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The use of whey protein particles in gluten-free bread production, the effect of particle stability

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ABSTRACT

Wheat dough has unique properties for bread making due to its elastic and strain hardening behaviour. A mesoscopically structured whey protein particle system possesses those elastic and strain hardening properties when mixed with starch to a certain extent. However, the extensibility is lower and the particles are more stable than gluten particles upon kneading, probably due to a too high degree of internal crosslinking. This study describes the relation between the number of disulphide bonds of a mesoscopic whey protein particle suspension blocked by NEM treatment and the resulting properties of a dough and bread prepared with that suspension. This study shows that the properties of the particle network are influenced by the ability to form disulphide bonds. Our study shows that a certain amount of disulphide bonds is essential, but too many disulphide bonds can lead to too stiff dough and poorer bread properties.

1. Introduction

With the increasing numbers of people intolerant to gluten, the need is rising for high-quality gluten-free bread. Replacing or removing gluten is not trivial, because gluten has unique desirable properties. Those properties of gluten are difficult to mimic with other components or cereals (Ribotta et al., 2004). Gluten-free breads are typically made using a batter. However, the resulting breads often possess poor properties with respect to the bread volume and the crumb structure. Besides, gluten-free breads typically stale rapidly after baking (Arendt, Morrissey, Moore, & Dal Bello, 2008). In many gluten-free recipes, ingredients such as polysaccharides are added to improve the properties of gluten-free bread through a high bulk viscosity (Demirkesen, Mert, Sumnu, & Sahin, 2010). A high bulk viscosity can improve the volume of the gluten-free breads, but due to a lack of elasticity, stability of gas cell against disproportionation remains limited (Kloek, van Vliet, & Meinders, 2001; Mills, Wilde, Salt, & Skeggs, 2003). The ability of wheat dough to retain gas is related to the rheological properties, such as viscoelasticity, and strain hardening (Khatkar, Bell, & Schofield, 1995; Kokelaar, van Vliet, & Prins, 1996). The strain hardening behaviour of dough is often correlated with baking performance (van Vliet, 2008).

The viscoelastic and strain hardening properties of dough originates from the gluten network that give rise to elasticity. The gluten is able to recover after breakage upon deformation (Cornec, Popineau, & Lefebvre, 1994; Don, Lichtendonk, Plijter, van Vliet, & Hamer, 2005; Li, Dobraszczyk, & Schofield, 2003; Shewry, Halford, Belton, & Tatham, 2002). The glutenin macro polymer (GMP) fraction is generally accepted to be the gluten fraction that provides the greatest contribution to these elastic and strain hardening properties (Don, Lichtendonk, Plijter, & Hamer, 2003; Lindsay & Skerritt, 1999). Although it comprises only 2–4% of the wheat flour, the GMP fraction is very important in bread making (Peighambardoust, van der Goot, Hamer, & Boom, 2005; Wieser, 2007).

In previous articles (van Riemsdijk, van der Goot, Boom, & Hamer, in press; van Riemsdijk, Pelgrom, van der Goot, Boom, & Hamer, 2011; van Riemsdijk, Sprakel, van der Goot, & Hamer, 2010), we showed some promising results to substitute gluten with a gluten-free protein source (whey protein) structured into mesoscopic (~20 μm) protein particles. We demonstrated that a suspension containing those whey protein particles displays elastic properties (van Riemsdijk et al., 2010).

Mixing these particles with starch and water gave rise to wheat dough-like properties including strain hardening behaviour (van Riemsdijk, Pelgrom, et al., 2011). Breads with a specific volume of 3.7 ml/g were obtained after baking this gluten-free dough (van Riemsdijk, van der Goot, et al., in press).

Not withstanding the similarities, normal wheat dough and whey protein particle dough also differed. Compared to wheat

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dough, the particle dough showed a lower mixing tolerance (mixing tolerance was 96% for wheat dough and 83% for the particle dough, analysed with a Farinograph) and showed less resistance to extension (strain at fracture was 1.4 for wheat dough and 0.7 for the particle dough – the stress at fracture was 37.5 kN/m^2 for wheat dough and 2.7 kN/m^2 for the particle dough, both analysed with extensional tests in a Texture Analyser) (van Riemsdijk, Pelgrom, et al., 2011). These differences in the rheological behaviour can (partly) explain why the breads prepared with whey protein particles have more ruptures than a dough with gluten (According to C-cell experiments 4% of the gluten rich bread is ruptured and 6% of the particle dough is ruptured) (van Riemsdijk, van der Goot, et al., in press). In addition, the particles used in the gluten-free recipe showed no signs of disruption after kneading. Previous research on glutenin particles showed that those particles are deformable and show a reduction in particle size upon dough mixing (Don et al., 2005; Peressini, Peighambardoust, Hamer, Sensidoni, & van der Goot, 2008). Also, glutenin particles have a high ability to reform which is related with the viscoelastic behaviour of dough (Don et al., 2005). Thus, the particle network formed by the whey protein particles differs from the network present in wheat dough especially in a number of properties. Apparently, the whey protein particles are too rigid.

The strength of the particles is most likely related to the protein concentration in the particles, and to the number of disulphide bonds present in the particles. The protein concentration in GMP dispersions is ~1.2% (w/w) (Don et al., 2005), which is 10 fold lower than the protein concentration in whey protein particles, which is ~12% (w/w). The amount of disulphide bonds per mol is higher for the glutenin proteins than for the whey proteins. Comparing the protein percentage in the particles and the amount of disulphide bonds present in gluten (~60 μM/g dry weight (Beveridge, Toma, & Nakai, 1974)) and in whey protein (~120 μM/g dry weight (Nakai & Lichan, 1985)), we conclude that the total amount of disulphide bonds/particle is much higher with whey protein particles. This high amount of disulphide bonds could be a cause for the fact that the whey protein particles are more rigid than gluten.

In this study we investigate the influences of the amount of disulphide bonds on dough and bread properties. The amount of disulphide bonds was controlled by blocking (part of) the reactive thiol groups of whey proteins with *N*-ethylmaleimide (NEM). The aim therefore is to provide a better insight in the similarities and differences between the whey protein particle network and the gluten network in dough.

2. Experimental section

2.1. Preparation of protein structures

A whey protein (WP) solution was transformed into WP particles using a cold gelation method. The particles were prepared using a two step procedure. First, a 9% (w/w) WP (Davisco Foods International Inc., USA) solution was heated at 68 °C for 2.5 h to form small WP aggregates. Then, the WP aggregates were mixed with locust bean gum (Danisco Holland BV, The Netherlands) and subsequently gelled with Glucono-delta-lacton (GDL, Sigma Chemicals, The Netherlands).

To investigate the effect of disulphide bonds on the WP particle behaviour, the reactive thiol groups of the WP aggregates were blocked with *N*-ethylmaleimide (NEM). Analysis of the effect of the thiol-blocking with Ellman's reagent showed that treatment of a 9% (w/w) WP aggregate solution with 2.25 mM NEM blocked 94 ± 2% of the accessible thiol groups of the WP aggregates. Therefore, three different concentrations of NEM were selected 2.25 mM, 1.13 mM and 0.56 mM, and added to a 9% (w/w) protein aggregate solution. The reaction with NEM was allowed to proceed at room temperature for at least 30 min. The preparation of particles was similar to the particle preparation without blocking of the reactive thiol groups. We also included a sample in which NEM was added after particle formation, but before dough processing. The amount of NEM added in this procedure was similar to the amount used to block 94 ± 2% of the accessible thiol groups of the WP aggregates. In this case, the intact disulphide bonds in the WP particles will not be influenced by NEM, but disulphide bonds that break during dough mixing cannot be reformed.

2.2. Preparation of dough mixtures

Non-yeasted gluten-free dough mixtures were prepared by mixing wheat starch (Sigma Chemicals, the Netherlands), NaCl (Merck, Germany) and the WP-locust bean gum suspensions in a Farinograph dough mixer for 3 min at a speed of 63 rpm and a temperature of 30 °C. The protein concentration in the mixture was 2.5% (w/w db), the locust bean gum concentration was 0.4% (w/w db), the salt concentration was 2.5% (w/w db) and the moisture content was 47% (w/w).

Yeasted gluten-free dough mixtures were prepared through mixing starch, salt, WP-locust bean gum suspension, dried active bakery yeast (Algist Bruggeman Co., Belgium) and D-glucose (Sigma Chemicals, the Netherlands) in a Farinograph dough kneader for 3 min using a mixing rate of 63 rpm and a temperature of 30 °C. The final protein concentration was 2.4% (w/w db), the final locust bean gum concentration was 0.4% (w/w db), the salt concentration was 2.4% (w/w db), the glucose concentration was 1.1% (w/w db), the yeast concentration was 1.9% (w/w db) and the water percentage was 46% (w/w). Two baking tins of 18 cm^2 (top)/15 cm^2 (bottom) × 3 cm were filled with 30 g dough. The dough was proved in a climate chamber at 35 °C and 85% RH for 100 min. Addition of NEM had no influence on the CO_2 produced by the yeast. A dough ball (5 g) with 0 mM NEM and a dough ball (5 g) with 2.25 mM NEM produced both ~3.5 ml CO_2/g dough during proving. After proving, the dough mixtures were baked in a pre-heated automated kitchen bread machine at ~200 °C for 35 min. The breads were produced in duplicate.

2.3. Analysis of dough mixtures

2.3.1. Structural analysis

The WP suspensions were non-covalently labelled with Rhodamine B (Sigma Chemicals, The Netherlands) to visualise the protein structure before and after dough preparation with Confocal Laser Scanning Microscopy (CLSM – LSM 510, Zeiss, Oberkochen, Germany). After protein structuring, the WP suspensions were transferred into a two well-chambered cover glass (Nunc, Naperville, IL, USA), where Rhodamine B was added before visualising.

Visualisation after dough processing was done by separating the WP particles from the dough using the following procedure. First, the starch present in the dough was dissolved by heating a ten times diluted dough solution at 80 °C for 5 min. Then, the WP particles were separated by centrifugation at 1000×*g* for 3 min. The gel layer formed was diluted and transferred into two well-chambered cover glasses, where it was stained with Rhodamine B. To check if the separation procedure influenced the WP particle structure, we performed two additional experiments. The effect of the heat treatment on the protein structure was excluded by heating a WP particle sample at 80 °C immediately after preparation. No differences in the structure were visible after heating. The effect of starch was excluded by including an extra separation step in a WP particle dough sample. After heating, the gluten-free dough was incubated with Amylase p500 (Gist-Brocades) for 3 h, and separated by centrifugation at 1000×*g* for 3 min. Full conversion of