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Effect of pH and mixing ratios on the synergistic enhancement of Bambara groundnut-whey protein gels

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ABSTRACT

Plant and animal-based protein mixtures are increasingly recognised as a new group of functional ingredients offering novel structuring capabilities. When combining new sources of plant and animal proteins, it is important to gain a mechanistic understanding of such mixtures to enable their use as food ingredients. In this study, we have investigated the synergistic enhancement of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) protein isolates [BGN-PI] when combined with whey protein isolates [WPI] in heat-induced gelled mixtures. Mixed proteins were characterised for their rheological and structural properties at 12% (w/w) total protein concentrations at three mixing ratios (70:30, 50:50 and 30:70) and three pH values (pH 3, 5 and 7), with comparison to the single protein systems. At acidic pH (pH 3) WPI dominated the gel formation of the mixed gels with BGN-PI having no effect, whilst close to the isoelectric point of both proteins at pH 5, BGN-PI lowered the gel strength of the mixtures. Synergistic enhancement was observed at pH 7, where independent of the BGN-PI concentration in the mixtures, the mixed gels were characterised with similar high gel strengths comparable to that of the single BGN-PI. Hence, BGN-PI dominated the elasticity of the mixed gel networks at neutral pH.

1. Introduction

In recent years, plant proteins have become of increasing interest from not only a nutritional perspective, but also as a means of creating a more sustainable and secure food supply (Day, 2013; Nadathur, Wanasundara, & Scanlin, 2017). Pulses which are an important group of plant proteins, were shown to reduce on average 40% of carbon emissions and 17% of land use per kg of proteins, when compared to milk (Calles, Xipsiti, & del Castello, 2019; Nijdam, Rood, & Westhoek, 2012). As such, there is also an increasing trend to establish the protein functionality of pulse (and other legume) crops in admixture with animal-derived proteins (Ben-Harb et al., 2018; Comfort & Howell, 2002; Wong, Vasanthan, & Ozimek, 2013).

However, when blending plant and animal-based proteins it is important to understand the mechanical and structural properties of these blends, considering that challenges in textural and other sensorial properties can arise (Ainis, Ersch, & Ipsen, 2018; Jose, Pouvreau, & Martin, 2016). Soy proteins as commercially available plant proteins have been extensively studied for their rheological behaviour in combination with whey proteins, which are an important group of proteins well-known for their gelation functionality in foods. The addition of soy to whey protein systems at different ratios resulted in gels with similar gel strengths, but with visibly different microstructures (McCann, Guyon, Fischer, & Day, 2018). As also shown by Roesch and Corredig (2005), the concentration of soy to whey proteins is an important factor in the gelation kinetics, with soy present in lower amounts in the mixtures behaving in a similar manner as the single whey protein gels, whereas at higher concentrations there is a difference in gel network development. Similarly, Jose et al. (2016) have shown that both soy and whey proteins contributed to the formation of a gel network when mixed at different ratios, however noting the decrease in gel strength upon increasing concentrations of soy proteins in the mixed protein gels. In comparison to soy proteins, research on other plant proteins and specifically pulse proteins in admixture with whey proteins and their effect on gelation functionality, is limited (Ainis et al., 2018, 2019; Wong et al., 2013). Hence, considering again the shift towards sustainable and "climate-smart crops" (Calles et al., 2019; Nadathur et al., 2017), it becomes increasingly important to not only diversify our

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diets with untapped protein sources such as indigenous pulses and ancient grains, but to also establish the functionality of such proteins as food ingredients.

As a high-quality protein pulse crop, Bambara groundnut (*Vigna subterranea* (L.) Verdc.) [BGN] proteins have shown promising gelling behaviour upon heat-induced gelation. As shown in our previous work (Diedericks, de Koning, Jideani, Venema, & van der Linden, 2019), BGN seeds are primarily composed of the globulins (comprised of vicilin and legumin) and albumin storage proteins, with vicilin forming the major protein fraction (46 g/100 g total protein). These storage proteins are globular in nature, with vicilins characterised as trimeric proteins (M_w ~150–170 kDa) devoid of disulphide bonds, whereas legumins (M_w ~300–400 kDa, hexameric) and albumins (M_w ~5–80 kDa, heterodimeric) are characterised by disulphide-linked subunits (Boye, Zare, & Pletch, 2010; Shewry, Napier, & Tatham, 1995). Furthermore, we have also shown that vicilin controls the gelation behaviour of the mixture of BGN proteins obtained from isoelectric precipitation (Diedericks, Shek, Jideani, Venema, & Van der Linden, 2020). To further explore the potential of Bambara groundnut as a plant protein source, the aim of this study was to determine the effect of BGN proteins in admixture with whey proteins, in terms of the rheological properties and network structures of the resulting gels. The single BGN and whey proteins were investigated and compared to the mixed protein systems, at varying pH and at different mixing ratios. As such, we could gain insights into the structuring ability of BGN proteins in mixed systems and establish the synergistic enhancement under the conditions investigated.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) (BiPro JE-099-2-420) with a protein content of 97.7% (specified by manufacturer) was obtained from Davisco Foods International Inc. (Le Sueur, USA). Black-eye Bambara groundnut seeds were obtained from Thusano Products (Louis Trichardt, Limpopo, South Africa) and processed into defatted flour as previously described (Diedericks et al., 2019). All chemicals used were of analytical grade.

2.2. Extraction of Bambara groundnut protein isolates and solutions preparation

Bambara groundnut protein isolate (BGN-PI) was extracted from defatted black-eye flour with the isoelectric precipitation method as described earlier (Diedericks et al., 2020). Briefly, the flour was dispersed in deionised water at a ratio of 1:10 (w/v) and the pH adjusted to 9.5 with 1 M NaOH before being stirred for 2 h at 4 °C. The solubilised proteins were obtained by centrifugation (Avanti J-26 XP, Beckman Coulter, USA) at 4000 *g* (30 min, 4 °C) in the supernatant, which were then adjusted to pH 4 with 1 M HCl for isoelectric precipitation. The precipitated proteins were recovered in the residue after a second centrifugation step and readjusted to pH 7, before being freeze-dried as the protein isolates. The protein content of the BGN-PIs was 72.6 ± 1.7 g/100 g as determined with the Dumas nitrogen combustion method (N x 5.7).

Stock solutions of WPI and BGN-PI were prepared in deionised water to reach at least 12% w/w final protein concentrations at pH 3, 5 or 7 (adjusted with 1 M NaOH or 1 M HCl), under continuous overnight stirring at 4 °C. Following dissolution, the stock solutions were mixed at three ratios of WPI to BGN-PI (70:30, 50:50 and 30:70) to a total protein concentration of 12% w/w. All samples were stored at 4 °C in the presence of 0.02% (w/w) NaN_3 to prevent microbial spoilage.

2.3. Determination of free thiol groups

The accessible thiol groups in WPI and BGN-PI were determined with the Ellman's assay (Ellman, 1959) as described in the protocol by Aitken and Learmonth (2009). Briefly, 10 mM of 5,5′-dithiobis(2-nitrobenzoicacid) (DTNB) were dissolved in 0.1 M phosphate buffer at pH 8 and a volume of 100 μl added to a cuvette containing 3 ml denaturing buffer (6 M guanidinium chloride in 0.1 M Na_2HPO, pH 8) for absorbance reading at 412 nm with a Cary 60 UV–Vis spectrophotometer (Agilent Technologies Inc., USA). The protein solution (100 μl) was then added to the cuvette and after thorough mixing, the absorbance read at 412 nm. A reference cuvette was prepared in the same manner containing 100 μl reaction buffer (0.1 M phosphate, pH 8) without DTNB. The absorbance values determined at 412 nm were used to calculate the thiol concentrations,[1] using an extinction coefficient of 13600 M/cm (Cornacchia, Forquenot De La Fortelle, & Venema, 2014).

2.4. Visual observation and microstructure of protein gels

A volume of 12 ml of the stock solutions and their mixtures at the different ratios were prepared in pre-lubricated 30 ml cups. These samples were subjected to heat-induced gelation by heating in a water bath at 95 °C for 30 min, after which they were cooled at room temperature for 1 h and overnight at 4 °C. Following cooling, the cups were inverted to visually evaluate the gels which were formed.

Scanning electron microscopy (SEM) was used to evaluate the microstructure of the single and mixed WPI and BGN-PI gels. Gels were prepared in pre-lubricated 10 ml syringes after which it was prepared for SEM imaging as previously described for BGN protein gels. In brief, gels were cut into pieces and cross-linked in aqueous glutaraldehyde solution (2.5% v/v) for 8 h, after which the glutaraldehyde was stepwise removed with deionised water and ethanol under gentle rotation. The gel pieces were then dried through critical point drying (Leica Automated Critical Point Dryer EM CPD300, Leica, Austria), the dried pieces fractured and attached to the sample holders with Carbon Adhesive (Electron Microscopy Sciences, USA). The solvent was subsequently evaporated and the samples sputter-coated with a 15 nm thick layer of Tungsten (MED 020, Leica, Austria), before analysis in a field emission SEM (Magellan 400, FEI, The Netherlands). The SEM was operated at a working distance of 4 mm with 2 kV and 13 pA SE detection.

2.5. Small deformation rheology

Dynamic oscillatory measurements were performed in a controlled stress rheometer (MRC302, Anton Paar, Austria) fitted with a sand-blasted concentric cylinder (CC17) geometry. The single BGN-PI and WPI, and their mixtures at 12% (w/w) total protein solutions were measured at varying pH, whilst the single BGN-PI were also measured at pH 7 at the corresponding concentrations as added in the mixtures (i.e. 3.6%, 6% and 8.4% w/w). The solutions were heated from 20 °C to 95 °C at 3 °C/min, held at 95 °C for 30 min, followed by cooling to 20 °C (3 °C/min) and a final holding step for 25 min at 20 °C. A thin layer of paraffin oil was placed on top of the samples during measurement to avoid evaporation. Rheological parameters were recorded at constant strain (1%) within the linear viscoelastic regime and a frequency of 1 Hz.

In addition, the same measurements were performed for single WPI, single BGN-PI and the 6% WPI/6% BGN-PI mixture at pH 7 to which *n*-ethylmaleimide (NEM) was added. NEM is a thiol blocking agent which is used to evaluate the role of disulphide bonds during gelation (Alting, Hamer, De Kruif, & Visschers, 2000), and was added to the protein solutions in excess of 10× or 20× the free thiol groups present in the protein isolates.

[1] Calculated according to the Beer-Lambert Law: $A = \varepsilon cl$, where A is the absorbance, ε the extinction coefficient, c the concentration (mol/L) and l the optical path length (cm) (Aitken & Learmonth, 2002, 2009).

3. Results and discussion

3.1. Visual observation and microstructural characterisation of single and mixed whey and Bambara groundnut protein gels

Gel formation occurred in all protein systems (12% w/w total protein concentrations) at all pH values, with distinct physical differences and no visible observation of syneresis as shown in Fig. 1. The single WPI gels at pH 7 were transparent with a smooth surface, whilst at pH 3 the gels were transparent but with an irregular and sticky surface. This is in agreement to previous observations for whey protein gels, which at low pH below and at neutral pH above the isoelectric point (pI ≈ 4.9) are known to form fine-stranded networks with respectively rigid strands or flexible curved strands (Cornacchia et al., 2014; Foegeding, 2006; Langton & Hermansson, 1992). At pH 5 the single WPI gels were opaque and white, which is attributed to the low electrostatic repulsion near the pI, resulting in the formation of particulate gel networks (Ako, Nicolai, Durand, & Brotons, 2009; Foegeding, 2006). In contrast to the WPI gels, single BGN-PI gels were opaque and white to slight yellowish at all pH values. The turbidity and crumbly nature of these gels could be attributed to the presence of larger aggregates/irregularly-shaped particles in the BGN-PI gel networks (Jose et al., 2016; Renkema, Lakemond, De Jongh, Gruppen, & Van Vliet, 2000), which as shown for lupine protein isolates are typically induced by freeze drying (Berghout, Venema, Boom, & van der Goot, 2015). The mixed protein gels at pH 3 and pH 7 were visually characteristic of both single protein systems, whereas at pH 5 at all mixing ratios the gels were visually comparable to the single BGN-PI gel.

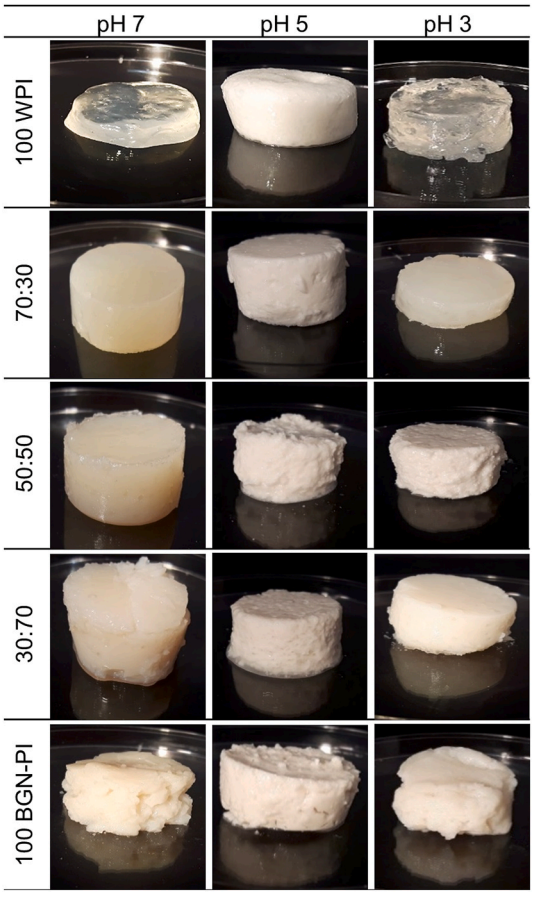

Fig. 1. Physical appearance of single and mixed whey and Bambara groundnut protein gels (12% w/w total protein concentrations) at varying pH. The ratios correspond to the following concentrations: 70:30–8.4% WPI/3.6% BGN-PI, 50:50–6% WPI/6% BGN-PI, 30:70–3.6% WPI/8.4% BGN-PI.

The microstructures of the gels at pH 7 were further investigated with SEM. As shown in Fig. 2, the gel microstructures appeared homogeneous at larger length scales and became increasingly coarser at smaller length scales. Comparing the single WPI and BGN-PI gels, the BGN-PI gel network appeared to form coarser aggregates with large pores, whilst a more uniform network structure with some strands were visible in the WPI gels (see supplementary material for additional SEM images at varying magnifications). These are however small differences which do not directly explain the vast differences between the single protein gels as observed on a macroscopic scale. As already alluded to in our discussion above, freeze-dried BGN-PI contains large insoluble materials which, independent of pH, are subsequently also present in the resultant gel network. These large materials would scatter more light as such causing the observed turbidity. It is thus expected that at higher magnifications (at smaller length scales) that structural differences between the single protein systems would become more prominent. Similar microstructures to the single BGN-PI systems were observed for the mixed protein gels, although appearing more dense with smaller pores at increasing BGN-PI to WPI concentration ratio. BGN-PI can thus replace WPI at varying concentrations without largely affecting the gel network structures. In comparison, Roesch and Corredig (2005) observed distinct differences in gel microstructures of soy and whey protein mixtures, where soy proteins caused phase separation at higher ratios (70:30) or large particulate aggregates at low ratios (30:70) in mixed gels with 10% (w/w) total protein concentrations.

3.2. Rheological behaviour of single and mixed whey and Bambara groundnut protein gels

The heat-induced gelation kinetics in terms of the storage modulus G′ of WPI and BGN-PI gels and their mixtures, at varying pH, are shown in Fig. 3. At neutral pH, single BGN-PI gels were characterised with higher elasticity compared to single WPI gels. These differences in gel strength can be attributed to the type (non-covalent vs covalent disulphide bonds) and extent of interactions occurring in the gel networks, as reported for other whey and plant protein mixtures (Ainis et al., 2018; McCann et al., 2018). In these studies however, the gel strength at pH 7 of the plant proteins (soy or rapeseed) was lower compared to that of WPI at the same concentrations, which was attributed to predominantly non-covalent hydrophobic or hydrogen interactions occurring in the plant proteins and disulphide interactions in WPI. The higher gel strength of single BGN-PI in our study could thus be indicative of the extent of disulphide interactions contributing to the gel network. As reported by Kudre and Benjakul (2014), the reduction in sulfhydryl groups and increasing disulphide content of BGN-PI upon heating were evident of aggregate formation through disulphide interactions. Considering that vicilins as the major storage proteins present in BGN seeds are devoid of disulphide bonds amongst the subunits (Diedericks et al., 2019), such interactions would be driven by the sulphur-rich albumin and legumin fractions. In addition, considering that electrostatic repulsion is also known to contribute to gel formation of denatured proteins (Ako et al., 2009), these interactions could also contribute to the gel network of BGN-PI which at pH 7 is furthest from the isoelectric point (pI = 4.3). The G′ development of the mixed gels at pH 7, at all mixing ratios, were initially similar to that of the WPI gels, before sharply increasing to reach a similar gel strength as the BGN-PI gels. These results are in agreement to the observed microstructures, which for the mixed protein systems closely resembled the network structure of the single BGN-PI gel with coarser aggregates. The effect of the single BGN-PI at the corresponding concentrations to which they were added to the mixture, was also determined. As shown in Fig. 4, these single BGN-PI gels were characterised with lower elasticity compared to the mixtures. It would thus appear that for the concentrations measured, BGN-PI is able to exert a strengthening effect on the gel networks. The hypothesis by Ainis et al. (2018), i.e. that the rheological responses of mixed protein systems are governed by the protein which forms a

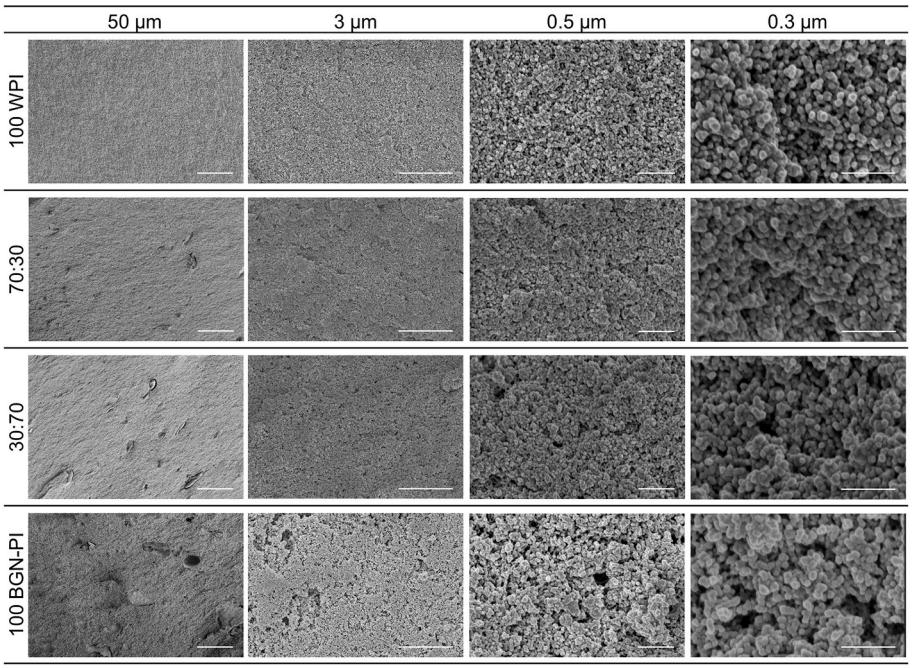

Fig. 2. Microstructures of single and mixed whey and Bambara groundnut protein gels at pH 7 and 12% (w/w) total protein concentrations as imaged with scanning electron microscopy. Scale bars correspond to 50 μm, 3 μm, 0.5 μm and 0.3 μm respectively.

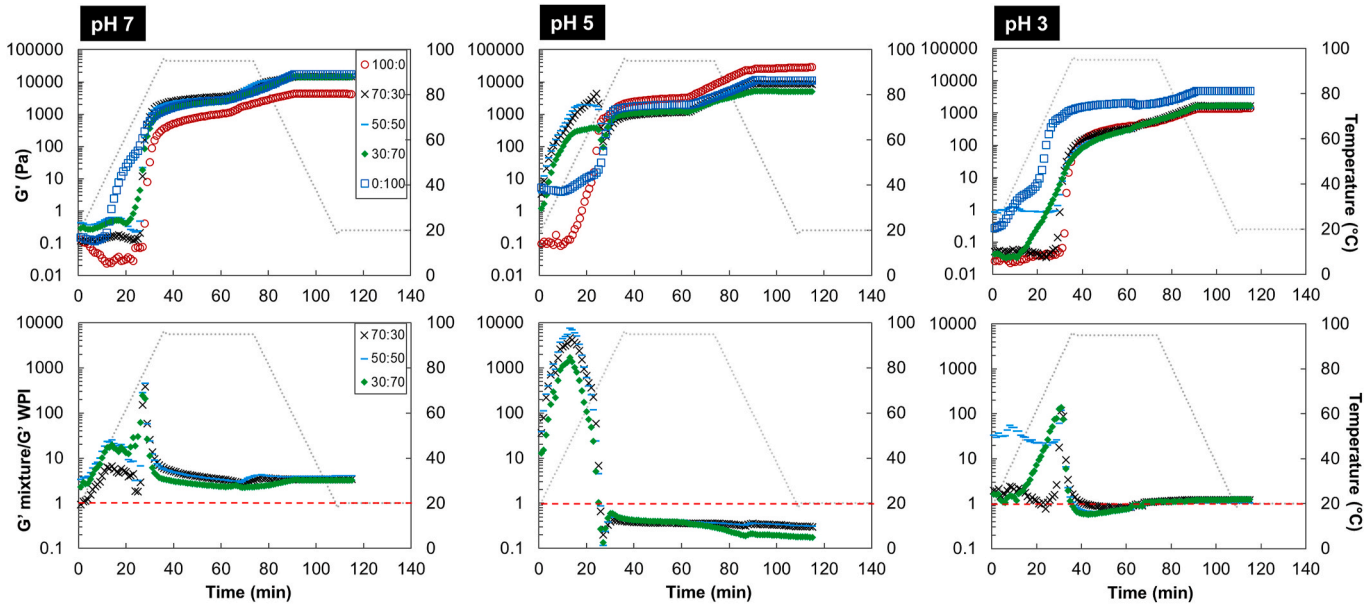

Fig. 3. (Top row) Elastic moduli (G′) development of heat-induced whey and Bambara groundnut protein gels at different mixing ratios at pH 7 (left), pH 5 (middle) and pH 3 (right). The mixing ratios correspond to the following protein concentrations: 100:0–12% WPI, 70:30–8.4% WPI/3.6% BGN-PI, 50:50–6% WPI/6% BGN-PI, 30:70–3.6% WPI/8.4% BGN-PI, 0:100–12% BGN-PI. (Bottom row) Degree of synergy in the corresponding systems; dashed line indicates a ratio of 1 for G′ mixture to G′ WPI.

stronger network, could thus to some extent be applied to our systems (at pH 7). In addition, the sharp increase in G′ of the mixed gels occurs close to the gelation point (i.e. the G'/G" cross-over point) of WPI (see Table 1).

In mixtures of soy and whey proteins, the coinciding gelation points of the single WPI and the mixed protein systems were attributed to the denaturation of the major whey protein fraction, β-lactoglobulin (Jose et al., 2016; McCann et al., 2018). As such it is evident that whey proteins also participate in the network formation of the mixed protein systems at neutral pH. This observation is also confirmed by the deformation of the network structures under applied strain (Jose et al., 2016), as shown in Fig. 5. The linear viscoelastic region (LVE) of the mixed gels at the 8.4% WPI/3.6% BGN-PI and 6% WPI/6% BGN-PI mixing ratios closely resembled that of the single WPI gels (strain up to 100%). The 3.6% WPI/8.4% BGN-PI gels were characterised with an LVE between that of single BGN-PI (strain around 10%) and single WPI.

Similarly at pH 3, single BGN-PI gels are characterised with higher gel strengths in comparison to single WPI gels (Fig. 3). At pH 3, both WPI and BGN-PI are below their isoelectric points where non-covalent hydrogen bonds and hydrophobic interactions, as opposed to stronger

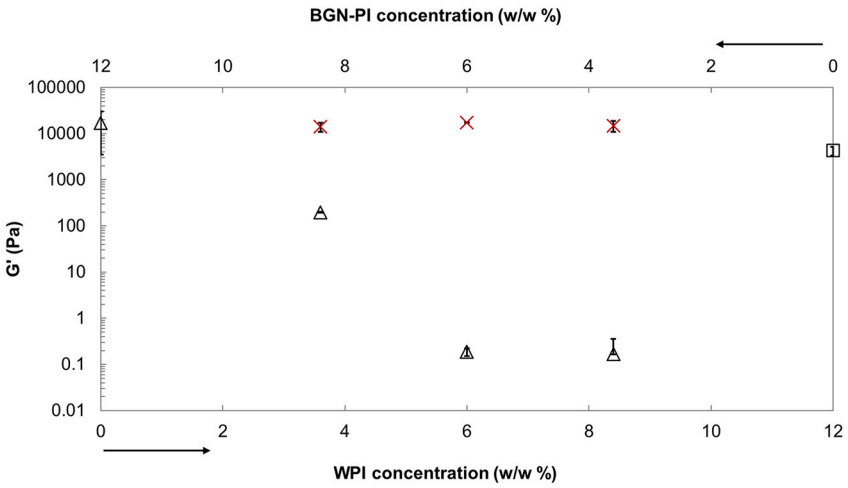

Fig. 4. Elastic moduli (G′) at pH 7 after cooling at 20 °C of single Bambara groundnut proteins (Δ) with concentrations indicated on the top axis and the single whey proteins (□) with concentrations indicated on the bottom axis. The mixed WPI/BGN-PI (x) systems are indicated at the corresponding concentrations of WPI and BGN-PI. The arrows indicate increasing concentrations and the error bars indicate the standard deviations.

Table 1
Gelation point (G'/G" crossover point) parameters of single and mixed whey and Bambara groundnut protein systems at various pH conditions.[a]

	WPI 12%w/w	70:30	50:50	30:70	BGN-PI 12%w/w
pH 7					
G' (Pa)	8.0	11.9	12.5	2.3	2.4
Time (min)	25.0	23.3	23.3	21.6	12.9
Temperature (°C)	93.6	88.4	88.5	83.3	57.4
pH 5					
G' (Pa)	1.9	8.7	12.5	7.9	4.1
Time (min)	16.4	1.7	1.7	3.4	6.9
Temperature (°C)	67.7	22.5	22.6	28.7	39.2
pH 3					
G' (Pa)	3.4	7.8	3.0	1.5	2.0
Time (min)	28.9	26.9	25.9	22.4	9.5
Temperature (°C)	95.1	95.5	96.2	85.9	47.0

[a] WPI: whey protein isolate, BGN-PI: Bambara groundnut protein isolate. Mixed protein systems correspond to the following concentrations: 70:30–8.4% WPI/3.6% BGN-PI, 50:50–6% WPI/6% BGN-PI, 30:70–3.6% WPI/8.4% BGN-PI.

disulphide bonds, are expected to dominate (Otte, Zakora, & Qvist, 2000; Shimada & Cheftel, 1989). This is highlighted in the final gel strength of the single protein systems, which at pH 3 is much lower compared to the higher pH values. The onset of gelation of the mixed protein systems (at all mixing ratios) coincided with that of single WPI (see Table 1), whilst their gelation profiles and final gel strengths also closely corresponded to that of WPI. This is evident that WPI dominates the elasticity in the mixed protein systems at pH 3, with BGN-PI having no effect. In comparison at pH 5, WPI gels were characterised with the highest elasticity compared to single BGN-PI and mixed protein systems (Fig. 3). At pH 5, WPI is close to its isoelectric point and the high gel strengths can be attributed to a combination of covalent disulphide bonds and hydrophobic interactions (Cornacchia et al., 2014; Otte et al., 2000). The G′ development of the mixed protein gels at the 8.4% WPI/3.6% BGN-PI and 6% WPI/6% BGN-PI mixing ratios, resembled that of the single WPI gels, whilst their final gel strengths were similar to that of the single BGN-PI gels. The lowest elasticity was determined in the mixture where BGN-PI was in excess (3.6% WPI/8.4% BGN-PI), which implies a negating effect on gel strength upon increasing BGN-PI concentrations. As shown in Table 1, the onset of gelation of the mixed protein systems at pH 5 occurred almost immediately at the start of the temperature sweep (22.5–28.7 °C), with a sharp increase in G′ reaching an initial maximum around 80 °C, followed by a sudden decrease and gradual increase to similar gelation profiles of the single systems. In sharp contrast, the onset of gelation for the single protein systems occurred at higher temperatures, 39.2 °C and 67.7 °C for BGN-PI and WPI, respectively. This initial elasticity behaviour of the mixtures could be attributed to aggregate formation resulting from electrostatic interactions between WPI and BGN-PI before heating, where at pH 5 both proteins are close to their isoelectric points (Nicolai, Britten, & Schmitt, 2011). At pH 5 both proteins still have a net charge, with BGN-PI (pI = 4.3) being more positively charged than WPI (pI ≈ 4.9). The strength of this attraction is dictated not only by the net charge, but also by the charge distribution on the proteins. As such, even proteins which both have a small, positive net charge, can attract each other through electrostatic interactions. The aggregation rate is further

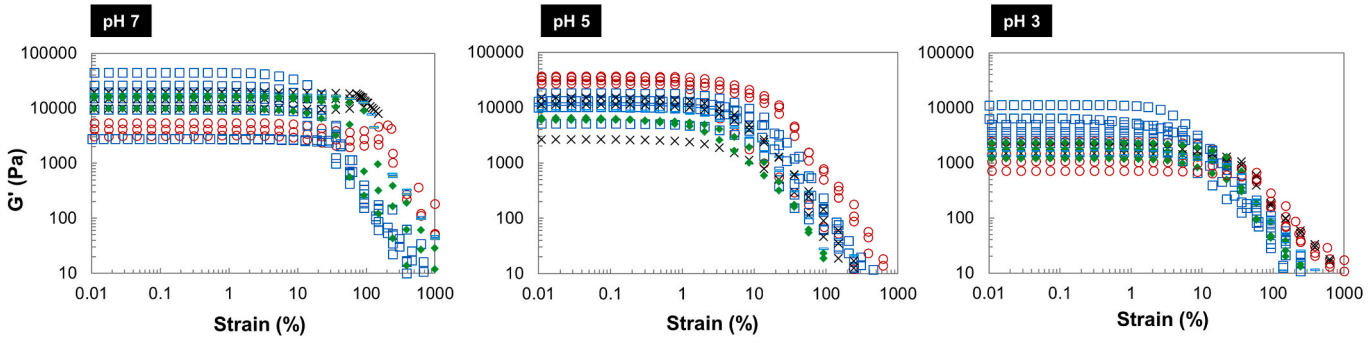

Fig. 5. Strain sweep of heat-induced whey and Bambara groundnut protein gels at different mixing ratios at pH 7 (left), pH 5 (middle) and pH 3 (right). Symbols correspond to: o—12% WPI, x—8.4% WPI/3.6% BGN-PI, —6% WPI/6% BGN-PI, ♦—3.6% WPI/8.4% BGN-PI, □—12% BGN-PI.

enhanced upon increasing temperatures, resulting in the formation of the initial gel network which are disrupted close to the denaturation temperature (~83 °C) of β-lactoglobulin (Ainis et al., 2018), as seen in the sudden drop in G'. In addition, when comparing the LVE of the gels at pH 5 and pH 3 to that at neutral pH, it is evident that the deformation under an applied strain of the gel networks are pH dependent. The limiting strain was around 10% for the gels at lower pH (Fig. 5), indicating the higher strain sensitivity and brittle nature of these gels (Langton & Hermansson, 1992; Stading & Hermansson, 1991).

Furthermore, as previously reported for other plant and whey protein systems, synergistic effects can be quantified by relating the final G′ of the mixtures to either the G' of the protein being replaced, or to the sum of the separate effects produced by the individual proteins at corresponding concentrations (Ainis et al., 2018; Jose et al., 2016; Wong et al., 2013). To evaluate if synergies occurred upon mixing of WPI and BGN-PI at 12% (w/w) total protein concentrations, the relation $S = \frac{G'mixture}{G'WPI}$ as proposed by (Wong et al., 2013) was used, where the ratio S is determined after heat treatment and indicative of the degree of synergism between the proteins. $S \neq 1$ is defined as the presence of one protein exerting an effect on the other protein in the mixture, whilst $S > 1$ is defined as synergy in the mixed protein system (Ainis et al., 2018). Wong et al. (2013) defined $S < 1$ as negative enhancement, which can be interpreted as either non-synergistic effects or antagonistic effects. The degree of synergy at the different mixing ratios for all pH values are also shown in Fig. 3. Synergistic effects were observed at pH 7 for all mixed protein systems. Compared to whey and pea protein mixtures (10% w/v total protein) at pH 6 and pH 8 where the highest synergies were observed at the lowest pea protein concentration (ratio 8:2 WPI to pea) (Wong et al., 2013), BGN-PI can replace WPI at higher concentrations whilst positively influencing the gel strength. At pH 5, the S ratio of all mixtures were below 1 with the lowest synergy observed for the gel containing BGN-PI in excess; hence confirming the negating effect of BGN-PI on the gel strength at this pH. The S ratio at pH 3 for all mixed protein systems was close to 1, which indicates that BGN-PI had no effect on the gelation behaviour of the mixtures. Ainis et al. (2018) observed a similar trend for rapeseed and whey protein mixtures, where at pH 3 the S ratio was close to 1 and the elasticity of the mixtures compared closely to that of the single WPI gel.

3.3. *Effect of disulphide interactions on gelation kinetics of single and mixed whey and Bambara groundnut protein systems at pH 7*

Disulphide interactions are known to stabilise whey protein gel networks at neutral or alkaline pH, as such contributing to higher gel strengths under these conditions (Shimada & Cheftel, 1989). The high elasticity of the single and mixed protein systems at pH 7, was therefore indicative of the role of disulphide interactions in the formation of the gel networks. To determine if disulphide bridges were indeed present and their effect on gel formation, the single proteins and the 6% WPI/6% BGN-PI mixed system were subjected to heat-induced gelation in the presence of the thiol blocking agent, NEM. As shown in Fig. 6, overall lower gel strengths were observed at a concentration of NEM in 10× excess of the free thiol concentrations quantified per protein system, i.e. 9.2 μmol/g protein and 28.2 μmol/g protein for BGN-PI and WPI, respectively. The gelation profiles of the single BGN-PI and the mixed protein system with NEM remained similar to the control gels, whereas the initial gelation kinetics of the single WPI appeared to deviate from the control. This observation was more prominent for single WPI gels where NEM was present at higher concentrations, resulting in a decrease in elasticity during initial gel formation. Heat-induced gel formation for whey proteins is generally reported as a two-stage process, i.e. disulphide interactions which forms the initial junction zones through sulfhydryl/disulphide interchange reactions followed by strengthening of the gel network through non-covalent interactions (Alting et al., 2000; Shimada & Cheftel, 1989). These results confirm the important role of disulphide interactions in gel formation of WPI gels, which to a lesser extent also influenced the gel networks of single BGN-PI and the 6% WPI/6% BGN-PI mixed protein system. The influence of NEM on BGN-PI could be attributed to various factors, as also found for cultivar-specific pea protein isolates where the addition of NEM resulted in either a decrease or increase of the gel elasticity. The differences in gel behaviour in these pea protein systems were ascribed to the reactive residues' spatial proximity upon unfolding of the legumins, or to increased repulsive forces amongst the vicilin α-subunits at high concentrations (O'Kane, Vereijken, Gruppen, & Van Boekel, 2005). Roesch and Corredig (2005) also studied the effect of NEM on soy and whey protein mixtures in a ratio of 70:30 (1.4% w/v total protein concentration) and observed that both disulphide bridging and non-covalent interactions were important for complex formation. It can thus be concluded that disulphide interactions contribute to the gel formation of both the single and mixed protein gels.

4. Conclusions

The addition of BGN-PI to WPI at different mixing ratios was shown to synergistically enhance gel formation at neutral pH, independent of the concentration of BGN-PI in the mixtures, as also evident from the similar gel microstructures of the mixed gels to that of single BGN-PI. This apparent beneficial influence of BGN-PI on whey proteins at neutral pH is noteworthy, considering that plant proteins are known to have a negating effect on gel strengths when replacing whey proteins at higher concentrations. The role of disulphide interactions was shown to be important in the network formation of single WPI gels; to a much larger extent compared to single BGN-PI and mixed protein gels. Under

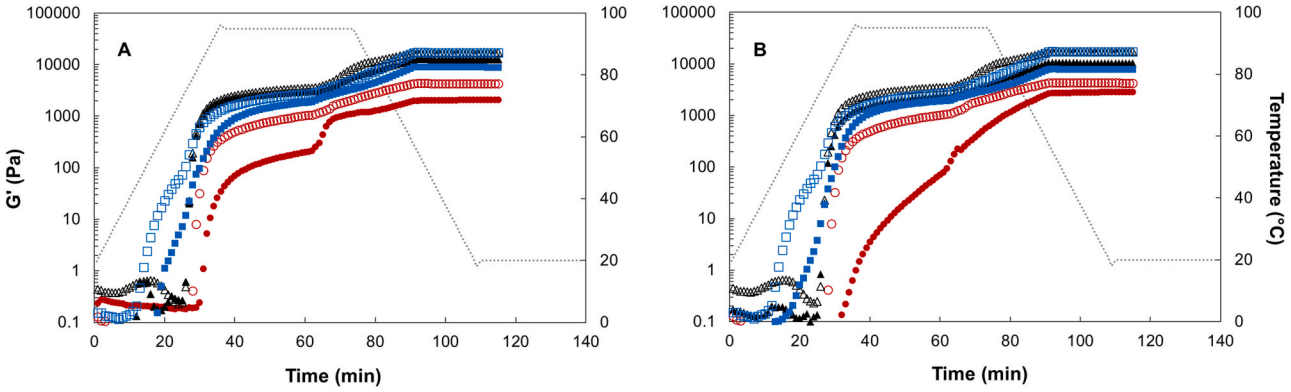

Fig. 6. The effect of NEM in (A) 10x excess and (B) 20x excess of thiol concentrations on the elastic moduli (G′) development of heat-induced single and mixed whey and Bambara groundnut protein gels at pH 7. Symbols correspond to: o—12% WPI, □—12% BGN-PI, Δ—6% WPI/6% BGN-PI without NEM; filled symbols correspond to the same protein concentrations with NEM.

acidic conditions, BGN-PI had either no effect on the viscoelastic properties of mixed protein gels (pH 3) or at increasing concentrations in the mixed protein systems resulted in lower gel strengths (pH 5). Hence, it can be concluded that BGN-PI as a novel plant protein has the ability to enhance or change the viscoelastic behaviour of gel networks when combined with whey proteins.

Author statement

Claudine Diedericks: conceptualisation, investigation, formal analysis, validation, writing—original draft, writing—review and editing.

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Declaration of competing interest

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Appendix A. Supplementary data

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