**Relating the structural, air-water interfacial and foaming properties of
wheat (*Triticum aestivum* L.) gliadin and maize (*Zea mays* L.) zein based
nanoparticle suspensions**

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1. INTRODUCTION

Air-in-water or oil-in-water dispersions such as foams and emulsions determine the texture and organoleptic properties of food products such as sauces, margarines, salad dressings, meringues, chocolate mousses, coffee foams and many more. Traditionally, foams and emulsions are stabilized by low molecular mass surfactants [1, 2] or proteins [1, 3]. Their amphiphilicity allows them to adsorb at oil-water or air-water interfaces and to thereby exert stabilizing effects. Alternatively, interfaces may be stabilized by solid particles. Such mechanism has been introduced in the early 1900s [4, 5] and is often referred to as Pickering stabilization. Upon adsorption of particles at an interface, they form a strong mechanical barrier which results in very high resistance against coalescence and disproportionation [6, 7, 8, 9]. However, not all particles adsorb at fluid interfaces equally effectively as they must have some affinity for both phases of the dispersion [10].

The affinity of a particle for the phases (α and β) of a dispersion is characterized by its contact angle ($\theta_{\alpha\beta}$) at the interface [7]. When $\theta_{\alpha\beta} = 90^\circ$, a particle has an equal affinity for both phases. Once adsorbed, the energy needed for a spherical particle to desorb from an interface between two fluids is given by

$$E = \pi r^2 \gamma_{\alpha\beta} (1 - |\cos \theta_{\alpha\beta}|)^2$$

in which r is the radius of the particle and $\gamma_{\alpha\beta}$ the surface tension between both immiscible phases [7, 9]. Provided that the contact angle is between 30° and 150° and that the particles have dimensions in a 1 nm to 1 μm range, the desorption energy is several orders of magnitude larger

than the thermal energy of Brownian motion. This results in such particles being practically irreversibly adsorbed [7, 8, 10].

Thus far, research on particle-stabilized emulsions and foams has mostly focused on particles based on silica [11] or latex [12]. However, such particles can be used neither in pharmaceutical nor in food systems. Over the past few years, the research focus in this field has shifted towards food-grade, biodegradable particles. Examples of such particles include modified fat crystals and polysaccharides [13]. However, proteins (which in many instances are quite surface-active and possess inherent molecular flexibility) are a very promising class of food-grade material for producing nanoparticles (NPs) or microparticles [14].

Animal proteins are used in a variety of food systems. While they have excellent functional and nutritional properties, their production is associated with high costs [15] and environmental impacts [16, 17, 18]. As a result, interest in and research on plant proteins and their functional properties have risen substantially over the past decade [19, 20]. As part of this general trend, the possibility to use them to produce particles has been investigated. The focus thus far has often been on encapsulating active compounds for controlled release or improved stability throughout the digestion tract [21]. However, as described above, such particles can also stabilize foams and emulsions.

A very promising approach for producing protein particle dispersions is liquid anti-solvent precipitation [21]. In such procedure, the quality of the solvent in which a protein is dissolved, is decreased by adding a second liquid. This leads to controlled protein aggregation and under appropriate conditions to homogeneously dispersed NP suspensions [21]. Other advantages of such approach include its high reproducibility and tunability in terms of particle size. Furthermore,

no specialized equipment is required and the technique is up-scalable and industrially feasible [21]. Protein NPs produced this way include, amongst others, those from wheat gliadins [22, 23, 24, 25], maize zeins [26, 27, 28, 29], sorghum kafirins [30] and whey proteins [22].

Several studies have dealt with the use of such particles for stabilizing interfaces in a food context. de Folter et al. [27] were amongst the first to report on zein NP stabilized oil-in-water emulsions. However, their stability was not as high as brought about by other stabilizing agents. It has been suggested that zein NPs are too hydrophobic too efficiently stabilize oil-water interfaces. Several modification strategies to alter both particle hydrophobicity and colloidal stability in food system relevant conditions have been described. These include addition of surfactants [31], tannic acid [29, 32], gum arabic [33], chitosan [34] or sodium caseinate [35]. Similarly, NPs based on wheat gliadins have been used to stabilize emulsions [23] and foams [25, 36]. Such gliadin NP stabilized dispersions are relatively stable, although the colloidal stability of the particles themselves under food system relevant conditions is still quite low [37].

In this area, there are still several knowledge gaps. First, most studies using plant protein based NPs for interfacial stabilization were on emulsions rather than on foams. Second, the structure-function relation of these particles should be further explored. Third, much is still to be learned about the behavior of such particles at interfaces. As described above, rigid solid particles can exert 'Pickering' type stabilization, while dissolved proteins adsorb and form viscoelastic films at the interface. It largely remains to be investigated how protein based NPs behave at such interfaces.

In the present paper we investigate the air-water interface stabilization by NPs based on the plant prolamins gliadin and zein and focus on the differences between wheat gliadin and zein based

NPs (WGNPs and MZNPs respectively) both in terms of structural features [size, surface charge, surface hydrophobicity (SH)] and their ability to stabilize air-water interfaces. As endogenously present lipids may impact particle formation and/or NP functionality, a defatting procedure was employed to study the impact of these lipids. Finally, mixed gliadin-zein NPs were studied to better understand the relationship between structure and function of protein based NPs. A thorough and comprehensive approach such as the one used here is imperative for a better understanding of the structure-function relationship of protein based NP suspensions and their behavior in food systems.

2. MATERIALS AND METHODS

2.1 Materials

Wheat gluten powder was kindly provided by Tereos Syral (Aalst, Belgium) and maize zein powder was from Sigma-Aldrich (Bornem, Belgium). They respectively contained 79.7% (N x 5.7) and 94.5% (N x 6.25) protein on dry matter (dm) basis as determined using an adaptation of AOAC Official Method 990.03 [38] to an EA1108 Elemental Analyzer (Carlo Erba/Thermo Scientific, Waltham, MA, USA). Their respective moisture contents [determined with AACCI International Method 44-19.01 [39]] were 5.3% and 5.0% on dm basis. Gliadin was extracted from wheat gluten using 70.0% v/v aqueous ethanol. Extracts were obtained in a gluten to solvent ratio of 1:10 (w:v) by shaking at room temperature for 60 min followed by centrifugation (12,000 *g* for 10 min) and filtration of the supernatant. Ethanol was removed by rotary evaporation under partial vacuum

at 40 °C and the resultant suspension was frozen with liquid nitrogen and freeze dried. The resulting gliadin powder had a protein content of 86.5% (determined as above, N x 5.7) on dry matter basis. All chemicals, solvents and reagents were from Sigma-Aldrich unless specified otherwise. All filtrations were with Whatman (Maidstone, UK) paper filters (pore size 4-7 µm).

2.2 Defatting of gliadin and zein powders

Gliadin and zein powders (see section 2.1) were defatted based on a protocol described by Bligh & Dyer [40]. Samples (300 mg) were accurately weighed in a test tube, after which 2.0 mL chloroform, 2.0 mL methanol and 1.8 mL deionized water were sequentially added. After addition of each individual solvent, the test tubes were vigorously shaken. After centrifugation [20 min, 500 *g*, room temperature (23 °C)], a lower lipid containing chloroform phase and an upper non-lipid material containing methanol-water phase separated by a residue could be distinguished. The upper and lower phases were isolated separately in tared test tubes and the extraction was repeated on the residue, after which the chloroform and methanol-water phases from both extractions were each pooled. The chloroform was evaporated with a rotary vacuum concentrator (Martin Christ, Osterode am Harz, Germany) and the resultant lipid extract accurately weighed. The methanol-water phases were added back to the pooled residues. The methanol was removed through rotary evaporation under partial vacuum at 40 °C. The defatted gliadin and zein were then obtained by subsequent freeze-drying as outlined above. An identical procedure was also performed on gliadin and zein powders in which both the chloroform and methanol-water phases were added back to the extracted residue. This served to

investigate the impact of the solvent treatment associated with the defatting procedure on the non-lipid gliadin and zein powder constituents. Such samples are further referred to as ‘treated’ samples. Both defatted and treated samples were prepared in six-fold and pooled prior to use for NP production.

2.3 Production of nanoparticles

WGNPs and MZNPs were obtained by liquid anti-solvent precipitation. Stock solutions containing 2.50% gliadin or zein protein (prepared from their powders as well as from their defatted or treated powders, see section 2.2) were prepared in 70.0% v/v aqueous ethanol. Also prepared were stock solutions containing 1.88% gliadin protein and 0.063% zein protein or 1.25% gliadin and 1.25% zein protein in 70.0% v/v aqueous ethanol. To produce particles, an aliquot (2.0 mL) of the respective stock solutions was added dropwise over a 10 s period above the center of a beaker (internal diameter 6.5 cm) containing 28.0 mL deionized water while continuously stirring at 440 rpm. The particle suspension was then stirred for an additional 30 s. The resulting suspensions were diluted with 4.67% v/v aqueous ethanol to obtain a protein concentration of 0.10% for further analyses. All further mentions of NPs based on gliadin (WGNPs) and zein (MZNPs) stock solutions, defatted or treated gliadin (D-WGNPs and T-WGNPs) or zein (D-MZNPs and T-MZNPs) stock solutions or mixed gliadin-zein (e.g. WGNP₇₅MZNP₂₅ refers to a sample based on a stock solution of 1.88% gliadin and 0.063% zein protein) stock solutions refer to such diluted suspensions. WGNP and MZNP suspensions had pH values of 6.0 and 4.5, respectively. WGNP

suspensions at pH 4.5 and MZNP suspensions at pH 5.5 were also prepared by adding 0.1 M HCL or 0.1 M NaOH to freshly prepared particle suspensions.

2.4 Measurement of particle size, polydispersity and zeta potential

Particle size distribution and polydispersity-index (PDI) measurements were based on dynamic light scattering and zeta potential (ZP) measurements on laser Doppler electrophoresis. All above described NP suspensions (see section 2.3) were transferred to a disposable capillary zeta cell (Malvern Instruments, Malvern, United Kingdom) to then determine the particle size distribution and ZP in a Malvern Zetasizer Nano ZS. Particle size measurements yielded scattering patterns which were converted into a correlation function which provides information on the diffusion coefficients of the dispersions. The latter then allowed calculating particle size distributions with the Stokes-Einstein relationship using the Malvern Zetasizer software. For ZP measurements an electrical field was applied which caused particles to move at a rate which depends on their charge. The resulting scattering pattern allowed determining the electrophoretic mobility and the ZP value, the latter with the above mentioned software. Measurements were at room temperature (23 ± 2 °C). The viscosity of 4.67% v/v ethanol was 1.0038 mPa.s [calculated based on Khattab, Bandarkar, Fakhree & Jouyban [41]], its refractive index was 1.335 [extrapolated from experimental values obtained by Belda, Herraes & Diaz [42]], and its dielectric constant 76.2 [calculated as in Akerlof [43]].

2.5 Fluorescence measurements

NP surface characteristics were assessed using intrinsic and extrinsic fluorescence measurements. Intrinsic fluorescence was based on the emission of fluorescent light by tryptophan and tyrosine residues in the proteins. All above described NP suspensions (see section 2.3) at a concentration of 0.01% protein were transferred to a quartz cuvette and introduced in a Fluoromax 4 fluorospectrophotometer (Horiba Jobin Yvon, Edison, NJ, USA). The excitation wavelength was 280 nm with a 2 nm slit width and the emission spectrum was recorded from 290 to 410 nm with the same slit width. The spectrum of 4.67% v/v ethanol was subtracted from spectra obtained for samples. Extrinsic fluorescent measurements were performed by using the uncharged hydrophobic fluorescent probe 6-propionyl-2-dimethylaminonaphthalene (PRODAN) and the recorded maximum emission intensities were indicative for the SH of the NPs. A 2.0 mL aliquot of each of the above described NP suspensions (see section 2.3) at a concentration of 0.03% protein were incubated for 5 min in the dark with 10 μ L 0.1025 mM PRODAN in methanol. These samples were transferred to a quartz cuvette and analyzed by fluorospectrophotometry as above. The excitation wavelength was 365 nm with a 4 nm slit width and the emission spectrum recorded from 390 to 590 nm with the same slit width. The spectrum of 4.67% v/v ethanol incubated with PRODAN in the same way was subtracted from all sample spectra. All measurements were conducted at a protein concentration which rendered a fluorescent signal in the linear range. Measurements were conducted at room temperature (23 ± 2 °C).

2.6 Foaming characteristics

Foaming properties were determined with the stirring test used earlier by Wouters et al. [44]. An aliquot (50.0 mL) of the above described (section 2.3) NP suspensions (0.10% protein in 4.67% v/v

ethanol) was placed in a graduated glass cylinder (internal diameter 60.0 mm) in a water bath at 25 °C. After equilibration for 15 min, the NP suspension was 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 of WGNP and MZNP suspensions were measured after 2, 4, 10, 15, 30, 45, 60, 120, 240, 480 and 1,440 min after the start of stirring. Foam heights of all other samples were measured up to 60 min after the start of stirring. This was sufficient to distinguish between samples. Foam volumes (in mL) were calculated from foam heights and the cylinder internal diameter. The foaming capacity was assessed as the foam volume 2 min after the start of whipping, while foam stability was assessed as the evolution of the foam volume over time.

2.7 (Oscillating) pendant drop measurements

(Oscillating) pendant drop measurements were performed in a procedure similar to that of Wouters et al. [45]. All above described (section 2.3) NP suspensions (0.10% protein in 4.67% v/v ethanol) were introduced in a Theta optical tensiometer (Biolin Scientific Attension, Stockholm, Sweden) to create a pendant drop with a fixed volume of 8 μ L. In a first type of measurement, surface tension γ of the pendant drop was recorded over the course of 10 min based on drop shape analysis and a Young-Laplace fit. During the measurements, images were recorded at one frame per 7 s. In a second type of measurement a sinusoidal oscillation of the drop (5 cycles) at 0.5 Hz with an amplitude set at 1.00 in the OneAttension software (Biolin Scientific Attension) was performed at several time points after drop creation. The set amplitude corresponded to a

volume change of $\pm 1 \mu\text{L}$ and was within the linear regime. During such oscillations, images were recorded at 7 frames per 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, at which point the measurements were concluded. Based on drop shape analysis during oscillation, the surface dilatational modulus E could be determined. E is the variation in surface tension γ per unit relative change in surface area (A) [46, 47]. The following relation holds

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

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 [46, 47].

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

Both surface dilatational elastic and viscous moduli E' and E'' are reported here. After each measurement, the device was thoroughly cleaned and the surface tension of pure water was checked to be $72.0 \pm 0.5 \text{ mN/m}$ before initiating the next measurement.

2.8 Statistical analyses

All determinations of particle size, ZP, fluorescent measurements, foaming properties and oscillating pendant drop measurements were at least in four-fold. Error bars and values represent standard deviations from the mean. All 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 Structural and functional characteristics of gliadin and zein based nanoparticles

Table 1 shows average values for the size (Z-average), PDI and ZP of WGNP and MZNP suspensions, immediately after production. WGNP and MZNP suspensions had pH 6.0 and pH 4.5 respectively and are further referred to as pH unadjusted samples. All particles were stable (no changes in size, PDI or ZP; data not shown) for at least 24 hours after production. Nonetheless, all analyses were carried out on freshly produced NP suspensions. pH unadjusted WGNPs were larger ($P < 0.05$) than pH unadjusted MZNPs while the latter had a higher ($P < 0.05$) PDI value and thus a less homogeneous particle size distribution than the former. Furthermore, pH unadjusted MZNPs had much higher ($P < 0.05$) ZP than pH unadjusted WGNPs. As mentioned, pH unadjusted WGNP and MZNP suspensions had pH values of 6.0 and 4.5, respectively, which likely explains the higher ZP of the latter. For the sake of rational comparison between samples, Table 1 also shows size, PDI and ZP of WGNPs at pH 4.5 and MZNPs at pH 5.5. The latter pH rather than pH 6.0 was chosen and deemed a valid basis for comparison because MZNP precipitate at a pH of about 5.7. Indeed, MZNPs at pH 5.5 were larger and had much lower ZP ($P < 0.05$) than at pH 4.5. This of course is related to the proximity to the point-of-zero-charge at pH 5.5. Interestingly, MZNPs at pH 5.5 had a more homogeneous size distribution than at pH 4.5. Similar observations were made for WGNPs. They had higher PDI and higher ZP ($P < 0.05$) at pH 4.5 than at pH 6.0. Nevertheless,

there was no significant ($P > 0.05$) difference in size between WGNPs at pH 4.5 or pH 6.0. Also, the ZP of WGNPs at pH 4.5 was still substantially lower ($P < 0.05$) than that of MZNPs at the same pH.

Figure 1 shows intrinsic and extrinsic fluorescence spectra of WGNP [pH 4.5 and pH 6.0 (pH unadjusted)] and MZNP [pH 4.5 (pH unadjusted) and pH 5.5]. Excitation of intrinsically present fluorophores in the protein (such as tryptophan and tyrosine) results in fluorescence of which both the intensity and the wavelength of maximal intensity provide information on the local environment of these fluorophores and thus on the conformational or aggregation state of the protein NPs [48]. Typically, exposure of proteins to 280 nm light results in excitation of both tyrosine and tryptophan residues.

In the present case, pH unadjusted WGNPs showed a maximal fluorescence intensity at about 352 nm, which is typical for the emission by tryptophan [49]. At about 310 nm, which is close to the wavelength at which tyrosine typically emits light after excitation at 280 nm [49], only a slight inflection in the fluorescence spectrum was observed. Thus, the fluorescent signal of WGNPs is dominated by tryptophan residues even though tryptophan and tyrosine respectively make up about 0.4% and 2.0% (on a molar basis) of the amino acids in the wheat prolamin fraction [50]. This can be explained by the lower molar absorptivity of tyrosine compared to that of tryptophan. Furthermore, tyrosine is sensitive to quenching in the presence of tryptophan [49]. For these reasons, the fluorescence by tyrosine is usually much lower than that of tryptophan when both are present.

266 In contrast, the fluorescence spectrum of pH unadjusted MZNP showed maximum emission
267 intensity at 305 nm, which, as mentioned earlier, is consistent with light emission by tyrosine after
268 excitation at 280 nm [49]. This of course is due to the absence of tryptophan residues in the
269 primary structure of zein proteins [51], while tyrosine residues comprise about 3.6% (on a molar
270 basis) of the amino acids in zein prolamins [50].

271 Interestingly, there were no differences in maximum fluorescence intensity or maximum intensity
272 wavelength for WGNPs at pH 4.5 and pH 6.0. However, MZNPs at pH 5.5 had a higher ($P < 0.05$)
273 fluorescence intensity than at pH 4.5. MZNPs were larger at pH 5.5 than at pH 4.5 (Table 1).
274 Possibly, swelling of MZNPs in proximity of the point-of-zero-charge altered their tyrosine
275 fluorescent signal. Indeed, Förster distances for resonance energy transfer between tyrosine
276 residues of 0.9-1.6 nm have been reported [49]. Considering that the average size of MZNPs was
277 approximately 97 nm at pH 4.5 and approximately 155 nm at pH 5.5 (Table 1), it is reasonable to
278 assume that energy transfer between tyrosine residues occurred to a lesser extent at pH 5.5,
279 resulting in lower ($P < 0.05$) fluorescence at pH 4.5 than at pH 5.5. That WGNPs at pH 4.5 and 6.0
280 had rather similar sizes (Table 1) and fluorescent emission spectra (Figure 1a) to some extent
281 supports this hypothesis.

282 Extrinsic fluorescent signals were obtained by PRODAN binding to hydrophobic patches at the
283 surface of the NPs [52, 53]. Doing so shifted the maximum emission intensity wavelength of
284 unbound PRODAN (at about 520 nm) to about 450 nm [53]. pH unadjusted MZNPs had much
285 higher ($P < 0.05$) PRODAN-induced fluorescence intensity and thus SH than pH unadjusted WGNPs
286 (Figure 1b). Furthermore, differences in pH did not cause significant differences ($P > 0.05$) in

fluorescence intensity or in the wavelengths of maximum intensity both for WGNPs and MZNPs. MZNPs thus had higher SH than WGNPs at both pHs.

Figure 2a shows the foaming properties of suspensions of WGNPs at pH 4.5 and pH 6.0 (pH unadjusted) and of MZNPs at pH 4.5 (pH unadjusted) and pH 5.5. WGNPs at pH 6.0 had both high foaming capacity as well as extremely high foam stability (section 2.6). Indeed, even after 24 hours almost 60% of the foam initially formed during stirring remained. In contrast, no foam could be formed with the pH unadjusted MZNP suspension at pH 4.5. At pH 5.5, stirring a MZNP suspension resulted in a limited amount of foam. This foam was very unstable and had already disappeared after 10 min. Stirring of a WGNP suspension at pH 4.5 or pH 6.0 resulted in similar foaming capacities, but the stability of the former foam was somewhat lower than that of the latter. Thus, overall, both for WGNPs and MZNPs foaming capacity and foam stability seemed to be higher at the higher pH. Nonetheless, the difference in foaming properties between WGNPs, which resulted in stable foams, and MZNPs, which had very poor foaming properties, was substantial.

Figure 2 also shows surface tension (b), E' (c) and E'' (d) values over the course of 10 min for WGNP (at pH 4.5 and 6.0) and MZNP (at pH 4.5 and 5.5) suspensions. In general, equilibrium surface tension had not yet been reached after 10 min, although the various curves approached a plateau value (Figure 2b). Nevertheless, there were no major differences in surface tension of the different samples. Only the surface tension curve of WGNP at pH 4.5 was slightly higher than that of the other samples, but the difference was minor. Thus, possible differences in E' and E'' of the different samples cannot be attributed to differences in surface pressure but are solely caused by inherent differences in rheological properties of the adsorbed protein material. Indeed,

E' and E'' provide information on the dilatational rheology of the air-water interface and thus on the viscoelasticity of the adsorbed layer thereat. E' values were higher than E'' values for all samples (Figure 2c-d) implying that the adsorbed layer's interfacial behavior was elastic rather than viscous. Figure 2c-d further shows that, most notably for WGNP, E' and E'' increased over time, suggesting the formation of a viscoelastic protein film at the interface. A similar observation was made in a very recent study. Our findings are indeed in line with those of Peng et al. [25] who reported that adsorption of gliadin based NPs at the air-water interface is accompanied by formation of an adsorbed layer with high E values. Interestingly, all this could be related to the foaming characteristics of WGNP and MZNP suspensions. Indeed, samples with high E' values (WGNP suspensions) also had high foaming capacity and foam stability. In general, increases of E' and E'' for protein solutions is typically associated with continuous adsorption and mutual interaction of protein molecules at the interface which results in a viscoelastic protein film. Additionally, high E values of such adsorbed protein films are often related to the protein's ability to form stable foams [3]. Here, similar observations were made for proteins occurring as a suspension of NPs of which the interfacial behavior has not been thoroughly described earlier. Indeed, very few studies in literature deal with the topic. When solid inert particles adsorb at the interface, generally no coherent elastic layer is formed at the interface. However, for NPs based on the proteins used here, this apparently is the case.

In summary, when adsorption of NPs at the interface results in high foaming capacity and foam stability, this is accompanied by the build-up of an adsorbed protein film characterized by high E values in dilatational rheological measurements. Indeed, WGNP suspensions had excellent

foaming properties and high interfacial E values, while MZNP suspensions resulted in very poor foaming and low E values. However, the underlying cause for these observations remains unclear. A likely explanation is that this difference arises from the much higher SH of MZNPs than of WGNPs, as shown by fluorescence spectroscopy, especially since differences in pH and thus surface charge were not be the determining factor.

In what follows, we explore whether the endogenous lipids in wheat gliadin and maize zein powders might be at the basis for the difference in their SH but, more importantly, also of their ability to stabilize air-water interfaces.

3.2 Role of endogenous wheat and maize lipids

Gliadin and zein powders contained 4.65% and 6.79% lipids on dry matter basis, respectively. The impact of these endogenous lipids on particle formation was investigated by defatting the powders prior to particle production. To assess the impact of the solvent treatment associated with the defatting procedure on the other, non-lipid, constituents present in these powders, 'treated' samples were also investigated (see section 2.2).

Table 2 shows that there were no significant differences in average size between the regular, defatted and treated WGNPs. The same held true for MZNPs. Indeed, PDI values of WGNPs and MZNPs were also not significantly ($P > 0.05$) affected by defatting or 'treatment' of the samples. ZP values of WGNPs, D-WGNPs and T-WGNPs were also not significantly different ($P > 0.05$).

The only notable difference here was observed for D-MZNPs, which had significantly lower ($P < 0.05$) ZP than MZNPs and T-MZNPs. Figure 3a-b shows intrinsic and extrinsic fluorescence spectra of regular, defatted and treated WGNP and MZNP suspensions. There was impact neither on

maximum fluorescence intensity nor on maximum intensity wavelength of the defatting or treatment procedure on WGNP or MZNP suspensions ($P > 0.05$). This was the case both for intrinsic and PRODAN-induced fluorescence.

Overall, neither defatting nor the solvent treatment associated therewith seemed to have a notable impact on particle properties (size, PDI, ZP and SH). It thus seems that endogenous lipids do not play a crucial role in particle formation, as has been suggested [24]. Furthermore, treatment with chloroform or methanol had no impact on the proteins in the gliadin and zein powders in terms of their ability to aggregate into NPs when subjected to an anti-solvent precipitation procedure.

Figure 4a clearly illustrates that WGNP samples consistently had better foaming properties than MZNP samples. Some foam could be formed by stirring a D-MZNP suspension but after 10 min no foam remained. Stirring of MZNPs and T-MZNPs did not result in any foam. In contrast, WGNPs, D-WGNPs and T-WGNPs all led to similarly high initial foam volumes as well as high foam stabilities. In an attempt to differentiate between the different WGNP samples, an additional foaming test was performed at lower protein concentration (0.030%, Figure 5a). Here, it seemed that D-WGNP foams possibly had slightly higher stability than those of WGNPs and T-WGNPs, although differences were limited at best. Moreover, the foaming characteristics of MZNPs at pH 5.5 were slightly better than at pH 4.5. To further assess the possible impact of pH, Figure 5b also shows an additional foam test in which defatted and treated MZNP were tested at pH 5.5. However, this also did not result in improved foaming characteristics.

Figure 4b also shows the evolution of surface tension over time for the different regular, treated and defatted samples. In line with what was observed in Figure 2b, surface tension curves of regular and treated WGNPs and MZNPs were very similar. The curves of D-WHNPs and D-MZNPs were characterized by slightly higher surface tensions, which indicates that lipids at least to some extent were present at the air-water interface. More interestingly, foaming properties of the different defatted, treated and regular WGNPs and MZNPs seemed in line with their behavior in interfacial dilatational experiments (Figure 4c-d). Indeed, both E' and E'' values over a 10 min time span of all MZNP samples were substantially lower than E values of the different WGNP samples. While there seemed to be no notable differences between the respective E' and E'' values of WGNPs and T-WGNPs, E' and E'' of D-WGNPs increased more pronouncedly over time than did those of WGNPs and T-WGNPs. That D-WGNPs had higher E' and E'' values than WGNPs is in line with what is known about the co-occurrence of proteins and lipids at air-water interfaces. Indeed, while proteins adsorb, mutually interact and thereby form a viscoelastic protein film, lipids form a highly mobile fluid monolayer [2]. These two different interfacial phenomena counteract each other when both proteins and lipids co-occur at an interface. This usually results in low interfacial stability [54]. Here, an increase in E' and E'' as a result of removing the lipids from the samples was noted. This suggests that even though lipids do not directly affect particle formation (see above) they do to some extent weaken the adsorbed protein film at the air-water interface. That only slight differences in foam stability between D-WGNPs and WGNPs were observed suggests that this effect is rather small in the present system. Nonetheless, that a similar phenomenon as that described for proteins and lipids in solution seems to occur here may imply that the protein particles upon adsorption at least to some extent disintegrate and 'unfold' at the interface and

mutually interact to form a viscoelastic protein film. For the different MZNP samples this was not observed probably because E' and E'' of MZNPs were too low to start off with.

Thus, the presence of endogenous lipids could not explain the differences in the ability of WGNPs and MZNPs to stabilize air-water interface. Considering that particle size and surface charge (see section 3.1) could not explain these differences, they must arise from differences in the inherent hydrophobicity of the proteins (see Figure 3a-b). Indeed, it has been proposed that zein protein based NPs rapidly aggregate at interfaces, thereby leading to low surface coverage [55]. Similarly, pH unadjusted zein based NPs are poor emulsion stabilizers presumably because of their high hydrophobicity [32]. Nonetheless, removing the lipids from gliadin powder supported the observation that for these protein based NPs to efficiently stabilize interfaces, they need to be able to adsorb, unfold and mutually interact at the interface, which is the case for WGNPs but not for MZNPs.

In a final part, mixed gliadin and zein based NPs were produced to assess whether such hybrid particles have improved functionality at the air-water interface. Doing so also allowed further testing our hypothesis regarding the formation of a viscoelastic protein film at the air-water interface and its relation to foam stability.

3.3 Mixed gliadin-zein nanoparticles

Hybrid gliadin-zein particles were produced by replacing 25% or 50% of the gliadin protein in the stock solution by zein protein (see section 2.3). Regular WGNP and MZNP in this section are referred to as WGNP₁₀₀ and MZNP₁₀₀, respectively. As already mentioned, WGNP₁₀₀ had higher average size than MZNP₁₀₀. Upon replacement of 25% of gliadin by zein protein material (resulting

420 in WGNP₇₅MZNP₂₅), the average particle size decreased significantly ($P < 0.05$). When even more
421 zein was present in the stock solution used to produce WGNP₅₀MZNP₅₀ and MZNP₁₀₀, the particle
422 size seemed to decrease even further, although it was not significantly ($P > 0.05$) different from
423 that of WGNP₇₅MZNP₂₅. As could be expected, an opposite trend was observed for the PDI values
424 of the different mixed particles. Indeed, MZNP₁₀₀ had higher PDI than WGNP₁₀₀, while
425 WGNP₇₅MZNP₂₅ had a PDI similar to that of WGNP₁₀₀ and that of WGNP₅₀MZNP₅₀ did not differ
426 significantly ($P > 0.05$) from those of any of the other samples. Finally, ZP of MZNP₁₀₀ was much
427 higher ($P < 0.05$) than those of the other samples, which had similar ZP values.

428 Figure 6a-b shows that also in terms of intrinsic and extrinsic (PRODAN induced) fluorescence, a
429 gradual transition from WGNP₁₀₀ towards MZNP₁₀₀ was observed. Indeed, while MZNP₁₀₀ and
430 WGNP₁₀₀ had maximum emission intensities at wavelengths of about 305 nm and 350 nm,
431 respectively, the mixed gliadin-zein NPs resulted in a fluorescent signal at both wavelengths with
432 intensities intermediate to those of the WGNP₁₀₀ and MZNP₁₀₀ suspensions. Similarly, while
433 MZNP₁₀₀ had much higher SH as assessed by PRODAN-induced fluorescence than WGNP₁₀₀, the
434 hydrophobicity of WGNP₇₅MZNP₂₅ and WGNP₅₀MZNP₅₀ was intermediate between those of these
435 two. Thus, it seemed that by using a stock solution containing both gliadin and zein proteins
436 particles could be produced with surface characteristics intermediate to those of the gliadin and
437 zein only based NPs. Nevertheless, a similar trend was not fully observed in terms of the foaming
438 characteristics of the mixed gliadin-zein NPs (Figure 7a). While at first glance it may look as if
439 WGNP₇₅MZNP₂₅ and WGNP₅₀MZNP₅₀ display foaming characteristics intermediate to those of
440 WGNP₁₀₀ and MZNP₁₀₀, the hybrid particles had performed worse than expected. Indeed, the

foam stability of WGNP₇₅MZNP₂₅ was substantially lower than that of WGNP₁₀₀ while that of WGNP₅₀MZNP₅₀ was even almost as poor as that of MZNP₁₀₀.

Figure 5a illustrates that this was not what we expected. Indeed, a WGNP₁₀₀ suspension containing 0.03% protein had excellent foam stability. That WGNP₇₅MZNP₂₅ and WGNP₅₀MZNP₅₀ still consisted of 75% and 50% of gliadin protein and that their foaming characteristics were tested at 0.10% protein implies that the zein proteins in the mixed gliadin-zein NPs had a detrimental effect on their functionality at the air-water interface, even if the hybrid NPs had SH intermediate to that of WGNP₁₀₀ and MZNP₁₀₀ particles. Similarly, E' and E'' values of WGNP₇₅MZNP₂₅ were substantially lower than those of WGNP₁₀₀, while E' and E'' of WGNP₅₀MZNP₅₀ were rather similar to those of MZNP₁₀₀ (Figure 7c-d). Figure 7b shows that no differences in the evolution of surface tension over time of the different mixed particles, which indicates that the observed differences in E' and E'' are due to inherent differences in film properties rather than to differences in surface pressure. All this is of course in line with the observed foaming properties of these mixed gliadin-zein NPs. It thus seems that when NPs contain zein this results in a weakly viscoelastic protein film and thus in low foam stability. This finding not only renders fundamental insights in the behavior of protein based NPs at interfaces, but possibly presents some interesting application potential. Indeed, hydrophobic particles, such as those made of hydrophobized silica are useful in anti-foaming applications [56, 57, 58]. However, to the best of our knowledge there are no published reports on edible protein based anti-foaming agents, which may be relevant for food systems. Our findings suggest that MZNPs may have such application potential.

4. CONCLUSIONS

463
464 WGNPs were larger and had a more homogeneous size distribution than MZNPs, while the latter
465 had higher ZP than the former. These differences could partly be ascribed to differences in pH,
466 although MZNPs at low pH still had much higher ZP than WGNPs at low pH. More important is
467 that MZNPs had much higher SH than WGNPs, regardless of the pH value. Defatting prior to
468 particle production revealed that wheat and maize endogenous lipids did not impact size and
469 surface charge of the NPs produced. More importantly, no impact on the SH of the NPs was
470 observed. Thus, the impact of endogenous lipids was limited at best and the high SH of the MZNPs
471 could be ascribed solely to the proteins present. It should be noted here that the properties of
472 the obtained NPs evidently depend on the properties of the protein material from which they are
473 made. Here, WG proteins were extracted from a commercial wheat gluten sample, while MZ
474 proteins were used as supplied (see section 2.1). The pre-treatment of these protein sources
475 might to some extent determine the characteristics of the particles they result in. Future research
476 to further investigate this would be desirable. Regardless, WGNPs here had excellent foaming
477 capacity and foam stability, while barely any foam could be formed with MZNPs. The foaming
478 properties of mixed gliadin-zein particles with intermediate SHs were worse than expected based
479 on their relatively high gliadin protein content. Thus, SH surely is not the only factor which
480 determines the air-water interfacial properties of plant proteins based NPs. Moreover, the
481 presence of zein proteins in NPs resulted in poor foam stability when these particles adsorbed at
482 air-water interfaces. Interestingly, all these observations regarding foaming characteristics could
483 be related to the build-up of a viscoelastic protein film at the interface upon adsorption of NPs.
484 Indeed, WGNPs had much higher E values than MZNPs, which was in line with the superior

foaming properties of the former. This suggested that for a suspension of NPs based on gliadins or zeins to be able to efficiently stabilize interfaces, they need to at least partially disintegrate and spread at the interface, thereby mutually interacting and forming a coherent viscoelastic protein film. This in itself is a worthwhile observation as it shows that the Pickering effect by which rigid NPs typically stabilize interfaces might not be the sole mechanism in play in systems where NP suspensions are produced from flexible surface-active biopolymers. We have shown that the structural (size, surface charge, surface hydrophobicity) properties of protein based NPs to a large extent but not fully explain their air-water interfacial characteristics. This illustrates the importance of thoroughly characterizing both the structural and functional properties in order to better understand their behavior in food systems. In a follow-up paper, we will conduct an in-depth investigation on how WGNPs adsorb and mutually interact at air-water interfaces and how this relates to their structural properties. Even if more research in this area is needed, it was clearly shown that controlled aggregation of some plant proteins, leading to a homogeneously distributed dispersion of NPs, is a valid strategy for improving the foaming properties of such otherwise largely insoluble and therefore poorly functional proteins in aqueous systems.

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REFERENCES

- (1) M.A. Bos, T. van Vliet, Interfacial rheological properties of adsorbed protein layers and surfactants: A review, *Adv. Colloid Interface Sci.* 91 (2001) 437-471.
- (2) P. Wilde, A. Mackie, F. Husband, P. Gunning, V. Morris, Proteins and emulsifiers at liquid interfaces, *Adv. Colloid Interface Sci.* 108 (2004) 63-71.
- (3) B.S. Murray, Rheological properties of protein films, *Curr. Opin. Colloid Interface Sci.* 16 (2011) 27-35.
- (4) S.U. Pickering, Cxvii.-emulsions, *Journal of the Chemical Society, Transactions* 91 (1907) 2001-2021.
- (5) W. Ramsden, 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 (1903) 156-164.
- (6) R. Aveyard, B.P. Binks, J.H. Clint, Emulsions stabilised solely by colloidal particles, *Adv. Colloid Interface Sci.* 100–102 (2003) 503-546.
- (7) B.P. Binks, Particles as surfactants—similarities and differences, *Curr. Opin. Colloid Interface Sci.* 7 (2002) 21-41.
- (8) S. Lam, K.P. Velikov, O.D. Velev, Pickering stabilization of foams and emulsions with particles of biological origin, *Curr. Opin. Colloid Interface Sci.* 19 (2014) 490-500.
- (9) I. Tavernier, W. Wijaya, P. Van der Meeren, K. Dewettinck, A.R. Patel, Food-grade particles for emulsion stabilization, *Trends Food Sci. Technol.* 50 (2016) 159-174.
- (10) J. Xiao, Y. Li, Q. Huang, Recent advances on food-grade particles stabilized pickering emulsions: Fabrication, characterization and research trends, *Trends Food Sci. Technol.* 55 (2016) 48-60.
- (11) B.P. Binks, S.O. Lumsdon, Stability of oil-in-water emulsions stabilised by silica particles, *Physical Chemistry Chemical Physics* 1 (1999) 3007-3016.
- (12) B.P. Binks, S.O. Lumsdon, Pickering emulsions stabilized by monodisperse latex particles: Effects of particle size, *Langmuir* 17 (2001) 4540-4547.
- (13) E. Dickinson, Biopolymer-based particles as stabilizing agents for emulsions and foams, *Food Hydrocolloid.* 68 (2017) 219-231.
- (14) E. Dickinson, Food emulsions and foams: Stabilization by particles, *Curr. Opin. Colloid Interface Sci.* 15 (2010) 40-49.
- (15) J.L. Lusk, F.B. Norwood, Some economic benefits and costs of vegetarianism, *Agricultural and Resource Economics Review* 38 (2009) 109-124.
- (16) N. Alexandratos, J. Bruinsma. World agriculture: Towards 2030/2050. Prospects for food, nutrition, agriculture and major commodity groups. An fao perspective. Interim report. Food and Agriculture Organisation. **2006**,
- (17) M. Herrero, P. Gerber, T. Vellinga, T. Garnett, A. Leip, C. Opio, H.J. Westhoek, P.K. Thornton, J. Olesen, N. Hutchings, H. Montgomery, J.F. Soussana, H. Steinfeld, T.A. McAllister, Livestock and greenhouse gas emissions: The importance of getting the numbers right, *Anim. Feed Sci. Technol.* 166-67 (2011) 779-782.
- (18) L. Reijnders, S. Soret, Quantification of the environmental impact of different dietary protein choices, *American Journal of Clinical Nutrition* 78 (2003) 664S-668S.

549 (19) L. Day, Proteins from land plants – potential resources for human nutrition and food security,
 550 Trends Food Sci. Technol. 32 (2013) 25-42.

551 (20) A.G.B. Wouters, I. Rombouts, E. Fierens, K. Brijs, J.A. Delcour, Relevance of the functional
 552 properties of enzymatic plant protein hydrolysates in food systems, Compr. Rev. Food. Sci. Food
 553 Saf. 15 (2016) 786-800.

554 (21) I.J. Joye, D.J. McClements, Production of nanoparticles by anti-solvent precipitation for use
 555 in food systems, Trends Food Sci. Technol. 34 (2013) 109-123.

556 (22) M.G. Corradini, M. Demol, J. Boeve, R.D. Ludescher, I.J. Joye, Fluorescence spectroscopy as a
 557 tool to unravel the dynamics of protein nanoparticle formation by liquid antisolvent precipitation,
 558 Food Biophysics 12 (2017) 211-221.

559 (23) Y.-Q. Hu, S.-W. Yin, J.-H. Zhu, J.-R. Qi, J. Guo, L.-Y. Wu, C.-H. Tang, X.-Q. Yang, Fabrication and
 560 characterization of novel pickering emulsions and pickering high internal emulsions stabilized by
 561 gliadin colloidal particles, Food Hydrocolloid. 61 (2016) 300-310.

562 (24) I.J. Joye, V.A. Nelis, D.J. McClements, Gliadin-based nanoparticles: Fabrication and stability
 563 of food-grade colloidal delivery systems, Food Hydrocolloid. 44 (2015) 86-93.

564 (25) D. Peng, W. Jin, J. Li, W. Xiong, Y. Pei, Y. Wang, Y. Li, B. Li, Adsorption and distribution of edible
 565 gliadin nanoparticles at the air/water interface, J. Agric. Food Chem. 65 (2017) 2454-2460.

566 (26) N. Chatsisvili, A.P. Philipse, B. Loppinet, R.H. Tromp, Colloidal zein particles at water-water
 567 interfaces, Food Hydrocolloid. 65 (2017) 17-23.

568 (27) J.W.J. de Folter, M.W.M. van Ruijven, K.P. Velikov, Oil-in-water pickering emulsions stabilized
 569 by colloidal particles from the water-insoluble protein zein, Soft Matter 8 (2012) 6807-6815.

570 (28) A.R. Patel, E.C.M. Bouwens, K.P. Velikov, Sodium caseinate stabilized zein colloidal particles,
 571 J. Agric. Food Chem. 58 (2010) 12497-12503.

572 (29) Y. Zou, J. Guo, S.-W. Yin, J.-M. Wang, X.-Q. Yang, Pickering emulsion gels prepared by
 573 hydrogen-bonded zein/tannic acid complex colloidal particles, J. Agric. Food Chem. 63 (2015)
 574 7405-7414.

575 (30) J. Xiao, X.a. Wang, A.J. Perez Gonzalez, Q. Huang, Kafirin nanoparticles-stabilized pickering
 576 emulsions: Microstructure and rheological behavior, Food Hydrocolloid. 54 (2016) 30-39.

577 (31) Y. Zou, Z. Wan, J. Guo, J. Wang, S. Yin, X. Yang, Modulation of the surface properties of protein
 578 particles by a surfactant for stabilizing foams, RSC Advances 6 (2016) 66018-66026.

579 (32) Y. Zou, C. van Baalen, X. Yang, E. Scholten, Tuning hydrophobicity of zein nanoparticles to
 580 control rheological behavior of pickering emulsions, Food Hydrocolloid. 80 (2018) 130-140.

581 (33) L. Dai, C. Sun, Y. Wei, L. Mao, Y. Gao, Characterization of pickering emulsion gels stabilized
 582 by zein/gum arabic complex colloidal nanoparticles, Food Hydrocolloid. 74 (2018) 239-248.

583 (34) L.J. Wang, S.W. Yin, L.Y. Wu, J.R. Qi, J. Guo, X.Q. Yang, Fabrication and characterization of
 584 pickering emulsions and oil gels stabilized by highly charged zein/chitosan complex particles
 585 (zccps), Food Chem. 213 (2016) 462-469.

586 (35) Y.M. Feng, Y. Lee, Surface modification of zein colloidal particles with sodium caseinate to
 587 stabilize oil-in-water pickering emulsion, Food Hydrocolloid. 56 (2016) 292-302.

588 (36) D. Peng, W. Jin, C. Tang, Y. Lu, W. Wang, J. Li, B. Li, Foaming and surface properties of gliadin
 589 nanoparticles: Influence of ph and heating temperature, Food Hydrocolloid. (2018).

590 (37) I.J. Joye, V.A. Nelis, D.J. McClements, Gliadin-based nanoparticles: Stabilization by post-
 591 production polysaccharide coating, Food Hydrocolloid. 43 (2015) 236-242.

592 (38) AOAC. Official methods of analysis. Method 990.03., Association of Official Analytical
 593 Chemists: Washington, DC, USA, 1995.

594 (39) AACCI. Aacc international method 44-19-01. Moisture - air-oven method, drying at 135°C,
 595 American Association of Cereal Chemists, 2018.

596 (40) E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Canadian
 597 Journal of Biochemistry and Physiology 37 (1959) 911-917.

598 (41) I.S. Khattab, F. Bandarkar, M.A.A. Fakhree, A. Jouyban, Density, viscosity, and surface tension
 599 of water+ethanol mixtures from 293 to 323K, Korean J. Chem. Eng. 29 (2012) 812-817.

600 (42) R. Belda, J.V. Herraiz, O. Diez, A study of the refractive index and surface tension synergy of
 601 the binary water/ethanol: Influence of concentration, Physics and Chemistry of Liquids 43 (2005)
 602 91-101.

603 (43) G. Akerlof, Dielectric constants of some organic solvent-water mixtures at various
 604 temperatures, Journal of the American Chemical Society 54 (1932) 4125-4139.

605 (44) A.G.B. Wouters, I. Rombouts, M. Legein, E. Fierens, K. Brijs, C. Blecker, J.A. Delcour, Air–water
 606 interfacial properties of enzymatic wheat gluten hydrolyzates determine their foaming behavior,
 607 Food Hydrocolloid. 55 (2016) 155-162.

608 (45) A.G.B. Wouters, I. Rombouts, E. Fierens, K. Brijs, C. Blecker, J.A. Delcour, B.S. Murray, Foaming
 609 and air-water interfacial characteristics of solutions containing both gluten hydrolysate and egg
 610 white protein, Food Hydrocolloid. 77 (2018) 176-186.

611 (46) E.H. Lucassen-Reynders, J. Benjamins, V.B. Fainerman, Dilational rheology of protein films
 612 adsorbed at fluid interfaces, Curr. Opin. Colloid Interface Sci. 15 (2010) 264-270.

613 (47) E.H. Lucassen-Reynders, D.T. Wasan, Interfacial viscoelasticity in emulsions and foams, Food
 614 Structure 12 (1993) 1-12.

615 (48) A. Ghisaidoobe, S. Chung, Intrinsic tryptophan fluorescence in the detection and analysis of
 616 proteins: A focus on Förster resonance energy transfer techniques, Int. J. Mol. Sci. 15 (2014)
 617 22518–22538.

618 (49) J.R. Lakowicz. Protein fluorescence. In *Principles of fluorescence spectroscopy*, Lakowicz,
 619 J.R., Eds.; Springer US, 2006, 529-575.

620 (50) H.-D. Belitz, W. Grosch, P. Schieberle. Food chemistry, fourth; Springer-Verlag: Berlin,
 621 Germany, 2009; 563-616; 670-745 p.

622 (51) R. Shukla, M. Cheryan, Zein: The industrial protein from corn, Industrial Crops and Products
 623 13 (2001) 171-192.

624 (52) N. Alizadeh-Pasdar, E.C.Y. Li-Chan, Comparison of protein surface hydrophobicity measured
 625 at various pH values using three different fluorescent probes, J. Agric. Food Chem. 48 (2000) 328-
 626 334.

627 (53) A. Chakrabarti, Fluorescence of spectrin-bound prodan, Biochemical and Biophysical
 628 Research Communications 226 (1996) 495-497.

629 (54) J. Maldonado-Valderrama, J.M.R. Patino, Interfacial rheology of protein–surfactant mixtures,
 630 Curr. Opin. Colloid Interface Sci. 15 (2010) 271-282.

631 (55) Y. Zou, Z. Wan, J. Guo, J. Wang, S. Yin, X. Yang, Tunable assembly of hydrophobic protein
 632 nanoparticle at fluid interfaces with tannic acid, Food Hydrocolloid. 63 (2017) 364-371.

633 (56) S.I. Karakashev, M.V. Grozdanova, Foams and antifoams, Adv. Colloid Interface Sci. 176-177
 634 (2012) 1-17.

635 (57) K. Koczko, J.K. Koczko, D.T. Wasan, Mechanisms for antifoaming action in aqueous systems
636 by hydrophobic particles and insoluble liquids, J. Colloid Interface Sci. 166 (1994) 225-238.
637 (58) R.J. Pugh, Foaming, foam films, antifoaming and defoaming, Adv. Colloid Interface Sci. 64
638 (1996) 67-142.

639

TABLE 1

Table 1: Z-average (nm), polydispersity-index and zeta potential (mV) values of wheat gliadin and maize zein nanoparticle (NP) suspensions (WGNPs and MZNPs, respectively) at pH 4.5 and 6.0 (pH unadjusted) for WGNPs and pH 4.5 (pH unadjusted) and 5.5 for MZNPs. Capital letters represent significant ($P < 0.05$) differences of a given parameter between different samples.

	pH	Z-average (nm)	Polydispersity-index	Zeta potential (mV)
WGNPs	4.5	132.5 ± 12.6^A	0.201 ± 0.036^A	21.3 ± 3.7^B
	6.0 (pH unadjusted)	140.2 ± 15.5^A	0.102 ± 0.029^B	15.5 ± 1.2^C
MZNPs	4.5 (pH unadjusted)	96.6 ± 5.4^B	0.186 ± 0.031^A	44.4 ± 4.6^A
	5.5	154.7 ± 28.8^A	0.115 ± 0.05^B	16.7 ± 2.0^{BC}

TABLE 2

Table 2: Z-average (nm), polydispersity-index and zeta potential (mV) values of wheat gliadin and maize zein nanoparticle (NP) suspensions (WGNPs and MZNPs, respectively) prepared from non-defatted, treated (T) or defatted (D) gliadin and zein powders. Capital letters represent significant ($P < 0.05$) differences of a given parameter between different samples.

	Z-average (nm)	Polydispersity-index	Zeta potential (mV)
WGNPs	139.6 ± 6.6^A	0.111 ± 0.034^C	13.9 ± 2.3^C
T-WGNPs	132.8 ± 6.2^A	0.149 ± 0.011^{BC}	15.6 ± 1.1^C
D-WGNPs	126.4 ± 3.3^A	0.141 ± 0.028^{BC}	14.3 ± 0.9^C
MZNPs	86.1 ± 15.5^B	0.215 ± 0.007^A	42.7 ± 3.4^A
T-MZNPs	75.8 ± 13.0^B	0.242 ± 0.043^{AB}	36.9 ± 4.8^{AB}
D-MZNPs	76.6 ± 18.1^B	0.209 ± 0.060^{AB}	30.9 ± 3.2^B

TABLE 3

Table 3: Z-average (nm), polydispersity-index and zeta potential (mV) values of nanoparticle (NP) suspensions based on 100% gliadin (WGNP₁₀₀), 75% gliadin and 25% zein (WGNP₇₅MZNP₂₅), 50% gliadin and 50% zein (WGNP₅₀MZNP₅₀) or 100% zein (MZNP₁₀₀). Capital letters represent significant ($P < 0.05$) differences of a given parameter between different samples.

	Z-average (nm)	Polydispersity-index	Zeta potential (mV)
WGNP₁₀₀	134.9 ± 12.9 ^A	0.137 ± 0.040 ^B	15.9 ± 2.3 ^B
WGNP₇₅MZNP₂₅	109.0 ± 7.7 ^B	0.121 ± 0.018 ^B	19.6 ± 2.8 ^B
WGNP₅₀MZNP₅₀	92.0 ± 10.0 ^B	0.179 ± 0.066 ^{AB}	19.9 ± 3.8 ^B
MZNP₁₀₀	94.1 ± 13.5 ^B	0.214 ± 0.016 ^A	44.2 ± 3.0 ^A

FIGURE CAPTIONS

Figure 1: 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 gliadin and zein nanoparticle (NP) (WGNPs and MZNPs, respectively) suspensions at pH 4.5 and 6.0 (pH unadjusted) for WGNP and 4.5 (pH unadjusted) and 5.5 for MZNP. A.U. are arbitrary units.

Figure 2: Foaming and air-water interfacial properties of gliadin and zein nanoparticle (NP) (WGNP and MZNP, respectively) suspensions (at 0.10% w_{protein}/v) at pH 4.5 and 6.0 (pH unadjusted) for WGNPs and 4.5 (pH unadjusted) and 5.5 for MZNPs. The top graph represents the foam volume over time after vigorous stirring of the different samples (a) while the bottom graphs represent the evolution of surface tension (γ) (b) surface dilatational elastic (E') (c) and viscous (E'') (d) moduli over time, obtained through oscillatory drop shape tensiometry.

Figure 3: 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 gliadin and zein nanoparticle (NP) (WGNP and MZNP, respectively) suspensions. NPs were produced from non-defatted gliadin and zein powders or from their defatted (D) or solvent treated (T) counterparts. A.U. are arbitrary units.

Figure 4: Foaming and air-water interfacial properties of gliadin and zein nanoparticle (NP) (WGNP and MZNP, respectively) suspensions containing 0.10% protein. Nanoparticles (NPs) were

produced from non-defatted gliadin and zein powders or from their defatted (D) or solvent treated (T) counterparts. The top graph represents the foam volume over time after vigorous stirring of the different samples (a) while the bottom graphs represent the evolution of surface tension (γ) (b), surface dilatational elastic (E') (c) and viscous (E'') (d) moduli over time, obtained through oscillatory drop shape tensiometry.

Figure 5: Foaming properties of gliadin and zein nanoparticle (NP) (WGNP and MZNP, respectively) suspensions. NPs were produced from non-defatted gliadin and zein powders or from their defatted (D) or solvent treated (T) counterparts. The top graph represents the foam volume over time of pH unadjusted WGNP, D-GNP and T-GNP suspensions containing 0.03% protein (a) while the bottom graph represents the foam volume of MZNP, D-MZNP and T-MZNP suspensions containing 0.10% protein at pH 5.5 (b).

Figure 6: 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 nanoparticle (NP) suspensions produced with protein stock solutions containing only wheat gliadin (WGNP₁₀₀), only maize zein (MZNP₁₀₀), 75% gliadin and 25% zein (WGNP₇₅MZNP₂₅) or 50% gliadin and 50% zein (WGNP₅₀MZNP₅₀). A.U. are arbitrary units.

Figure 7: Foaming and air-water interfacial properties of nanoparticle (NP) suspensions containing 0.10% protein produced with stock solutions containing only wheat gliadin (WGNP₁₀₀), only maize zein (MZNP₁₀₀), 75% gliadin and 25% zein (WGNP₇₅MZNP₂₅) or 50% gliadin and 50%

715 zein (WGNP₅₀MZNP₅₀). The top graph represents the foam volume over time after vigorous
716 stirring of the different samples (a) while the bottom graphs represent the evolution of surface
717 tension (γ) (b), surface dilatational elastic (E') (c) and viscous (E'') (d) moduli over time obtained
718 through oscillatory drop shape tensiometry.

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FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7