Colloidal Protein Particles Can Be Used to Develop a Gluten-free Bread

- Demand for high-quality gluten-free products is growing.
- Colloidal protein particles can mimic gluten functionality in a dough-like mixture.
- Protein particles show potential for development of next-generation gluten-free products.

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Gluten intolerance is a well-known disorder that affects almost 1% of the population (9). The demand for high-quality gluten-free products is increasing because the only means of managing gluten intolerance is to follow a gluten-free diet. This explains industrial as well as scientific interest in methods to replace gluten in a broad range of products. Bread is an example of an important product in which gluten must be replaced for consumers with gluten intolerance.

Currently, a number of routes are pursued when developing gluten-free breads. The first route focuses on the use of gluten-free cereals such as oats, corn, and buckwheat (3,14,23–25). Optimization of bread volume, texture, and staling through alterations in the composition or processing of gluten-free formulations has been investigated. The use of pseudocereal flours, such as quinoa and buckwheat, instead of potato starch also has shown good results, as evidenced by a large number of gas cells in the bread and a soft bread crumb (3). The bread volumes reported for gluten-free cereal flours vary from 1.6 mL/g (3,14) to 4.3 mL/g (25), although larger volumes are often accompanied by larger holes in the bread. Other studies have explored protein modification using enzyme, heat, or high-pressure treatment. The impact of ingredient additions (primarily hydrocolloids and emulsifiers) to gluten-free starches and flours has been studied as well (7,17,20–22,35). Increased bread volume and decreased staling rates often are reported in combination with a softer bread texture. The effects of additions are related to the water hydration and gelation capacity of the ingredients added (21,35). Additions have resulted in bread volumes that vary from 1.4 mL/g (7) to 3.8 mL/g (22). Fermentation to obtain sourdough (15) and detoxification (26) is another alternative for producing high-quality gluten-free breads.

Generally, the gluten-free formulations described above possess batter-like properties. In a batter, gas cell stabilization is based on stabilization through a high bulk viscosity obtained by hydrocolloid addition and starch gelatinization (6). In a wheat dough system, gas cell stabilization is obtained through the elasticity provided by a protein network. Because lack of elasticity often leads to coalescence and disproportionation of gas cells (16,19), it would be interesting to develop a gluten-free mixture with dough-like properties, including viscoelastic and strain-hardening properties, to investigate whether this improves gas cell stabilization and other bread properties. A method was developed to create a gluten-free mixture with dough-like properties using colloidal protein particles.

Why Protein Particles?

Dough made from wheat flour has unique mechanical properties in terms of viscoelasticity and strain hardening. The latter is an important indicator of the gas-holding capacity and breadmaking properties of dough (10). Strain-hardening properties are provided by gluten in the dough, which forms a network when the dough is kneaded (34). The gluten network is very complex, and the complete structure is still unclear. There are different theories that describe the relationship between gluten structure and the resulting properties. These theories vary in their hypotheses concerning the major factor(s) determining gluten network properties (4,5,12,18,33). Hamer and van Vliet (11) and Don et al. (8) hypothesized that mesoscopic (10–100 μm) glutenin particles are the main building blocks in gluten. The rheological properties of dough are determined by the interactions between, and the properties of, these mesoscopic-sized particles. These particles consist of high and low molecular weight glutenin subunits and are plasticized by gliadins. Changes in molecular composition, therefore, influence protein particle properties.

Based on the idea that gluten network properties at least partially originate from a particulate structure at the colloidal scale, we decided to explore whether a colloidal particle structure created from a noncereal protein source could form a particle network with viscoelastic properties. In addition, we studied the behavior of a mixture composed of a whey protein particle suspension and (wheat) starch. In the final step, breads were baked using a process

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similar to the traditional breadmaking process. A schematic overview of the whole process is given in Figure 1.

Creation of a Protein Particle Dispersion

Nonwheat proteins do not have the natural ability gluten has to form a protein network spontaneously when dough is kneaded. We found that a colloidal protein particle dispersion, prepared by gelling a phase-separating mixture, possessed elastic behavior (28–30). Moreover, an elastic protein network was obtained using two different proteins, gelatin and whey protein, provided that the proteins were structured into particles. This was a remarkable observation given the differences at the molecular scale between the two proteins. This suggests a more generic effect—colloidal protein particles can form an elastic network in suspension. Next, we tested the behavior of the gelatin protein particles in a mixture containing wheat starch. As with a standard dough, the mixture was kneaded, using a farinograph, to form a continuous protein network. Mixing starch and gelatin particles resulted in a dough with a protein structure comparable to the structure of a dough containing gluten. The mesostructures of a standard dough containing gluten and a gluten-free dough containing gelatin particles are depicted in Figure 2. The results showed that changing the mesoscopic properties of the proteins helped them to mimic the structure of gluten.

Comparable results were obtained using whey protein particles in locust bean gum. Addition of this suspension to a starch slurry also transformed it into a material with elastic and strain-hardening properties. Because these particles were heat stable (in contrast to gelatin, which melts when heated), we further investigated the behavior of whey protein particles as a gluten analogue in baking applications.

Bread Obtained After Baking

As stated earlier, protein particles can be used to transform a starch slurry into a dough-like material, which is why we baked a bread using a process similar to an existing baking process (Fig. 1). Production of the gluten-free breads started with the production of the protein particles. After production of the particles, the particle suspension was mixed with starch, water, yeast, and sugar. Working with the resulting dough, we applied steps similar to those in a standard bread-baking process (27). This was unique because most gluten-free formulations result in a batter-like material rather than a dough-like material. Using this dough-like material, we baked small bread loaves in a slightly adapted kitchen baking machine. The results were remarkably good considering the low protein content, as can be seen in Figure 3 (32). The overall protein content was limited to 2.4% due to the limitations of the protein particle production method.

The next question was whether a good protein particle mesostructure was sufficient to produce a good quality bread or whether the molecular properties of the protein also influenced the breadmaking properties. Compared with gluten, whey protein had a greater ability to form internal cross-links and could form about twice the number of disulfide linkages. This could have consequences for the stiffness of the particles, which could possibly be too rigid. For this reason, we investigated the effect of reducing the ability of whey to form disulfide linkages. In this project, a nonfood chemical (*N*-ethlylmaleimide [NEM]) was used, although food-grade alternatives might exist (e.g., bisulfite). Remarkably, partial blocking of the sulfide groups led to a dough with more strain hardening and bread with more volume and smaller bubbles (Fig. 3) (31). Based on these results, we concluded that the new method using protein particles is a very promising route for producing gluten-free breads. The results are especially interesting when the bread composition is considered: the bread contained protein (2.4 wt%), starch, yeast, salt, and sugar. This means that ample opportunities exist to improve the gluten-free bread using, for example, existing bread improvers.

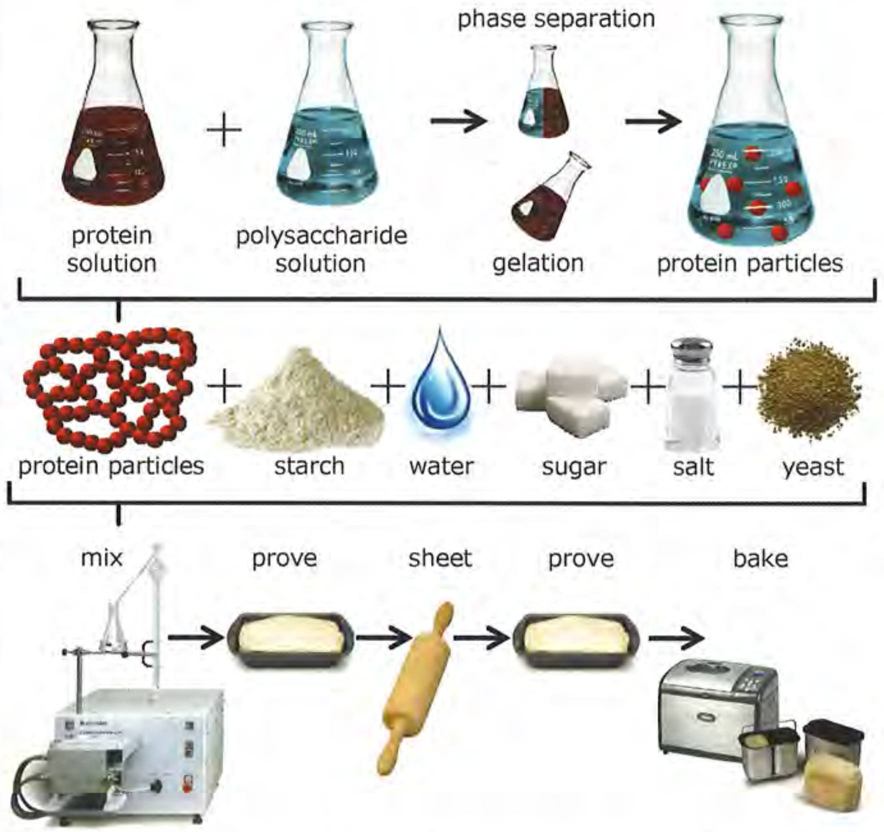


Fig. 1. Schematic overview of the novel method used to produce gluten-free breads.

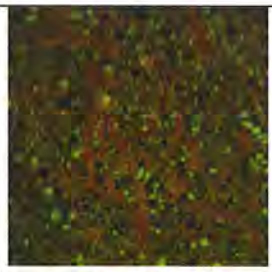
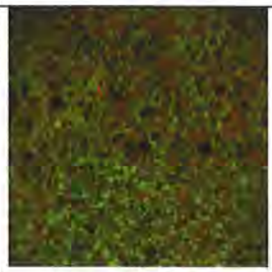
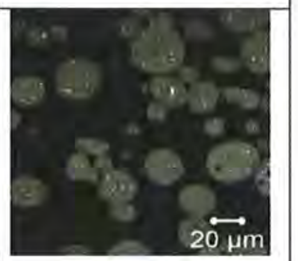
	Morphology of mixed dough	Morphology of proteins before dough mixing
Soissons protein percentage in dough is 10%		Glutenin structure is still unknown
Mixture of starch and gelatin particles protein percentage in dough is 4%		

Fig. 2. Confocal laser scanning microscopy (CLSM) images of the morphology of gluten-containing (Soissons) and gluten-free doughs after mixing (starch granules are green; proteins are red) and CLSM images of the morphology of proteins before gluten-free dough mixing (proteins are white).

Industrial Applications

Application of the method described above to the production of real bread products will require further development and optimization. One of the main issues to be solved is the supply of protein particle suspensions. As described above, the protein particles are formed in a liquid system, which is very susceptible to microbial spoilage. This means that the particle dispersion must be sterilized or dried. Sterilization is only possible if the physical properties of the protein particles do not change. We tested the heat stability of the liquid and found it to be heat stable up to 80°C. This suggests that pasteurization is possible, allowing a shelf life of up to several weeks. Heating at even higher temperatures might lead to additional cross-links in the protein, thereby changing the properties. We tested freeze-drying, which unfortunately led to a protein powder that could not be rehydrated sufficiently to obtain the original protein particle properties.

Another possibility would be to design a protocol that can be used by the manufacturers themselves. In this study, we produced the particles using a versatile method based on phase separation. At the same time, we also tested a more straightforward method to create a protein particle dispersion. In this method the protein was gelled and subsequently broken into smaller pieces and suspended in locust bean gum solutions. Baking tests showed very promising results considering the simple production method. It should be noted, however, that the protein particle method gave better results. We attributed this to the more homogeneous particle size distribution and smaller average particle size. Further optimization of the milling process for the protein gel might overcome this problem and provide additional benefits because the preparation process for gel particles from a gel is more flexible. For example, the protein concentration can be adjusted easily (1,2). A cold-set whey protein gel can be prepared at a higher concentration (limited to ≈12%, wt/wt [13]) than whey protein particles (limited to ≈3%, wt/wt). In addition, protein particles originating from a gel do not require the presence of a polysaccharide. Consequently, the influence of a polysaccharide could be investigated further and optimized in a gel system. The size (and size distribution) of the gel particles could be altered by adjusting milling or grinding conditions. After some adjustments have been made, creating protein particles by grinding a protein gel might become an attractive and robust method to produce a gluten-free dough.

Understanding Structure-Function Properties

In addition to its potential applications, the protein particle system presented here can also be used to further study the physical properties of wheat dough. It could possibly be used as a model system to help clarify the structure-function relationships in more detail and identify which physical properties are influenced by molecular properties and which are influenced by colloidal structures inside the protein network. The protein particle suspension is flexible because it is possible to control the particle size, shape, and mechanical properties. There are still clear differences between the particles presented here and the gluten particles found in wheat dough. For example, disruption and reformation due to dough kneading and resting was not clearly observed for the protein particles. One of the challenges, therefore, would be to modify the protein particles so this property is captured as well. By doing so, our understanding of the interaction at the colloidal and molecular scales can be improved.

Conclusions

Colloidal protein particles possess promising properties that can be used for the development of next-generation gluten-free products. A mixture containing protein particles and starch produced a dough-like material with strain-hardening and viscoelastic properties. Using a standard baking process, which included a sheeting step, we were able to make good quality breads with good volume, small gas cells, and a limited number of holes.

The next steps toward real world applications require a further understanding of the properties of the colloidal protein par-

	Final bread	
Soissons protein percentage in dough is 10%		
Mixture of starch and whey protein particles protein percentage in dough is 2.4%		
Mixture of starch and whey protein particles prepared with 0.56 mM NEM protein percentage in dough is 2.4%		

Fig. 3. Comparison of gluten-containing (Soissons) and gluten-free breads. The bread preparation method has been described previously (27,31,32). The depicted gluten-free breads were prepared without sheeting. NEM = *N*-ethlylmaleimide.

ticles and their resulting functional properties. In addition, the particle concept must be translated into an industrially feasible process that produces the particles in an efficient and robust manner.

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