraction, of the filler particles [18]. More recently, Koh and Kasapis [19] reported an increase in the network strength of gelatin by incorporating random or orientated microcrystalline cellulose fibres. However, there has been scant literature on the effect of grain particles, containing high levels of dietary fibre, on the structural properties of protein gels. The aim of the present work, therefore, is to investigate the inclusion of varying particle size and level of oat fibre in gelatin and discuss the network morphology and phase topology of the generated composite system.

2. Materials and Methods

2.1. Materials

Gelatin was purchased from Sigma Aldrich (MO, USA) with a Bloom value of 310. It was a high quality first extract from the acidic extraction of porcine skin (Type A) with an average molecular mass of 80,000. The material was in a powder form with a light yellow appearance, and the supplier determined the isoelectric point of about 8.0.

Oat samples were kindly donated by Sanitarium Health and Wellbeing Company (Cooranbong, Australia). They contain 60.2% total carbohydrate, 1% total sugar, 9.9% fat, 5.2% ash, 16.8% protein, and 6.9% moisture. The total dietary-fibre content of the oat particles is 29.3%, with the insoluble dietary fibre accounting for 23.4%. The three particle size distributions ($d_{(v,0.9)}$) used in this work were 28.2, 82.9 and 182.2 μm. According to the supplier, the particle size analysis utilised light scattering with a refractive index of 1.33 since water was both the dispersion medium and dispersed phase.

α-Amylase, amyloglucosidase, proteases, ethanol and hexane were supplied by Sigma Aldrich (MO, USA). Sodium phosphate dibasic anhydrious (Na2HPO4), sodium phosphate monobasic monohydrate (NaH2PO4), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck Millipore (Germany).

2.2. Sample Preparation

Protein solutions of 2, 3, 5, 7, 10, 15, 20 and 25% (w/w) were prepared by dissolving gelatin in distilled water with gentle stirring at ambient temperature and stored overnight to facilitate hydration. The following day, dispersions were heated to 58°C with gentle stirring on a hot plate until clear solutions was obtained; the hydration temperature of gelatin during sample preparation and subsequent analysis in single systems or in mixture with oat particles never exceeded 60°C.

Single preparations of oat particles were prepared by dispersing oat particles in distilled water at ambient temperature with continuous stirring for 5 min. Binary preparations involved mixing appropriate amounts of samples prepared as described earlier at 40°C to produce final compositions of 2% gelatin and 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0% (w/w) oat particles.

2.3. Lipid Extraction

The lipids in oat particles were extracted according to the method of Oomah *et al.* [20] with modifications. Ten grams of oat particles were suspended in 50 mL of 99.8% hexane with continuous agitation followed by vacuum filtration. The residue was suspended again in 99.8% hexane followed by another vacuum filtration. The liquid parts from two filtrations were combined and transferred into a round-bottom flask for rotary evaporation (Büchi rotavapor-R200, Switzerland) at a pressure of 60 kPa and temperature of 40°C. Samples were transferred into DSC pans for thermal analysis.

2.4. Enzymatic Digestion

This was carried out according to the method described by Bunzel *et al.* [21] with modifications. Defatted oat particles were subjected to enzymatic digestion by α-amylase and amyloglucosidase to remove starch and proteases to remove protein. Five grams of the defatted sample were suspended in 150 ml of sodium phosphate buffer (0.08 M, pH 6.0). For starch removal, α-amylase (375 μL), was added and kept in a 50°C water bath for 25 min with gentle agitation every 5 min. The pH was adjusted to 4.5 with HCl (0.325 M) and a 1500 μL amyloglucosidase was added to the sample followed by incubation at 50°C for 30 min with constant agitation. In the case of protein removal, the pH of defatted sample was adjusted to 7.5 with NaOH (0.275 M) and 150 μL of proteases was added to the sample followed by incubation at 50°C for 30 min with constant agitation. Next, the sample was washed twice with 70°C water (30 mL), 95% (v/v) ethanol (30 mL) followed by vacuum filtration each time. It was then kept in a 45°C vacuum oven overnight for further drying.

2.5. Methods

Small deformation oscillatory measurements in-shear were performed with ARG-2, which is a controlled strain rheometer with a magnetic thrust bearing technology (TA instruments New Castle, DE). A 40 mm diameter parallel plate geometry and 2 mm gap were employed throughout the experimentation. This type of mechanical analysis determines the elastic (storage modulus, G') and viscous modulus (loss modulus, G'') component of the network, complex viscosity (η^*) and a measure of the 'phase lag' δ ($\tan \delta = G''/G'$).

Samples were loaded onto the preheated Peltier plate (40°C) with their edges being covered in silicone fluid (50 cS) to minimise moisture loss in the course of experimentation. They were then cooled to 10°C at 1°C/min followed by isothermal run for 3 hr and a frequency sweep from 0.1 to 100 rad/s at 10°C. A constant angular frequency and strain of 1 rad/s and 1%, respectively, were maintained throughout the experimental routine. Determination of the viscoelastic region was achieved through strain sweep tests from 0.1 to

100% at 10°C and 1 rad/s. Experiments were replicated three times and mean values with one standard-deviation error bars are shown in Figure 5.

Differential scanning calorimetry was carried out using Setaram microDSC-III (Caluire, France). Approximately 800 mg of sample was filled into the DSC pan and sealed; a reference pan was also prepared with distilled water of equal weight. For measurement of the lipid sample, the reference pan was prepared with 99.8% hexane. The samples were stabilised for 30 min at 40°C to eliminate the effect of thermal history, cooled from 40 to 0°C at 0.5°C/min, stayed there for 30 min followed by heating to 95°C at the same scan rate. Triplicate data were obtained and mean data ± standard deviation are reported in Table 1.

Micrographs of single preparations of gelatin and oat particles, and binary mixtures thereof were obtained using Philips XL30 SEM (Sussex, England). The samples for imaging were freeze-dried and gold plated preparations under a high-vacuum mode. An accelerating voltage of 30 kV was used to produce microscopic images of these conductive samples, thus assisting in the characterisation of network morphology.

2.6. Statistical Analysis

All experimental tests were performed in triplicate, and data were subjected to two-way analysis of variance using IBM SPSS Statistic 21 software (IBM Corporation, Somers, NY, USA). Significant differences were defined as $P < 0.05$ with the Duncan test.

3. Results and Discussion

3.1. Structural Morphology of Gelatin/Oat Particle Mixtures

Prior to work on the mixtures, recording the structural behaviour of the gelatin sample of this investigation is needed to serve as a baseline for possible changes in network morphology with the addition of oat particles. Figure 1 reproduces a positive relationship between storage modulus and concentration of the protein, which on a double logarithmic scale is a good linear fit. Curing of the gel at 10°C for 3 hr results in well developed networks ranging in mechanical strength from about 0.67 to 110 kPa at 2 and 25% (w/w) gelatin in preparations. It is understood that network formation involves the construction of a triple helix consisting of three left-handed helices (α–chains) wound around each other into a right-handed super-helix [22]. Helices, as opposed to aggregation, are the main drive of structure formation, which is initiated with a β-bend bringing together two strands of the same molecule [23].

Figure 1 suffices as an index of convenience for identification of the concentration effect on gelatin's mechanical strength in relation to the ensuing discussion. Our immediate purpose was to incorporate oat in the gelatin system and find out whether a positive trend in network strength also develops with increasing concentration of the grain particle. Figure 2 depicts isothermal runs of 2% gelatin with increasing levels of grain addition at a mean particle size ($d_{(v,0.9)}$) of 182.2 µm following controlled cooling at 1°C/min from 40 to 10°C. Structure development of the single gelatin preparation and gelatin/oat mixtures was monitored over a period of 3 hr at this temperature.

The storage modulus of single gelatin preparations increases rapidly at first and then levels off gradually towards the end of the isothermal run. Thus the kinetics of network formation is rapid at the beginning followed by a process of near equilibrium at longer times. Gelatin networks develop dynamically due to the ongoing reconfiguration of the three-stranded super helices with time of observation at a given experimental temperature [24].

This increasing pattern of storage modulus over time is also apparent in Figure 2 for each mixture of the protein with varying concentrations of oat particles (0 to 4% w/w). However, there is an immediate drop in the values of G' with grain addition, which continues unabated from about 0.67 kPa for 2% gelatin gels to 0.24 kPa for mixtures containing 3.5% oat for this particle size ($d_{(v,0.9)}$ = 182.2 µm). A further oat addition of 4% sees a recovery in storage modulus recording reproducible values of about 0.34 kPa at the end of the isothermal routine. This type of behaviour is distinct from the incremental progression in the mechanical strength as a function of gelatin concentration in Figure 1 and requires rationalisation.

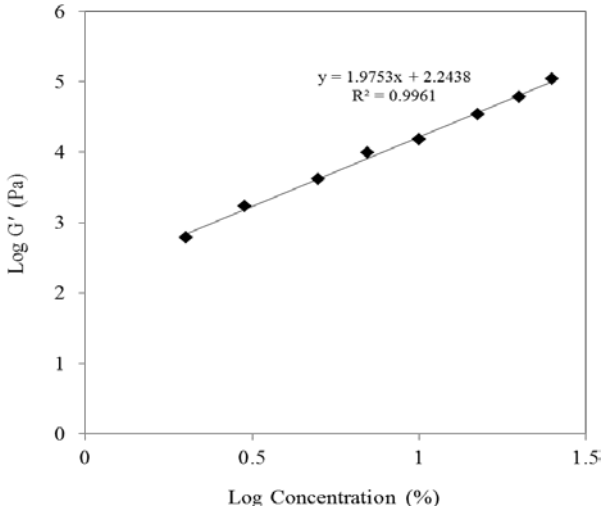

Figure 1. Standard curve of *G'* as a function of gelatin concentration at 2, 3, 5, 7, 10, 15, 20 and 25%

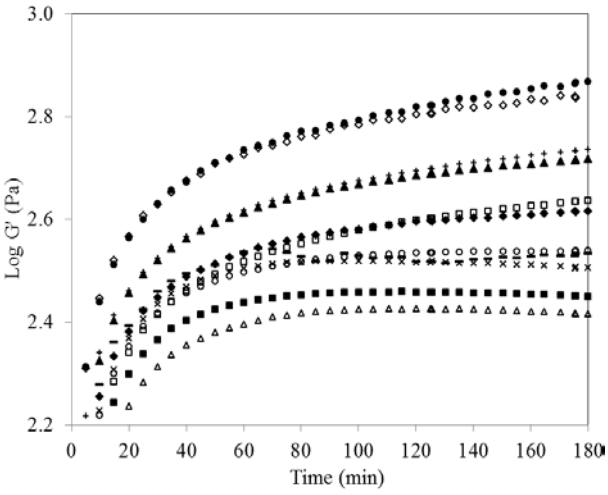

Figure 2. Time sweep of *G'* for 2% gelatin with 0 (●), 0.1 (◇), 0.3 (+), 0.5 (▲), 1.1 (□), 1.5 (♦), 2 (○), 2.5 (—), 3 (■), 3.5 (Δ) and 4% (x) oat particles at a scan rate of 1°C/min (the mean size of oat particles is 182.2 µm)

In doing so, we took advantage of additional rheological tests in relation to timescale and magnitude of applied deformation available in biopolymer research [25,26]. Figure 3 reproduces mechanical spectra of gelatin/oat mixtures over a wide range of oscillatory frequency (0.1 to 100 rad/s). Values of storage modulus remain flat within the experimental window of observation and well above those of the viscous component (tan δ ~ 0.009). Further, complex viscosity of the mixtures decreases continuously with a gradient of about -1 in the double logarithmic plot. Clearly, this is a typical viscoelastic behaviour of systems dominated by the small-deformation properties of the gelatin network.

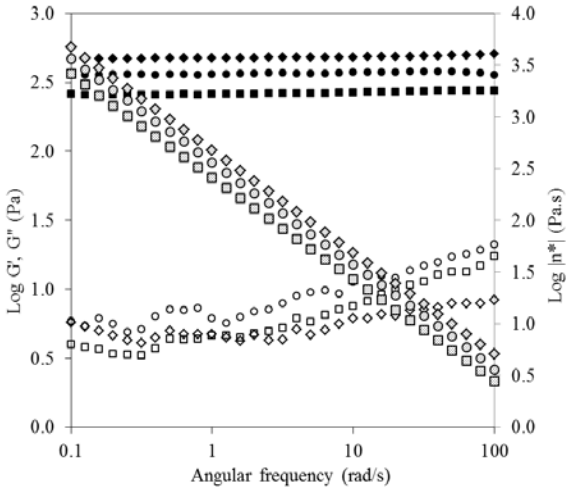

Figure 3. Frequency sweep of *G′* (closed symbols), *G″* (open symbols) and |η*| (dotted symbols) for 2% gelatin with 0.5 (diamond), 3.5 (square), and 4% (circle) oat particles (the mean size of oat particles is 182.2 μm)

Figure 4 illustrates the applied strain counterpart for the gelatin/oat mixtures of the preceding paragraph. All systems exhibit a long linear viscoelastic region, which is typical of gelatin assemblies at concentrations above the gel point [27]. Increasing of storage modulus values at amplitudes in excess of 10% is due to gel hardening where the gelatin helices align in the direction of the strain vector. Eventually the network is fractured at strain levels in excess of 100% yielding a catastrophic fall in the values of G'. In both Figure 3 and Figure 4, the pattern of modulus variation as a function of oat addition from 0.5 to 3.5 and 4% is congruent with that in Figure 2. Similar isothermal, frequency and strain sweeps were recorded for the other two particle size distributions, $d_{(v,0.9)}$ of 28.2 and 82.9 μm (results are not shown here), to document the prevalence of the gelatin network albeit moderated by the presence of oat particles.

Collection of all experimental data, following controlled cooling, isothermal run and frequency sweep, and plotting of the solid-like component of the gelatin network as a function of oat particle concentration and size distribution is illustrated in Figure 5. It is evident that for each particle size distribution, values of G' decline with oat addition to a minimum followed by an upward trend in the concentrated formulations. Binary mixtures prepared from fibre particles with $d_{(v,0.9)}$ of 28.2 μm show a turning point at around 1.5% oat, while fibres with $d_{(v,0.9)}$ of 82.9 and 182.2 μm exhibit such concentration thresholds at around 2.5 and 3.5% oat, respectively.

Modification in the particle size of dietary fibres is known to influence the functional properties of the material, especially the water sorption capacity. For example, Strange and Onwulata [28] reported an increase in the water absorption index (WAI) of oat fibres from 188 to 301 when the mean particle size was reduced from 180 to 106 μm. Smaller fibre particles possess a larger surface area that allows them to exhibit higher water sorption capacity. These are able to interfere effectively with the incipient formation of the gelatin network leading to a reduction in the overall mechanical response. Similar patterns of shear modulus development are observed for larger particles where the turning points lie at higher oat concentrations.

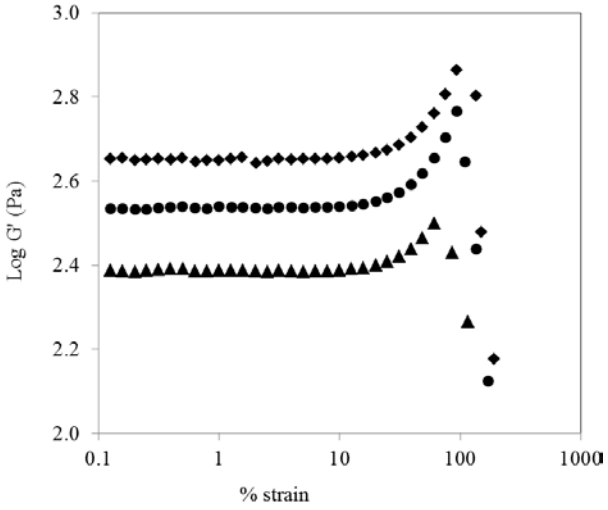

Figure 4. Strain sweep of *G′* for 2% gelatin with 0.5 (♦), 3.5 (▲) and 4 (●) oat particles (the mean size of oat particles is 182.2 μm)

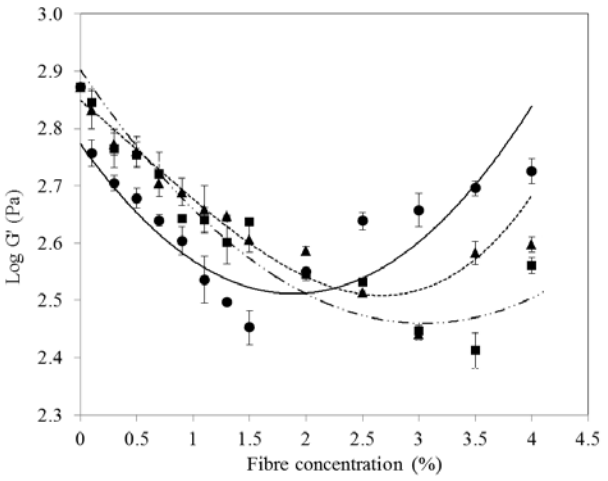

Figure 5. *G′* variation of 2% gelatin as a function of oat particle concentration for three size distributions, i.e. 28.2 (●,—), 82.9 (▲ , ---) and 182.2 μm (■ , —··—)

In all cases, however, there is considerable recovery in the mechanical strength of the gelatin network in the concentrated regime (e.g. at 4% fibre in the mixture). This outcome indicates extensive steric exclusion (and water partition) between the two polymeric constituents leading to an effective (final) gelatin concentration in its phase that is higher than the original addition of 2% protein in the mixture. Consequently, the rigidity of the protein phase and that of the mixture is reinforced following the turning point recorded in Figure 5. Competition for water between two polymeric components in a mixture is a common occurrence and in the case of whey protein-

agarose gels, the proportion of solvent associated with the globular-protein phase increases rapidly at slightly acidic and alkaline pH where it exhibits a higher degree of unfolding compared to its pI related pH of around 5.0 [16].

3.2. Calorimetric Observations on the Structural Characteristics of the Gelatin/Oat Mixtures

In conjunction with mechanical spectroscopy, complementary evidence from micro differential scanning calorimetry was sought to provide a firm footing on the structural behaviour of our systems. The technique is able to record the position and magnitude of the temperature band associated with a first order thermodynamic transition and calculate the exothermic or endothermic change in enthalpy (ΔH) from the area under the thermal event [29,30].

Figure 6a depicts typical exothermic profiles as a function of increasing oat concentration ($d_{(v,0.9)}$ of 182.2) for the mixtures with gelatin that were cooled to 0 from 40°C at 0.5°C/min. The heat flow traces of the single gelatin preparation show a peak representing the coil-to-helix transition of the polymer with a maximum (midpoint) temperature of 17.0°C. In comparison, the corresponding process in the mixtures becomes increasingly gradual with additional levels of oat, as indicated by the broader exothermic events. These also culminate progressively at lower temperatures, which for 4% (w/w) oat in the mixture is about 13.0°C. Observations are according to experience from results in Figure 2, where longer timescales are required for structure development with increasing inclusions of oat particles in the gelatin gel. Further, the presence of a single peak during the cooling routine indicates that there are no consequential specific interactions between gelatin and grain constituents.

Subsequent heating of single and mixed systems from 0 to 95°C at the same scan rate produces endothermic events illustrated in Figure 6b for oat at the highest particle size ($d_{(v,0.9)}$ of 182.2). Relatively sharp peaks are recorded for the helix-to-coil transition of gelatin that signify the cooperative nature of the melting process. Again, endothermic peaks broaden up with increasing grain addition, but the maximum heat flow temperature for all samples remains unaffected by the concentration of oat to culminate at about 30°C. Thermograms were further analysed to estimate changes in enthalpy that are summarised in Table 1. There is a clear reduction in ΔH values from about 0.38 to 0.06 J/g for exothermic scans, as the concentration of oat increases from 0 to 4%. Similar trends in ΔH are recorded for endothermic scans, i.e. from about 0.61 to 0.21 J/g with oat addition to the mixture. We also obtained congruent results to those in Figure 6(a-b) and Table 1 for samples made of 2% gelatin and oat addition with mean particle size of 28.2 and 82.9 μm (results are not shown here). Clearly, incorporation of oat particles to the aqueous gelatin solution retards the temperature induced kinetics of the reversible coil⇔helix transition, which is further reflected in a three dimensional structure of reduced rigidity in Figure 5.

3.3. Thermal Behaviour of Oat Particles

The absence of noticeable calorimetric events in Figure 6(a-b) attributed to constituents of the oat particle prompted us to examine them separately from gelatin. In doing so, oat particles with mean size distribution of 182 μm were first defatted and then subjected to enzymatic digestion for progressive removal of starch and protein. Lipid extracts and aqueous solutions of 2% defatted oat particles were characterised as for the binary mixtures with gelatin, i.e. cooled from 40 to 0°C followed by heating to 95°C at 0.5°C/min.

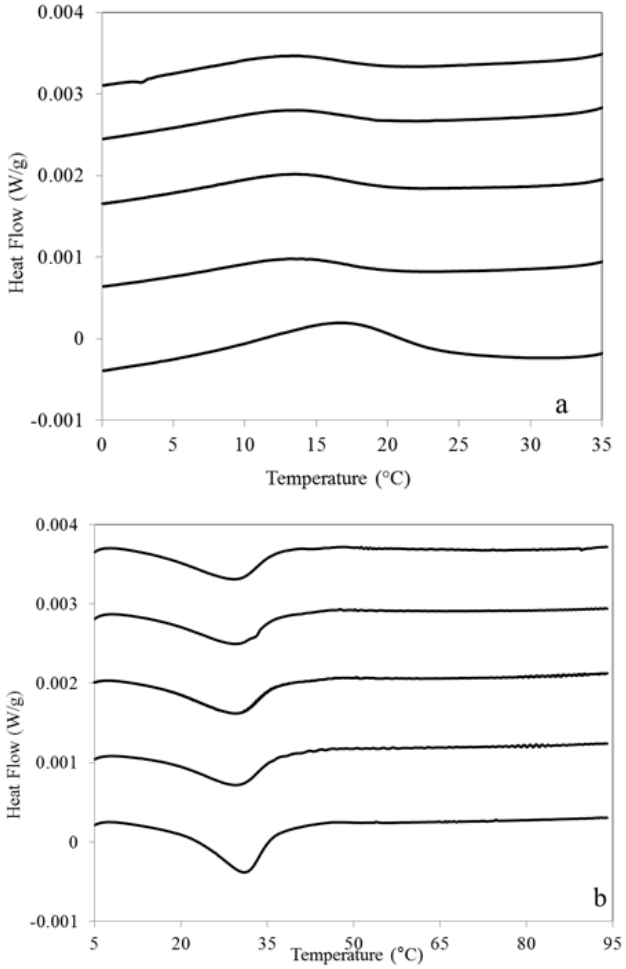

Figure 6. Micro DSC cooling (a) and heating (b) profiles for 2% gelatin with 0, 1, 2, 3 and 4% oat particle (182.2 μm) arranged successively upwards

Table 1. Change in enthalpy during cooling and heating of 2% gelatin samples with varying concentrations of oat particles (the size distribution of oat particles is 182.2 μm)

Sample	ΔH (J/g)	
	Exothermic reaction	Endothermic reaction
2% gelatin	0.376 ± 0.012^{a}	0.610 ± 0.053^{a}
2% gelatin + 1% oat particles	0.093 ± 0.005^{b}	0.265 ± 0.011^{b}
2% gelatin + 2% oat particles	0.073 ± 0.008^{bc}	0.249 ± 0.005^{b}
2% gelatin + 3% oat particles	0.060 ± 0.006^{d}	0.231 ± 0.001^{b}
2% gelatin + 4% oat particles	0.056 ± 0.002^{cd}	0.212 ± 0.010^{b}

Data are expressed as mean ± standard deviation
Means followed by the same letters (a-d) within the same column were not significantly different (P<0.05).

Thermograms from the cooling routine of these materials fail to show any noticeable peaks, which, however, wasn't the case upon subsequent treatment. Thus, heating of lipids extracted from oat yields a melting event in Figure 7 that occurs over the same temperature range with the deconvolution of gelatin, i.e. from 15 to 35°C. Similar peaks in terms of temperature band are recorded

for the untreated, and defatted starch-free samples. The appearance of peaks at this temperature range should indicate the chain-melting transition of naturally occurring lipid in oat particles, which may form complexes with protein.

No peaks are observed in defatted and protein-free samples, and defatted, starch-free and protein-free samples, an outcome that further supports the idea of a non-extractable lipid fraction in complexation with the protein of oat particles. Literature has documented such interactions in model systems of lipid and membrane proteins [31]. Thermal denaturation of oat proteins, mainly globulins, has been recorded at temperature higher than 100°C [32]. Starch gelatinisation occurs at higher temperature than lipid melting, typically within the range of 55 to 70°C [33], but it has not been observed presently due to the low amount of oat sample (2% w/w) used for analysis.

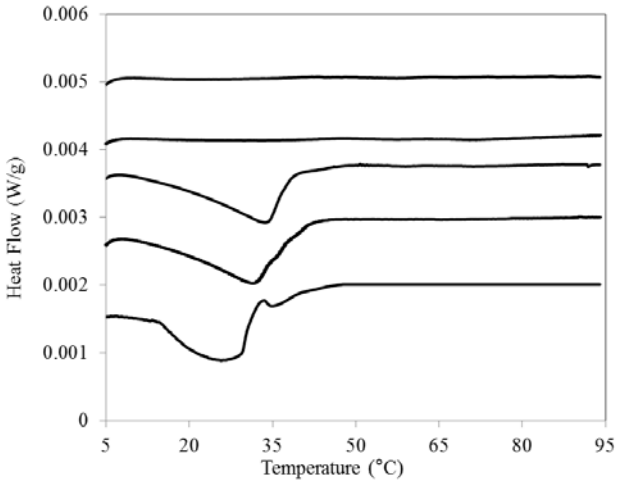

Figure 7. Micro DSC heating profiles of aqueous 2% oat particles with size distribution of 182 µm that were subjected to a defatting process and enzymatic digestion to remove progressively lipid, starch and protein; successively upwards: i) lipid from the sample, ii) untreated sample, iii) defatted and starch-free sample, iv) defatted and protein-free sample, v) defatted, starch-free and protein-free sample

3.4. Microscopy Images of the Gelatin/Oat Particle Matrix

Scanning electron microscopy was employed to provide tangible evidence of the phase morphology in gelatin gels in the presence of varying levels of oat fibre ($d_{(v,0.9)}$ of 182.2 µm). Figure 8a reproduces a typical particle-like assembly of oat samples with various degrees of agglomeration. Gelatin gels, on the other hand, form a regular superhelical assembly of high porosity, due to the relatively low gelation rate of this investigation (2°C/min), leading to a bulky but smooth network development with rubbery consistency (Figure 8b and [34]). A fine-stranded network was observed when aqueous gelatin solutions were cooled with ultrarapid rates in the order of 10^5 °C/sec and kept under vacuum at -100°C to sublimate ice crystals [35].

Introduction of oat at varying concentrations alters the microscopic characteristics of the gelatin structure, which, however, remains the continuous phase. Both Figure 8c and Figure 8d, i.e. in the presence of 1 and 2% oat sample, show a less well defined, stranded network of the protein. Incorporation of oat particles at the 3% level disrupts the gelatin matrix that appears to be irregular with high-volume cavitations in Figure 8e. In the concentrated regime of 4% oat addition, the regularity in the supramolecular assembly of the protein is partially restored with an accompanied increase in polymeric density (Figure 8f). Overall, microscopy images support the idea of a phase separated gelatin network whose rigidity is directly affected by the amount of oat particle inclusions.

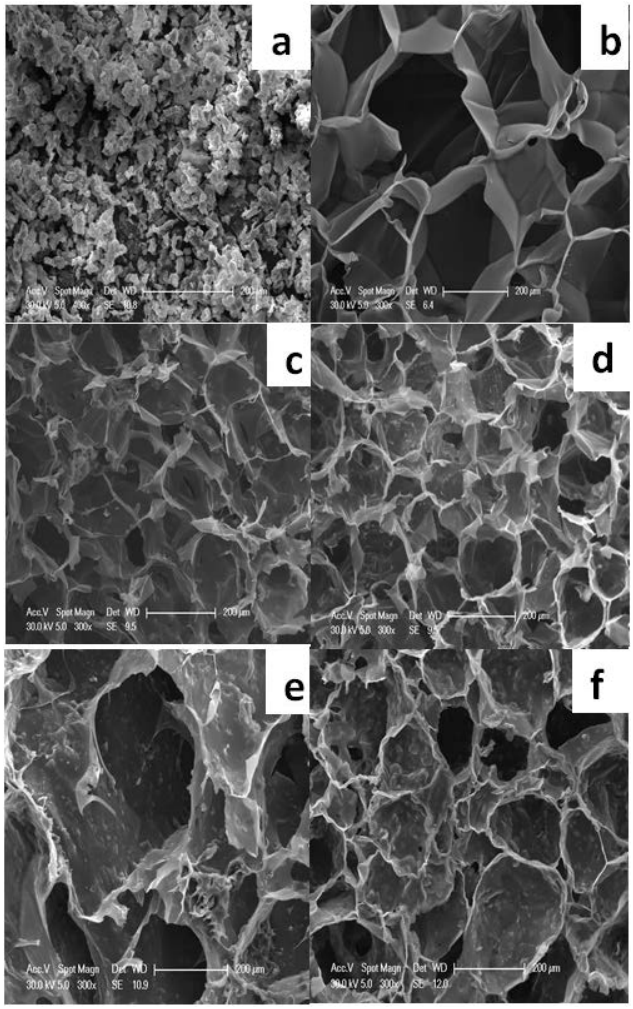

Figure 8. SEM micrographs of (a) 2% oat particles, (b) 2% gelatin, (c) 2% gelatin with 1% oat particles, (d) 2% gelatin with 2% oat particles, (e) 2% gelatin with 3% oat particles and (f) 2% gelatin with 4% oat particles (the mean size of oat particles is 182.2 µm)

4. Conclusion

The present work is part of an effort to better understand the phase behaviour in biopolymer mixtures that include considerable amounts of food-grade insoluble dietary fibre. This particular aspect is under researched although phase separation in protein-polysaccharide mixtures is, in general, well understood. Work enjoys the opportunity to examine both the effect of concentration and size distribution of oat particles in mixtures. In contrast to the previously reported results on gelatin/microcrystalline cellulose systems, where network reinforcement was observed, addition of oat particles causes an immediate drop in the mechanical strength of the protein gels, and the phenomenon is accelerated at

small particle sizes. This outcome was rationalised with differential calorimetry measurements, which demonstrated a considerable reduction in the kinetics of the forward reaction from coil to helix in gelatin molecules with increasing levels of oat addition. At the most concentrated regime examined presently, extensive steric exclusion between gelatin and polymeric oat constituents recovers some of the protein's network strength. Results may serve as a baseline of behaviour for further explorations in the fundamental and technological aspects of insoluble fibre containing binary mixtures.

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References

[1] Codex Alimentarius, Guidelines on nutrition labelling (2013).

[2] Cho SS and Prosky L, Complex carbohydrates: Definition and analysis, in *Complex carbohydrates*, ed by Cho SS, Prosky L and Dreher M. Marcel Dekker, New York, (1999).

[3] Slavin JL, Jacobs D. and Marquart L, Grain Processing and Nutrition. *Crit Rev Food Sci Nutr* 40: 309-326 (2000).

[4] Guillon F and Champ M, Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Res Int* 33: 233-245 (2000).

[5] Chau CF, Wen YL and Wang YT, Improvement of the functionality of a potential fruit insoluble fibre by micron technology. *Int J Food Sci Technol* 41: 1054-1060 (2006).

[6] Alqahtani NK, Ashton J, Katopo L, Haque E, Jones OAH and Kasapis S, Consistency of UHT beverages enriched with insoluble fibre during storage. *Bioactive Carbohydrates and Dietary Fibre* 4: 84-92 (2014).

[7] Anderson JW, Smith BM and Gustafson NJ, Health benefits and practical aspects of high-fiber diets. *Am J Clin Nutr* 59: 1242S-1247S (1994).

[8] Tinker LF, Schneeman BO, Davis PA, Gallaher DD and Waggoner CR, Consumption of prunes as a source of dietary fiber in men with mild hypercholesterolemia. *Am J Clin Nutr* 53: 1259-1265 (1991).

[9] Collar C and Angioloni A, An approach to structure-function relationships of polymeric dietary fibres in foods: significance in breadmaking applications, in *Dietary Fibre: New Frontiers For Food and Health*, ed by van der Kamp JW and McCleary BV. Wageningen Academic Publishers, Wageningen (2010).

[10] Narchi I, Vial C and Djelveh G, Effect of protein–polysaccharide mixtures on the continuous manufacturing of foamed food products. *Food Hydrocoll* 23: 188-201 (2009).

[11] Schrieber R and Gareis H, *Gelatine handbook - theory and industrial practice*. Wiley-VCH Verlag, Weinheim, Germany (2007).

[12] Haug IJ and Draget KI, Gelatin, in *Handbook of Food Protein*, ed by Phillips GO and Williams PA. Woodhead Publishing, Cambridge (2011).

[13] Almrhag O, George P, Bannikova A, Katopo L, Chaudhary D and Kasapis S, Investigation on the phase behaviour of gelatin/agarose mixture in an environment of reduced solvent quality. *Food Chem* 136: 835-842 (2013).

[14] Fang Y, Li L, Inoue C, Lundin L and Appelqvist I, Associative and Segregative Phase Separations of Gelatin/κ-Carrageenan Aqueous Mixtures. *Langmuir* 22: 9532-9537 (2006).

[15] Harrington JC and Morris ER, Conformational ordering and gelation of gelatin in mixtures with soluble polysaccharides. *Food Hydrocoll 23*: 327-336 (2009).

[16] Katopo L, Kasapis S and Hemar Y, Segregative phase separation in agarose/whey protein systems induced by sequence-dependent trapping and change in pH. *Carbohydr Polym* 87: 2100-2108 (2012).

[17] Anderson VJ and Jones RAL, The influence of gelation on the mechanism of phase separation of a biopolymer mixture. *Polymer* 42: 9601-9610 (2001).

[18] Richardson RK, Robinson G, Ross-Murphy SB and Todd S, Mechanical spectroscopy of filled gelatin gels. *Polymer Bull* 4: 541-546 (1981).

[19] Koh LW and Kasapis S, Orientation of short microcrystalline cellulose fibers in a gelatin matrix. *Food Hydrocoll* 25: 1402-1405 (2011).

[20] Oomah BD, Mazza G. and Przybylski R, Comparison of flaxseed meal lipids extracted with different solvents. *LWT-Food Sci Technol* 29:654-658 (1996).

[21] Bunzel M, Ralph J, Marita JM, Hatfield RD and Steinhart H, Diferulates as structural components in soluble and insoluble cereal dietary fibre. *J Sci Food Agric* 84: 653-660 (2001).

[22] Djabourov M, Lechaire JP, and Gaill F, Structure and rheology of gelatin and collagen gels. *Biorheology* 30: 191 (1993).

[23] Hawkins K, Lawrence M, Williams PR, and Williams RL, A study of gelatin gelation by Fourier transform mechanical spectroscopy. *J Non-Newtonian Fluid Mech* 148: 127-133 (2008).

[24] Fonkwe LG, Narsimhan G, and Cha AS, Characterization of gelation time and texture of gelatin and gelatin–polysaccharide mixed gels. *Food Hydrocoll* 17: 871-883 (2003).

[25] Tabilo-Munizaga G and Barbosa-Canovas GV, Rheology for the food industry. *J Food Eng* 67: 147-156 (2005).

[26] Kasapis S, Phase separation in biopolymer gels: a low- to high-solid exploration of structural morphology and functionality. *Crit Rev Food Sci Nutr* 48: 341-359 (2008).

[27] Gilsenan PM and Ross-Murphy SB, Viscoelasticity of thermoreversible gelatin gels from mammalian and piscine collagens. *J Rheol* 44: 871-883 (2000).

[28] Strange ED and Onwulata CI, Effect of particle size on the water sorption properties of cereal fibers. *J Food Qual* 25: 63-73 (2002).

[29] Mousia Z, Farhat IA, Blachot JF and Mitchell JR, Effect of water partitioning on the glass-transition behaviour of phase separated amylopectin–gelatin mixtures. *Polymer* 41: 1841-1848 (2000).

[30] Rahman MS, State diagram of foods: Its potential use in food processing and product stability. *Trends Food Sci Technol* 17: 129-141 (2006).

[31] McElhaney RN, Differential scanning calorimetric studies of lipid-protein interactions in model systems. *Biochim Biophys Acta* 864: 361-421 (1986).

[32] Harwalkar VR and Ma CY, Study of thermal properties of oat globulin by differential scanning calorimetry. *J of Food Sci* 52: 394-398 (1987).

[33] Stevenson DG, Eller FJ, Jane J, and Inglett GE, Structure and physicochemical properties of defatted and pin-milled oat bran concentrate fractions separated by air-classification. *Int J Food Sci Technol* 43: 995-1003 (2008).

[34] Almrhag O, George P, Bannikova A, Katopo L, Chaudhary D, and Kasapis S, Phase behaviour of gelatin/polydextrose mixtures at high level of solids. *Food Chem* 134: 1938-1946 (2012).

[35] Djabourov M, Leblond J and Papon P, Gelation of aqueous gelatin solutions. I. Structural investigation. *J Phys* 49: 319-332 (1988).