Food Hydrocolloids 25 (2011) 1744–1750

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

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

Lieke E. van Riemsdijk [a,b], Atze J. van der Goot [a,*], Rob J. Hamer [b]

[a] Laboratory of Food Process Engineering, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands
[b] Laboratory of Food Chemistry, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 4 January 2011
Accepted 23 March 2011

Keywords:
Gluten-free
Whey protein
Mesoscopic
Disulphide

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

* Corresponding author. Tel.: +317 480852; fax: +317 482237.
E-mail address: Atzejan.vanderGoot@wur.nl (A.J. van der Goot).

0268-005X/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2011.03.017

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

the starch was confirmed using iodine staining. For this purpose a 0.05 M iodine (Merck) solution was used. No difference in the structure was visible with and without amylase incubation. The average particle diameter was calculated by measuring the mean diameter of eight particles.

2.3.2. Small deformation measurements strain sweeps

The small deformation behaviour of dough was measured with a Paar Physica MCR 301 (Anton Paar, Austria) stress-controlled rheometer, equipped with a serrated plate/plate geometry (diameter 25 mm − gap 1 mm) and a solvent trap. Before the strain was logarithmically increased from 0.001% to 400%, samples were rested for 15 min to allow relaxation of the stresses induced during sample loading. The tests were done with a constant frequency of 1 Hz and a temperature of 25 °C.

2.3.3. Large deformation measurements, uniaxial extension tests

The large deformation behaviour of dough was measured with a Texture Analyser (Instron-5564Series-Table-Model-Systems-Twin-column-design, Canton USA), equipped with a Kieffer dough-and-gluten extensibility rig and a 50 N load cell. The dough was moulded into trapezium-shaped strips using a Kieffer mould coated with silicon oil. The samples were allowed to rest inside the mould at 25 °C and 90% RH for 45 min before the sample strips (18×16 mm^2) were elongated using a deformation rate of 3.3 mm/s. At least three samples for each dough type were tested. The force–displacement curves were transformed into stress–strain data as described by Dunnewind, Sliwinski, Grolle, and Van Vliet (2004), taking into account that most of the samples had a negligible banding distance, and assuming a constant volume. The stress (σ) at fracture, the Henky strain (ϵ) at fracture stress and the apparent strain hardening coefficient (n) were determined. The strain hardening coefficient was determined by applying an exponential fit on the $\sigma-\epsilon$ curve in the Henky strain ranging from 20 to 95% of fracture strain.

2.3.4. Bread analysis

After baking, the breads were cooled to room temperature before they were further analysed. Bread volume was determined with the rapeseed displacement method (AACC-2000 method 10-05). The structure of bread was visualised by photographic imaging of the whole breads and bread slices. From each bread type a representative slice is both used as photographic representation and for C-cell analysis. The structure of the bread crumbs was evaluated using the C-Cell Bread Imaging System. The parameters used for the crumb characterisation are the average cell diameter (mm), and the area of holes (%). A smaller average cell diameter reflects a finer crumb structure. A larger area of holes reflects a lack of elasticity, and consequently a poor gas cell stabilisation.

3. Results

The effect of NEM treatment on the particle shape and size was investigated using CLSM. Fig. 1 shows the microscopic images of the WP particles before dough processing and after dough processing and isolation, using different NEM concentrations. The microscopic images of the WP particles before dough processing confirm that the WP particles with and without NEM treatment have a similar shape (van Riemsdijk, Snoeren, van der Goot, Boom, & Hamer, 2011). The size before dough processing is similar for the untreated particles (17 ± 4 μm), and the particles treated with 2.25 mM NEM (15 ± 3 μm). This similarity in size is in line with our earlier observation that NEM addition does not influence the particle formation process (van Riemsdijk, Snoeren, et al., 2011). However, our data show that particle size is influenced by the NEM treatment at intermediate concentrations. The average size before dough processing of the WP particles treated with 1.13 mM NEM or 0.56 mM NEM was larger (26 ± 5 μm and 31 ± 9 μm respectively) than the average size of the WP particles without NEM treatment.

The effects of mixing on the particle shape and size were also investigated using CLSM. When interpreting these results it is important to be aware of possible side effects of the separation procedure. Particles isolated from dough were subjected to heat (80 °C), this was required to gelatinise and enzymatically remove the starch. Nevertheless some remarkable differences in the shape of the WP particles were observed after mixing. The untreated WP particles retained their size (16 ± 4 μm), and showed only slight particle deformation (less spherical) upon dough processing.

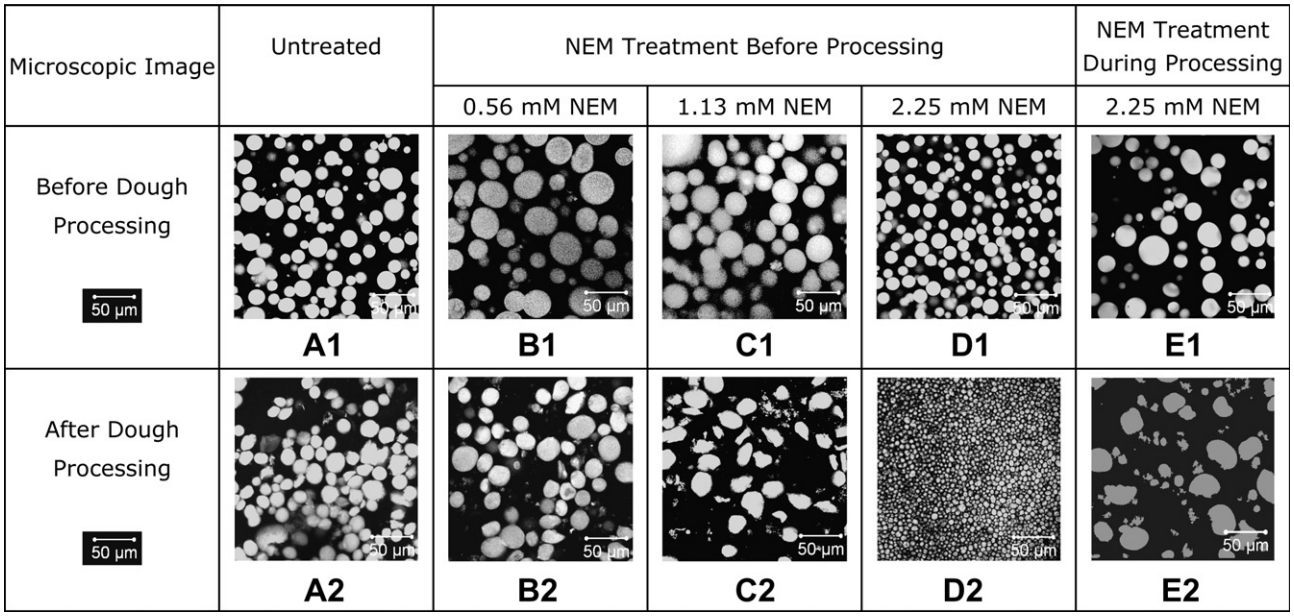

Fig. 1. Overview of the structure of whey protein particles before and after dough processing. The particles vary in the NEM treatment. A 9% (w/w) protein aggregate solution was untreated or treated with 0.56 mM NEM, 1.13 mM NEM or 2.25 mM NEM before particle formation.

Dough prepared with WP particles treated with 1.13 mM NEM or 0.56 mM NEM resulted in particle deformation and break-up, leading to the appearance of fragments and a decrease in particle size (to 21 ± 5 μm and 16 ± 4 μm respectively) and irregularities in shape. Dough prepared with an even higher amount of NEM (2.25 mM NEM) resulted in a significant particle size reduction after dough processing. Here, the size decreased to 4 ± 1 μm. When NEM was added after preparation, but during dough mixing, only disulphide bonds that break during processing will be affected. Our results reveal differences between NEM addition before WP particle formation and NEM addition during dough processing (compare Fig. 1 D2 and E2). The WP particles in which NEM was added during dough processing were clearly deformed and had lost their spherical shape. Besides, the mixing led to marked changes in particle size distribution, from a single distribution (19 ± 5 μm) to a bimodal distribution with both small (10 ± 4 μm) and large particles (36 ± 3 μm).

The mixing behaviour of a dough gives some information about the stability of the protein network. Fig. 2 depicts the peak consistency and the consistency after 3 min mixing for the gluten-free dough mixtures. The more NEM was used the larger the value for the peak consistency of the dough mixture (3.3 Nm for untreated particles, 3.4 for particles treated with 0.56 mM NEM 3.6 for particles treated with 1.13 mM NEM and 4.0 Nm for particles treated with 2.25 mM NEM). Although the NEM treatment gave an increase in the peak consistency, the torque value at the end of the mixing is lower for the dough mixture prepared with 2.25 mM NEM (1.9 Nm) than for the other dough mixtures (2.7–2.9 Nm).

The small deformation properties of the dough mixtures are depicted in Table 1. The loss factor of all mixtures was between 0.1 and 0.2, indicating that the mixtures were firm. The strength of the mixtures differed however. The storage and loss moduli of the dough mixture prepared with WP particles treated with 0.56 mM NEM or 1.13 mM NEM was higher (storage modulus was $\sim 22 \times 10^3$ Pa and the loss modulus was 4.3×10^3 Pa) compared to dough prepared with untreated WP particles (15×10^3 Pa and 4.3×10^3 Pa respectively). The increase in the moduli can be a result

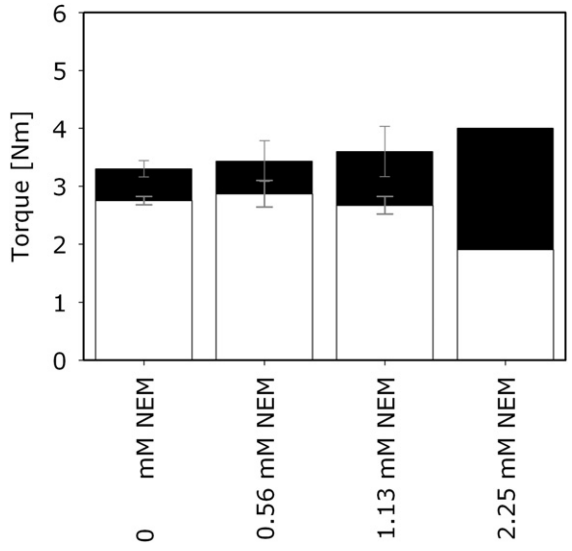

Fig. 2. Torque values during mixing in a Farinograph of starch and whey protein particles. The particles vary in the NEM treatment. A 9% (w/w) protein aggregate solution was untreated or treated with 0.56 mM NEM, 1.13 mM NEM or 2.25 mM NEM before particle formation. The white bars mark the torque values after mixing (3 min) dough, the black bars mark the torque values at the peak of the Farinograph curve. Different letters indicate statistically significant differences.

Table 1
Storage modulus, loss modulus and loss factor of dough samples under small deformation measurements in a rheometer. The dough mixtures are prepared of starch and whey protein particles. The dough mixtures are prepared with particles that vary in the NEM treatment. A 9% (w/w) protein aggregate solution was untreated or treated with 0.56 mM NEM, 1.13 mM NEM or 2.25 mM NEM before particle formation.

NEM treatment	Storage modulus (Pa)	Loss modulus (Pa)	Loss factor (−)
0 mM NEM	$15.2 \times 10^3 \pm 0.0 \times 10^3$	$2.0 \times 10^3 \pm 0.0 \times 10^3$	0.13 ± 0.00
0.56 mM NEM	$23.4 \times 10^3 \pm 6.1 \times 10^3$	$4.3 \times 10^3 \pm 0.9 \times 10^3$	0.18 ± 0.01
1.13 mM NEM	$20.1 \times 10^3 \pm 0.7 \times 10^3$	$4.3 \times 10^3 \pm 0.1 \times 10^3$	0.22 ± 0.01
2.25 mM NEM	$8.8 \times 10^3 \pm 1.5 \times 10^3$	$1.8 \times 10^3 \pm 0.3 \times 10^3$	0.20 ± 0.00

of the higher phase volume of the particles. A dough prepared with WP particles treated with 2.25 mM NEM gave lower moduli (storage modulus was 8.8×10^3 Pa and the loss modulus was 1.8×10^3 Pa) than the other dough mixtures.

The final bread properties can be often related with the large deformation behaviour of the dough. Especially, the strain hardening is related to the gas cell stabilisation (Tronsmo et al., 2003). The large deformation properties of the dough mixtures are depicted in Table 2. The results show that the NEM treatment of the WP particles had a relatively small impact on strain at fracture of the WP particle dough mixtures. There is a slight decrease visible in the strain at fracture when more NEM is used. Although there is no significant correlation observed. These results have to be interpreted carefully. The WP particle dough mixtures lack a component which increases the viscous behaviour such as gliadins. Adding gliadins increases the strain at fracture of a dough (Uthayakumaran, Newberry, Keentok, Stoddard, & Bekes, 2000), hence the lack of gliadins can explain the low strain at fracture of the particle dough. The lack of gliadins resulted in a different behaviour upon deformation compared to normal wheat dough. As a result, the strips of the mixtures broke at the hook rather than in the middle of the sample. Most likely, this effect will lead to an underestimation of the actual strain at break.

We could observe that NEM treatment influenced the stress at fracture and the strain hardening behaviour significantly. The dough mixtures prepared with WP particles treated with 0.56 mM NEM showed the highest value for the stress at fracture (3.2 kN/m^2). A smaller or larger NEM concentration resulted in a lower stress at fracture (2.7 kN/m^2 for untreated WP particles and 1.1 kN/m^2 and 0.5 kN/m^2 for the dough mixtures prepared with WP particles treated with 1.13 mM NEM and 2.25 mM NEM respectively). An optimum was also visible for the strain hardening behaviour. Dough with untreated WP particles showed a limited strain hardening (1.2). The strain hardening has the highest value for the dough prepared with WP particles treated with 0.56 mM NEM (2.3). Strain hardening decreased again if more NEM was used (1.9). Dough prepared with WP particles treated with 2.25 mM NEM showed no strain hardening.

Table 2
Stress at fracture, strain at fracture and strain hardening value of dough samples under extensional tests in a Texture Analyser. The dough mixtures are prepared of starch and whey protein particles. The dough mixtures are prepared with particles that vary in the NEM treatment. A 9% (w/w) protein aggregate solution was untreated or treated with 0.56 mM NEM, 1.13 mM NEM or 2.25 mM NEM before particle formation.

NEM treatment	Henky strain at fracture (−)	Stress at fracture (kN/m^2)	Strain hardening (−)
0 mM NEM	0.7 ± 0.1	2.7 ± 0.1	1.2 ± 0.2
0.56 mM NEM	0.6 ± 0.0	3.2 ± 0.3	2.3 ± 0.3
1.13 mM NEM	0.5 ± 0.0	1.1 ± 0.3	1.9 ± 0.2
2.25 mM NEM	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 1.0

Besides mixing and rheological experiments, the gas holding ability of the mixtures is measured. First, we demonstrated that NEM treatment did not affect the activity of the yeast. Differences observed will therefore be due to differences in gas holding capacity. Under the conditions used (time, temperature, amount of yeast) the average gas production was ~3.5 ml/g dough. The dough mixtures were then used to bake breads. The bread prepared with 0.56 mM NEM gave the bread with the most attractive appearance (though we realise that this is a subjective visual observation). This bread had a nice cap, while other breads had a more cubic shape. Fig. 3 depicts the volumes of the bread obtained by the different WP particle mixtures. Overall, the bread volume became smaller when the particles were treated with NEM. The specific volume of the untreated WP particle bread was 3.7 ml/g, the specific volume of the NEM treated WP particle breads was lower 2.8–3.0 ml/g.

Photographic images of the breads are depicted in Fig. 4. These images show that the height of the bread prepared with untreated WP particles was lower than the bread prepared with WP particles treated with 0.56 mM NEM. C-cell experiments confirm that the maximum height is larger, but the average height is lower for the bread prepared with WP particles treated with 0.56 mM NEM. The bread prepared with untreated WP particles is more cubic, while the breads prepared with WP particles treated with 0.56 mM NEM had a cap. Another difference between the breads is the colour. The bread prepared with untreated WP particles had a darker crust compared to the breads prepared with WP particles that are treated with NEM. The differences in bread shape and crust colour can have different causes (e.g. rate of water evaporation, relative humidity during baking (Purlis & Salvadori, 2009; Vanin, Lucas, & Trystram, 2009)).

The gas cell structures are visible in Fig. 4. In almost all breads, ruptures were visible. The bread with the lowest amount of ruptures is the bread prepared with WP particles treated with 0.56 mM NEM (3% of the total bread volume). The bread with the highest amount of ruptures is the bread prepared with WP particles treated with 2.25 mM NEM (11% of the total bread volume). The other two breads (untreated WP particles and WP particles treated with 1.13 mM NEM) had a comparable amount of ruptures (6% and 7% of the total bread volume respectively). The average diameter of the gas cells is the largest for the bread prepared with WP particles treated with 2.25 mM NEM (2.9 mm). The bread prepared with untreated WP particles had a slightly larger diameter (2.5 mm) than the breads prepared with WP particles treated with 0.56 mM NEM and 1.13 mM NEM (2.2 mm).

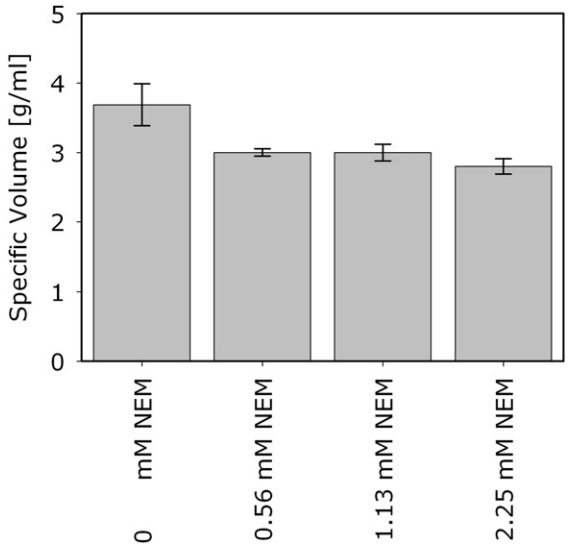

Fig. 3. Specific volume (ml/g) of bread prepared of starch and whey protein particles. The breads are prepared with particles that vary in the NEM treatment. A 9% (w/w) protein aggregate solution was untreated or treated with 0.56 mM NEM, 1.13 mM NEM or 2.25 mM NEM before particle formation.

4. Discussion

In previous articles (van Riemsdijk, van der Goot, et al., in press; van Riemsdijk, Pelgrom, et al., 2011) we have demonstrated that a mesoscopically structured whey protein dispersion can be used as a substitute for gluten in the preparation of a dough and a leavened bread (van Riemsdijk, van der Goot, et al., in press; van Riemsdijk, Pelgrom, et al., 2011). We have demonstrated that the whey particles are quite stable, certainly in comparison with wheat glutenin particles, which are disrupted during mixing (Don et al., 2005; Peressini et al., 2008).

The amount of disulphide bonds can have an effect on the phase volume of the WP particles. Too many cross-links prevent an increase in phase volume of the particles. Removing or blocking part of the reactive thiol groups will induce the phase volume. Consequently, the protein particles will behave more elastically. The increase in phase volume can have different causes e.g. swelling, or the formation of a more loosely packed particle structure. The volume increase is almost 8 times, which suggests that swelling cannot be the only reason. Further research is needed to completely unravel how the NEM addition affects the particle size.

This study aims at clarifying the importance of disulphide bonds on the behaviour of whey particles in a dough system. Since the separation procedure can have side effects, we focussed on the main changes in the structure of the particles. The results presented here show that WP particles without NEM treatment and WP particles treated with 0.56 mM NEM or 1.13 mM NEM can withstand the forces during dough mixing, although some deformation and break-up occurs. Only when the WP particles are treated with 2.25 mM NEM (blocking the ability to stabilise particles by the formation of disulphide bonds), the intra-particle interactions are not sufficient to prevent disruption of particles upon dough mixing. Nevertheless, these broken particles still form a cohesive dough in combination with starch, although resulting dough and bread-making properties are deteriorated. The dough has a lower consistency after dough mixing (Fig. 2), the small deformation moduli are lower (Table 1), the strain hardening behaviour has disappeared (Table 2) and the gas bubble stability was reduced (as is clear from the lower volume and the large amount of ruptures and cracks, Figs. 3 and 4).

The effect of complete blocking of the reactive thiol groups via NEM addition has a similar effect on wheat gluten dough and the gluten-free dough mixtures studied here (Belitz, Kieffer, Seilmeier, & Wieser, 1986; Peressini et al., 2008). In both materials, the final consistency and the mixing tolerance decreases. The initial high peak consistency of gluten after NEM treatment was related to the depolymerisation, which initially increases the water hydration capacity, and consequently the viscosity of the dough (Peressini et al., 2008). A decrease in stress at fracture, strain at fracture and the strain hardening behaviour upon NEM treatment was also observed in wheat dough (Peressini et al., 2008). A complete blocking of the reactive thiol groups weakened the gluten, and consequently weakened the dough (Belitz et al., 1986). All of the effects mentioned above were also found for WP particle dough in case of complete blocking of the reactive thiol groups, suggesting that the WP particle system creates dough-like properties in a similar manner as gluten does in wheat dough.

A main difference between dough and the gluten-free mixtures is related to the apparent absence of breakage of particles when no

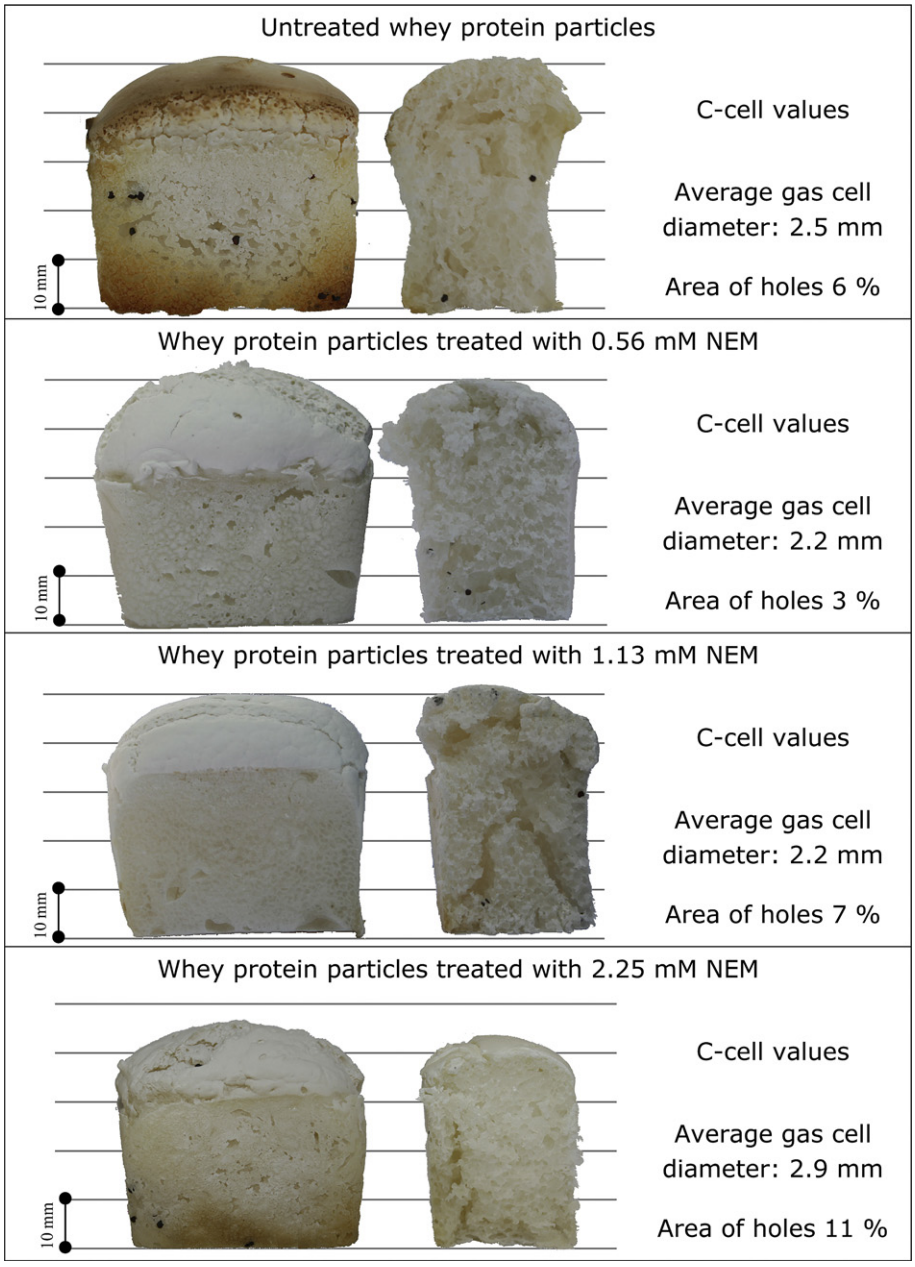

Fig. 4. Photographic images of bread prepared of starch and whey protein particles. The breads are prepared with particles that vary in the NEM treatment. A 9% (w/w) protein aggregate solution was untreated or treated with 0.56 mM NEM, 1.13 mM NEM or 2.25 mM NEM before particle formation.

NEM was added. The fact that the particles kept their original size if the thiol groups were not blocked can have two causes. First, the fact that the amount of disulphide bonds in whey protein particles is high compared to the amount in a GMP dispersion results in a high mechanical strength of the particles. Second, the high concentration of thiol groups will allow fast (re-)formation of disulphide bonds in case these are broken due to shear forces onto the particles. We performed an experiment in which NEM was added during dough mixing to provide further understanding of the effect of dough mixing on the particles.

Our observations show that after dough mixing the WP particles with NEM addition during dough mixing, were not identical to particles present in the dough without NEM addition (Fig. 1 A2 and E2). After mixing, small particles as well as larger particles were observed. The small particles suggesting particle break-up. The large particles suggesting an increase in phase volume. From the increased phase volume, we conclude that dough mixing results in rupture of (part of) the disulphide bonds, NEM prevent reformation of these bonds, and as a result the number of bonds will decrease. The reduced number of disulphide bonds in the particles will weaken the particles, which could explain the particle break-up observed.

The additional volume fraction of the particles treated with 0.56 mM NEM might explain why the dough prepared with these WP particles has a mixing consistency, strain hardening behaviour and stress at fracture that approaches wheat dough better than those of the other WP particle dough mixtures. Also the increased elasticity of the particles might play a role.

Unfortunately, NEM is not a food-grade components. This implies that another procedure is necessary to control the amount

of disulphide interactions in the WP particles. An option could be to alter the heating conditions during the preparation of the aggregates. That results in a different reactivity of the protein particles (Purwanti et al., 2011). Alternatively, another protein type with a lower amount of disulphide interactions could be used. In that case ovalbumin, seems a promising candidate.

5. Conclusions

The present study confirms the potential of mesoscopic protein particle networks to imitate gluten properties. Despite the simple composition and low protein concentration the dough already showed important similarities (e.g. strain hardening behaviour) to wheat dough. The present study focuses on the role of the mechanical stability of particles as affected by internal crosslinking. By chemically affecting disulfide bond formation, we demonstrated the role of disulphide bonds, not only in the formation of such particles, but also in determining their mechanical stability, phase volume and ability to form a viscoelastic network. This phenomenon can be used to further improve dough and bread-making properties of mesoscopically structured non-gluten proteins.

Acknowledgement

The authors thank Derk Mudde (Nederlands Bakkerij Centrum, Wageningen) for the C-cell measurements. This research was financially supported by the Graduate School VLAG.

References

Arendt, E. K., Morrissey, A., Moore, M. M., & Dal Bello, F. (2008). Gluten-free breads. In E. K. Arendt, & F. Dal Bello (Eds.), *Gluten-free cereal products and beverages* (pp. 289–319). San Diego: Academic Press.

Belitz, H. D., Kieffer, R., Seilmeier, W., & Wieser, H. (1986). Structure and function of gluten proteins. *Cereal Chemistry, 63*(4), 336–341.

Beveridge, T., Toma, S. J., & Nakai, S. (1974). Determination of SH- and SS-groups in some food proteins using Ellman's reagent. *Journal of Food Science, 39*(1), 49–51.

Cornec, M., Popineau, Y., & Lefebvre, J. (1994). Characterisation of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. *Journal of Cereal Science, 19*(2), 131–139.

Demirkesen, L., Mert, B., Sumnu, G., & Sahin, S. (2010). Rheological properties of gluten-free bread formulations. *Journal of Food Engineering, 96*(2), 295–303.

Don, C., Lichtendonk, W., Plijter, J. J., & Hamer, R. J. (2003). Glutenin macropolymer: a gel formed by glutenin particles. *Journal of Cereal Science, 37*(1), 1–7.

Don, C., Lichtendonk, W. J., Plijter, J. J., van Vliet, T., & Hamer, R. J. (2005). The effect of mixing on glutenin particle properties: aggregation factors that affect gluten function in dough. *Journal of Cereal Science, 41*(1), 69–83.

Dunnewind, B., Sliwinski, E. L., Grolle, K., & Van Vliet, T. (2004). The Kieffer dough and gluten extensibility rig: an experimental evaluation. *Journal of Texture Studies, 34*(5–6), 537–560.

Khatkar, B. S., Bell, A. E., & Schofield, J. D. (1995). The dynamic rheological properties of glutens and gluten sub-fractions from wheats of good and poor bread making quality. *Journal of Cereal Science, 22*(1), 29–44.

Kloek, W., van Vliet, T., & Meinders, M. (2001). Effect of bulk and interfacial rheological properties on bubble dissolution. *Journal of Colloid and Interface Science, 237*(2), 158–166.

Kokelaar, J. J., van Vliet, T., & Prins, A. (1996). Strain hardening properties and extensibility of flour and gluten doughs in relation to breadmaking performance. *Journal of Cereal Science, 24*(3), 199–214.

Li, W., Dobraszczyk, B. J., & Schofield, J. D. (2003). Stress relaxation behavior of wheat dough, gluten, and gluten protein fractions. *Cereal Chemistry, 80*(3), 333–338.

Lindsay, M. P., & Skerritt, J. H. (1999). The glutenin macropolymer of wheat flour doughs: structure-function perspectives. *Trends in Food Science & Technology, 10*(8), 247–253.

Mills, E. N. C., Wilde, P. J., Salt, L. J., & Skeggs, P. (2003). Bubble formation and stabilization in bread dough. *Food and Bioproducts Processing, 81*(C3), 189–193.

Nakai, S., & Lichan, E. (1985). Structure modification and functionality of whey proteins – quantitative structure-activity relationship approach. *Journal of Dairy Science, 68*(10), 2763–2772.

Peighambardoust, S. H., van der Goot, A. J., Hamer, R. J., & Boom, R. M. (2005). Effect of simple shear on the physical properties of glutenin macro polymer (GMP). *Journal of Cereal Science, 42*(1), 59–68.

Peressini, D., Peighambardoust, S. H., Hamer, R. J., Sensidoni, A., & van der Goot, A. J. (2008). Effect of shear rate on microstructure and rheological properties of sheared wheat doughs. *Journal of Cereal Science, 48*(2), 426–438.

Purlis, E., & Salvadori, V. O. (2009). Modelling the browning of bread during baking. *Food Research International, 42*(7), 865–870.

Purwanti, N., Smiddy, M., van der Goot, A. J., de Vries, R., Alting, A., & Boom, R. M. (2011). Modulation of rheological properties by heat-induced aggregation of whey protein solution. *Food Hydrocolloids, 25*(6), 1482–1489.

Ribotta, P. D., Ausar, S. F., Morcillo, M. H., Perez, G. T., Beltramo, D. M., & Leon, A. E. (2004). Production of gluten-free bread using soybean flour. *Journal of the Science of Food and Agriculture, 84*(14), 1969–1974.

van Riemsdijk, L. E., van der Goot, A. J., Boom, R. M..Hamer, R. J. Preparation of gluten-free bread using a meso-structured whey protein particle system. *Journal of Cereal Science*, in press, doi:10.1016/j.jcs.2011.02.006.

van Riemsdijk, L. E., Pelgrom, P. J. M., van der Goot, A. J., Boom, R. M., & Hamer, R. J. (2011). A novel method to prepare gluten-free dough using a meso-structured whey protein particle system. *Journal of Cereal Science, 53*(1), 133–138.

van Riemsdijk, L. E., Sprakel, J., van der Goot, A. J., & Hamer, R. J. (2010). Elastic networks of protein particles. *Food Biophysics, 5*(1), 41–48.

van Riemsdijk, L. E., Snoeren, J. P. M., van der Goot, A. J., Boom, R. M., & Hamer, R. J. (2011). New insights on the formation of colloidal whey protein particles. *Food Hydrocolloids, 25*, 333–339.

Shewry, P. R., Halford, N. G., Belton, P. S., & Tatham, A. S. (2002). The structure and properties of gluten: an elastic protein from wheat grain. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 357*(1418), 133–142.

Tronsmo, K. M., Magnus, E. M., Baardseth, P., Schofield, J. D., Aamodt, A., & Færgestad, E. M. (2003). Comparison of small and large deformation rheological properties of wheat dough and gluten. *Cereal Chemistry, 80*(5), 587–595.

Uthayakumaran, S., Newberry, M., Keentok, M., Stoddard, F. L., & Bekes, F. (2000). Basic rheology of bread dough with modified protein content and glutenin-to-gliadin ratios. *Cereal Chemistry, 77*(6), 744–749.

van Vliet, T. (2008). Strain hardening as an indicator of bread-making performance: a review with discussion. *Journal of Cereal Science, 48*(1), 1–9.

Vanin, F. M., Lucas, T., & Trystram, G. (2009). Crust formation and its role during bread baking. *Trends in Food Science & Technology, 20*(8), 333–343.

Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology, 24*(2), 115–119.