Cereal protein based nanoparticles as agents stabilizing air-water and oil-water interfaces in food systems

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ABSTRACT (100-120 words)

There has been a recent surge of interest in the use of food-grade nanoparticles (NPs) for stabilizing food foams and emulsions. Cereal proteins are a promising raw material class to produce such NPs. Studies thus far have focused mostly on wheat gliadin and maize zein based NPs. The former are effective interfacial stabilizing agents, while the latter due to their high hydrophobicity generally result in poor interfacial stability. Several strategies to modify the surface properties of wheat gliadin and maize zein NPs have been followed. In many instances, this resulted in improved foam or emulsion stability. Nonetheless, future efforts should be undertaken to gain fundamental insights in the interfacial behavior of NPs, to further explore NP surface modification strategies, and to validate the use of NPs in actual food systems.

Abbreviations and symbols: O/W – oil-in-water; W/O – water-in-oil; ϕ –oil fraction of the emulsion; PZC – point-of-zero-charge; WG – wheat gliadin; MZ – maize zein; HIPE – high internal phase emulsions; NP – nanoparticle; SDS – sodium dodecyl sulfate.

Keywords: Gliadin; Zein; Nanoparticle; Foam; Emulsion

1. Introduction

As the crop class with the highest annual world-wide production, cereals are an extremely important source of protein in the human diet (Table 1) [1]. The average protein content of various cereals typically ranges from 8.5% up to 14.5% on a dry matter basis (Table 1) [2,3]. Cereal proteins contribute to the structure of a wide range of cereal based foods. The most notable examples are of course gluten proteins, the storage proteins of wheat, which are indispensable in the production of wheat based food products because of their ability to form viscoelastic dough upon hydration and mixing [4,5]. Cereal crops contain a large variety of proteins. Osborne in 1907 introduced a classification scheme to distinguish between plant proteins based on their sequential extractability in several media [6]. Table 1 provides an overview of estimated average levels of albumins (proteins extractable in water), globulins (proteins extractable in diluted salt solutions), prolamins (proteins extractable in aqueous alcohol solutions) and glutelins (proteins extractable in diluted acid/base solutions) for the most produced cereal crops world-wide. It is clear that the most abundant cereal proteins are prolamins and glutelins. While they thus lack solubility and functionality in aqueous systems, this also poses opportunities. One such opportunity is to use them for producing micro- or nano-sized aggregates which have at least some degree of colloidal stability in aqueous media. A consideration that can be made here is that studies dealing with such systems very often employ the term ‘nanoparticles’ (NPs). It can be questioned whether such a term should be used when referring to these nano-sized aggregates made up from rather flexible biopolymers. Indeed, they likely have soft matter-like behavior rather than that of solid, inorganic particles such as those made from silica or gold [7●].

Furthermore, one could argue that any aggregate consisting of several molecules could be considered to be a NP. The aggregated systems considered in this paper generally vary in size in a 50 nm to several hundred nm range. Considering the above, we here for the sake of conformity still use the term NP for the different aggregated protein (and polysaccharide) based systems discussed here. The concept of protein based NPs has most often been exploited for drug delivery applications or the encapsulation of bioactive molecules. Indeed, cereal prolamins such as maize zeins (MZ) and wheat gliadins (WG) have been widely used as material for producing biocompatible carrier nanoparticles (NPs). Studies in this field are abundant, have been excellently reviewed on several occasions [8-11] and therefore will not be the focus of the present review.

However, cereal protein based NPs may also be used to provide structure and texture in food systems. It has long been known that inert, rigid particles such as those based on silica or latex have the ability to very efficiently stabilize air-water or oil-water interfaces (Pickering stabilization) [7●]. Such particles can evidently not be used in food applications, which has incited an increasing interest in biodegradable food-grade NPs as interfacial stabilizers [12]. Given the abundance of cereal protein and the relatively low environmental impact associated with their production [13], cereal proteins may be a very promising raw material to produce NPs as interfacial stabilizing agents for food industry. Here, we offer a view on the state-of-the-art of cereal protein based NPs and their ability to stabilize interfaces and put forward some perspectives for research.

2. Cereal based nanoparticles

There are various ways to produce protein based NPs. The most often exploited strategy, also for the studies discussed below, is via liquid anti-solvent precipitation. In such method, the solvent quality of a protein solution is decreased which leads to controlled aggregation and the formation of a homogeneously distributed NP dispersion [14]. The technique has been described as very reproducible, easily scalable and fairly cheap [14]. It is widely used on an industrial scale for pharmaceutical applications [14]. All this makes it an interesting technology for possible food applications. We will not in detail discuss the methodologies used in each of the studies discussed below, but Table 2 outlines their research strategies and main results. Among the first to report on cereal protein based NPs for food dispersion stabilization were de Folter *et al.* [15●●]. They used MZNP to stabilize O/W emulsions [with a soy bean oil fraction ($\phi_{soy\ bean\ oil}$)=0.50]. Such emulsions have relatively high stability (up to two weeks after production) but only at low ionic strengths (<10 mM) and at pH values far from the point-of-zero-charge (PZC) of the MZs (about pH 6.5) [15]. Elsewhere, MZNP were employed to stabilize W/O emulsions with water fractions up to 0.30 [16]. However, such emulsions have only very limited stability [16]. Similarly, very low stability (less than one hour) was reported for O/W emulsions ($\phi_{fish\ oil}$=0.30) stabilized by MZNPs [17]. It has also been reported that MZNPs cannot effectively stabilize O/W emulsions ($\phi_{corn\ oil}$=0.80) [18]. On the other hand, Zou *et al.* [19] produced MZNPs by an acetic acid solution – water based anti-solvent precipitation procedure. While these MZNPs can stabilize O/W emulsions ($\phi_{corn\ oil}$=0.50), it should be mentioned that about 20-25% v/v acetic acic was still present in these emulsions, which may limit their applicability. Remarkably, after three days of storage, such emulsions had transformed into a gel-like solid, which the authors attributed to

inter-droplet hydrophobic interactions between MZs [19]. Thus, dispersions stabilized by MZNPs seem to have limited stability at best. In contrast, O/W emulsions ($\phi_{corn\ oil}$=0.50) based on WGNPs are stable for up to three months at pH values in a 4.0 to 9.0 range, but tend to coalesce at pH 3.0 [20●●]. Liu *et al.* in a very similar set-up demonstrated that microfluidization of WGNP stabilized O/W emulsions ($\phi_{algal\ oil}$=0.50) led to subsequent gliadin protein cross-linking resulting in a highly structured emulsion gel [21]. Hu *et al.* showed that at $\phi_{corn\ oil}$=0.80, similar emulsion gels were formed [20●●]. Indeed, when the volume fraction of oil exceeds 0.74, high internal phase emulsions (HIPEs), which often behave like elastic solids (gels) rather than like viscous fluids, as well as display shear thinning behavior, are obtained [22]. Such rheological behavior poses some interesting opportunities in food industry, for example in replacing solid fats as texture providers by liquid oils. Indeed, Liu *et al.* [23] showed that O/W HIPEs ($\phi_{sunflower\ oil}$=0.76) stabilized by wheat gluten NPs have rheological properties comparable to those of an egg based mayonnaise. Alternatively, Peng *et al.* [24●] have used WGNP to stabilize foams. Such particles resulted in high foaming capacity and stability, even easily out-performing ovalbumin and sodium caseinate when tested under the same conditions. In a follow-up study by the same authors, it was found that at pH 3.0, WGNPs display poor foaming properties, while at pH 5.8 (close to their point-of-zero-charge) they possess the excellent foaming properties described earlier [25]. Finally, O/W emulsions ($\phi_{soy\ bean\ oil}$=0.30-0.80) stabilized by sorghum kafirin NPs have relatively high stability and can be used for controlled release of curcumin [26,27].

Thus, cereal protein based NPs clearly have some potential to stabilize food dispersions. However, much progress in terms of the stability of such dispersions can still be made. Indeed, it has been put forward that for example MZNPs are quite hydrophobic which might, to an extent, be

unfavorable for their adsorption at interfaces [28●]. The surface modification of NPs is a very promising strategy to alter their air-water or oil-water interfacial characteristics.

3. Surface modified cereal based nanoparticles

Several approaches have been exploited to alter the surface properties of cereal based NPs. These efforts have focused solely on WGNPs and (most often) MZNPs. Many studies have focused on polysaccharides as modification agents. Such hydrophilic molecules can alter the hydrophilic-lipophilic balance of NPs. Indeed, because of the often very high hydrophobicity of the proteins used, the resulting NPs are in some instances not suitable as interface stabilizing agents (see above). Furthermore, because of the relatively high molecular mass and high charge density of charged polysaccharides, adsorption of hybrid protein-polysaccharide NPs might result in improved interface stability via additional steric or electrostatic stabilization. Table 3 outlines the modification strategies and main results of the studies discussed below. In the case of WGNPs, three studies dealing with hybrid WG-chitosan NPs have been reported. Yuan *et al*. [29] studied O/W emulsions ($\phi_{corn\ oil}$=0.10-0.80) stabilized by WG-chitosan NPs produced via simultaneous anti-solvent precipitation of WG and chitosan. Such emulsions at low ionic strength and low pH (2.9) have rather low stability due to the high surface charge and therefore low affinity of the particles for the oil phase. At pH 4.0 and 5.0 or at elevated ionic strengths (> 20 mM NaCl), such emulsions are stable for up to two months after production. The same particles efficiently stabilize O/W HIPEs ($\phi_{corn\ oil}$=0.80) with solid-like characteristics [29]. Similarly, Zeng *et al.* [30] used WG-chitosan NPs to stabilize O/W HIPEs ($\phi_{corn\ oil}$=0.83). Such emulsions have a higher yield

stress than WGNP stabilized emulsions [30]. Moreover, in a very similar set-up, Zhou *et al.* [31] reported that O/W HIPEs ($\phi_{algal\ oil}$=0.75) could be efficiently stabilized by WG-chitosan NPs and that such emulsions can be an efficient delivery vehicle for curcumin. As mentioned, some efforts to reduce the high surface hydrophobicity of MZNPS have been undertaken. In an approach similar to that described above for WGNPs, Wang *et al.* [32] used hybrid MZ-chitosan NPs produced via simultaneous anti-solvent precipitation to stabilize O/W emulsions ($\phi_{n\text{-}tetradecane}$=0.20-0.70). While at $\phi_{n\text{-}tetradecane}$=0.10-0.30 stable emulsions could be formed, emulsions at higher ϕ values were not stable. This is in contrast with what was found for WGNPs and WG-chitosan NPs. These are suitable for stabilizing HIPEs (ϕ>0.74). In a similar approach, Zhou *et al.* [18] used hybrid MZ-pectin NPs produced via simultaneous anti-solvent precipitation to stabilize O/W HIPEs ($\phi_{corn\ oil}$=0.80). Such HIPEs are very stable but only at low pH values (3.8). This illustrates the importance of electrostatic interactions between positively charged MZ proteins and negatively charged pectin molecules. Using a different approach, Soltani *et al.* [17] produced coarse O/W emulsions ($\phi_{fish\ oil}$=0.30) based on MZNPs. When they then added a pectin solution, the emulsion stability improved from less than one hour to 60 days. Subsequent addition of laccase, which results in oxidation of feruloyl groups and therefore cross-linking of pectin chains, and Ca^{2+} ions transformed the emulsions into emulgels [17]. Dai *et al.* [33] used hybrid MZ-propylene glycol alginate NPs to stabilize emulsions with different ϕ values ($\phi_{medium\ chain\ triglyceride\ oil}$=0.30-0.75). At low ϕ (ϕ=0.30), creaming was observed while at intermediate ϕ (ϕ=0.60), stable emulsions were formed. However, upon further increasing ϕ (ϕ=0.70-0.75), some oiling off was observed, indicating that these MZ-propylene glycol alginate NPs were not suitable for stabilizing HIPEs. However, in a follow-up study, a ternary system consisting of NPs composed of MZ-

propylene glycol alginate-rhamnolipids was used to stabilize O/W emulsions at ϕ=0.75. Such emulsions were stable under a wide range of food processing conditions [34]. In another ternary NP system described by Sun *et al.* [35], zein was dissolved in water at pH 12.5. Sodium caseinate and propylene glycol alginate were then added. The latter due to partial alkaline hydrolysis led to a decrease of pH, which resulted in co-precipitation and thus formation of hybrid MZ-sodium caseinate-propylene glycol alginate NPs. These ternary NPs efficiently stabilize O/W emulsions ($\phi_{soy\ bean\ oil}$=0.40-0.80). Samples with higher ϕ (HIPEs) have a gel-like texture which resembles that of mayonnaise [35●].

Taking a step back from such ternary systems, Feng *et al*. [36] described hybrid MZ-sodium caseinate NPs produced by depositing sodium caseinates at the surface of the MZNPs as a result of electrostatic interactions. This approach resulted in O/W emulsions ($\phi_{canola\ oil}$=0.50) with higher stability than those stabilized solely by MZNPs [36]. However, due to the electrostatic nature of the MZ-sodium caseinate interactions, the resistance of such emulsions towards changes in ionic strength or pH of the medium is limited. Others have used gum Arabic to modify the properties of MZNPs in a similar way [37,38]. Indeed, addition of a gum Arabic solution after particle production resulted in deposition of negatively charged gum Arabic molecules onto the positively charged NP surface. Dai *et al.* [37] reported that MZ-gum Arabic NPs have contact angles closer to neutral wettability than is the case for the unmodified MZNPs. Indeed, the former resulted in stable (up to 30 days) O/W emulsions ($\phi_{medium\ chain\ triglyceride\ oil}$=0.50-0.70) with elastic gel-like characteristics. Li *et al.* [38] reported similar findings but also noted that such emulsions lost stability when 150 mM NaCl was added, which further illustrates the electrostatic nature of the interaction between the MZNPs and the gum Arabic. Finally, several studies by the group of the

same main author have dealt with the use of tannic acid to alter the surface properties of MZNPs [28,39-43]. In a more fundamental study on the air-water interfacial behavior of such hybrid MZ-tannic acid NPs, Zou *et al.* [41] reported that MZNPs rapidly aggregate at air-water interfaces which led to low surface coverage. In contrast, the more hydrophilic MZ-tannic acid NPs adsorb more gradually and form ordered interfacial structures with higher surface coverage. Elsewhere, different ratios of zein to tannic acid were used to produce MZ-tannic acid NPs with varying hydrophobicity [28●]. This allowed tuning the rheological properties of O/W emulsions ($\phi_{sunflower\ oil}$=0.05-0.60) [28●,42]. While such particles can stabilize emulsions, they are not suitable as foam stabilizers [40]. To overcome this, small levels (0.6 mM) of the anionic surfactant sodium dodecyl sulfate (SDS) were used to induce fractal aggregation of MZ-tannic acid NPs [40]. The authors suggest that negatively charged SDS molecules interact with the positively charged MZ-based NPs thereby allowing controlled aggregation of such NPs. It is however unclear whether the intact NPs aggregate in an orderly fashion, or whether their integrity is lost after which the constituents they were made up from form larger aggregates. Nevertheless, these aggregated MZ-tannic acid NPs display high foaming capacity and foam stability [40].

4. Knowledge gaps and perspectives

The above clearly illustrates that there has been a recent surge of interest in stabilizing food dispersions by cereal protein based NPs. Indeed, each of the 27 research papers discussed here has been published in the last six years. Most efforts have focused on NPs based on WGs and MZs produced via anti-solvent precipitation. WGNPs provide excellent foam and emulsion stability.

On their own, MZNPs are less efficient at stabilizing interfaces. Several strategies, often involving the use of polysaccharides, have been put forward to alter the surface properties of such NPs. Figure 1 visually represents the different strategies to produce and modify cereal protein based NPs. Such modification strategies in many instances have substantially improved the stability of emulsified systems. A notable application of cereal protein based NPs is the stabilization of HIPEs. Such systems have the potential to provide alternative textures in a wide range of food systems, but are usually prone to phase inversion or require substantial amounts of emulsifier to keep them from destabilizing. WG and modified MZ NPs seem able to efficiently stabilize such systems. A point of attention might be that while several studies report on the rheological behavior of such emulsions in the linear viscoelastic regime, they do not directly address their stability when subjected to shear at larger strains. Indeed, this in some cases is an issue for Pickering emulsions [44]. Regardless, despite the substantial efforts described here, major steps are still to be taken in future research on cereal protein based NPs and their ability to stabilize interfaces.

(i) Thus far, research has been mostly focused on WGs and MZs. Other cereal protein sources may prove to be valuable sources of raw materials to produce NPs.

(ii) Most research has focused on O/W emulsions. The potential of cereal protein based NPs as foaming agents largely remains unexplored.

(iii) The Pickering mechanism by which rigid particles stabilize interfaces has been put forward in the above studies as the main mechanism. However, the NPs used are protein-based and not at all rigid and inert and may thus behave differently. More fundamental research into the interfacial behavior and more specifically, into the structure-function relationship, of such NPs is needed.

(iv) Further efforts should be undertaken to modify the surface properties of NPs. For instance, covalent linkage of NPs to other biomolecules may not only alter their interfacial characteristics but also improve their colloidal stability in aqueous systems. This could provide a basis for using them in other applications. Whether such modifications would ever be allowed to be implemented in food industry is beyond the scope of the present manuscript. Much would seem to depend on the reaction conditions which are needed to make them occur and whether such modifications are already spontaneously occurring in food processing.

(v) Validation of the applicability of cereal protein based NPs as interfacial stabilizers in actual food systems is still necessary.

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REFERENCES

1. FAOSTAT: **Production statistics of the Food and Agriculture Organisation of the United Nations**. 2016, Accessed on **December 13 2018**. http://faostat.fao.org.
2. Belitz H-D, Grosch W, Schieberle P: *Food chemistry* edn fourth. Berlin, Germany: Springer-Verlag; 2009.
3. Jambunathan R, Singh U, Subramanian V: **Grain quality of sorghum, pearl millet, pigeon-pea, and chick-pea**. In *Interfaces between Agriculture, Nutrition, and Food Science*; 1984:32-47.
4. Delcour JA, Joye IJ, Pareyt B, Wilderjans E, Brijs K, Lagrain B: **Wheat gluten functionality as a quality determinant in cereal-based food products**. *Annual Review of Food Science and Technology* 2012, **3**:469-492.
5. Goesaert H, Brijs K, Veraverbeke WS, Courtin CM, Gebruers K, Delcour JA: **Wheat flour constituents: How they impact bread quality, and how to impact their functionality**. *Trends in Food Science & Technology* 2005, **16**:12-30.
6. Osborne TB: *The proteins of the wheat kernel.* Washington: Carnegie Inst.; 1907.
7. Binks BP: **Particles as surfactants—similarities and differences**. *Current Opinion in Colloid & Interface Science* 2002, **7**:21-41.
   - Excellent review article dealing with the differences between colloidal particles and amphiphilic surfactants in terms of their ability and mechanism to stabilize air-water and oil-water interfaces. The focus lies on rigid, inert particles, but many of the concepts put forward in this article are highly relevant for the cereal protein based nanoparticles discussed here.
8. Malekzad H, Mirshekari H, Sahandi Zangabad P, Moosavi Basri SM, Baniasadi F, Sharifi Aghdam M, Karimi M, Hamblin MR: **Plant protein-based hydrophobic fine and ultrafine carrier particles in drug delivery systems**. *Critical Reviews in Biotechnology* 2018, **38**:47-67.
9. Joye IJ, McClements DJ: **Biopolymer-based delivery systems: Challenges and opportunities**. *Current Topics in Medicinal Chemistry* 2016, **16**:1026-1039.
10. Tarhini M, Greige-Gerges H, Elaissari A: **Protein-based nanoparticles: From preparation to encapsulation of active molecules**. *International Journal of Pharmaceutics* 2017, **522**:172-197.
11. Fuchs S, Coester C: **Protein-based nanoparticles as a drug delivery system: Chances, risks, perspectives**. *Journal of Drug Delivery Science and Technology* 2010, **20**:331-342.
12. Dickinson E: **Biopolymer-based particles as stabilizing agents for emulsions and foams**. *Food Hydrocolloids* 2017, **68**:219-231.
13. Day L: **Proteins from land plants – potential resources for human nutrition and food security**. *Trends in Food Science & Technology* 2013, **32**:25-42.
14. Joye IJ, McClements DJ: **Production of nanoparticles by anti-solvent precipitation for use in food systems**. *Trends in Food Science & Technology* 2013, **34**:109-123.
15. de Folter JWJ, van Ruijven MWM, Velikov KP: **Oil-in-water pickering emulsions stabilized by colloidal particles from the water-insoluble protein zein**. *Soft Matter* 2012, **8**:6807-6815.

●● This study was the first to focus on the stabilization of O/W emulsions by cereal protein based nanoparticles. Maize zeins were used to produce nanoparticles. Limited emulsion stability was achieved, but only at pH values far from the point-of-zero-charge of the zein proteins, and at low ionic strengths.

16. Rutkevičius M, Allred S, Velev OD, Velikov KP: **Stabilization of oil continuous emulsions with colloidal particles from water-insoluble plant proteins**. *Food Hydrocolloids* 2018, **82**:89-95.

17. Soltani S, Madadlou A: **Two-step sequential cross-linking of sugar beet pectin for transforming zein nanoparticle-based pickering emulsions to emulgels**. *Carbohydrate Polymers* 2016, **136**:738-743.

18. Zhou F-Z, Huang X-N, Wu Z-l, Yin S-W, Zhu J-h, Tang C-H, Yang X-Q: **Fabrication of zein/pectin hybrid particle-stabilized pickering high internal phase emulsions with robust and ordered interface architecture**. *Journal of Agricultural and Food Chemistry* 2018, **66**:11113-11123.

19. Zou Y, Pan R, Wan Z, Guo J, Wang J, Yang X: **Gel-like emulsions prepared with zein nanoparticles produced through phase separation from acetic acid solutions**. *International Journal of Food Science & Technology* 2017, **52**:2670-2676.

20. Hu Y-Q, Yin S-W, Zhu J-H, Qi J-R, Guo J, Wu L-Y, Tang C-H, Yang X-Q: **Fabrication and characterization of novel pickering emulsions and pickering high internal emulsions stabilized by gliadin colloidal particles**. *Food Hydrocolloids* 2016, **61**:300-310.

●● The first paper to report on the stabilization of high internal phase emulsions (HIPEs) by wheat gliadin based nanoparticles. Such HIPEs exhibit gel-like rheological properties and can be a promising textural alternative to solid fats in a wide range of food products. HIPEs often require high levels of surfactants to stabilize and are prone to phase inversion. Thus, cereal protein based nanoparticles represent a promising new class of agents to stabilize such systems.

21. Liu X, Liu Y-Y, Guo J, Yin S-W, Yang X-Q: **Microfluidization initiated cross-linking of gliadin particles for structured algal oil emulsions**. *Food Hydrocolloids* 2017, **73**:153-161.

22. Leal-Calderon F, Thivilliers F, Schmitt V: **Structured emulsions**. *Current Opinion in Colloid & Interface Science* 2007, **12**:206-212.

23. Liu X, Guo J, Wan Z-L, Liu Y-Y, Ruan Q-J, Yang X-Q: **Wheat gluten-stabilized high internal phase emulsions as mayonnaise replacers**. *Food Hydrocolloids* 2018, **77**:168-175.

24. Peng D, Jin W, Li J, Xiong W, Pei Y, Wang Y, Li Y, Li B: **Adsorption and distribution of edible gliadin nanoparticles at the air/water interface**. *Journal of Agricultural and Food Chemistry* 2017, **65**:2454-2460.

● The first paper to report on unmodified cereal protein based nanoparticles to stabilize foams. Wheat gliadin nanoparticles were obtained via anti-solvent precipitation and were found to be efficient air-water interfacial stabilizing agents.

25. Peng D, Jin W, Tang C, Lu Y, Wang W, Li J, Li B: **Foaming and surface properties of gliadin nanoparticles: Influence of ph and heating temperature**. *Food Hydrocolloids* 2018.

26. Xiao J, Wang Xa, Perez Gonzalez AJ, Huang Q: **Kafirin nanoparticles-stabilized pickering emulsions: Microstructure and rheological behavior**. *Food Hydrocolloids* 2016, **54**:30-39.

27. Xiao J, Li C, Huang Q: **Kafirin nanoparticle-stabilized pickering emulsions as oral delivery vehicles: Physicochemical stability and in vitro digestion profile**. *Journal of Agricultural and Food Chemistry* 2015, **63**:10263-10270.
28. Zou Y, van Baalen C, Yang X, Scholten E: **Tuning hydrophobicity of zein nanoparticles to control rheological behavior of pickering emulsions**. *Food Hydrocolloids* 2018, **80**:130-140.
- By using different ratios of zein to tannic acid, the hydrophobicity of the obtained particles could be tuned. This in turn was exploited to tweak the stability and rheological behavior of the obtained O/W emulsions.

29. Yuan DB, Hu YQ, Zeng T, Yin SW, Tang CH, Yang XQ: **Development of stable pickering emulsions/oil powders and pickering HIPEs stabilized by gliadin/chitosan complex particles**. *Food & Function* 2017, **8**:2220-2230.
30. Zeng T, Wu Z-l, Zhu J-Y, Yin S-W, Tang C-H, Wu L-Y, Yang X-Q: **Development of antioxidant pickering high internal phase emulsions (HIPEs) stabilized by protein/polysaccharide hybrid particles as potential alternative for phos**. *Food Chemistry* 2017, **231**:122-130.
31. Zhou FZ, Zeng T, Yin SW, Tang CH, Yuan DB, Yang XQ: **Development of antioxidant gliadin particle stabilized pickering high internal phase emulsions (HIPEs) as oral delivery systems and the in vitro digestion fate**. *Food & Function* 2018, **9**:959-970.
32. Wang LJ, Yin SW, Wu LY, Qi JR, Guo J, Yang XQ: **Fabrication and characterization of pickering emulsions and oil gels stabilized by highly charged zein/chitosan complex particles (ZCCPS)**. *Food Chemistry* 2016, **213**:462-469.
33. Dai L, Zhan X, Wei Y, Sun C, Mao L, McClements DJ, Gao Y: **Composite zein - propylene glycol alginate particles prepared using solvent evaporation: Characterization and application as pickering emulsion stabilizers**. *Food Hydrocolloids* 2018, **85**:281-290.
34. Dai L, Yang S, Wei Y, Sun C, McClements DJ, Mao L, Gao Y: **Development of stable high internal phase emulsions by pickering stabilization: Utilization of zein-propylene glycol alginate-rhamnolipid complex particles as colloidal emulsifiers**. *Food Chemistry* 2019, **275**:246-254.
35. Sun C, Gao Y, Zhong Q: **Properties of ternary biopolymer nanocomplexes of zein, sodium caseinate, and propylene glycol alginate and their functions of stabilizing high internal phase pickering emulsions**. *Langmuir* 2018, **34**:9215-9227.
- The first report of a ternary nanoparticle system to be used for interfacial stabilization, in which nanoparticles consisted of maize zein, sodium caseinate and propylene glycol alginate. This resulted in emulsions with high stability in a wide range of food processing conditions, as opposed to particles based solely on maize zeins.

36. Feng YM, Lee Y: **Surface modification of zein colloidal particles with sodium caseinate to stabilize oil-in-water pickering emulsion**. *Food Hydrocolloids* 2016, **56**:292-302.
37. Dai L, Sun C, Wei Y, Mao L, Gao Y: **Characterization of pickering emulsion gels stabilized by zein/gum arabic complex colloidal nanoparticles**. *Food Hydrocolloids* 2018, **74**:239-248.
38. Li J, Xu X, Chen Z, Wang T, Lu Z, Hu W, Wang L: **Zein/gum arabic nanoparticle-stabilized pickering emulsion with thymol as an antibacterial delivery system**. *Carbohydrate Polymers* 2018, **200**:416-426.

39. Zou Y, Guo J, Yin S-W, Wang J-M, Yang X-Q: **Pickering emulsion gels prepared by hydrogen-bonded zein/tannic acid complex colloidal particles**. *Journal of Agricultural and Food Chemistry* 2015, **63**:7405-7414.
40. Zou Y, Wan Z, Guo J, Wang J, Yin S, Yang X: **Modulation of the surface properties of protein particles by a surfactant for stabilizing foams**. *RSC Advances* 2016, **6**:66018-66026.
41. Zou Y, Wan Z, Guo J, Wang J, Yin S, Yang X: **Tunable assembly of hydrophobic protein nanoparticle at fluid interfaces with tannic acid**. *Food Hydrocolloids* 2017, **63**:364-371.
42. Zou Y, Yang X, Scholten E: **Rheological behavior of emulsion gels stabilized by zein/tannic acid complex particles**. *Food Hydrocolloids* 2018, **77**:363-371.
43. Zou Y, Zhong J, Pan R, Wan Z, Guo J, Wang J, Yin S, Yang X: **Zein/tannic acid complex nanoparticles-stabilised emulsion as a novel delivery system for controlled release of curcumin**. *International Journal of Food Science & Technology* 2017, **52**:1221-1228.
44. Whitby CP, Fischer FE, Fornasiero D, Ralston J: **Shear-induced coalescence of oil-in-water pickering emulsions**. *Journal of Colloid and Interface Science* 2011, **361**:170-177.

TABLE 1

Table 1: Annual worldwide production (2016, in million metric tonnes) and typical average protein contents (expressed on dry matter basis) of various cereals. Relative amounts of albumins, globulins, prolamins and glutelins in various cereals (expressed as percentage of the total protein present).

Cereal	Annual production[1]	Protein content (%)[2]	Relative amounts			
			Albumin (%)[2]	*Globulin (%)*[2]	*Prolamin (%)*[2]	*Glutelin (%)*[2]
Maize	1,060	10.5	4.0	2.8	47.9	45.3
Wheat	750	13.5	14.7	7.0	32.6	45.7
Rice	741	8.5	10.8	9.7	2.2	77.3
Barley	141	12.0	12.1	8.4	25.0	54.5
Sorghum	64	11.8	17.4		25.2	57.4
Millet	28	12.1	18.2	6.1	33.9	41.8
Oats	23	14.5	20.2	11.9	14	53.9
Triticale	15	11.0	44.4	10.2	20.9	24.5
Rye	13	10.5	4.0	2.8	47.9	45.3

[1] Based on data by the Food and Agricultural Organization of the United Nations [1]

[2] Based on Belitz *et al.* [2] and Jambunathan *et al.* [3].

388 **TABLE 2**

389 **Table 2:** Protein source used, the nanoparticle (NP) production strategy, the type of dispersion stabilized and the main results of studies

390 dealing with stabilization of foams and emulsions by unmodified cereal protein based NPs. Abbreviations and symbols are as follows:

391 O/W– oil-in-water; W/O – water-in-oil; ϕ – the oil fraction of the emulsion; PZC – point-of-zero-charge; WG – wheat gliadin; MZ –

392 maize zein; HIPE – high internal phase emulsion.

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Protein	Production strategy	Type of dispersion	Main results	Reference
Zein	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{soy\ bean\ oil}$ = 0.50)	Emulsions were relatively stable for up to two weeks but only at low ionic strength and at pH values far from the PZC.	[15●●]
Zein	Anti-solvent precipitation (ethanol-water)	W/O emulsions ($\phi_{soy\ bean\ oil}$ = 0.70)	Emulsions had very limited stability (hours).	[16]
Zein	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{fish\ oil}$ = 0.30)	Emulsions had very low stability (less than one hour).	[17]
Zein	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{corn\ oil}$ = 0.80)	No stable emulsions could be formed.	[18]

Zein	Anti-solvent precipitation (acetic acid solution-water)	O/W emulsion ($\phi_{corn\ oil}$ = 0.50)	After three days, emulsions were transformed into a gel-like solid which suggests droplet flocculation caused by zein interactions.	[19]
Gliadin	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{corn\ oil}$ = 0.50)	Emulsions were stable for up to 3 months at pH 4.0-9.0 but unstable at pH 3.0.	[20●●]
Gliadin	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{corn\ oil}$ = 0.80)	HIPEs were obtained with stability for up to two months.	[20●●]
Gliadin	Anti-solvent precipitation (ethanol-water)	Foam	Foaming capacity and stability were higher than for ovalbumin or sodium caseinate under the same conditions.	[24●]
Gliadin	Anti-solvent precipitation (ethanol-water)	Foam	High foaming capacity and foam stability at pH 5.8 but low foaming capacity and stability at pH 3.0.	[25]
Gliadin	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{algal\ oil}$ = 0.80)	Solid-like emulgels were obtained by microfluidization after prior formation of coarse emulsions.	[21]
Wheat gluten	Dissolution in acetic acid/ethanol, subsequent emulsification and ethanol removal	O/W emulsion ($\phi_{sunflower\ oil}$ = 0.76)	Rheological properties comparable to those of egg mayonnaise were obtained (solid-like).	[23]

Kafirin	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{sunflower\ oil}$ = 0.30-0.70)	Rheological properties of the obtained emulsions can be tuned by altering their compositions.	[26]
Kafirin	Anti-solvent precipitation (ethanol-water)	O/W emulsion ($\phi_{vegetable\ oil}$ = 0.20-0.80)	The emulsions can be used for controlled release of curcumin.	[27]

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TABLE 3

Table 3: Protein source used, the nanoparticle (NP) production strategy, the type of dispersion stabilized and the main results of studies dealing with the stabilization of foams and emulsions by modified cereal protein based NPs. Abbreviations and symbols are as follows: O/W– oil-in-water; W/O – water-in-oil; ϕ – the oil fraction of the emulsion; PZC – point-of-zero-charge; WG – wheat gliadin; MZ – maize zein; HIPE – high internal phase emulsions.

Protein	Modifier	Production/modification strategy	Type of dispersion	Main results	Reference
Gliadin	Chitosan	Simultaneous protein-polysaccharide anti-solvent precipitation	O/W emulsion ($\phi_{corn\ oil}$ = 0.50-0.90)	Stable HIPEs were obtained up to ϕ=0.83. NPs with chitosan resulted in emulsions with a higher yield stress than NPs without chitosan.	[30]
Gliadin	Chitosan	Simultaneous protein-polysaccharide anti-solvent precipitation	O/W emulsion ($\phi_{corn\ oil}$ = 0.50)	At low pH (2.9) and low ionic strength, emulsions were unstable. At pH 4.0-5.0 or at	[29]

				elevated ionic strengths (> 20 mM NaCl), they were stable for up to two months.	
Gliadin	Chitosan	Simultaneous protein-polysaccharide anti-solvent precipitation	O/W emulsion ($\phi_{corn\ oil}$ = 0.80)	Stable HIPEs with solid-like behavior were obtained.	[29]
Gliadin	Chitosan	Simultaneous protein-polysaccharide anti-solvent precipitation	O/W emulsion ($\phi_{corn\ oil}$ = 0.75)	Stable HIPEs with solid-like behavior were obtained and served as delivery vehicle for curcumin.	[31]
Zein	Chitosan	Simultaneous protein-polysaccharide anti-solvent precipitation	O/W emulsion ($\phi_{n\text{-}tetradecane}$ = 0.20-0.70)	Stable emulsions were obtained at ϕ=0.10-0.30, but at higher ϕ, unstable emulsions or even phase inversion was observed.	[32]
Zein	Pectin	Anti-solvent precipitation, emulsification and subsequent pectin addition	O/W emulsion ($\phi_{fish\ oil}$ = 0.30)	Emulsions without pectin were not stable. In the presence of pectin, they were stable for up to 60 days. Simultaneous addition of laccase and Ca^{2+} ions led to a solid-like gel.	[17]

Zein	Pectin	Simultaneous protein-polysaccharide anti-solvent precipitation	O/W emulsion ($\phi_{corn\ oil}$ = 0.80)	Emulsions were very stable, but only at pH values (3.8) far from the PZC of the zeins, illustrating the importance of electrostatic zein-pectin interactions.	[18]
Zein	Propylene glycol alginate	Simultaneous protein-polysaccharide anti-solvent precipitation	O/W emulsion ($\phi_{medium\ triglyceride\ oil}$ = 0.30-0.75)	At low ϕ, some creaming was observed. At ϕ=0.60, no phase separation occurred and stable emulsions were obtained. At ϕ=0.70-0.75, some oiling off was observed.	[33]
Zein	Propylene glycol alginate – rhamnolipid	Simultaneous protein-polysaccharide-lipid anti-solvent precipitation	O/W emulsion ($\phi_{medium\ triglyceride\ oil}$ = 0.75)	HIPEs were obtained with high stability in a wide range of food processing conditions (temperature, ionic strength, pH).	[34]
Zein	Propylene glycol alginate – sodium caseinate	Simultaneous protein-polysaccharide-lipid precipitation based on pH cycling	O/W emulsion ($\phi_{soy\ bean\ oil}$ = 0.40-0.80)	Stable emulsions were formed at all ϕ values. At higher ϕ, the obtained HIPEs had gel-like textures.	[35●]

Zein	Sodium caseinate	Particle production via anti-solvent precipitation and subsequent electrostatic deposition of sodium caseinate at the particle surface	O/W emulsion ($\phi_{canola\ oil}$ = 0.50)	Emulsions stabilized by MZ-sodium caseinate NPs had higher stability than those stabilized by MZNPs	[36]
Zein	Gum Arabic	Anti-solvent precipitation and subsequent polysaccharide addition	O/W emulsion ($\phi_{medium\ triglyceride\ oil}$ = 0.50-0.70)	Stable emulsions at relatively high ϕ were obtained with elastic gel-like rheological properties.	[37]
Zein	Gum Arabic	Anti-solvent precipitation and subsequent polysaccharide addition	O/W emulsion ($\phi_{soy\ bean\ oil}$ = 0.60)	Stable emulsions at relatively high ϕ were obtained but they lost stability upon addition of 150 mM NaCl, illustrating the electrostatic nature of the interaction between zein and gum Arabic.	[38]
Zein	Tannic acid	Simultaneous protein-tannic acid anti-solvent precipitation after heating	O/W emulsion ($\phi_{corn\ oil}$ = 0.50)	MZ-tannic acid NPs had much higher emulsion stability than MZNPs. The ratio of	[39,43]

		the aqueous ethanol solution (solvent phase)		zein to tannic acid could be used to tweak emulsion properties.	
Zein	Tannic acid	Simultaneous protein-tannic acid anti-solvent precipitation after heating the aqueous ethanol solution (solvent phase)	Air-water interface	MZNPs without tannic acid resulted in low air-water interface coverage due to high tendency of MZNPs to rapidly aggregate at the interface. MZ-tannic acid NPs adsorbed more gradually and formed more ordered interfacial structures.	[41]
Zein	Tannic acid	Simultaneous protein-tannic acid anti-solvent precipitation after heating the aqueous ethanol solution (solvent phase)	O/W emulsion ($\phi_{sunflower\ oil}$ = 0.30-0.50)	Altering the zein to tannic acid ratio and therefore the hydrophobicity of MZ-tannic acid NPs allowed controlling the rheological properties of the emulsions.	[28●]
Zein	Tannic acid	Simultaneous protein-tannic acid anti-solvent precipitation after heating	O/W emulsion ($\phi_{sunflower\ oil}$ = 0.05-0.60)	Altering the zein:tannic acid ratio and therefore the hydrophobicity of MZ-tannic acid NPs allowed exerting control over the rheological properties of obtained emulsions.	[42]

		the aqueous ethanol solution (solvent phase)			
Zein	Tannic acid – sodium dodecyl sulfate	Simultaneous protein-tannic acid anti-solvent precipitation and subsequent addition of sodium dodecyl sulfate	Foam	Addition of sodium dodecyl sulfate led to fractal aggregation of MZ-tannic acid NPs, which strongly improved their foaming properties.	[40]

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FIGURE 1

Figure 1: Common strategies for producing and modifying cereal protein based nanoparticles (NPs) for stabilizing oil-water (O-W) or air-water (A-W) interfaces. A. Protein dissolved in the solvent phase is added to the anti-solvent phase resulting in unmodified NPs. B. Protein dissolved in the solvent phase is added to the anti-solvent phase after which a solution of modifying agent is added, which is thereby deposited on the surface of the pre-formed NPs. C. Protein dissolved in the solvent phase is added to an anti-solvent phase which contains dissolved modifying agent resulting in hybrid NPs consisting of protein and the modifying agent. D. Protein and modifying agent dissolved in the solvent phase are simultaneously added to the anti-solvent phase, resulting in hybrid NPs consisting of protein and the modifying agent.