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**Relating the composition and air/water interfacial properties of
wheat, rye, barley, and oat dough liquor**

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Abstract

Gas cell stabilization in dough by its aqueous phase constituents is arguably more important in non-wheat than in wheat dough due to weaker protein networks in the former. Dough liquor (DL), a model for the dough aqueous phase, was isolated from fermented wheat, rye, barley, and oat doughs by ultracentrifugation. DL composition (protein, lipid, arabinoxylan, β -glucan) and air/water interfacial functionality [foaming, viscosity, surface tension, surface dilatational modulus (E)] were related to bread quality. Poor foaming and low E of wheat DL were ascribed to lipids and proteins co-occurring at the interface. Nonetheless, the presence of a gluten network resulted in high-quality wheaten breads. Homogeneous and heterogeneous crumb structures of rye and barley breads, respectively, were attributed to high and low E values of their respective DLs. High lipid content and low surface tension of oat DL indicated a lipid-dominated interface, which may explain the heterogeneous crumb structure of oat breads.

KEYWORDS

Dough liquor; proteins; lipids; non-starch polysaccharides; bread making

1. Introduction

Wheat (*Triticum aestivum* L.) bread making typically starts by mixing water, flour, yeast, and salt. Air cells are incorporated while kneading these constituents to form dough. They expand during fermentation due to carbon dioxide production by the yeast. Wheat gluten proteins form a viscoelastic network in which starch granules are embedded and which physically stabilizes the expanding gas cells (Sroan, Bean, & MacRitchie, 2009). However, already in the early stages of fermentation, discontinuities start to develop in the gluten-starch matrix, which leaves gas cell surfaces only surrounded by a thin liquid film (Gan, Angold, Williams, Ellis, Vaughan, & Galliard, 1990).

Fermenting bread dough can thus be regarded as foam [*i.e.* a dispersion of a gas (air) in a liquid (water)] and is thus thermodynamically unstable. It is susceptible to two major destabilization mechanisms, *i.e.* disproportionation and coalescence (Damodaran, 2005; Murray, 2007). During bread making these processes are slowed down as the air/water interfaces in dough are directly stabilized by proteins and lipids and indirectly by non-starch polysaccharides (NSPs) in the liquid films surrounding gas cells and thus present in the dough aqueous phase (Gan, Ellis, & Schofield, 1995; Sroan & MacRitchie, 2009). In what follows, the term 'interface' is used throughout this manuscript to indicate the dough air/water interface.

Proteins and lipids can diffuse to and adsorb at the interface and thereby lower surface tension. Once adsorbed at the interface, proteins unfold and orient their more hydrophobic amino acid regions towards the air phase. While these conformational rearrangements occur, proteins mutually interact and thereby form coherent viscoelastic films around gas cells (Murray, 2007). Proteins can not only form such films, they can also stabilize gas cells by sterically or electrostatically hindering other gas cells from approaching each other (Murray, 2007). In contrast to proteins, lipids stabilize an interface by forming a highly fluid monolayer. When the lipid concentration at an interface is locally reduced, *e.g.* by stretching the gas cell surface, adsorbed lipids migrate to areas of low surface

concentration to restore the concentration gradient. This phenomenon is known as the Gibbs-Marangoni effect (Damodaran, 2005). In addition to the direct stabilization by proteins and lipids, NSP can indirectly stabilize foams. When solubilized in the dough aqueous phase, they increase its viscosity and thereby may decelerate drainage of liquid from the aqueous film between gas cells. In turn, this can delay gas cell coalescence (Courtin & Delcour, 2002). Furthermore, NSP have been suggested to interact with proteins adsorbed at the interface, thereby modifying their interfacial characteristics (Sarker, Wilde, & Clark, 1998; Primo-Martín, Hamer, & De Jongh, 2006).

A representative fraction of the aqueous phase of dough can be isolated by ultracentrifugation and is further referred to as dough liquor (DL) (Baker, Parker, & Mize, 1946). MacRitchie (1976) studied the effect of the gravitational force on the water content of the pellet after centrifugation and concluded that a gravitational force of 100,000 *g* for a reasonable time is sufficient to separate the doughs' insoluble from its doughs' soluble phase. Several research groups have studied the composition (Salt, Robertson, Jenkins, Mulholland, & Mills, 2005; Pauly, Pareyt, Fierens, & Delcour, 2014; Gerits, Pareyt, Masure, & Delcour, 2015; Salt, González-Thuillier, Chope, Penson, Tosi, Haslam, et al., 2018), the foaming, and/or the surface-active properties (MacRitchie & Gras, 1973; MacRitchie, 1976; Sahi, 2003; Primo-Martín, Hamer, & De Jongh, 2006; Salt, Wilde, Georget, Wellner, Skeggs, & Mills, 2006; Pauly, Pareyt, Fierens, & Delcour, 2014; Turbin-Orger, Della Valle, Doublier, Fameau, Marze, & Saulnier, 2015; Salt, et al., 2018) of wheat DL. Although the wheat cultivars and experimental set-ups (such as bread making recipe and ultracentrifugation conditions) strongly differed in these studies, overall it was established that the interfaces in wheat dough are unstable as they are occupied by a mixture of proteins and lipids. Indeed, mixed protein-lipid interfaces are unstable as both constituents disrupt each other's interfacial stabilization mechanism (cfr. supra) (Wilde, 2000).

Today's consumers are increasingly aware of the potential health benefits of bread consumption. While most non-wheat cereals have higher dietary fiber and essential protein amino acid(s) levels than wheat (Dewettinck, Van Bockstaele, Kühne, Van de Walle, Courtens, & Gellynck, 2008), non-

wheat breads are of lower quality in terms of specific loaf volume and crumb structure than wheat breads, because they lack the high quality viscoelastic protein network which pure wheat dough has. This implies that the above described mechanism whereby dough aqueous phase constituents stabilize gas cells during late fermentation and early baking is probably more important in non-wheat than in wheat doughs. Today, no studies are available which deal with the composition or functionality of the fraction obtained from fermented non-wheat dough by ultracentrifugation. Hence, the potential of non-wheat flour constituents to stabilize dough interfaces during fermentation is not well understood. Against this background, we here isolated DL not only from fermented wheat, but also from fermented rye, barley, and oat doughs and related their foaming, viscosifying, and surface-active (decrease of surface tension over time and surface dilatational modulus) properties to their chemical composition [protein, lipid, arabinoxylan (AX), and β -glucan contents as well as the lipid population (i.e. the relative amounts of non-polar [free fatty acids (FFA) and triacylglycerols (TAG)] and polar (glycolipids and phospholipids) lipids present in the DL samples]. Furthermore, these findings were related to wheat, rye, barley and oat bread loaf specific volume and crumb structure. The present work thus aimed at studying whether the composition and functionality of the aqueous phase of non-wheat dough determines the quality of non-wheat breads. In addition, increasing knowledge regarding the constituents that stabilize (or destabilize) the interfaces in fermenting non-wheat dough will provide a solid basis for selecting non-wheat flour for manufacturing nutrient dense breads.

2. Materials & Methods

2.1 Materials

Commercial bread wheat flour was from Dossche Mills (Deinze, Belgium). It contained 13.6% moisture, 12.9% dry matter (dm) protein, 1.3% dm lipid, 2.1% dm total AX, 0.2% dm β -glucan, and

0.6% dm ash. Rye kernels (cultivar Dukato) from AVEVE (Merksem, Belgium) were conditioned to 16.0% moisture and subsequently roller milled in-house using a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill. Rye flour contained 13.6% moisture, 4.0% dm protein, 0.7% dm lipid, 4.2% dm total AX, 1.1% dm β -glucan, and 0.4% dm ash. Barley grains (cultivar Sebastian) were from Cargill (Herent, Belgium). They were conditioned to 16.0% moisture prior to roller milling (mill specifications as above). Barley flour contained 12.6% moisture, 6.4% dm protein, 1.6% dm lipid, 1.0% dm total AX, 2.3% dm β -glucan, and 0.9% dm ash. Commercial oat flour was from Raisio Nutrition (Raisio, Finland) and contained 10.2% moisture, 13.3% dm protein, 7.4% dm lipid, 1.4% dm total AX, 2.7% dm β -glucan, and 1.5% dm ash. The methods used to determine the composition of the different flours are outlined in section 2.2.1. Sugar, salt, and fresh compressed yeast (AB Mauri, Dordrecht, Nederland) were bought in a local supermarket. All other chemicals, solvents, and reagents were from Sigma-Aldrich (Bornem, Belgium; 2-propanol, acid-washed sand, triethyl amine, β -D-allose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucose, benzoic acid, sodium borohydride, 2-octanol, and ethyl acetate), Thermo Fisher Scientific [Aalst, Belgium; acetic acid (used for lipid analysis), cellulose filters, hexane, chloroform, methanol, bromophenol blue, trifluoroacetic acid, 1-methylimidazole, and ethanol absolute], or VWR International [Oud-Heverlee, Belgium; ammonia, potassium hydroxide, acetic acid (used for AX analysis), acetic anhydride, and anhydride sodium sulfate] and of analytical grade. All solvents used for the lipid extraction and analysis were of high-performance liquid chromatography (HPLC) grade.

2.2 Methods

2.2.1 Flour composition

All analyses described in this section were executed in triplicate. Moisture and ash levels of flour were determined with (AACCI) Methods 44-19.01 and 08-01.01, respectively. Flour protein content was determined using an adaptation of AOAC method 990.03 (AOAC, 1995) to an automated Dumas protein analysis system (Vario Max Cube, Elementar, Hanau, Germany). Nitrogen to protein

conversion factors were 5.70 for wheat and rye flour, 6.25 for barley flour, and 5.80 for oat flour. Flour lipid contents were determined gravimetrically as the sum of free and bound lipids as in Melis, Pauly, Gerits, Pareyt, and Delcour (2017). Free lipids were extracted with hexane while bound lipids were then extracted using water-saturated butanol followed by a purification step (Bligh & Dyer, 1959) to remove non-lipid material (primarily protein). AX levels were the sum of xylose and arabinose levels [corrected for the presence of arabinose originating from arabinogalactan peptide (arabinose to galactose ratio 0.7) for wheat, rye, and barley] multiplied by 0.88 to correct for the incorporation of water during hydrolysis. Xylose and arabinose levels were determined in a gas chromatography procedure in which flour samples were sequentially subjected to acid hydrolysis, reduction of monosaccharides to alditols and subsequent conversion to alditol peracetates (Courtin, Roelants, & Delcour, 1999). β -Glucan levels were colorimetrically measured using a Megazyme (Bray, Ireland) protocol, which is also available as (AACCI) Method 32.23-01. In essence, samples are incubated with lichenase enzyme and hydrolysed to completion with β -glucosidase. The D-glucose produced is assayed using a glucose oxidase/peroxidase reagent.

2.2.2 Isolation of dough liquor

As was already mentioned in the introduction, DL is a representative model system for the aqueous phase of a dough and can be isolated by ultracentrifugation (Baker, Parker, & Mize, 1946; MacRitchie, 1976). Wheat dough optimum water level and mixing time were determined using (AACCI) Methods 54-21.02 and 54-40.02, respectively. As in Finney (1984), flour (100.0 g), deionized water (49.0 ml), sugar (6.0 g), compressed fresh yeast (5.3 g), and salt (1.5 g) were mixed in a pin mixer (National Manufacturing, Lincoln, NE, USA) for 4 min. Rye, barley, and oat flour weights were 265, 255, and 236 g, respectively. The corresponding levels of water in these recipes were 75%, 80%, and 90% w/w on flour basis. Based on Hager, Bosmans, and Delcour (2014), flour, deionized water, sugar (6.0%), fresh yeast (5.3%), and salt (1.5%) (all weight percentages on flour basis) were mixed in a KitchenAid (St. Joseph, MI, USA) KPM5 mixer (60 s at speed 1 and 120 s at speed 4). Shortening was

not used in any of the recipes to avoid lipid contamination in DL samples. For all doughs, fermentation took place in a fermentation cabinet [30.0 °C, 90% relative humidity (National Manufacturing), 126 min]. Wheat doughs were sheeted thrice during fermentation (at roll distances of 4.8 mm after 52 and 77 min and 7.9 mm after 90 min). Fermented samples (ca. 15.0 g, accurately weighed) of the different doughs were transferred to ultracentrifuge tubes (38 mL thick polycarbonate tubes, Beckman Coulter, Brea, CA, USA), centrifuged at 165,000 *g* (20 °C, 60 min) (L7 Ultracentrifuge, Beckman Coulter) (Pauly, Pareyt, Fierens, & Delcour, 2014), and directly placed on ice to minimize potential endogenous enzymatic activity. The supernatants, i.e. DLs, were collected in tarred amber-colored vials and weighed. For compositional analyses (section 2.2.3), DLs were freeze-dried and stored at -18.0 °C until analysis. For surface tension (section 2.2.5), dilatational rheology (section 2.2.5), and foaming (section 2.2.6) experiments, freshly isolated DLs were stored at -80.0 °C until analysis.

2.2.3 Composition of dough liquor

DL protein content was determined with an adaptation of AOAC method 990.03 (AOAC, 1995) to an automated 1108 Elemental Analyser (Carlo Erba, Hindley Green, UK) for microgram scale samples. The nitrogen to protein conversion factor was 6.25 for all DL samples. DL lipid levels were determined according to Bligh and Dyer (1959). Chloroform, methanol, and milli-Q water were sequentially added to about 250 mg of freeze-dried DL in a ratio 1.0/1.0/0.9. After centrifugation (500 *g*, 20 min, room temperature), three phases were distinguished: a lower chloroform phase containing lipids, a middle phase containing DL residue, and an upper methanol-water phase with (mainly) protein. The upper phase was discarded and the lower collected. DL residue was extracted twice more with chloroform. The solvent was evaporated from the combined chloroform phases and lipid levels were gravimetrically quantified. The lipid composition was analyzed at constant mass basis (3.0 mg lipids/ml iso-octane) using HPLC combined with an evaporative light scattering detector (ELSD) as in Melis, Pauly, Gerits, Pareyt, and Delcour (2017). Injection volumes were 3.0 µl for TAG

and FFA and 20.0 μ l for glycolipids and phospholipids. AX and β -glucan levels of freeze-dried DL were determined using gas chromatography and colorimetry, respectively, as described above for flour samples (section 2.2.1).

2.2.4 Foaming properties of dough liquor

Fresh DL samples were diluted with deionized water to a concentration of 5.00 mg protein/ml. A whipping test was carried out to determine DL foaming behavior based on Wouters, Rombouts, Legein, Fierens, Brijs, Blecker, and Delcour (2016). Diluted DL aliquots (50 mL) were poured in a graduated glass cylinder (internal diameter: 60 mm) and whipped for 130 sec at 2,000 rotations/min (rpm) using a rotating propeller (outer diameter: 45.0 mm, thickness: 0.4 mm, diameter cylindrical bar: 6.0 mm) in a water bath at 30 °C. Immediately after whipping, the propeller was removed and the glass cylinder covered with Parafilm M (Bemis, Neenah, WI, USA) to minimize foam disruption by air circulation. The foaming capacity is the foam volume measured 180 sec after the start of whipping. The foam volume was monitored over 60 min and was calculated based on the foam height and the diameter of the graduated cylinder. The rate and extent of the decline of foam volume in time is a measure of foam stability. Foaming properties were single measurements on DLs from three separate isolation experiments.

2.2.5 Apparent viscosity of dough liquor

Fresh DL samples were diluted with deionized water to a concentration of 5.00 mg protein/ml. The apparent viscosity was measured at room temperature using a Brookfield (Brookfield Ametek, Middleboro, MA, USA) DV-III Ultra viscometer equipped with a small sample adapter. An aliquot (6.7 ml) was accurately transferred to the sample chamber and allowed to equilibrate for 60 sec after insertion of a cylindrical spindle SC4-18 (diameter: 17.48 mm, side length: 31.72 mm). The spindle was then rotated at 75 rpm (corresponding to a shear rate of 99/s). Viscosity values were recorded every 30 sec over the course of 3 min. Apparent viscosities were expressed as average values and were single measurements on DLs from two separate isolation experiments.

2.2.6 Surface-active properties of dough liquor

Fresh DL samples were diluted with deionized water to a concentration of 5.00 mg protein/ml. The decrease of surface tension over time and the surface dilatational modulus (E) were measured at ambient temperature using a Theta optical tensiometer (Attension, Biolin Scientific, Stockholm, Sweden). Diluted DL samples were introduced in the device to create a pendant drop with a fixed volume (8.0 μ l). Surface tension was monitored over 900 sec (with 1.4 frames recorded/sec in the first 300 sec and 0.14 frames recorded/sec in the last 600 sec). Immediately afterwards, the droplet was sinusoidally oscillated (volume amplitude of approximately 1 μ l or 12.5% of droplet volume) at 0.8 Hz (with 6.9 frames recorded/sec) for 10 cycles. E was calculated by drop shape analysis and Fourier transformation and is the change in surface tension γ relative to the change in the natural logarithm of the surface area A :

$$E = \Delta\gamma / \Delta \ln A$$

After each measurement, the device was thoroughly cleaned and the surface tension of pure water was verified to be 72.0 ± 0.50 mN/m before initiating a next measurement. Both surface tension and dilatational rheology measurements were conducted in duplicate on DLs from two separate isolation experiments.

2.2.7 Bread making

The recipes and procedures used to make bread doughs are outlined in section 2.2.2, with the following exceptions. After the third sheeting step (roll distance of 7.9 mm after 90 min) of wheat dough (total weight 161.8 g), it was rolled into a cylinder, placed in a slightly greased rectangular baking tin (length: 12.9 cm, width: 6.3 cm, height: 5.7 cm), and proofed in the fermentation cabinet for 36 min. It was then baked in a rotary oven (National Manufacturing) for 24 min at 215 °C. Rye, barley, and oat doughs (total weight 200.0 g each) were placed in slightly greased rectangular baking tins (length: 14.3 cm, width: 7.9 cm, height: 5.7 cm) and allowed to ferment for 30 min. Such short

fermentation times were used to avoid dough collapse. The doughs were baked in a Condilux deck oven (Hein, Strasse, Luxembourg) for 45 min at 195 °C. Two hours after baking, breads were weighed and their volumes measured using a Volscan Profiler (Stable Microsystems, Godalming, Surrey, UK). The specific loaf volume is the volume divided by mass. Breads were stored at 23 °C for 24 hours prior to crumb structure analysis. Bread making trials were performed in duplicate on two different days.

2.2.8 Crumb structure image analysis

The crumb structure of bread loaves was analyzed using two-dimensional image. An electric slicer (Affettatrice Slicer 30N, Galesecca, Italy) was used to collect center crumb pieces with a thickness of 1.0 cm from rye and barley breads. Oat bread slices were cut manually because their crumb structure was brittle. The surface of bread slices was digitized using a flatbed scanner (Scanjet 3800, Hewlett-Packard, Beijing, China). An image of 30 x 30 mm at the center of each slice was recorded for further analysis. It was stored in .jpg format (254 x 255 pixels) and had a resolution of 300 dots/inch. The conversion of grayscale to binary images was based on the algorithm of Otsu (1979). These were then subjected to the Image Processing Toolbox of Matlab 8.0 (Mathworks, Natick, MA, USA). The crumb structure parameters analyzed were mean cell area, number of cells/cm², and two-dimensional porosity (*i.e.* ratio of the area of cells over the total area). Crumb structure analyses were done on four bread loaves from two breads of each formula. Thus, eight samples of each bread formula were analyzed.

2.2.9 Statistical analysis

One-way analysis of variance was combined with Tukey's honest significant difference test to identify significant differences ($\alpha < 0.05$) for several variables using the statistical software JMP pro 12 (SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1 Chemical composition and lipid population of dough liquor

Table 1A shows the chemical composition of DL samples from fermented wheat, rye, barley, and oat doughs. Wheat DL had the highest protein content (17% dm), followed by those of DLs from rye (14% dm), barley (9% dm), and oat (8% dm) (**Table 1A**). As DL is aqueous salt solution, the proteins in DL are probably for the most part albumins and globulins. However, using confocal laser scanning microscopy, Li, Dobraszczyk, and Wilde (2004) also identified gliadins at the gas cell interfaces in wheat dough. For more information on wheat DL proteins, the interested reader is referred to Salt, Robertson, Jenkins, Mulholland, and Mills (2005) and Pauly, Pareyt, Fierens, and Delcour (2014). In line with our expectations, the AX contents of rye (15% dm) and wheat (9% dm) DLs were high, as opposed to those from barley and oat (3% dm in both cases). Oat and barley DLs contained the highest levels of β -glucan (11% and 8% dm, respectively) (**Table 1A**), followed by rye (5% dm) and wheat (1% dm) DLs. The populations of AX and β -glucan in DL are most likely those that are water-extractable from flour. The interested reader is referred to Courtin and Delcour (2002), Vinkx and Delcour (1996), and Izydorczyk and Dexter (2008) for additional information on the structure and classification of wheat AX, rye AX, and barley and oat β -glucan, respectively. While the lipid content of oat DL (24% dm) (**Table 1A**) was exceptionally high, wheat, rye, and barley DLs had relatively low lipid levels (0.8, 2.0, and 0.2% dm, respectively). As already mentioned, these lipids may play a role in gas cell stabilization in dough. In addition to their concentration in DL, their structure also determines their surface activity (Gerits, Pareyt, & Delcour, 2014; Gerits, Pareyt, Masure, & Delcour, 2015).

Figure 1 shows HPLC-ELSD elution profiles of lipid extracts of the different DLs to identify which lipid classes were present in the different DL samples. Absolute quantification of specific lipid classes was not possible due to variation in detector response between the different lipid classes [see also Melis, Pauly, Gerits, Pareyt, and Delcour (2017)]. However, we could compare the relative contents of different DL samples for a given lipid class. Levels of TAG decreased in the order oat, wheat, rye, and

barley DLs (**Figure 1A**). Wheat DL had slightly higher FFA levels than rye and barley DLs, whereas oat DL contained only traces of FFA. While the polar glycolipids and phospholipids were abundantly present in wheat and rye DLs, only very low levels were detected in DLs isolated from barley and oat doughs (**Figure 1C and 1D**). However, it is of note that lipid extracts of DL were diluted to a constant lipid extract mass basis of 3.0 mg lipid extract/ml iso-octane prior to injection into the HPLC. Thus, although we detected only very low polar lipid levels in oat DL, the total polar lipid content in oat DL may still be similar to or even higher than that in wheat and rye DLs as the total lipid content in the former was much higher than that in the latter (**Table 1**). Both glyco- and phospholipids were detected in the lipid elution profiles of water-saturated butanol extracts of barley and oat flours (data not shown). Their absence in the dough aqueous phase implies that during kneading they interact with other flour constituents and/or that they become entrapped within the dough matrix. In wheat dough, polar lipids interact with gluten proteins (McCann, Small, Batey, Wrigley, & Day, 2009). They may also become physically entrapped in the gluten-starch matrix (Pomeranz & Chung, 1978). Glycolipids of wheat and rye DLs were mainly digalactosyldiacylglycerols (DGDG) and, to a lesser extent, monogalactosyldiacylglycerols (MGDG). No large differences were observed in peak areas of MGDG or DGDG between wheat and rye DLs. The most abundant phospholipid classes in both wheat and rye DLs were N-acyl phosphatidylethanolamine (NAPE), phosphatidylcholine (PC) and their respective lysolipid forms, N-acyl lysophosphatidylethanolamine (NALPE) and lysophosphatidylcholine (LPC). Wheat DL contained higher NAPE levels than rye DL. While the levels of NALPE and LPC were similar in wheat and rye DLs, PC was more prevalent in the latter. As PC represents the most common yeast phospholipid (data not shown), part of PC in the DLs probably originated from the yeast. The lipid composition of wheat DL was in line with those described by Gerits, Pareyt, Masure, & Delcour (2015) and Salt et al. (2018).

Apart from lipids, proteins, and NSPs, the aqueous phase of cereal doughs most likely also contains mono-, di- and oligosaccharides and small levels of salt, water-soluble minerals, and water-soluble vitamins. It should be mentioned that, during dough fermentation yeast produces organic acids such

as succinic acid which lower the pH from 6.0 in freshly mixed dough to roughly 5.0 in fermented dough (Jayaram, Cuyvers, Lagrain, Verstrepen, Delcour, & Courtin, 2013). Furthermore, in previous work by our group (Pauly, Pareyt, Fierens, & Delcour, 2014) the composition and foaming properties of DL isolated from fermented and non-fermented wheat doughs were compared. It was established that yeast releases surface-active components from the gluten-starch matrix, as reflected by higher protein and AX levels. Yeast also had a positive effect on the foaming properties of wheat DL (Pauly, Pareyt, Fierens, & Delcour, 2014). However, in the present study, the same amount of yeast (5.3 % w/w on flour basis) was added to each bread making recipe. In addition, pH measurements of the different DLs showed that the pH of wheat, rye, barley, and oat DLs only ranged from 4.73 to 4.89 and thus were very similar. Therefore, we do not expect differences related to the addition of yeast between the different bread types and will not consider this further.

Some if not all of the above mentioned constituents in the dough liquid film probably contribute to the stabilization of gas cells in bread dough. In what follows, the functional properties of the different DL samples are studied and related to their composition.

3.2 Foaming properties of dough liquor

We here compared the foaming, viscosifying, and surface-active properties of DL samples on a constant protein basis, as proteins are likely key constituents of DL in the context of their ability to stabilize dough interfaces. Because the different undiluted DL samples varied in protein content (**Table 1A**), their dilution to a constant protein concentration had consequences for the concentration of the other constituents. **Table 1B** lists protein, lipid, AX, and β -glucan concentrations and protein-to-lipid ratios of the different DL samples after dilution to a concentration of 5.00 mg protein/ml.

Figure 2 shows foam volume over time plots of diluted (5.00 mg protein/ml) wheat, rye, barley, and oat DLs. Rye and barley DLs produced significantly ($P < 0.05$) more foam (80 and 77 ml, respectively) than wheat DL (24 ml). Foams from rye and barley DLs were quite stable, with remaining foam

volumes being 25 and 33 ml, respectively, after 60 min. Wheat DL foam was relatively unstable, resulting in a low foam volume of 6 ml after 60 min. Regretfully, no foam could be produced with oat DL at 5.00 mg protein/ml, due to the very high apparent viscosity of the sample (see section 3.3). Therefore, a lower concentration of 2.50 mg protein/ml was assessed. At 2.50 mg protein/ml, oat DL produced 25 ml of foam (data not shown). This was a relatively low reading when compared to those of rye and barley DL foams at 5.00 mg protein/ml. However, these oat DL foams were exceptionally stable, with a remaining foam volume of 17 ml after 60 min (data not shown).

Interestingly enough, additional experiments at a concentration of 0.50 mg protein/ml showed that both the foaming capacity and foam stability of wheat DL were substantially better (data not shown) than at 5.00 mg protein/ml. This seems counter-intuitive, as an increase in concentration of surface-active constituents would be expected to be accompanied by improved foaming characteristics. Indeed, for all other DL samples, this was the case (data not shown). The detrimental impact of increasing protein concentration on wheat DL foams suggests that lipids impair the foaming of wheat DL proteins. Indeed, although the protein-to-lipid ratio (**Table 1B**) on a mass basis remained constant with increasing protein concentration, the same ratio on a molar basis decreased substantially because of the low molecular mass of lipids. Thus, a molar protein-to-lipid ratio may exist above which lipids interfere with the foaming of wheat DL protein. These observations are in line with previous studies which suggested that when proteins and lipids co-occur at the interface, this results in poor stability (MacRitchie & Gras, 1973; MacRitchie, 1976; Sahi, 2003; Primo-Martín, Hamer, & De Jongh, 2006; Salt, Wilde, Georget, Wellner, Skeggs, & Mills, 2006; Salt, et al., 2018).

In summary, rye and barley DL constituents seem to have more potential to stabilize dough interfaces during fermentation than those of wheat or oat flour. These observations can likely be explained by differences in apparent viscosity of the different DL samples (see section 3.3) and/or by differences in the structure and properties of the adsorbed interfacial layer formed at the interface (see section 3.4).

3.3 Apparent viscosity of dough liquor

Diluted (5.00 mg protein/ml) DLs from rye and oat were highly viscous (> 40 mPa.s, *i.e.* the upper detection limit of the applied spindle at 75 rpm) in comparison with diluted DLs of wheat (6.8 mPa.s) and barley (1.7 mPa.s). Measurements with the same spindle at 10 rpm revealed that the apparent viscosity of rye DL (153 ± 15 mPa.s) was lower than that of oat DL (221 ± 6 mPa.s).

Viscosity mainly depends on the concentration and structure of the NSPs present in the different DL samples. The arabinose/xylose mass ratio of rye DL AX (0.56) was higher than that of AX in wheat DL (0.45). The lower and higher AX levels in diluted wheat and rye DLs (**Table 1B**), respectively, most likely are at the basis of their relatively low and high respective apparent viscosities. Diluted barley DL contained less β -glucan than diluted oat DL. However, although the apparent viscosity of the former was indeed much lower than that of the latter, this difference was too large to be fully explained by differences in β -glucan concentration. It can probably be ascribed to structural differences [such as molecular weight and molar ratio of trisaccharide over tetrasaccharide units (Izydorczyk & Dexter, 2008)] between barley and oat β -glucans. Besides NSPs, mono-, di-, and oligosaccharides (such as dextrins) may also increase the viscosity of DL. However, we expect the impact of NSPs on the viscosity of DL to be much more pronounced than that of mono-, di-, and oligosaccharides.

It is well known that high bulk viscosity stabilizes foam by delaying gas cell coalescence (Damodaran, 2005). Thus, the high apparent viscosities of diluted rye and oat DLs may partially explain the high stability of their foams (see section 3.2). This was not the case for barley DL. It had good foaming characteristics, but low apparent viscosity. Hence, proteins seem to be the main responsible for the high stability of barley DL foams (see section 3.2). Finally, the low apparent viscosity of wheat DL may contribute to the low stability of its foam (see section 3.2).

3.4 Surface-active properties of dough liquor

Figure 3 shows surface tension over time plots and E values of the diluted (5.00 mg protein/ml) DLs as measured with the pendant drop method (see section 2.2.6). The rate and extent at which surface tension decreases over time is a measure for the continuous diffusion to and adsorption at the interface of surface-active DL constituents. As a point of reference, the equilibrium surface tension of an interface stabilized by globular proteins can be as low as 47 mN/m, whereas that of an interface stabilized by low molecular mass surfactants, such as lipids, can be as low as 22 mN/m (Bos & Van Vliet, 2001). After 15 min, hanging droplets were subjected to sinusoidal volume oscillations, from which E, a measure for the viscoelasticity of the adsorbed films, could be calculated (**Figure 3**) (see section 2.2.6). High E values indicate that a strong viscoelastic protein film was formed at the interface. The latter can often be related to high foam stability as it delays gas cell coalescence and disproportionation (Murray, 2007).

The surface tension decrease of diluted barley DL was the least pronounced (a reading of 50 mN/m after 900 sec was noted). Surface tension over time plots of wheat and rye DLs were rather similar and showed a sharp decrease in surface tension in the first 150 sec after which a plateau value of 40 mN/m was reached. Finally, oat DL constituents reduced the surface tension to the greatest extent (34 mN/m after 900 sec). E values of rye (168 mN/m) and oat (132 mN/m) DLs were significantly ($P < 0.05$) higher than those of wheat (27 mN/m) and barley (34 mN/m) DLs.

The relatively high surface activity (low surface tension value) and low E value of wheat DL indicate the presence of wheat lipids at the interface and their interference with the formation of a viscoelastic protein network. Wheat DL indeed had high levels of polar lipids (such as DGDG, NAPE, NALPE, and PC, see section 3.1) that are surface-active (Gerits, Pareyt, & Delcour, 2014; Gerits, Pareyt, Masure, & Delcour, 2015). The formation of a mixed protein-lipid interface also explains the low foaming capacity and foam stability of wheat DL at 5.00 mg protein/ml (see section 3.2). Rye DL had a much higher lipid level (0.72 mg/ml) than wheat DL (0.24 mg lipids/ml) (**Table 1B**). Both had

very similar lipid compositions, with an abundance of polar lipids. However, NAPE was only present in very low levels in rye DL (see section 3.1) (**Figure 1D**). Still, rye DL had a high E value and its foaming characteristics were much better than those of wheat DL. This suggested that proteins dominate its interface. Thus, rye DL proteins seemed to be more surface-active than wheat DL proteins. The ability of rye DL proteins to form a strong viscoelastic film at the interface in combination with the high apparent viscosity of rye DL (see section 3.3) most likely explain the high foaming capacity and foam stability of rye DL (see section 3.2). Considering that barley DL only had a low lipid content (0.12 mg/ml, **Table 1B**), little if any polar lipids, a low surface activity (relatively high surface tension values), and excellent foaming properties, its interface was likely dominated by protein rather than by lipid. However, the relatively low E value of barley DL was rather unexpected as it reflects that the protein film had low viscoelasticity. Moreover, as already stated in section 3.3, the high stability of barley DL foams could not be attributed to the viscosity of the bulk phase. Hence, other phenomena are responsible for the excellent foaming characteristics of barley DL. We hypothesize that adsorbed proteins stabilize neighboring gas bubbles in foams made from barley DL by steric and/or electrostatic effects, rather than by forming a highly viscoelastic protein film at the interface. The high surface activity (low surface tension values) of oat DL and the high lipid content point to lipids predominating the interface (Bos & Van Vliet, 2001; Damodaran, 2005). However, oat DL contained much less polar (and thus surface-active) lipid than non-polar lipid (**Figure 1**). Probably, TAG, which were present in extremely high concentrations in oat DL, also adsorbed at the interface of oat DL (**Figure 1A**). The presence of lipids at the interface probably also explains the poor foaming capacity of oat DL at 2.50 mg protein/ml (see section 3.2). Interestingly, oat DL still had a high E value at 5.00 mg protein/ml (**Figure 3**). As lipid monolayers at an interface generally have low E values (Bos & Van Vliet, 2001; Damodaran, 2005) and as lipids interfere with the formation of viscoelastic films of adsorbed proteins, which generally result in high E values, this was not expected.

In what follows, we put forward two hypotheses to explain the formation of a highly viscoelastic interface predominantly occupied by lipids. First, synergistic effects may occur between oat DL

proteins and lipids. For wheat, synergistic effects between puroindolines and lipids were found to depend on the lipid-binding capacity of the protein, the type of lipid, and the lipid-to-protein molar ratio (Wilde, Clark, & Marion, 1993). Although oat contains puroindoline-homologues (data not shown), their functional properties remain to be investigated. Second, the E value of an interface derived from an oscillating pendant drop measurement (see section 2.2.6) likely depends on the rate at and extent to which surface-active constituents are exchanged between the bulk phase and the interface upon subsequent compression and dilatation. Hence, with lipids abundantly present in the bulk phase, this can result in high E values provided they rapidly diffuse to and adsorb at the deforming interfacial layer. The E value of an interface predominated by lipids likely also depends on the type of lipid monolayer formed (Lucassen-Reynders, 1993). This in turn relies on packing of the lipid molecules at the interface and, thus, on their molecular structure. For example, unsaturated FFA form expanded monolayers, while saturated FFA give rise to a condensed monolayer (Lucassen-Reynders, 1993; Gerits, Pareyt, & Delcour, 2014). The latter is more tightly packed and would result in a higher E value than that of an expanded monolayer.

In summary, rye and oat flour constituents seem to have more potential to stabilize their dough interfaces during fermentation than those of wheat or barley flour. For wheat, rye, and oat DLs, E values of the film at the interface were in line with their foam stabilities. Other phenomena seem to be responsible for the excellent foaming of barley DL. In the following paragraph, the functionality of the different DLs will be related to the specific loaf volume and crumb structure of their respective breads.

3.5 Bread properties

To obtain bread with a high loaf volume and a homogeneous crumb structure, gas cells need to be stabilized until the fluid-like foam structure of dough is transformed into the solid open sponge structure of bread. The latter of course is caused by starch gelatinization and protein polymerization. The stability of gas cells in dough depends on (i) the extent to which the viscoelastic protein network

can be stretched upon extension (due to gas cell expansion during fermentation and early baking) and (ii) the ability of dough aqueous phase constituents to stabilize the air/water interface surrounding the gas cells.

In the case of wheat bread, the viscoelastic gluten network rather than adsorption of dough aqueous phase constituents at the interface is the main responsible for gas cell stabilization and, hence, for the high quality of wheat breads. Indeed, in spite of the poor foaming and surface-active properties of wheat DL as a result of mixed protein-lipid interfaces, breads prepared with wheat flour had a high specific loaf volume ($3.5 \text{ cm}^3/\text{g}$) and a fine and homogeneous crumb structure (**Table 2**). This evidently questions the importance of gas cell stabilization in wheat dough by its aqueous phase constituents. However, in the case of non-wheat breads, the flour proteins do not form a viscoelastic network upon hydration and kneading the way wheat gluten proteins do. This suggests that gas cell stabilization by dough aqueous phase constituents is more important in non-wheat than in wheat dough. There seemed to be no relationship between DL functionality and bread loaf specific volume. Indeed, rye, barley, and oat breads had much lower specific volumes than wheat breads, regardless of their DL functionality. Thus, the development of a viscoelastic protein network is probably essential to obtain bread with a high loaf volume. However, in terms of crumb structure, a relationship between DL functionality and crumb homogeneity was observed. Indeed, we believe that the homogeneous crumb structure of rye breads, which had significantly ($P < 0.05$) smaller gas cells than wheat breads (**Table 2**), is related to the high viscosity of the rye dough aqueous phase and the formation of a strong viscoelastic protein film at the interface of the gas cells. Furthermore, although barley breads had a relatively high specific loaf volume ($2.6 \text{ cm}^3/\text{g}$), gas cells in their crumb were significantly ($P < 0.05$) larger than those in wheat or rye breads (**Table 2**). Because of the excellent foaming properties of barley DL, we had not expected the crumb of barley breads to be heterogeneously grained. Of course, although the foaming test used here has merits, bread dough foams are still different as gas cells in dough continuously expand due to carbon dioxide production by the yeast. The formation of a strong viscoelastic interfacial protein film during fermentation is

likely of utmost importance for withstanding the increase in interfacial area caused by gas cell expansion in systems lacking the wheat viscoelastic gluten-starch matrix. This way, the low E value of barley DL (**Figure 3**) may be at the basis of the heterogeneous crumb structure of barley breads. Finally, despite the high viscosity and high E value of oat DL, oat breads had a heterogeneous crumb structure (**Table 2**). This was in line with the poor foaming characteristics of oat DL and can likely be explained by lipids dominating the interface surrounding the gas cells in oat dough (which also explains the high E value, as noted in section 3.4).

4. Conclusions

The present work aimed at studying whether the composition and functionality of the aqueous phase of non-wheat dough, as assessed by the composition (proteins, lipids, AX, and β -glucan) and functionality (Foaming, viscosifying, and surface-active properties) of DL, are predictive for the quality of non-wheat breads. First, it was established that, in spite of the excellent DL functionality of several of the non-wheat doughs, the loaf volumes of the corresponding breads were still much lower than those of wheat breads. This implies that the formation of a viscoelastic protein network during dough making is essential for bread loaf volume. Second, it was demonstrated that the functionality of the aqueous phase in non-wheat doughs plays an important role in determining the crumb structure of the resulting breads.

Figure 4 schematically represents our hypotheses on the interfacial compositions of wheat, rye, barley, and oat DLs. The poor foaming properties of **wheat** DL in combination with the formation of a weak viscoelastic film at the interface pointed to the co-existence of proteins and lipids at the

interface. That wheat bread still had a high loaf volume and a homogeneous crumb structure, despite the poor functionality of wheat DL, is of course due to the presence of the viscoelastic gluten network, and evidently questions the relevance of gas cell stabilization in wheat dough by its aqueous phase constituents. **Rye** flour constituents had the highest potential to stabilize dough interfaces during fermentation. This was ascribed to the ability of rye water-extractable AX to form a highly viscous bulk solution and the presence of a strong viscoelastic protein film at the interfaces surrounding the gas cells. Both phenomena most likely postponed coalescence and disproportionation of neighboring gas cells during fermentation and early baking. Indeed, rye bread had a homogeneous crumb structure. Regardless of the good foaming characteristics of **barley** DL, barley breads had a heterogeneous crumb structure with large gas cells. This was associated with the low viscosity of barley DL and the weak viscoelasticity of the protein film at the interface. Despite the high viscosity of **oat** DL, oat breads had a heterogeneous crumb structure. The latter could be explained by lipids predominating the interface of oat DL because of its high lipid content, its low foaming capacity, and its exceptionally high surface-activity.

The present comprehensive study on the compositional, foaming, viscosifying, and surface-active properties of rye, barley, and oat DLs has provided a better understanding of gas cell stabilization in such doughs. It can thus serve as a basis for modifying the dough aqueous phase composition to optimize non-wheat cereal dough gas cell stability. An example could be the addition of water-soluble proteins with excellent surface-active properties, such as plant-based hydrolysates (Wouters, et al., 2016) to bread making recipes. Furthermore, in situ modification of flour endogenous lipids by lipases (Gerits, Pareyt, & Delcour, 2014; Gerits, Pareyt, Masure, & Delcour, 2015; Schaffarczyk, Østdal, Matheis, & Koehler, 2016) may be another promising strategy to improve DL functionality.

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Declarations of interest

None.

ABBREVIATIONS

AX	Arabinoxylan
DGDG	Digalactosyldiacylglycerols
DL	Dough liquor
dm	Dry matter
E	Surface dilatational modulus
FFA	Free fatty acids
LPC	Lysophosphatidylcholine
MGDG	Monogalactosyldiacylglycerols
NALPE	N-acyl lysophosphatidylethanolamine
NAPE	N-acyl phosphatidylethanolamine
NSP	Non-starch polysaccharides
PC	Phosphatidylcholine
SG	Steryl glycosides
TAG	Triacylglycerols

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TABLE CAPTIONS**Table I**

(A) Chemical composition [dry matter (dm) basis] of dough liquor (DL) isolated from fermented wheat, rye, barley, and oat doughs. Column values with the same lower case letter are not significantly different ($\alpha = 0.05$). Error values represent the deviation from the mean for duplicate measurements of two separate DL isolation experiments. (B) Protein, lipid, arabinoxylan (AX), and β -glucan concentration and protein-to-lipid ratio (on mass basis) of wheat, rye, barley, and oat dough liquors (DL), diluted to a concentration of 5.00 mg protein/ml.

Table II

Specific loaf volume and crumb structure parameters of breads prepared with wheat, rye, barley, and oat flour. Column values with the same lower case letter are not significantly different ($\alpha = 0.05$). Specific loaf volume error values represent the deviation from the mean for duplicate measurements of two separate bread making trials. Crumb structure measurements were executed on eight slices originating from two different breads.

FIGURE CAPTIONS

Figure I

Elution profiles of purified lipid extracts of dough liquor (DL) isolated from fermented doughs prepared with wheat, rye, barley, and oat flour, diluted to a concentration of 3.00 mg lipid/ml iso-octane. Figures **A** and **B** show the elution profiles of triacylglycerols (TAG) and free fatty acids (FFA), respectively, while the elution profiles of glyco- and phospholipids are presented in **C** and **D**, respectively. Abbreviations: MGDG, monogalactosyldiacylglycerols; SG, sterol glycosides; DGDG, digalactosyldiacylglycerols; NAPE, N-acyl phosphatidylethanolamine; NALPE, N-acyl lysophosphatidylethanolamine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine. The elution profiles represent means of duplicates of two separate DL isolation experiments.

Figure II

Foam volume as a function of time of diluted (5.00 mg protein/ml) dough liquor (DL) isolated from fermented wheat, rye, barley, and oat doughs. Error bars represent the deviation from the mean for single measurements of three separate DL isolation experiments.

Figure III

Evolution of surface tension as a function of time at and surface dilatational moduli (E) of the interface of a pendant drop of diluted (5.00 mg protein/ml) dough liquor (DL) isolated from fermented wheat, rye, barley, and oat doughs. The data represent means of duplicates of two separate DL isolations. Values with the same lower case letter are not significantly different ($\alpha = 0.05$). Error values represent the deviation from the mean for duplicate measurements of two separate DL isolation experiments.

Figure IV

Schematic representation of the air/water interface composition of dough liquor (DL) isolated from fermented wheat, rye, barley, and oat doughs. Abbreviations: E, surface dilatational modulus; AX, arabinoxylan.

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A

DL source	Protein (% dm)	Arabinoxylan (AX) (% dm)	β -glucan (% dm)	Lipid (% dm)
Wheat	16.7 a (± 0.3)	9.0 a (± 0.1)	0.8 a (± 0.1)	0.8 a (± 0.1)
Rye	13.8 b (± 1.2)	15.1 b (± 0.2)	4.9 b (± 0.1)	2.0 a (± 0.3)
Barley	8.5 c (± 0.1)	2.7 c (± 0.0)	8.2 c (± 0.5)	0.2 a (± 0.0)
Oat	8.3 c (± 0.1)	2.7 d (± 0.1)	11.4 d (± 0.5)	23.6 b (± 2.9)

B					
DL source	Proteins (mg/ml)	Lipids (mg/ml)	Protein-to-lipid ratio (-) (mg/ml)	AX	β - glucan (mg/ml)
Wheat	5.00	0.24	20.8	2.69	0.24
Rye	5.00	0.72	6.9	5.47	1.78
Barley	5.00	0.12	42.5	1.59	4.82
Oat	5.00	15.30	0.33	1.75	6.87

Flour source	Specific loaf volume (cm ³ /g)	Mean cell area (mm ²)	Number of cells/cm ² (-)	Two-dimensional porosity (%)
Wheat	3.5 a (± 0.2)	0.34 a (± 0.1)	97 a (± 10)	36.7 ab (± 4.6)
Rye	2.1 b (± 0.3)	0.26 b (± 0.0)	113 b (± 14)	34.0 b (± 2.9)
Barley	2.6 c (± 0.1)	0.49 c (± 0.1)	76 c (± 7)	39.6 a (± 3.5)
Oat	2.1 b (± 0.1)	0.51 c (± 0.1)	69 c (± 7)	36.3 ab (± 3.5)

Highlights

1. *Dough liquor (DL) constituents contribute to gas cell stabilization*
2. *Composition and functionality of wheat, rye, barley, and oat DLs were studied*
3. *DL constituents determine the crumb structure of the non-wheat breads*
4. *Mixed protein-lipid interfaces result in poor DL functionality*
5. *Rye DL constituents are potent air/water interface stabilizers*