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AUTHOR STATEMENT

Arno G.B. Wouters: Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Funding Acquisition. **Iris J. Joye:** Conceptualization, Writing – Review & Editing, Funding Acquisition. **Jan A. Delcour:** Writing – Review & Editing, Funding Acquisition

Understanding the air-water interfacial behavior of suspensions of wheat gliadin nanoparticles

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Abbreviations and symbols used: 6-propionyl-2-dimethyl-amino-naphthalene (PRODAN); disulfide (SS); dry matter (dm); Fourier Transform Infra-Red (FTIR); mercury cadmium telluride (MCT); nanoparticle (NP); one way analysis of variance (ANOVA); polydispersity-index (PDI); scanning electron microscopy (SEM); sodium dodecyl sulfate (SDS); surface area (A); surface dilatational elastic modulus (E'); surface dilatational viscous modulus (E''); surface tension (σ); transmission electron microscopy (TEM); thiol (SH); wheat gliadin (WG); wheat gliadin nanoparticle (WGNP); zeta potential (ZP).

ABSTRACT

The low solubility of many plant proteins (such as those of cereals) is a main obstacle preventing their use for stabilizing food foams and emulsions. Protein based nanoparticle suspensions hold promise for stabilizing such systems. Here, we shed light on how wheat gliadin based nanoparticles (WGNPs) behave at air-water interfaces, which at present remains largely unknown. At pH 4.0 and pH 6.0, WGNPs display very poor and excellent foam stability and result in interfacial films with low and high viscoelasticity, respectively. Fourier Transform Infra-Red and fluorescence spectroscopy revealed substantial differences neither in structural nor in surface properties of WGNPs, nor in WGNP morphology at varying pH values ranging between 4.0 and 6.0, implying that the differences in interfacial behavior originate at the interface during or after adsorption of WGNPs. Cryo scanning electron microscopy imaging of foams stabilized by WGNPs showed that at pH 4.0 and pH 6.0 NP-like structures and a more coherent film are present at the interface, respectively. This is consistent with the higher viscoelasticity of adsorbed interfacial films at pH 6.0 than that at pH 4.0. Foam fractionation revealed that proteins in foams produced at pH 6.0 contain a substantial amount of intermolecular disulfide bonds. Thus, the excellent foam stability of WGNPs at pH 6.0 may at least to some extent be ascribed to formation of a covalently cross-linked protein network at the air-water interface.

Keywords: *Wheat, Gliadin, Nanoparticles, Foam, Air-water interface*

1. INTRODUCTION

Food foams and emulsions are traditionally stabilized by low molecular weight surfactants or proteins. Examples include meringue, mayonnaise, milk foam (on coffee) and cake. Such dispersions often contain animal proteins. Their abundant use is in part due to their favorable nutritional profile and their enjoyable organoleptic properties but mostly to their excellent functional properties (Day, 2013). However, the production of animal protein is more expensive and has a greater environmental impact than that of plant protein (Henchion, Hayes, Mullen, Fenelon, & Tiwari, 2017; Herrero et al., 2011).

Plant-based protein rich side-streams originate from various industrial processes, as is the case in the isolation of starch from maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Mulder, 2010; Van Der Borght, Goesaert, Veraverbeke, & Delcour, 2005) or the isolation of oil from soy (*Glycine max*) beans (Rodrigues, Coelho, & Carvalho, 2012). The low solubility of the proteins in these side-streams, be it due to their innate structure or to their pre-treatment, is often a major obstacle preventing their successful use for stabilizing food dispersions. Strategies for inducing or improving functionalities of plant protein have been researched. A promising strategy is to produce dispersions of nano-sized protein aggregates, hereafter referred to as nanoparticles (NPs), with high colloidal stability in aqueous systems (Joye & McClements, 2013; Wouters & Delcour, 2019). Such NPs may stabilize food foams and emulsions. An important concept in this regard is that of Pickering stabilization whereby interfaces are stabilized by adsorption of hard, rigid NPs (Binks, 2002; Pickering, 1907; Ramsden, 1903). Provided that such rigid NPs have sizes

within a 1 nm to 1 μm range and that they have a contact angle at the interface of both dispersed phases in a 30° to 150° range, their interfacial adsorption is practically irreversible (Tavernier, Wijaya, Van der Meeren, Dewettinck, & Patel, 2016; Xiao, Li, & Huang, 2016a). This then results in a strong steric barrier which protects the system from destabilization (Binks, 2002; Xiao et al., 2016a). The concept of interface stabilization by rigid NPs has been investigated for various types of NPs, including some based on silica (AlYousef, Almobarky, & Schechter, 2018; Binks & Lumsdon, 1999), latex (Binks & Lumsdon, 2001) or clays (Abend, Bonnke, Gutschner, & Lagaly, 1998). Such NPs are of course not suitable for many food or pharmaceutical applications. More recently, interest in the production of biopolymer based NPs has risen substantially (Joye & McClements, 2013). Proteins, due to their relatively high hydrophobicity, innate surface-activity and molecular flexibility may well be good raw materials for producing such NPs (Dickinson, 2010).

In many recent studies dealing with stabilization of emulsions by protein based NPs, a Pickering effect is assumed to be a major mechanism (de Folter, van Ruijven, & Velikov, 2012; Hu et al., 2016; Xiao, Wang, Perez Gonzalez, & Huang, 2016b; Zou, van Baalen, Yang, & Scholten, 2018). That NPs outperform the proteins they are made from indeed suggests that their particulate nature contributes to their functionality. However, this does not necessarily mean that other mechanisms are not at play. The notion that protein based NPs are likely to an extent deformable could suggest that they would indeed behave differently as is the case for rigid, inert NPs. Moreover, the behavior of protein based NPs at air-water interfaces has only been reported on in a few instances.

In a recent paper by our group (Wouters, Schaefer, Joye, & Delcour, 2019), the foaming and air-water interfacial properties of NPs based on wheat gliadins (WGs) and maize zeins were studied. One of the main conclusions of this work was that wheat gliadin nanoparticles (WGNPs) efficiently stabilize foams. A similar observation was earlier made elsewhere (Peng et al., 2017; Peng et al., 2018). More specifically, for WGNP suspensions at pH values close to their point-of-zero-charge (pH 6.0), excellent foam stability and an interfacial film with high surface dilatational moduli were observed. At pH 4.5, poor foam stability and films with low surface dilatational moduli were obtained (Wouters et al., 2019). Interestingly, that foam stability of WGNP suspensions seems to be related to formation of a viscoelastic film at the interface to some extent might imply – and only imply – unfolding, reorientation of protein molecules as well as lateral protein-protein interactions at the interface. While such behavior is typical for films made up by protein adsorbed from solution (Murray, 2011), it has not been investigated in the case of NPs. This would to some extent contradict the notion that NPs would merely exert a Pickering type effect. This phenomenon thus requires further in-depth investigation.

In the present paper, we set out to shed light on the behavior of WGNPs at air-water interfaces. The above described pH-dependent air-water interfacial behavior of WGNPs presents an excellent opportunity to investigate this. Here, variations of pH from 4.0 – at which poor foaming properties are expected – to 6.0 – at which good foaming properties are expected – was exploited as a tool

- (i) to investigate the relationship between the structural and air-water interfacial properties of WGNPs,

- (ii) to shed light on how WGNPs behave at air-water interfaces and whether mechanisms other than a Pickering effect can be considered important for interfacial stabilization, and
- (iii) to investigate the contribution of molecular changes [disulfide (SS) bond formation] in the protein population to interfacial stabilization

To achieve these goals, we here used a multi-disciplinary approach combining a thorough structural (size, surface charge, protein conformation, surface hydrophobicity, morphology) characterization of NPs, air-water interface dilatational measurements, visualization of NP-stabilized foam structures with cryo-SEM and a foam fractionation experiment to investigate changes in both the NP properties (size, surface charge, surface hydrophobicity) as well as molecular changes in the protein population due to air-water interfacial adsorption.

2. MATERIALS AND METHODS

2.1 Materials

Wheat gluten was kindly provided by Tereos Syral (Aalst, Belgium). It contained 79.7% protein (N x 5.7) on dry matter (dm) basis when determined using an adaptation of AOAC Official Method 990.03 (AOAC, 1995) to an EA1108 Elemental Analyzer (Carlo Erba/Thermo Scientific, Waltham, MA, USA). All other chemicals, solvents and reagents, unless otherwise specified, were from Sigma-Aldrich (Bornem, Belgium) and of at least analytical grade.

2.2 Gliadin extraction

WG was extracted from wheat gluten with a gluten-to-extraction solvent (70.0% v/v ethanol) ratio of 1:10 (w:v) by 60 min of shaking, centrifugation (12,000 *g* for 10 min) and filtration of the supernatant over Whatman (Maidstone, United Kingdom) paper filters (pore size 4-7 μm). The ethanol was removed by rotary evaporation under partial vacuum at 40 °C and the resultant suspension freeze-dried. The resulting WG powder had a protein content of 86.5% (N x 5.7) on dm basis, as determined with the method described in section 2.1.

2.3 Production of nanoparticles

WGNPs were obtained by liquid anti-solvent precipitation which relies on a decrease in solvent quality resulting in controlled aggregation of protein and thus the formation of a finely dispersed protein NP suspension. An aliquot (15.54 mL) of WG stock solution containing 4.50% protein/volume (4.50% w_p/v) in 70.0% v/v ethanol was slowly added at the center of a beaker containing 54.46 mL deionized water while continuously stirring at 440 rpm. The NP suspension was then stirred for an additional 30 s, the pH was gradually lowered to pH 4.0 by adding small amounts of 0.10 and 0.01 M HCl, and the ethanol was removed by rotary evaporation as above. An amount of deionized water equal to the amount of liquid removed by evaporation was added to obtain a WGNP suspension with a concentration of 1.0% w_p/v in water. pH values of WGNP suspensions at 1.0% w_p/v (see sections 2.7 and 2.9) or at 0.1% w_p/v (diluted with

deionized water) (see sections 2.4, 2.5, 2.6, 2.8 and 2.10) were then adjusted to pH 4.0, 4.5, 5.0, 5.5, 6.0 by adding small amounts of 0.10 M and 0.01 M HCl or 0.10 M and 0.01 M NaOH.

2.4 Measurement of particle size distribution and zeta potential

Measurements of particle size distribution and zeta potential (ZP) were on freshly produced WGNP suspensions (1.0% w_p/v in 15.5% v/v ethanol), WGNP suspensions after pH adjustment to 4.0 (1.0% w_p/v in 15.5% v/v ethanol), and WGNP suspensions obtained after solvent evaporation and water addition (1.0% w_p/v in water) (see section 2.3). Furthermore, WGNP suspensions at 0.1% w_p/v at pH 4.0, 4.5, 5.0, 5.5 and 6.0 (see section 2.3) were also analyzed. Particle size distribution and polydispersity-index (PDI) values were determined with dynamic light scattering while ZP measurements were made using laser Doppler electrophoresis. Analyses were executed as in Wouters et al. (2019). WGNP suspensions were transferred to disposable capillary zeta cells (Malvern Instruments, Malvern, United Kingdom) to subsequently determine NP size distributions and ZPs in a Zetasizer Nano ZS (Malvern). Particle size measurements resulted in scattering patterns which were converted to correlation functions. These gave information on the diffusion coefficients of the NP dispersions, which allowed calculating NP size distributions with the Stokes-Einstein relationship using Malvern Zetasizer software. For ZP measurements, an electrical field was applied, resulting in NPs moving at a rate dependent on their charge. The resulting scattering pattern allowed determining the electrophoretic mobility and subsequently a ZP value with Malvern Zetasizer software. Measurements were conducted at room temperature (23 ± 2 °C). Water has a viscosity of

0.8914 mPa.s (Khattab, Bandarkar, Fakhree, & Jouyban, 2012), a refractive index of 1.3326 (Belda, Herraiez, & Diez, 2005) and a dielectric constant of 78.7 (Akerlof, 1932). The viscosity (1.3020 mPa.s) and dielectric constant (70.3) of 15.5% v/v ethanol were calculated based on Khattab, Bandarkar, Fakhree & Jouyban (2012) and Akerlof (1932), respectively. Its refractive index (1.3405) was based on work by Belda, Herraiez & Diaz (2005).

2.5 Measurement of foaming characteristics

Foaming properties were determined with the stirring test of Wouters et al. (2016). Aliquots (50 mL) of all above described NP suspensions at 0.1% w_p/v at pH 4.0, 4.5, 5.0, 5.5 and 6.0 (section 2.3) were placed in a graduated glass cylinder (internal diameter 60.0 mm) in a water bath at 25 °C, allowed to equilibrate for 15 min, and stirred for 70 s at 2,000 rpm using an in-house made propeller (outer diameter 45.0 mm; thickness 0.4 mm). After stirring, the propeller was immediately removed and the glass cylinder sealed with Parafilm M (Bemis, Neenah, WI, USA) to avoid foam disruption by air circulation. Foam heights were measured 2, 4, 10, 15, 30, 45, 60, 120, 240 and 420 min after the start of stirring. Based on the foam height and the cylinder internal diameter, foam volume (in mL) was calculated. The foaming capacity was assessed as the foam volume 2 min after the start of whipping, while foam stability was assessed by evaluating the evolution of its volume in time.

2.6 Measurement of surface tension and interfacial dilatational rheology

WGNP suspensions containing 0.1% w_p/v at pH 4.0, 4.5, 5.0, 5.5 and 6.0 (section 2.3) were introduced in a Theta optical tensiometer (Biolin Scientific Attension, Stockholm, Sweden) to create a pendant drop with a fixed volume of 8 µL as in Wouters et al. (2019). Surface tension (γ) was determined over a time frame of 10 min by drop shape analysis and a Young-Laplace fit with OneAttension software (Biolin Scientific Attension). During γ measurements, one image per 7 s was recorded. In a separate measurement, at several time points after drop creation, the drop was sinusoidally oscillated (5 cycles) at 0.5 Hz with an amplitude set at 1.00 in the OneAttension software (Biolin Scientific Attension), which corresponded to a volume change of about 1 µL and was within the linear regime. During oscillation, images were recorded at 7 frames/s. The first oscillatory measurement took place 15 s after drop formation with subsequent measurements every 15 s thereafter until 5 min after drop formation. From that moment on, a measurement was performed every min until 10 min after drop formation. The surface dilatational modulus E was determined based on drop shape analysis during oscillation. E is the variation in γ per unit relative change in surface area (A) (Lucassen-Reynders, Benjamins, & Fainerman, 2010; Lucassen-Reynders & Wasan, 1993).

$$E = \frac{d\gamma}{d \ln A}$$

Wherein E is a viscoelastic complex quantity. It consists of a real surface dilatational elastic (E') and an imaginary dilatational viscous (E'') contribution, of which the latter is given by the product of a surface dilatational viscosity (η_d) and the frequency (ω) of the variation in A (Lucassen-Reynders et al., 2010; Lucassen-Reynders & Wasan, 1993).

192

$$E = E' + iE'' = E' + i\omega\eta_d$$

193

194 Both surface dilatational elastic and viscous moduli E' and E'' are reported here. After each
 195 measurement, the device was thoroughly cleaned and the γ of pure water was checked to be
 196 72.0 ± 0.5 mN/m before initiating the next measurement.

197

198 **2.7 Fourier transform infra-red spectroscopy**

199

200 Fourier Transform Infra-Red (FTIR) spectroscopy measurements were performed on WG in
 201 70.0% v/v ethanol and on WGNP suspensions at pH 4.0, 4.5, 5.0, 5.5 and 6.0 (see section 2.3) in
 202 water, all at concentrations of 1.0% w_p/v. Analyses were carried out using a sealed and
 203 desiccated (humidity <2%) Vertex 70 series spectrophotometer (Bruker Optics, Milton, ON,
 204 Canada) equipped with a KBr beamsplitter, a BioATRCeII accessory and a mercury cadmium
 205 telluride (MCT) detector cooled with liquid nitrogen. Prior to each measurement, a reference
 206 spectrum of the sample continuous phase (70.0% v/v ethanol or water at pH 4.0, 4.5, 5.0, 5.5
 207 and 6.0) was recorded and subtracted from the corresponding sample spectrum. For each
 208 absorbance spectrum, 128 scans were performed in the 800-3,000 cm⁻¹ region with a resolution
 209 of 4 cm⁻¹ and an aperture diameter of 3 mm. Opus Software (Bruker) was used for spectral
 210 acquisition and reference subtraction. Spectra were corrected for baseline, smoothened (8
 211 point Savitsky-Golay) and subjected to second derivative calculation in Origin software

(OriginLab Corporation, Northampton, MA, USA). Protein (NP) secondary structural characteristics were assessed by studying the amide I band region (1600-1700 cm^{-1}).

2.8 Fluorescence spectroscopy

Surface characteristics of NPs were assessed using intrinsic and extrinsic fluorescence measurements with a methodology similar to that of Wouters et al. (2019). WGNP suspensions containing 0.1% w_p/v at pH 4.0, 4.5, 5.0, 5.5 and 6.0 (see section 2.3) were further diluted to 0.01% w_p/v using deionized water corrected to the respective pH values of the samples.

Intrinsic fluorescence measurements were based on light emission by tryptophan and tyrosine residues in the protein structure. Diluted WGNP suspensions were transferred to a quartz cuvette and introduced in a Shimadzu (Kyoto, Japan) RF-5301PC fluorospectrophotometer. The excitation wavelength was 295 nm and emission was recorded from 300 to 450 nm both with a 5 nm slit width. A spectrum of water served as blank measurement and was subtracted from spectra of the samples.

Extrinsic fluorescence measurements were performed using 6-propionyl-2-dimethyl-amino-naphthalene (PRODAN) as fluorescent probe. The recorded maximum emission intensities are indicative for the surface hydrophobicity of the NPs. A 2.00 mL aliquot of each of the above described NP suspensions (see section 2.3) at a concentration of 0.01% w_p/v was incubated for 5 min in the dark with 10 μL PRODAN stock solution (0.1025 mM in methanol). These samples were transferred to a quartz cuvette and analyzed following excitation at 365 nm with a 5 nm slit width. The emission spectrum was recorded from 300 to 600 nm with a 5 nm slit width. A

spectrum of water containing PRODAN served as blank measurement and was subtracted from the spectra of the samples.

All measurements were conducted at a protein concentration which yielded a fluorescence signal in the linear range and at room temperature (23 ± 2 °C).

2.9 Transmission electron microscopy

The morphology of WGNPs was studied with transmission electron microscopy (TEM). An aliquot (10 µl) of WGNP suspensions containing 0.1% w_p/v at pH 4.0, 4.5, 5.0, 5.5 and 6.0 was loaded for 3 min on glow discharged copper grids of 400-mesh which were coated with formvar film (Agar Scientific, Stansted, United Kingdom). After sample adsorption, the excess sample was drained with filter paper. Then, samples were washed with MilliQ water and stained with 2.0% (w/v) uranyl acetate in MilliQ water for 45 s. Stained samples were washed a second time with MilliQ water and drained with filter paper. The grids were dried for 5 min at room temperature and examined using a JEM-1400 TEM (Jeol, Tokyo, Japan) instrument at 80 kV.

2.10 Cryo scanning electron microscopy

Cryo scanning electron microscopy (SEM) imaging was carried out to visualize structures at the air-water interfaces of foams produced from WGNP suspensions at 1.0% w_p/v at pH 4.0 and 6.0. Foams were produced as in section 2.5. Immediately afterwards, aliquots were placed in a sample holder and dipped in slush nitrogen under vacuum. The frozen samples were transferred to the sample preparation chamber of a Quorum (Quorum Technologies, Laughton, United

Kingdom) PP3010 cryo-unit, in which the frozen foams were fractured using an iced knife. Ice was removed by sublimation during 5 min at -90 °C and the resulting surface was sputter coated with Pt for 1 min at 10 mA. Samples were then transferred to an FEI [Field Electron and Ion Company (Thermo Fisher Scientific), Hillsboro, OR, USA] Quanta Field Emission Gun 250 SEM device. Images were obtained at an acceleration voltage of 5 kV.

2.11 Foam fractionation

GNP suspensions containing 0.1% w_p/v at pH 4.0, 4.5, 5.0, 5.5 and 6.0 (section 2.3) were subjected to the stirring procedure used to produce foams in section 2.7. After 10 min, the foam and liquid fractions were sampled. To assess the properties of the proteins in both fractions, the foams were (gently) disrupted by centrifugation at 2,000 *g* for 60 min. The protein concentrations of the original WGNP suspensions, the liquid and the foam fractions were measured. An aliquot of sample (150 µL) was added to 850 µL deionized water and 1.0 mL 2.0% (w/v) sodium dodecyl sulfate (SDS) to disintegrate all particulate structure and unfold and dissolve the proteins present. This allowed measuring the absorbance at 214 nm. After subtracting the absorbance of a blank [1.0 mL deionized water + 1.0 mL 2.0% (w/v) SDS] from the absorbance of the samples, the protein concentration was calculated based on a calibration curve made by measuring the absorbance of WGNP suspensions at concentrations of 0.0025, 0.0050, 0.0075 and 0.010% w_p/v with the same methodology. The protein concentrations of samples were expressed relative to those of the original WGNP suspensions of known protein concentrations. They were also measured under reducing conditions, which comprised replacing

2.0% (w/v) SDS (see section 2.9) by 2.0% (w/v) SDS containing 0.15% (w/v) dithiothreitol in the protocol. Furthermore, the intrinsic fluorescence signals of the original WGNP suspensions, the liquid fractions and the foam fractions at different pH values were measured as in section 2.5. Fluorescence spectra were corrected for the soluble protein content (under non-reducing conditions) in the different samples (see above).

2.12 Statistical analyses

Determinations of NP size, ZP, fluorescence spectroscopy, FTIR spectroscopy, foaming properties, γ , dilatational rheology and the foam fractionation were at least in four-fold. Cryo-SEM imaging was carried out in duplicate. Error bars or values in all figures and tables represent the standard deviations from the mean. Data was analyzed using statistical software JMP Pro 12 (SAS Institute, Cary, NC, USA). One way analysis of variance (ANOVA) was performed, followed by a Tukey multiple comparison test as post-hoc analysis to detect significant differences, both at a significance level $P = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Wheat gliadin nanoparticle production

NP properties (size, PDI, ZP) vary with the way they are produced (Joye, Nelis, & McClements, 2015). The protocol in section 2.3 to produce NPs differed from that of Wouters et al. (2019). We here made NP suspensions of higher protein concentration which contained no residual

ethanol. Such features are beneficial when using WGNPs in food systems. To allow for ethanol removal via rotary evaporation under partial vacuum, a pH correction to pH 4.0 was necessary as NP precipitation took place when ethanol removal was carried out at the native pH (pH 5.5) of the WGNP suspension immediately after its production. Table 1 shows average size (Z-average), PDI and ZP values of WGNPs immediately after production, after adjustment of the pH to 4.0 and after solvent removal and dilution to 1.0% w_p/v with deionized water. Adjustment of the pH to 4.0 resulted in a slight increase ($P < 0.05$) in NP size and a pronounced one ($P < 0.05$) in PDI while no effect ($P > 0.05$) on ZP was noted. Subsequent solvent removal and dilution to 1.0% w_p/v had no further impact ($P > 0.05$) on NP size and PDI, but led to a slight decrease in ZP, although this effect was limited at best. It should be mentioned the above mentioned precipitation only occurred during ethanol removal and that the pH of the resulting WGNP suspension in water could be readily adjusted to pH values ranging from 4.0-6.0.

3.2 Structural and functional properties of gliadin based nanoparticles

Figure 1a shows the foaming characteristics of WGNPs (at 0.1% w_p/v) as a function of pH. The foaming capacity of WGNPs hardly varied with pH. However, there were pronounced differences in foam stability. The highest foam stability was noted at pH 5.5 and at pH 6.0. It decreased in the order pH 5.5, 5.0, 4.5, and 4.0. While at pH 4.0 less than 15% of the initial foam volume remained after 30 min, at pH 6.0, more than 70% of the initial foam was still present 7 hours after foaming.

Figure 1b-d shows the evolution of γ , E' and E'' over a 10 min time span after drop formation. The curves of γ of WGNPs at pH 5.0, 5.5 and 6.0 were very similar. Those at pH 4.0 and 4.5 indicated a slightly lower surface activity of WGNPs at these pH readings than at pH 5.0, 5.5 and 6.0. However, the differences were limited. In contrast, there were pronounced differences in E' and E'' values. A gradual increase of E' and E'' over time was noted at higher pH values (pH 6.0, 5.5 and 5.5) but much less so at pH 4.5 and not at all pH 4.0, suggesting the build-up of a viscoelastic film over time at the former pH values. That E' values were generally higher than the accompanying E'' values indicates that the adsorbed interfacial films were elastic rather than viscous. The absolute values of E' values at a given time point were lower when pH was lower. Thus, as is generally the case for protein solutions (Murray, 2011), high surface dilatational moduli of WGNP suspensions here corresponded to high foam stabilities. This is in line with earlier findings by Wouters et al. (2019) that foam stabilities of WGNPs under different conditions correlate very well with the evolution of E' and E'' over time. It remains to be investigated whether and, if so, to which extent structural differences of WGNPs at different pH lie at the basis of these observations.

Figure 2 shows average sizes, PDI and ZP values of WGNPs (at 0.1% w_p/v). At pH 6.0, which is closest to the point-of-zero-charge of WGNPs, the average size was highest, while PDI and ZP values were lowest. Indeed, for the latter two, an increase was observed when pH decreased. For ZP, this was expected as proteins carry a greater charge at pH values further away from their point-of-zero-charge. That this was also the case for the PDI values is ascribed to swelling of NPs

at lower pH, which itself likely results from the greater NP charge density associated with low pH leading to a greater extent of electrostatic repulsion of protein molecules within the NPs. Whereas such effect was expected to be accompanied by an increase in WGNP size with decreasing pH, this was only partly the case. When the pH was lowered from 5.0 to 4.0, the above was indeed observed as WGNPs had higher average size at pH 4.0 than at pH 5.0. However, when it was lowered from 6.0 to 5.0, a decrease in average size was observed. This may be because when decreasing the charge density, and thus when moving from pH 5.0 to pH 6.0, more dense NPs were obtained which slightly grew in size due to attraction of protein in the medium. Regardless of the above, the average size of the NPs did not vary greatly with pH. Indeed, neither pronounced disintegration nor aggregation took place at any of the pH values tested.

Figure 3 shows the amide I band ($1600 - 1700\text{ cm}^{-1}$) of normalized FTIR spectra (Figure 3a) and the second derivative thereof (Figure 3b) of a 1.0% w_p/v WG solution in 70.0% v/v ethanol, as well as of 1.0% w_p/v WGNP suspensions in water at pH 4.0, 4.5, 5.0, 5.5 and 6.0. This band is the most sensitive spectral region to probe protein secondary structures (Kong & Yu, 2007). Thus, Figure 3 not only provides qualitative information on whether conformational changes occur during NP production, but also on whether protein conformation in WGNPs varies with pH. The here obtained FTIR spectra (Figure 3a) of both soluble WG and the WGNPs at varying pH values are rather similar. Their second derivatives allow better identification of differences in conformation under varying conditions. Indeed, Figures 3a-b mark some specific wavenumbers indicative for certain characteristic secondary structural elements, as reported by Kong & Yu (2007). For the WG solution, a clear negative peak at about 1680 cm^{-1} was observed (Figure 3b).

Also, at 1680 to 1670 cm^{-1} , the second derivative profile showed negative peaks. Negative peaks at these wavenumber values are characteristic for β -turns in protein secondary structures (Kong & Yu, 2007). At about 1655 cm^{-1} , an even more pronounced negative peak was observed which corresponds to α -helices (typically at 1656 cm^{-1}). That at 1648 cm^{-1} no negative peak was observed indicated that random coil structural features were not prominent in dissolved WG proteins. Some less pronounced (than was the case for the peaks in the 1680 – 1655 cm^{-1} region) negative peaks between 1636 and 1625 cm^{-1} indicated the presence of some β -sheets. These observations correlate very well with literature data. Indeed, ω -gliadins are rich in β -turns and contain only low levels of α -helices and β -sheets (Gianibelli, Larroque, MacRitchie, & Wrigley, 2001; Tatham & Shewry, 1985). Furthermore, α - and γ -gliadins have relatively high levels of β -turns and α -helices (Gianibelli et al., 2001; Tatham, Field, Smith, & Shewry, 1987; Tatham, Marsh, Wieser, & Shewry, 1990; Tatham & Shewry, 1985). Interestingly, the second derivative of the amide I band in the spectra of WGNP suspensions in water was very similar to that of WGs in 70.0% v/v ethanol. Only in the 1635-1620 cm^{-1} region, which is indicative for β -sheets, were slight differences between WGs and WGNPs noted, although differences were limited at best. Overall, there seemed to be no notable conformational changes in WG proteins as a result of anti-solvent precipitation. The second derivative profiles of the WGNP suspensions of different pH values tested were quite similar which implied that the conformation of WG proteins in the WGNPs did not change with pH.

Figure 4 deals with the surface characteristics of the WGNPs at various pH values, as assessed by intrinsic (Figure 4a) and extrinsic (Figure 4b) fluorescence measurements.

When intrinsically present fluorophores such as tryptophan and tyrosine are excited at a given wavelength, measurement of the intensity and the wavelength of maximum emission provide information on the local environment of the fluorophores and thus on the protein structure and conformation (Ghisaidoobe & Chung, 2014; Lakowicz, 2006). Here, excitation was at 295 nm and thus tryptophan only was excited (Lakowicz, 2006). There were no significant differences ($P > 0.05$) in the maximum emission wavelengths of WGNP suspensions at any of the pH values tested (Figure 4a). WGNPs at pH 6.0 had slightly higher ($P < 0.05$) emission intensity than at the other pH values. This could be indicative for slight changes in the NP structure and thus contribute to its size and surface characteristics. However, no gradual changes in the fluorescence intensity as function of pH were noted which contrasts to what was observed for the interfacial properties of WGNPs in the pH range studied (Figure 1). It is therefore not likely that this slightly higher emission intensity at pH 6.0 can be related to its foaming characteristics.

Binding of WGNPs to PRODAN provides an indication for their surface hydrophobicity. Figure 4b shows a blank measurement (PRODAN without WGNPs) as well as corrected (after subtracting the blank profile) emission spectra for WGNPs after binding with PRODAN at varying pH. Binding of PRODAN to WGNPs resulted in a pronounced shift of the maximum intensity wavelength from about 525 nm (for the blank) to about 450 nm (WGNPs and PRODAN), a known phenomenon (Chakrabarti, 1996). That there were no significant differences ($P > 0.05$) in the intensity of the peaks at 450 nm for the samples of different pH demonstrates that their surface hydrophobicity was similar.

Figure 5 shows representative TEM images of WGNPs at pH 4.0, 4.5, 5.0, 5.5 and 6.0 which allow (i) visualizing the obtained WGNPs and (ii) assessing whether pH impacts the WGNP morphology. First, it was concluded that at all pH values tested, a range in NP sizes was observed, consistent with the data obtained via dynamic light scattering (Figure 2). The WGNPs observed were rather spherical although their surface did not seem to be entirely smooth. Most importantly, there did not seem to be notable differences in WGNP morphology at different pH values.

Neither the analyses dealing with the structural features of the gliadin proteins present in WGNPs nor those of the surface characteristics of the WGNPs themselves, nor the WGNP morphology as assessed by TEM imaging could explain the substantial differences in interfacial characteristics at different pH. Indeed, pH for the most part affected neither the structural nor the surface characteristics of WGNPs. The above implies that the phenomenon which determines the differences in interfacial behavior of WGNP suspensions at varying pH finds its origin at the interface during or after the adsorption of WGNPs thereat. In what follows, we further explore this.

3.3 Unraveling the interfacial behavior of gliadin based nanoparticles

3.3.1 Visualization of air-water interfaces

A first approach to better understand how WGNPs behave at the air-water interface at different pH is to visualize such interfaces using cryo-SEM. Here, fresh foams with respectively poor and

excellent foam stability were produced from WGNP suspensions (at 1.0% w_p/v) at pH 4.0 and 6.0. Cryo-SEM sample preparation (see section 2.9) unavoidably to some extent affects the foam structure. Figures 6a-b show some typical, representative images of the observed structures. Remnants of gas cells are present as rather smooth surfaces, in between which some kind of matrix made up from strands of protein material can be seen (Figure 6a and 6b). This likely is WGNP protein material present in former liquid films between gas cells which has aggregated in strands due to removal of water during sample preparation. Figure 6b shows a structure which can be very well recognized as a Plateau border, a feature typically found in closely stacked foams. The obtained images show a representative adsorbed layer at remnants of air-water interfaces of the frozen foam. In a different topic of study, Hanazawa, Sakurai, Matsumiya, Mutoh & Matsumura (2018) have interpreted very similar images in a similar way. Figure 7 presents some representative images of foams produced from WGNP suspensions (at 1.0% w_p/v) at pH 4.0 and 6.0. Remarkably, the interfaces in foams at pH 4.0 were very different from those at pH 6.0. At pH 4.0, the interfaces were characterized by NP like structures embedded in a rather smooth film. The latter likely is made up from soluble protein material still present in the medium or protein material originating from partial unfolding of the NPs at the interface. In contrast, at pH 6.0, some remnants of particulate material could also still be distinguished. Nevertheless, NPs at pH 6.0 seemed to spread out and form a more coherent adsorbed layer than was the case at pH 4.0. These observations would be in line with WGNPs at pH 6.0 mutually interacting and forming a viscoelastic protein film at the interface upon adsorption, as put forward in section 3.2. That this seemed to occur much less at pH 4.0 is in line with the lower surface dilatational moduli at this pH 4.0 than at pH 6.0.

3.3.2 Foam fractionation

A second approach for studying the interfacial behavior of WGNPs is to prepare foam as in section 2.7, and to sample its liquid and foam fractions after 10 min. Comparison of the protein contents and the NP properties (size, PDI, ZP and intrinsic fluorescence) of the original WGNP suspensions and those of the foam and liquid fractions allows probing WGNP adsorption at the interface and structural changes associated therewith.

Figure 8 shows average size, PDI and ZP values of WGNPs (at 0.1% w_{protein}/v) at pH 4.0, 4.5, 5.0, 5.5 and 6.0 as well as of the foam and liquid fractions of the corresponding samples. The average sizes of the WGNPs in both the liquid and foam fractions did not differ ($P < 0.05$) from those in the original WGNP suspension. Only at pH 4.0 was the average size of the WGNPs in the foam fraction slightly lower ($P < 0.05$) than that in either the liquid fraction or the original WGNP suspension. PDI values of the WGNPs in the foam fraction at all pH values were slightly lower ($P < 0.05$) than those in the liquid fraction and in the original WGNP suspension. No differences ($P < 0.05$) in ZP were observed between the foam or liquid fractions and the original WGNP suspensions. In general, the combination of the whipping procedure and adsorption of WGNPs seemed not to impact the NP properties. However, that these measurements rely on light scattering implies that the average size, polydispersity and surface charge obtained only refer to the fraction of NPs in the suspension. Not all protein material previously present in WGNP form

is necessarily still present in that form. In addition, the protein concentration in the different fractions may vary.

Figure 9a shows the protein concentrations in the original WGNP suspensions at pH values of 4.0, 4.5, 5.0, 5.5 and 6.0 as well as those in the respective liquid and foam fractions. Protein concentrations (%) are expressed relative to the protein concentrations in the original WGNP suspensions before foam fractionation. These measurements were obtained in the presence of SDS which led to disintegration of the NPs and solubilization of the WGs and thus allowed quantification of the protein material via measurement of extinction at 214 nm (see section 2.10). At pH values 4.0, 4.5 and 5.0, no notable differences in the protein concentrations between the original WGNP suspensions and the liquid and foam fractions were observed. At pH 5.5 and, more pronouncedly, at pH 6.0, the protein concentration in the liquid fraction was lower than that in the original WGNP suspensions. While it makes sense that protein material would be concentrated in the foam fraction at the expense of that in the liquid fraction, no increase in the protein concentration of the foam fraction was found. Indeed, at pH 5.5 and pH 6.0, the protein concentration in the foam fraction was considerably lower than that in the original WGNP suspensions (Figure 9a). This implies that some of the protein was no longer extractable under the conditions used. Figure 9b shows the results of the same experiment as that depicted in Figure 9a, but under conditions in which dithiothreitol was present, and SS bonds were thus broken. As expected, the trends for the original WGNP suspension and the liquid fractions at all pH values were similar irrespective of whether dithiothreitol had been used. However, under reducing conditions, the protein concentrations in all foam fractions were

considerably higher than those of the original WGNP suspensions and more pronouncedly so at pH 6.0 and, although to a lesser extent, at pH 5.5 than at pH 5.0 or lower. Thus, the production of foam leads to a greater enrichment of protein in the foam fraction at higher than at lower pH values. The discrepancy in protein concentrations of the foam fractions when analyzed under non reducing and reducing conditions can be explained by the formation intermolecular SS bonds upon adsorption of the WGNPs at the air-water interface. That a similar trend was not observed when comparing the protein concentrations of the liquid fractions, which had undergone the same stirring test as the foam fractions, under non reducing and reducing conditions, confirms that these SS bonds were formed at the interface. This in itself is a notable observation. Only a few earlier literature reports, as mentioned below, are available on the formation of such bonds between proteins upon adsorption at air-water interface. Indeed, that they may contribute to improved foam stability was put forward in two studies in which thiolated α_{s1} -casein displayed better foam stability than its non-modified counterpart, which does not contain cysteine residues, as a result of the oxidation of thiol (SH) groups to form SS bonds (Okumura, Miyake, Taguchi, & Shimabayashi, 1989, 1990). For ovalbumin, which has free SH groups and cysteine residues involved in intramolecular SS bonds (Mine, 1995), a similar effect was observed. Indeed, adsorption of ovalbumin at the air-water interface leads to unfolding of the protein and subsequent SH-SS exchange reactions resulting in cross-linked molecules (Kitabatake & Doi, 1987; Lechevalier et al., 2003).

An important difference between these studies and the present one is that the above mentioned proteins at least have one free SH group, while the WGs used here only have cysteine residues involved in intramolecular SS bonds (α - and β -gliadins) and/or no cysteine

residues at all (ω -gliadins) (Delcour et al., 2012). That would imply that, regardless of possible conformational changes taking place upon adsorption at the interface, no SH-SS exchange reactions can take place. However, the WG used here was prepared from commercial wheat gluten which may well have contained traces of protein species having free SH groups. These might include proteins normally found in the albumin or globulin fractions of wheat or low molecular mass glutenins. The latter are structurally similar to some gliadins and have free SH groups (Delcour et al., 2012). Apparently, the presence of small levels of such proteins with free SH groups sufficed to induce intermolecular SS bond formation upon adsorption at the air-water interface. Of course, the above observations allow only to speculate on the identity of the protein species inducing this cross-linking effect via free SH groups in their structure. Although outside of the scope of the present work, an approach combining electrophoretic or chromatographic separation (under reducing and non-reducing conditions) of the proteins present in the foam fraction, combined with mass spectrometry would allow identification of the protein species responsible for inducing the SH-SS exchange reactions in which the present WGs are involved. Regardless, a similar effect was observed elsewhere, as SH-SS exchange reactions were observed for ovotransferrin only in the presence of trace amounts of ovalbumin (Lechevalier et al., 2003). To the best of our knowledge, the ability of WGs to form intermolecular SS bonds at the air-water interface (when a kick-starter molecule with free SH groups is present) has not been described in literature thus far. That this occurs more pronouncedly at high pH values is likely due to the lower extent of electrostatic repulsion in proximity to the point-of-zero-charge rather than to structural differences between WGNPs at different pH. Adsorbed WG molecules in the absence of strong electrostatic repulsion more

easily spread at the interface and mutually interact non-covalently and, apparently, also covalently. Another effect that might be at play is that the reactivity of SH groups increases with pH as the pKa value of the SH group is approached or surpassed (Singh & Whitesides, 1993). Although pKa values for cysteine residue in a protein molecule are approximately 8.5 (Poole, 2015), the local chemical environment of a SH group may result in different pKa values and thus reactivities. For instance, model peptides ranging in chain length from 10 to 22 amino acids can have cysteine SH group pKa values ranging from 7.4 to 9.2 (Bulaj, Kortemme, & Goldenberg, 1998). Elsewhere, it has been reported that DsbA, a periplasmic *Escherichia coli* oxidoreductase, has a SH group which shows high reactivity at pH values as low as pH 4.0 (Nelson & Creighton, 1994). It is thus not difficult to imagine that the protein conformation, the presence of a protein molecule within a NP as well as adsorption of such a protein NP at the interface can affect the chemical environment of SH groups and thus their pH-dependent reactivity. More research would be necessary to investigate whether and to which extent this is actually the case in the present context. Irrespective of the above, the formation of such SS bonds at the air-water interface obviously strengthens the degree to which protein-protein interactions occur thereat. Indeed, all this is in line with the earlier discussed high foam stability, the high viscoelasticity of the adsorbed films and the images of foam obtained at pH 6.0.

Finally, Figure 10 shows intrinsic fluorescence measurements of the original WGNP suspensions at pH values of 4.0, 4.5, 5.0, 5.5 and 6.0, as well as of their respective liquid and foam fractions. While this was not the case for the original WGNP suspensions and the liquid fractions, much higher fluorescence intensity was observed in the foam fractions at higher pH. Such increase in

fluorescence intensity may have been due to the tryptophan residues being affected less by quenching at higher pH. Indeed, tryptophan fluorescence intensity can be substantially reduced due to quenching as a result of electron transfer to locally present functional protein groups. Examples of functional groups which may quench tryptophan fluorescence include protonated histidine residues, the SH group of cysteine, amide groups and carboxyl groups (Callis, 2014; Corradini, Demol, Boeve, Ludescher, & Joye, 2017). It is clear that changes in the conformation of proteins and thus of the local environment of tryptophan residues can affect the observed fluorescence. Thus, the higher fluorescence of the foam fractions at higher pH could be due to the aforementioned unfolding of protein molecules from the WGNPs at the interface and the formation of a coherent adsorbed protein film. This would imply a more 'open' structure, where less quenching of tryptophan fluorescence occurs than in the WGNPs, in which protein functional groups are in close contact.

4. CONCLUSIONS

The goal of this paper was to in-depth study

- (i) to investigate the relationship between the structural and air-water interfacial properties of WGNPs,
- (ii) to shed light on how WGNPs behave at air-water interfaces and whether mechanisms other than a Pickering effect can be considered important for interfacial stabilization, and

- (iii) to investigate the contribution of molecular changes in the protein population to interfacial stabilization

That the foam stability and air-water interfacial characteristics of WGNPs strongly depend on pH allowed varying it to study these phenomena. At pH 4.0, very poor foam stability was observed, while excellent foam stability was achieved at pH 6.0. To achieve the set goals, we used an approach combining in-depth NP surface characterization, surface dilatational moduli measurements, visualization of NP-stabilized foams and a foam fractionation experiment to investigate NP properties after adsorption at an A-W interface. This allowed making the following novel observations and associated conclusions.

- The conformational characteristics of the proteins in WGNPs, the surface properties of the WGNPs themselves and the WGNP morphology as assessed by TEM do not vary much with pH. This implies that the phenomena determining the pH-dependent differences in interfacial behavior of WGNP suspensions originate at the interface during or after the adsorption of WGNPs thereat.
- The surface dilatational moduli of WGNPs are much higher at pH 6.0 than at pH 4.0. Cryo-SEM images of frozen foams at pH 4.0 and 6.0, which represented the worst and best foams tested, revealed that at the air-water interface, NP-like structures and a more coherent, continuous film are present at pH 4.0 and 6.0, respectively. This suggests unfolding and to some extent disintegration of WGNPs at the interface and the subsequent formation of an adsorbed film with high viscoelasticity sustained by lateral protein-protein interactions. Further evidence for this view was delivered by intrinsic

fluorescence spectroscopy data obtained from WGNP samples after their use in a foam fractionation experiment.

- A foam fractionation experiment revealed a much more pronounced increase in the level of protein material present in the foam fraction at pH 6.0 than at pH 4.0. Moreover, this was only observed when samples were analyzed under reducing conditions, which strongly suggests that a substantial amount of intermolecular SS bonds are formed between WG molecules upon adsorption at the interface at pH 6.0.
- It thus seems that the excellent foam stability of WGNPs at pH 6.0 can at least to some extent be ascribed to the formation of a covalently cross-linked protein network at the air-water interface.

The above poses some exciting opportunities for the inclusion of WGNPs as foam stabilizing agents in food products, which will of course require further research to validate whether WGNPs still display such functionality in actual food systems. That WG proteins potentially form SS-bonds upon adsorption at air-water interfaces has to the best of our knowledge not been described before. This merits further investigation and may lead to new insights in applications where WGs occur and gas cell stabilization is required (e.g. bread, cake, etc.).

The above mentioned approach to investigate which protein species would induce intermolecular SS bond formation in WGs can be a first step in this regard. In any case, we here have shown that the Pickering type stabilization which is often assumed to take place for protein based NPs, is not necessarily the dominant mechanism for interface stabilization in this context. Indeed, spreading and the formation of a strong protein film at the air-water

626 interface seems highly important for proper foam stabilization by WGNPs. Whether this is
627 the case for other protein based NPs remains to be investigated.

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REFERENCES

- Abend, S., Bonnke, N., Gutschner, U., & Lagaly, G. (1998). Stabilization of emulsions by heterocoagulation of clay minerals and layered double hydroxides. *Colloid and Polymer Science*, 276, 730-737.
- Akerlof, G. (1932). Dielectric constants of some organic solvent-water mixtures at various temperatures. *Journal of the American Chemical Society*, 54, 4125-4139.
- AlYousef, Z. A., Almobarky, M. A., & Schechter, D. S. (2018). The effect of nanoparticle aggregation on surfactant foam stability. *Journal of Colloid and Interface Science*, 511, 365-373.
- AOAC. (1995). Official methods of analysis. Method 990.03. Washington, DC, USA. Association of Official Analytical Chemists.
- Belda, R., Herraiz, J. V., & Diez, O. (2005). A study of the refractive index and surface tension synergy of the binary water/ethanol: Influence of concentration. *Physics and Chemistry of Liquids*, 43, 91-101.
- Binks, B. P. (2002). Particles as surfactants—similarities and differences. *Current Opinion in Colloid & Interface Science*, 7, 21-41.
- Binks, B. P., & Lumsdon, S. O. (1999). Stability of oil-in-water emulsions stabilised by silica particles. *Physical Chemistry Chemical Physics*, 1, 3007-3016.
- Binks, B. P., & Lumsdon, S. O. (2001). Pickering emulsions stabilized by monodisperse latex particles: Effects of particle size. *Langmuir*, 17, 4540-4547.
- Bulaj, G., Kortemme, T., & Goldenberg, D. P. (1998). Ionization–reactivity relationships for cysteine thiols in polypeptides. *Biochemistry*, 37, 8965-8972.
- Callis, P. R. (2014). Binding phenomena and fluorescence quenching. II: Photophysics of aromatic residues and dependence of fluorescence spectra on protein conformation. *Journal of Molecular Structure*, 1077, 22-29.

- Chakrabarti, A. (1996). Fluorescence of spectrin-bound prodan. *Biochemical and Biophysical Research Communications*, 226, 495-497.
- Corradini, M. G., Demol, M., Boeve, J., Ludescher, R. D., & Joye, I. J. (2017). Fluorescence spectroscopy as a tool to unravel the dynamics of protein nanoparticle formation by liquid antisolvent precipitation. *Food Biophysics*, 12, 211-221.
- Day, L. (2013). Proteins from land plants – potential resources for human nutrition and food security. *Trends in Food Science & Technology*, 32, 25-42.
- de Folter, J. W. J., van Ruijven, M. W. M., & Velikov, K. P. (2012). Oil-in-water pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein. *Soft Matter*, 8, 6807-6815.
- Delcour, J. A., Joye, I. J., Pareyt, B., Wilderjans, E., Brijs, K., & Lagrain, B. (2012). Wheat gluten functionality as a quality determinant in cereal-based food products. *Annual Review of Food Science and Technology*, 3, 469-492.
- Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science*, 15, 40-49.
- Ghisaidoobe, A., & Chung, S. (2014). Intrinsic tryptophan fluorescence in the detection and analysis of proteins: A focus on Förster resonance energy transfer techniques. *International Journal of Molecular Sciences*, 15, 22518–22538.
- Gianibelli, M. C., Larroque, O. R., MacRitchie, F., & Wrigley, C. W. (2001). Biochemical, genetic, and molecular characterization of wheat endosperm proteins. *Online Review by American Association of Cereal Chemists*.
- Hanazawa, T., Sakurai, Y., Matsumiya, K., Mutoh, T.-a., & Matsumura, Y. (2018). Effects of solid fat content in fat particles on their adsorption at the air–water interface. *Food Hydrocolloids*, 83, 317-325.
- Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., & Tiwari, B. (2017). Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. *Foods*, 6, 53.
- Herrero, M., Gerber, P., Vellinga, T., Garnett, T., Leip, A., Opio, C., Westhoek, H. J., Thornton, P. K., Olesen, J., Hutchings, N., Montgomery, H., Soussana, J. F., Steinfeld, H., & McAllister, T. A. (2011). Livestock and greenhouse gas emissions: The importance of getting the numbers right. *Animal Feed Science and Technology*, 166-67, 779-782.
- Hu, Y.-Q., Yin, S.-W., Zhu, J.-H., Qi, J.-R., Guo, J., Wu, L.-Y., Tang, C.-H., & Yang, X.-Q. (2016). Fabrication and characterization of novel pickering emulsions and pickering high internal emulsions stabilized by gliadin colloidal particles. *Food Hydrocolloids*, 61, 300-310.
- Joye, I. J., & McClements, D. J. (2013). Production of nanoparticles by anti-solvent precipitation for use in food systems. *Trends in Food Science & Technology*, 34, 109-123.
- Joye, I. J., Nelis, V. A., & McClements, D. J. (2015). Gliadin-based nanoparticles: Fabrication and stability of food-grade colloidal delivery systems. *Food Hydrocolloids*, 44, 86-93.
- Khattab, I. S., Bandarkar, F., Fakhree, M. A. A., & Jouyban, A. (2012). Density, viscosity, and surface tension of water+ethanol mixtures from 293 to 323K. *Korean Journal of Chemical Engineering*, 29, 812-817.
- Kitabatake, N., & Doi, E. (1987). Conformational change of hen egg ovalbumin during foam formation detected by 5,5'-dithiobis (2-nitrobenzoic acid). *Journal of Agricultural and Food Chemistry*, 35, 953-957.

- Kong, J., & Yu, S. (2007). Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochim Biophys Sin (Shanghai)*, 39, 549-559.
- Lakowicz, J. R. (2006). Protein fluorescence. In J. R. Lakowicz (Ed.), *Principles of fluorescence spectroscopy* (pp. 529-575): Springer US.
- Lechevalier, V., Croguennec, T., Pezennec, S., Guérin-Dubiard, C., Pasco, M., & Nau, F. (2003). Ovalbumin, ovotransferrin, lysozyme: Three model proteins for structural modifications at the air–water interface. *Journal of Agricultural and Food Chemistry*, 51, 6354-6361.
- Lucassen-Reynders, E. H., Benjamins, J., & Fainerman, V. B. (2010). Dilational rheology of protein films adsorbed at fluid interfaces. *Current Opinion in Colloid & Interface Science*, 15, 264-270.
- Lucassen-Reynders, E. H., & Wasan, D. T. (1993). Interfacial viscoelasticity in emulsions and foams. *Food Structure*, 12, 1-12.
- Mine, Y. (1995). Recent advances in the understanding of egg white protein functionality. *Trends in Food Science & Technology*, 6, 225-232.
- Mulder, W. J. (2010). Proteins in biomass streams. In Wageningen: Food & Biobased Research.
- Murray, B. S. (2011). Rheological properties of protein films. *Current Opinion in Colloid & Interface Science*, 16, 27-35.
- Nelson, J. W., & Creighton, T. E. (1994). Reactivity and ionization of the active site cysteine residues of dsba, a protein required for disulfide bond formation in vivo. *Biochemistry*, 33, 5974-5983.
- Okumura, K., Miyake, Y., Taguchi, H., & Shimabayashi, Y. (1989). Enhanced stability of protein foam due to disulfide bond formation just after foaming. *Agricultural and Biological Chemistry*, 53, 2029-2030.
- Okumura, K., Miyake, Y., Taguchi, H., & Shimabayashi, Y. (1990). Formation of stable protein foam by intermolecular disulfide cross-linkages in thiolated .Alpha.S1-casein as a model. *Journal of Agricultural and Food Chemistry*, 38, 1303-1306.
- Peng, D., Jin, W., Li, J., Xiong, W., Pei, Y., Wang, Y., Li, Y., & Li, B. (2017). Adsorption and distribution of edible gliadin nanoparticles at the air/water interface. *Journal of Agricultural and Food Chemistry*, 65, 2454-2460.
- Peng, D., Jin, W., Tang, C., Lu, Y., Wang, W., Li, J., & Li, B. (2018). Foaming and surface properties of gliadin nanoparticles: Influence of pH and heating temperature. *Food Hydrocolloids*, 77, 107-116.
- Pickering, S. U. (1907). Cxcvi.-emulsions. *Journal of the Chemical Society, Transactions*, 91, 2001-2021.
- Poole, L. B. (2015). The basics of thiols and cysteines in redox biology and chemistry. *Free Radical Biology and Medicine*, 80, 148-157.
- Ramsden, W. (1903). Separation of solids in the surface-layers of solutions and 'suspensions' (observations on surface-membranes, bubbles, emulsions, and mechanical coagulation). -- preliminary account. *Proceedings of the Royal Society of London*, 72, 156-164.
- Rodrigues, I. M., Coelho, J. F. J., & Carvalho, M. G. V. S. (2012). Isolation and valorisation of vegetable proteins from oilseed plants: Methods, limitations and potential. *Journal of Food Engineering*, 109, 337-346.
- Singh, R., & Whitesides, G. M. (1993). Thiol—disulfide interchange. In S. Patai & Z. Rappoport (Eds.), *Sulphur-containing functional groups* (pp. 633-658): John Wiley & Sons.

- Tatham, A. S., Field, J. M., Smith, S. J., & Shewry, P. R. (1987). The conformations of wheat gluten proteins, ii, aggregated gliadins and low molecular weight subunits of glutenin. *Journal of Cereal Science*, 5, 203-214.
- Tatham, A. S., Marsh, M. N., Wieser, H., & Shewry, P. R. (1990). Conformational studies of peptides corresponding to the coeliac-activating regions of wheat α -gliadin. *Biochemical Journal*, 270, 313-318.
- Tatham, A. S., & Shewry, P. R. (1985). The conformation of wheat gluten proteins - the secondary structures and thermal stabilities of alpha-gliadins, beta-gliadins, gamma-gliadins and omega-gliadins. *Journal of Cereal Science*, 3, 103-113.
- Tavernier, I., Wijaya, W., Van der Meeren, P., Dewettinck, K., & Patel, A. R. (2016). Food-grade particles for emulsion stabilization. *Trends in Food Science & Technology*, 50, 159-174.
- Van Der Borgh, A., Goesart, H., Veraverbeke, W. S., & Delcour, J. A. (2005). Fractionation of wheat and wheat flour into starch and gluten: Overview of the main processes and the factors involved. *Journal of Cereal Science*, 41, 221-237.
- Wouters, A. G. B., & Delcour, J. A. (2019). Cereal protein-based nanoparticles as agents stabilizing air-water and oil-water interfaces in food systems. *Current Opinion in Food Science*, 25, 19-27.
- Wouters, A. G. B., Rombouts, I., Legein, M., Fierens, E., Brijs, K., Blecker, C., & Delcour, J. A. (2016). Air-water interfacial properties of enzymatic wheat gluten hydrolyzates determine their foaming behavior. *Food Hydrocolloids*, 55, 155-162.
- Wouters, A. G. B., Schaefer, S., Joye, I. J., & Delcour, J. A. (2019). Relating the structural, air-water interfacial and foaming properties of wheat (*triticum aestivum* L.) gliadin and maize (*zea mays* L.) zein based nanoparticle suspensions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 567, 249-259.
- Xiao, J., Li, Y., & Huang, Q. (2016a). Recent advances on food-grade particles stabilized pickering emulsions: Fabrication, characterization and research trends. *Trends in Food Science & Technology*, 55, 48-60.
- Xiao, J., Wang, X. a., Perez Gonzalez, A. J., & Huang, Q. (2016b). Kafirin nanoparticles-stabilized pickering emulsions: Microstructure and rheological behavior. *Food Hydrocolloids*, 54, 30-39.
- Zou, Y., van Baalen, C., Yang, X., & Scholten, E. (2018). Tuning hydrophobicity of zein nanoparticles to control rheological behavior of pickering emulsions. *Food Hydrocolloids*, 80, 130-140.

TABLE 1

Table 1: Average size, polydispersity-index (PDI) and zeta potential (ZP) of a wheat gliadin (WG) nanoparticle (WGNP) suspension containing 1.0% protein/volume (1.0% w_p/v), the same after adjustment to pH 4.0 and after solvent removal via rotary solvent evaporation (RSE) and subsequent dilution to 1.0% w_p/v with deionized water. Lowercase letters represent significant (P < 0.05) differences of a given parameter between different samples.

	Average size (nm)	PDI (-)	ZP (mV)
WGNP	190.7 ± 10.6 ^b	0.067 ± 0.041 ^b	17.3 ± 1.9 ^a
WGNP - pH 4.0	220.6 ± 6.7 ^a	0.219 ± 0.029 ^a	18.3 ± 1.4 ^a
WGNP - pH 4.0 - RSE	212.8 ± 7.2 ^a	0.232 ± 0.023 ^a	14.8 ± 0.6 ^b

FIGURE CAPTIONS

Figure 1: Evolution in time of foam volume (a), surface tension (γ) (b), surface dilatational elastic (E') (c) and viscous (E'') (d) moduli of wheat gliadin nanoparticle (WGNP) suspensions containing 0.1% protein/volume (0.1% w_p/v) at pH 4.0, 4.5, 5.0, 5.5 and 6.0.

Figure 2: Average size, polydispersity-index (PDI) and zeta potential of wheat gliadin nanoparticle (WGNP) suspensions containing 0.1% protein/volume (0.1% w_p/v) at pH 4.0, 4.5, 5.0, 5.5 and 6.0. Lowercase letters represent significant ($P < 0.05$) differences of a given parameter between different samples.

Figure 3: Fourier Transform Infra-Red spectra (a) and the second derivative spectra thereof (b) of a wheat gliadin solution in 70.0% v/v ethanol and of wheat gliadin nanoparticle (WGNP) suspensions containing 1.0% protein/volume (1.0% w_p/v) at pH 4.0, 4.5, 5.0, 5.5 and 6.0. Characteristic wavenumbers for various secondary structure features are marked in the spectra. A.U. are arbitrary units.

Figure 4: Intrinsic (a) and extrinsic (b) [induced by binding of 6-propionyl-2-dimethylaminonaphthalene (PRODAN) to hydrophobic patches on the surface of proteins] fluorescence spectra of wheat gliadin nanoparticle (WGNP) suspensions containing 0.01% protein/volume (0.01% w_p/v), at pH 4.0, 4.5, 5.0, 5.5 and 6.0. A.U. are arbitrary units.

Figure 5: Representative transmission electron microscopy images of wheat gliadin nanoparticle (WGNP) suspensions containing 0.1% protein/volume ($0.1\% w_p/v$) at pH 4.0, 4.5, 5.0, 5.5 and 6.0. Two different magnifications (mag) are shown for the same region.

Figure 6: Representative cryo scanning electron microscopy images of foams produced from wheat gliadin nanoparticle (WGNP) suspensions containing 1.0% protein/volume ($1.0\% w_p/v$). Smooth remnants of gas cell interfaces and the matrix material in between gas cells (image obtained from foam produced at pH 4.0) (a) as well as a Laplace region, a typical feature for closely stacked foams (image obtained from foam produced at pH 6.0) (b) can be observed.

Figure 7: Representative cryo scanning electron microscopy images of foams produced from wheat gliadin nanoparticle (WGNP) suspensions containing 1.0% protein/volume ($1.0\% w_p/v$) at pH 4.0 and 6.0. Two different magnifications (mag) for the same region are shown.

Figure 8: Average size, polydispersity-index (PDI) and zeta potential of wheat gliadin nanoparticle (WGNP) suspensions containing 0.1% protein/volume ($0.1\% w_{\text{protein}}/v$) at pH 4.0, 4.5, 5.0, 5.5 and 6.0, as well as of their respective foam and liquid fractions as obtained by foam fractionation.

Figure 9: Protein concentrations, expressed relative to the protein concentration of the original wheat gliadin nanoparticle (WGNP) suspensions, of WGNP suspensions containing 0.1% protein/volume ($0.1\% w_{\text{protein}}/v$) at pH 4.0, 4.5, 5.0, 5.5 and 6.0, as well as of their respective

foam and liquid fractions as obtained by foam fractionation. Protein concentrations under non-reducing (a) and reducing (b) conditions are shown.

Figure 10: Intrinsic fluorescence spectra of wheat gliadin nanoparticle (WGNP) suspensions containing 0.01% protein/volume (0.01% w_{protein}/v) at pH 4.0, 4.5, 5.0, 5.5 and 6.0, as well as of their respective foam and liquid fractions obtained by foam fractionation. A.U. are arbitrary units.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

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FIGURE 6

FIGURE 7

FIGURE 8

FIGURE 9

FIGURE 10

HIGHLIGHTS

- Wheat gliadin nanoparticles (WGNPs) display excellent foam stability at pH 6.0 but not at pH 4.0
- WGNP surface properties and morphology in suspension did not vary much with pH
- Interfacial films with high viscoelasticity were formed at pH 6.0 but not at pH 4.0
- Disulfide bond formation occurred extensively upon interfacial adsorption at pH 6.0
- High foam stability at pH 6.0 is due to intermolecular cross-linking at the interface

CONFLICT OF INTEREST

None.