The role of lipids in determining the air-water interfacial properties of wheat, rye, and oat dough liquor constituents

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ABSTRACT

Bread is mainly made from wheat but also from other cereals such as rye and oats. We here report on the role of dough liquor (DL) proteins and lipids in determining the stability of gas cell air-water (A-W) interfaces in wheat, rye, and oat bread making. Surprisingly, most lipids in DLs of these cereals are nonpolar. Their main polar DL lipids are phospholipids. Lipids at wheat and rye DL stabilized A-W interfaces impair interactions between its proteins, as reflected by an increased A-W interfacial shear viscosity of the adsorbed film upon defatting. In contrast, removing most lipids from oat DL pronouncedly increased the A-W interface surface tension, demonstrating that lipids are the prominent adsorbed species.

1. Introduction

The stability of gas cells during fermentation and early baking is essential to obtain bread with high specific volume and a homogeneous crumb structure. In wheat (*Triticum aestivum* L.) bread making, gluten proteins form a viscoelastic network that during fermentation provides structural support to expanding gas cells (Delcour & Hosney, 2010). It has been proposed that particularly in the advanced stages of fermentation and early baking discontinuities develop in the gluten-starch matrix (Gan et al., 1990). At this point, lipids, proteins, and non-starch polysaccharides in the dough aqueous phase supposedly take over gas cell stabilization (Gan, Ellis, & Schofield, 1995; MacRitchie, 1976).

Non-wheat breads have a lower specific volume than wheat breads because they lack gluten proteins. This implies that the mechanism by which soluble dough aqueous phase constituents stabilize gas cells may be more important in non-wheat than in wheat doughs.

Some lipids and proteins can stabilize air-water (A-W) interfaces by directly adsorbing at them. Upon adsorption, they lower the surface tension (σ) of the A-W interface and, hence, the energy needed to create new interfacial area.

Lipids form a weakly viscoelastic fluid monolayer at the A-W interface. When their concentration is locally reduced, e.g. when gas cells expand during fermentation, adsorbed lipid molecules diffuse to re-

gions of lower surface concentration to restore the concentration gradient (Damodaran, 2005). The ability of surface-active molecules to respond to spatial gradients in surface concentration is described as the Marangoni effect (Marangoni, 1872).

When proteins adsorb at an A-W interface, they partially unfold, orient their more hydrophobic regions towards the air phase, mutually interact, and develop a strong viscoelastic film (Murray, 2007). Such continuous protein film provides a degree of physical stability to gas cells and can slow down merging of neighboring gas cells by exerting steric hindrance and/or as a result of electrostatic repulsion.

Of particular interest is that the interfacial stabilization by lipids and proteins is considered incompatible and, thus, that mixed protein-lipid A-W interfaces are inherently unstable (Wilde, 2000). Since lipids and proteins are both present in the aqueous phase of dough, they compete for adsorption at dough gas cell A-W interfaces. Thus, the protein to lipid ratio at such A-W interfaces inevitably determines their stability.

Dough liquor (DL) is considered to be a model for the aqueous phase of dough. It is isolated by ultracentrifugation (Baker, Parker, & Mize, 1946; MacRitchie, 1976). All studies available in literature in this context deal with wheat dough DL. The focus has been on studying the protein and/or lipid composition or A-W interfacial properties.

In terms of protein composition, albumins and globulins are the dominant protein fractions in wheat DL (Pauly, Pareyt, Fierens,

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& Delcour, 2014; Salt, Robertson, Jenkins, Mulholland, & Mills, 2005). Salt et al. (2005) identified 42 albumins and/or globulins in wheat DL and foam thereof with mass spectrometry. The most abundant proteins were β -amylase, tritin, serpin, and members of the α -amylase/trypsin inhibitor family (Salt et al., 2005). Particularly tritins and α -amylase/trypsin inhibitors were lost and enriched, respectively, in foam made from DL. Also of interest is that neither puroindolines nor prolamins have been detected in DL (Salt et al., 2005). More recently, Pauly et al. (2014) investigated whether dough fermentation changes the protein composition of wheat DL and foam made thereof. They identified the same proteins as reported by Salt et al. (2005) and found that fermentation increases the level of α -amylase/trypsin inhibitors in wheat DL. In contrast to Salt et al. (2005), they detected puroindolines in wheat DL and even observed that they were enriched in foam made from DL from fermented dough (Pauly et al., 2014). Although prolamins were not detected in wheat DL (Salt et al., 2005), they may to an extent also be present at dough gas cell A-W interfaces (Li, Dobraszczyk, & Wilde, 2004).

The total lipid (TL) population in wheat DL is made up by a heterogeneous mixture of nonpolar lipids (NPLs) [i.e. triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), and free fatty acids (FFAs)] and polar lipids (PLs) [i.e. glycolipids (GLs) and phospholipids (PhLs)] (Gerits, Pareyt, Masure, & Delcour, 2015; Janssen, Wouters, Pauly, & Delcour, 2018; Salt et al., 2018). Although MAGs and FFAs are more polar than DAGs and TAGs, we here also consider them to be NPL species. The most common GLs are monogalactosyldiacylglycerols (MGDGs) and digalactosyldiacylglycerols (DGDGs), whereas N-acyl phosphatidylethanolamines (NAPEs), phosphatidylethanolamines (PEs), phosphatidylcholines (PCs), and their respective lysoforms lyso-NAPEs (NALPEs), lyso-PE (LPEs), and lyso-PC (LPCs) are the most abundant PhL classes (Gerits et al., 2015; Janssen et al., 2018; Salt et al., 2018).

As to their impact on interfacial properties, it has been suggested that proteins and lipids co-occur at wheat DL constituent stabilized A-W interfaces and render them unstable (MacRitchie & Gras, 1973; MacRitchie, 1976; Pauly et al., 2014; Primo-Martín, Hamer, & de Jongh, 2006; Sahi, 2003; Salt et al., 2006, 2018; Turbin-Orger et al., 2015). However, the relevance of the above in the context of dough gas cell stability is rather unclear as in all of these studies measurements were conducted with diluted wheat DL and thus with lower surface-active constituent concentrations than those in dough.

No attention has been given to DLs isolated from doughs made with flour from cereals other than wheat. In recent work by our group (Janssen et al., 2018), some hypotheses were put forward with respect to the composition of A-W interfaces stabilized by constituents present in DL isolated from fermented wheat, rye, and oat doughs. It was reasoned that rye and oat DL stabilized A-W interfaces may be dominated by proteins and lipids, respectively. As the composition of dough gas cell A-W interfaces and the interplay of constituents thereat may be of crucial importance for dough gas cell stability both in wheat and non-wheat systems, we here set out to understand the surface-active characteristics of wheat, rye and oat DL constituents. The lipid species populations in wheat, rye, and oat DL extracts were analyzed with hydrophilic interaction liquid chromatography electrospray ionization tandem mass spectrometry (HILIC-ESI-MS/MS). Although the lipid population of wheat DL (apart from its NAPEs and NALPEs) has been reported (Salt, González-Thuillier et al. 2018), those of rye and oat DLs have – to the best of our knowledge – not been published.

In addition, we compared the A-W interfacial properties (i.e. extent and rate of diffusion to and adsorption at the A-W interface, and A-W interfacial shear rheology) of the constituents in control and defatted wheat, rye, and oat DLs.

The present work thus aimed to study the role of lipids and their interplay with other adsorbed DL constituents of wheat, rye, and oat

in determining DL A-W interfacial properties. Such knowledge is essential for rationally modifying the composition of the aqueous phase in wheat, rye, and oat doughs to improve their gas cell stabilization capabilities and, hence, their bread making quality.

2. Materials & methods

2.1. Materials

Crousti commercial bread wheat flour from Dossche Mills (Deinze, Belgium) contained 13.6% moisture, 12.9% protein [on dry matter (dm) basis], 1.4% lipid, 2.1% total arabinoxylan (AX) (of which 18.2% was water-extractable), 0.2% β -D-glucan, and 0.67% ash (all on dm basis). Rye kernels (cultivar Dukato) from AVEVE (Merksem, Belgium) were conditioned to 16.0% moisture and roller milled in-house using a Bühler (Uzwil, Switzerland) MLU-202 laboratory mill as in Delcour, Vanhamel, and De Geest (1989). Rye flour contained 13.6% moisture, 4.0% protein, 0.7% lipid, 4.2% total AX (of which 27.9% water-extractable), 1.1% β -D-glucan, and 0.41% ash. Commercial oat flour (10.2% moisture content) was from Raisio Nutrition (Raisio, Finland) and contained, 13.3% protein, 7.4% lipid, 1.4% total AX (of which 10.9% was water-extractable), 2.7% β -D-glucan, and 1.55% ash (all on dm basis). The interested reader is referred to Janssen, Wouters et al. (2018) for the methods used to determine these compositions. Sugar, salt, and fresh compressed yeast (AB Mauri, Dordrecht, Nederland) were purchased in a local supermarket. β -D-Allose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucose, benzoic acid, sodium borohydride, 2-octanol, and ethyl acetate were from Sigma-Aldrich (Bornem, Belgium); chloroform, methanol, bromophenol blue, trifluoroacetic acid, 1-methylimidazole, and absolute ethanol from Thermo Fisher Scientific (Aalst, Belgium); concentrated ammonia, potassium hydroxide, acetic acid, acetic anhydride, and anhydrous sodium sulfate from VWR International (Oud-Heverlee, Belgium); and ethyl acetate from Merck (Darmstadt, Germany). All chemicals were of at least analytical grade. The high-performance liquid chromatography grade SPLASH® LIPIDOMIX® Mass Spec Standard (#330707) as well as the MGDG, DGDG, and NAPE standards were from Avanti Polar Lipids (Alabaster, AL, USA).

2.2. Methods

2.2.1. Dough making and dough liquor isolation

Wheat, rye, and oat DLs were isolated as in Janssen, Wouters et al. (2018).

Wheat dough optimum water level and mixing time were determined using AACCI Methods 54–21.02 and 54–40.02, respectively. Flour (100.0 g), deionized water (54.73 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) during 210 sec. Rye or oat flour (265.0 g or 236.0 g, respectively), deionized water (200.9 mL or 232.3 mL, respectively), sugar (16.0 g or 14.8 g, respectively), fresh yeast (14.1 g or 13.1 g, respectively), and salt (4.0 g or 3.7 g, respectively) were mixed in a KitchenAid (St. Joseph, MI, USA) KPM5 mixer (60 s at speed 1 and 120 s at speed 4).

All doughs were fermented in a National Manufacturing fermentation cabinet (30.0 °C, 90% relative humidity, 126 min). Wheat doughs were sheeted in a National Manufacturing sheeter thrice during fermentation (roll distance 4.8 mm after 52 and 77 min and 7.9 mm after 90 min).

Fermented dough samples (ca. 15.0 g, accurately weighed) were centrifuged at 165,000 g (20 °C, 60 min) in 38 mL thick polycarbonate tubes (Beckman Coulter, Brea, CA, USA) with an L7 Ultracentrifuge (Beckman Coulter) and thereafter immediately placed on ice to minimize potential endogenous enzymatic activity. The supernatants, i.e. the DLs, were collected in tared amber-colored Falcon tubes,

weighed, freeze-dried, and stored at -18°C until analysis. These freeze-dried wheat, rye, and oat DLs contained about 95% dm and 5% moisture. The latter was taken into account when calculating isolation yields as well as protein, lipid, AX, and β -D-glucan levels (see section 2.2.2.1). For all other analyses, freeze-dried DL powders were dissolved (continuous stirring, 30 min, $23 \pm 2^{\circ}\text{C}$) in deionized water at 5.00 or 1.50 mg protein/ml (depending on the analysis) as outlined below, and are further referred to as 'control wheat, rye, and oat DLs' (Fig. 1).

2.2.2. Analysis of dough liquor composition

2.2.2.1. Protein, arabinoxylan, β -D-glucan, and lipid levels

The composition of the different DLs was determined as in Janssen, Wouters et al. (2018). Protein, AX, β -D-glucan, and lipid levels were determined in duplicate on DLs from each of two separate isolations. DL **protein** ($N \times 6.25$) content was determined with an adaptation of AOAC method 990.03 to an automated 1108 Elemental Analyser (Carlo Erba, Hindley Green, UK) for microgram scale samples. DL **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 (Loosveld, Grobet, & Delcour, 1997; Van den Bulck et al., 2005) DLs] 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 (Englyst & Cummings, 1984). DL **β -D-glucan** levels were measured colorimetrically using a Megazyme (Bray, Ireland) protocol also available as AACCI Method 32.23-01. In essence, samples were incubated with lichenase and hydrolyzed to completion with β -D-glucosidase. The D-glucose produced was assayed using the Megazyme glucose oxidase/peroxidase reagent. DL **TL** levels were determined gravimetrically after following the extraction protocol of Bligh and Dyer (1959). Chloroform, methanol, and milli-Q water were sequentially added to 250 mg freeze-dried wheat, rye, and oat DL powder in a ratio 1.0/1.0/0.9. Centrifugation (500 g, 20 min, room temperature) yielded three phases: a lower chloroform phase containing *inter alia* lipids, a middle phase containing the remaining DL material, and an upper methanol-water phase with (mainly) protein. The upper phase was discarded and the lower phase collected. The middle phase was extracted twice more with chloroform, methanol, and milli-Q water. After combining the three chloro-

form phases and evaporating the solvent, TL levels were gravimetrically quantified.

2.2.2.2. Mass spectrometric analysis of dough liquor lipid population

2.2.2.2.1. Sample preparation

For analyzing the TL species population of control wheat, rye, and oat DLs, TL extracts (25 μg) of wheat, rye, and oat DLs obtained with the protocol of Bligh and Dyer (1959) and of oat DL defatted with hexane (see section 2.2.3) were solubilized in 60 μL methanol containing 3.0 μL of the Mass Spec Standard (#330707) containing deuterium labeled TAG 15:0/18:1/15:0, DAG 15:0/18:1, MAG 18:1, cholesterol ester 18:1, LPC 18:1, PC 15:0/18:1, LPE 18:1, PE 15:0/18:1, phosphatidylglycerol (PG) 15:0/18:1, and phosphatidylinositol (PI) 15:0/18:1 species, and 5.0 μL of a mixture containing hydrogenated MGDG 36:0 (840526P, 10 mg powder, dissolved with chloroform at 62.50 pmol/ μL), hydrogenated DGDG 36:0 (840527P, 10 mg powder, dissolved with chloroform at 62.50 pmol/ μL), and NAPE 36:2 PE N-19:0 (10 mg powder, dissolved with chloroform at 46.63 pmol/ μL), as internal standards.

2.2.2.2.2. Lipid detection

Lipid species were detected by HILIC-ESI/MS/MS on a Nexera X2 UHPLC system (Shimadzu, Duisburg, Germany) coupled with a hybrid triple quadrupole/linear ion trap mass spectrometer (6500 + QTRAP system, AB Sciex, Framingham, MA, USA). HILIC separation was on an XBridge amide column (150 mm $\times$ 4.6 mm, 3.5 μm , Waters, Milford, MA, USA) at 35°C using mobile phase A [1.0 mM ammonium acetate in water-acetonitrile 5:95 (v/v)] and mobile phase B [1.0 mM ammonium acetate in water-acetonitrile 50:50 (v/v)]. The elution gradient was as follows: 0 – 6 min: 100% A, 0% B; 6 – 10 min: 94% A, 6% B; 10 – 11 min: 75% A, 25% B; 11 – 13 min: 2% A, 98% B; 13 – 19 min: 0% A, 100% B; 19 – 24 min: 100% A, 0% B; the flow rate 0.7 mL/min during the first 13 min and 1.5 mL/min afterwards. The instrument parameters were as follows: curtain gas: 35 psi; collision gas: 8 arbitrary units (medium); ion spray voltage: 5500 V/–4,500 V; temperature: 550°C ; ion source gas 1: 50 psi; ion source gas 2: 60 psi; declustering potential: 60 V/–80 V; entrance potential: 10 V/–10 V; collision cell exit potential: 15 V/–15 V. Lipid semi-quantification was performed by scheduled Multiple Reactions Monitoring (MRM). Lipid identification was based on typical neutral losses or the generation of fragment ions during collision induced dissociation in the tandem MS set-up.

2.2.2.2.3. Nonpolar lipid classes

TAGs and DAGs were measured in positive ion mode as ammonium adducts with MRM transitions based on the neutral loss of the following fatty acids (FAs): C16:0,

Fig. 1. Wheat, rye, and oat DLs were isolated from fermented doughs by ultracentrifugation and freeze-dried. These freeze-dried DL powders were then dissolved in water at 5.00 or 1.50 mg protein/mL and as such referred to as 'control wheat, rye, and oat DLs'. Wheat and rye DL powders defatted according to the protocol of Bligh and Dyer (1959), freeze-dried, and dissolved in water at 5.00 or 1.50 mg protein/mL are referred to as 'defatted wheat and rye DLs'. Oat DL powder defatted with hexane, freeze-dried, and dissolved in water at 5.00 or 1.50 mg protein/mL is referred to as 'defatted oat DL'. To assess the impact of the organic solvents used in both methods on the functionality of DL constituents other than lipids, wheat, rye, and oat DLs to which the respective solvent phases were added back after extraction were also prepared. These samples were freeze-dried and dissolved in water at 5.00 or 1.50 mg protein/mL. Such DL solutions are referred to as 'treated wheat, rye, and oat DLs'.

C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:2, C22:3, C22:4, C22:5, and C22:6. MAGs were measured in positive ion mode as protonated species with MRM transitions based on the neutral loss of glycerol.

2.2.2.2.4. Polar lipid classes PCs and LPCs were measured in negative ion mode as acetate adducts and PEs, LPEs, PGs, and PIs in negative ion mode as deprotonated species with MRM transitions based on the generation of FA C14:0, C14:1, C16:0, C16:1, C16:2, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:3, C20:4, C20:5, C22:0, C22:1, C22:2, C22:4, C22:5, and C22:6 fragment ions. The GLs and NA(L)PEs were measured in positive ion mode as ammonium adducts. Identification of MGDGs/MGMGs and DGDGs/DGMGs was based on the neutral loss of m/z 179.1 and m/z 341.1, respectively. Identification of NAPEs and NALPEs was based on the neutral loss of the following ammoniated N-fatty amide head group fragments: 396.3 (N-C16:0), 394.3 (N-C16:1), 392.3 (N-C16:2), 390.3 (N-C16:3), 424.3 (N-C18:0), 422.3 (N-C18:1), 420.3 (N-C18:2), 418.3 (N-C18:3), 416.3 (N-C18:4), 452.3 (N-C20:0), 450.3 (N-C20:1), 448.3 (N-C20:2), 446.3 (N-C20:3), and 444.3 (N-C20:4).

2.2.2.2.5. Data processing Peak integration was performed with the MultiQuant™ software version 3.0.3 (AB Sciex, Framingham, MA, USA). Lipid species were only reported when their peak area was at least three times larger than that of the blank (i.e. pure methanol). After subtraction of blank signals, lipid species signals were corrected for isotopic contributions and were divided by the appropriate standard signals for normalization and semi-quantification. Due to a lack of MGMGs, DGMGs, and NALPEs standards, MGDG, DGDG and NAPE standards were used instead. Finally, data were linked the absolute amount of lipid extract analyzed and expressed as nmol/g dm DL. HILIC-ESI-MS/MS analyses were duplicate measurements of lipid extracts of DLs from each of two separate isolations.

2.2.3. Defatting of dough liquor

2.2.3.1. Wheat and rye DLs Wheat and rye DL powders obtained as in section 2.2.1 were defatted with a procedure similar to the Bligh and Dyer (1959) protocol (see section 2.2.2.1) (Fig. 1). After three extractions, the combined methanol/water phases were added back to the middle phase to prevent loss of protein material and the solvent was evaporated. The middle phase was then freeze-dried, ground, and stored at -18°C until analysis. The sequential extractions with chloroform, methanol, and water may have impacted on the functionality of DL constituents other than lipids. To investigate the impact of such solvent treatments, DL samples to which both the chloroform and methanol/water phases were added back to the middle phase after extraction were also prepared (Fig. 1). After evaporation of the solvents, the DLs were again freeze-dried, ground, and stored at -18°C until analysis.

2.2.3.2. Oat DL Oat DL powder obtained as in section 2.2.1 was defatted with the above described Bligh and Dyer (1959) protocol. However, the freeze dried defatted DL powder obtained could not be dissolved in water in spite of extensive stirring, mild heating, and sonication. To avoid this lack of solubility, oat DL was partially defatted with hexane (Fig. 1). Hexane (5.0 mL) was added to 250 mg oat DL powder. The suspension was mechanically shaken [175 movements per min (rpm), 30 min, room temperature] and centrifuged (2,000 g, 15 min). The supernatant was collected and the extraction was repeated twice. The combined hexane phases were evaporated and lipid levels were weighed. Doing so resulted in extraction of about 80% of the TL in DL. The remaining DL material was then freeze-dried, ground, and stored at -18°C until analysis. As above, the impact of hexane extraction on the functionality of oat DL constituents other than lipids was assessed (Fig. 1). After three extractions, the combined hexane phases were added back to the remaining DL material, the solvent was evaporated, and the DLs were freeze-dried, ground, and stored at -18°C until analysis.

Each of the two solvent treatments and defatting procedures were performed in six-fold on DL powders from each of four separate isolations. Protein levels of these defatted and solvent treated freeze-dried wheat, rye, and oat DL powders were determined as described in section 2.2.2.1. Defatted and solvent treated freeze-dried wheat, rye, and oat DL powders were dissolved in deionized water at 1.50 or 5.00 mg protein/ml as described in section 2.2.1 and are further referred to as 'defatted wheat, rye, and oat DLs' and 'treated wheat, rye, and oat DLs', respectively.

2.2.4. Analysis of dough liquor air-water interfacial properties

The surface stress (σ_s) of rheologically complex A-W interfaces such as those of gas cells in bread dough is composed of surface tension [σ (T , T), which only depends on the excess concentration Γ and the temperature T] and surface extra stress (τ) contributions (Jaensson and Vermant, 2018):

$$\sigma_s = \sigma(\Gamma, T) + \tau$$

These contributions are not separated in maximum bubble pressure (section 2.2.4.1) and pendant drop (section 2.2.4.2) tensiometry measurements. Thus, if τ is not negligible, which is the case for the A-W interfaces studied in the present paper, the parameters obtained with each of these methods in fact represents σ_s rather than σ (Γ , T). Regardless, obtained σ_s values over time yield information about the diffusion and adsorption of DL constituents at A-W interfaces. Shear rheometry measurements (section 2.2.4.3) provide information about the magnitude of τ of the film formed at the A-W interface.

2.2.4.1. Maximum bubble pressure tensiometry The kinetics of diffusion and adsorption of DL constituents at the A-W interface were assessed at ambient temperature ($23 \pm 2^{\circ}\text{C}$) using a BP50 maximum bubble pressure tensiometer (Krüss, Hamburg, Germany) as in Wouters et al. (2018). Such device measures σ_s as a function of air bubble surface age (Janssen et al., 2018). Air bubbles were introduced in control, treated, and defatted wheat, rye, and oat DLs at 1.50 mg protein/mL at the tip of a disposable polypropylene capillary (inner diameter 0.4 mm). When the radius of the bubble equaled that of the capillary (r_{cap}), the pressure inside the bubble reached a maximum value, i.e. P_{max} . With the Young-Laplace equation, the difference between P_{max} and the initial hydrostatic pressure (P_0) was used to calculate σ_s :

$$\sigma_s = \frac{(P_{\text{max}} - P_0) r_{\text{cap}}}{2}$$

The σ_s was monitored over surface ages ranging between 15 ms and 5 s. The lag time was the surface age at which it had decreased to a value equal to 99.5% of that of the initial value of 72 mN/m (σ_s of water at ambient temperature). Hence, it is a measure of the rate of diffusion to and early stage adsorption (at which point σ_s has not yet decreased) of DL surface-active constituents at the A-W interface. A second parameter derived from a maximum bubble pressure measurement is the absolute value of the slope of the decrease of σ_s as a function of the logarithm of the magnitude of the surface age ($IS_{\text{ST-L}}$), which is indicative for the rate of continuous adsorption and rearrangement of DL surface-active constituents at the A-W interface. Diffusion and adsorption are continuous processes that cannot be discriminated as independent phenomena with the methodology used here. After each measurement, the capillary was replaced, the system calibrated, and σ_s of pure water verified to be 72.0 ± 0.50 mN/m before initiating a new measurement. Maximum bubble pressure measurements were performed in duplicate on DLs from each of two separate isolations.

2.2.4.2. Pendant drop tensiometry The decrease of σ_s over time was measured at ambient temperature ($23 \pm 2^{\circ}\text{C}$) using a Theta optical tensiometer (Attension, Biolin Scientific, Stockholm, Sweden) as in Janssen, Wouters et al. (2018). Control, treated, and defatted wheat, rye, and oat DLs at 5.00 mg protein/mL were introduced in the de-

vice to create a pendant drop with a fixed (8.0 μL) volume with monitoring σ_s over 900 s (with 1.4 frames recorded/s in the first 300 s and 0.14 frames recorded/s in the last 600 s). After each measurement, the device was thoroughly cleaned and σ_s of pure water was verified to be 72.0 ± 0.50 mN/m as above before initiating a new measurement. All pendant drop measurements were performed in duplicate on DLs from each of two separate isolations.

2.2.4.3. Stress-controlled interfacial shear rheometry The linear viscoelastic rheological material functions of DL stabilized A-W interfaces were measured in simple shear deformations with a stress-controlled rheometer (Discovery HR-3, TA Instruments, New Castle, DE, USA) equipped with the platinum double wall ring (DWR) geometry from TA Instruments (New Castle, DE, USA). The DWR had the following dimensions: inner radius: 34.50 mm, outer radius: 35.50 mm, ring width: 1.00 mm. Aliquots (18.80 mL) of control, treated, and defatted wheat, rye, and oat DLs at 1.50 mg protein/mL were gently transferred to a double wall Teflon sample holder. In this set up, DL surface-active constituents spontaneously adsorbed at the A-W interface. Time sweeps were performed for 126 min (i.e. the total fermentation time during the bread making protocol) at an oscillation frequency of 3.14 rad/s or 0.50 Hz and a σ_s amplitude of 16.23 $\mu\text{N/m}$, which was within the linear viscoelastic regime for all DLs (see supplementary information). Oscillatory frequency sweeps (0.50 – 5.00 Hz) revealed that the obtained moduli increased linearly with the applied frequency. The oscillation frequency was thus relevant. Experiments were performed at 21 °C to minimize evaporation. The Boussinesq (Bq) number, which represents the ratio between the interfacial shear viscosity and the bulk phase viscosity, was calculated to evaluate to which extent the bulk phase viscosity influenced the A-W interfacial viscosity measurements (Vandebriel, Franck, Fuller, Moldenaers, & Vermant, 2010). Because of the slow adsorption of constituents at the A-W interface in defatted wheat DL, Bq numbers were not calculated for the initial modulus of the complex surface shear viscosity ($|\eta_s^*|$), but with the magnitude of the $|\eta_s^*|$ value measured 25 min after positioning the ring at the A-W interface. As Bq numbers were lower than 100, the components of $|\eta_s^*|$ were corrected using an iterative solver to take into account the coupling of the flow in the subphase with the A-W interface (Renggli, Alicke, Ewoldt, & Vermant, 2020). This was essential to obtain the exact deformation profile and shear rates at the A-W interface. The steady state bulk phase shear viscosity was monitored at 21 °C as a function of the shear rate (logarithmic sweep between 1 and 1,000 s^{-1}) using a stress-controlled MCR501 rheometer (Anton Paar, Graz, Austria) equipped with a double-wall Couette geometry (DG26.7, inner radius 12.33 mm, outer radius 13.33 mm, bob length 40.00 mm). Thus, the magnitude of $|\eta_s^*|$ and the phase angle (δ_s) in Fig. 3 were calculated taking the subphase coupling into account. The $|\eta_s^*|$ can also be decomposed in an elastic part (η'' , which is out of phase with the deformation rate) and a viscous part (η' , which is in phase with the deformation rate). A-W interfacial shear rheology experiments were single (treated and defatted DLs) or duplicate (control DLs) measurements on DLs from each of two separate isolations.

2.2.5. Statistical analyses

Data were analyzed with statistical software JMP pro 12 (SAS Institute, Cary, NC, USA). Significant differences, at a significance level of $\alpha = 0.05$, were identified using one-way analysis of variance combined with a post-hoc Tukey's honest significant difference (HSD) test.

3. Results and discussion

3.1. Dough liquor chemical composition

Isolation yields as well as lipid, protein, AX, and β -D-glucan levels of DLs isolated from fermented wheat, rye, and oat doughs are given in Table 1. The isolation yields of DLs decreased in the order rye (10.4% of dough dm), oat (4.0% of dough dm), and wheat (3.4% of dough dm). Lipid levels were high in oat DL (29.6% of DL dm) and much lower in rye (1.5% of DL dm) and wheat (0.5% of DL dm) DLs. The protein levels decreased in the order wheat (16.9% of DL dm), rye (14.0% of DL dm), and oat (7.8% of DL dm) DLs. AX was evidently the major non-starch polysaccharide in wheat and rye DLs (9.0 and 15.1% of DL dm, respectively), while β -D-glucan was the most abundant one in oat DL (11.9% of DL dm).

Besides proteins, lipids, and NSPs, DL contains large amounts of mono-, di- and oligosaccharides (Primo-Martín et al., 2006; Salt et al., 2006; Turbin-Orger et al., 2015) and most likely also minor amounts of water-soluble minerals and vitamins (Belitz, Grosch, & Schieberle, 2009).

3.2. Dough liquor lipid population

The gravimetrically determined TL levels (in mg/g dm DL) of extracts of wheat, rye, and oat DLs obtained with the protocol of Bligh and Dyer (1959) are listed in Tables 2A and 2B. PL levels of a given DL were calculated by summing up the levels of all GL and PhL species analyzed with HILIC-ESI-MS/MS. The same approach was used to calculate the total level of NPLs of a given DL, which included TAGs, DAGs, and MAGs. We were not able to quantify FFAs and other NPL and PL classes that may have been present in the DL extracts. A remaining fraction was quantified by subtracting the PL and NPL levels (detected with HILIC-ESI-MS/MS) from the TL level (gravimetrically determined). Considering that the FFA level in DL extracts presumably is equal to or even higher than the total level of acylglycerols (Salt et al., 2017), FFAs are expected to be the dominant lipid class in this fraction. The levels (in nmol/g dm DL) of the different NPL and PL classes in extracts of the different DL samples (detected with HILIC-ESI-MS/MS) are also listed in Tables 2A and 2B. An overview of the most abundant acyl groups (for lysolipids) or acyl group combinations of the different lipid classes in these extracts is provided as supplementary information.

Table 1

Isolation yield [expressed on dough dry matter (dm) basis] as well as chemical composition of dough liquor (DL) [expressed on DL dry matter (dm) basis] isolated from fermented wheat, rye, and oat doughs. Column values with the same capital letter are not significantly different ($\alpha = 0.05$). Error values represent the deviation from the mean for duplicate measurements of each of two separate DL isolation experiments.

	Isolation yield (% of dough dm)		Lipid level (% of DL dm)		Protein level (% of DL dm)		Arabinoxylan (AX) level (% of DL dm)		β -D-glucan level (% of DL dm)	
Wheat DL	3.4 (± 0.4)	A	0.5 (± 0.0)	A	16.9 (± 0.4)	A	9.0 (± 0.1)	A	0.6 (± 0.1)	A
Rye DL	10.4 (± 0.8)	B	1.5 (± 0.1)	B	14.0 (± 0.7)	A	15.1 (± 0.2)	B	4.4 (± 0.4)	B
Oat DL	4.0 (± 0.2)	C	29.7 (± 0.5)	C	7.8 (± 0.3)	B	2.9 (± 0.1)	C	11.9 (± 0.6)	C

Table 2A

Gravimetrically determined total lipid (TL) level, expressed in mg/g dry matter (dm), of extracts of wheat, rye, and oat dough liquors (DLs) obtained with the protocol of Bligh and Dyer (1959) and in hexane extracts of oat DL. Polar lipid (PL) levels for a given DL were calculated by adding up the levels (in mg/g dm DL) of all glycolipid (GL) and phospholipid (PhL) species as determined with hydrophilic interaction liquid chromatography (HILIC) electrospray ionization (ESI) tandem mass spectrometry (MS/MS). A similar approach was used to calculate the nonpolar lipid (NPL) levels for a given DL. NPL classes included triacylglycerols, diacylglycerols, and monoacylglycerols. The remaining fraction, which contains free fatty acids amongst other lipid classes, was calculated by subtracting the total level of PLs as well as of NPLs (all in mg/g dm DL, detected with HILIC-ESI-MS/MS) from the TL level (in mg/g dm DL, determined gravimetrically). Error values represent the deviation from the mean for duplicate measurements of extracts of each of two separate DL isolation experiments. Values between brackets represent the level of a given lipid fraction (PL, NPL and remaining fraction) relative to the TL lipid level (100%).

(A)				
	Bligh and Dyer (1959)			Hexane
	Wheat DL	Rye DL	Oat DL	Oat DL
TLs	4.86 ± 0.24 (100%)	15.36 ± 0.60 (100%)	297.46 ± 1.89 (100%)	240.72 ± 0.90 (100%)
PLs	0.95 ± 0.28 (19%)	3.96 ± 0.23 (26%)	24.64 ± 3.54 (8%)	19.18 ± 3.52 (8%)
GLs	0.13 ± 0.06	1.38 ± 0.22	6.68 ± 1.35	3.69 ± 0.33
PhLs	0.82 ± 0.23	2.58 ± 0.17	17.96 ± 2.96	15.49 ± 3.36
NPLs	1.30 ± 0.20 (27%)	4.52 ± 0.54 (29%)	125.97 ± 8.66 (42%)	128.87 ± 6.75 (54%)
Remaining fraction	2.61 ± 0.19 (54%)	6.88 ± 0.81 (45%)	146.85 ± 10.73 (50%)	92.67 ± 10.25 (38%)

Table 2B

Levels (in nmol/g dm DL) of the different polar lipid (PL) classes as well as of the different nonpolar lipid (NPL) classes present in Bligh and Dyer (1959) extracts of wheat, rye, and oat dough liquors (DLs) and in hexane extracts of oat DL, detected with hydrophilic interaction liquid chromatography (HILIC) electrospray ionization (ESI) tandem mass spectrometry (MS/MS). Values represent the sum of all species detected for a given lipid class. Error values represent the deviation from the mean for duplicate measurements of extracts of each of two separate DL isolations. Row values with the same capital or lowercase letter are not significantly different ($\alpha = 0.05$). Abbreviations: TLs: total lipids; GLs: glycolipids; PhLs: phospholipids; TAGs: triacylglycerols; DAGs: diacylglycerols; MAGs: monoacylglycerols; MGMGs: monogalactosylmonoacylglycerols; DGMGs: digalactosylmonoacylglycerols; MGDGs: monogalactosyldiacylglycerols; DGDGs: digalactosyldiacylglycerols; LPCs, lysophosphatidylcholines; PCs, phosphatidylcholines; LPEs, lysophosphatidylethanolamines; PEs, phosphatidylethanolamines; PGs, phosphatidylglycerols; Pls, phosphatidylinositols; NALPEs, *N*-acyl lysophosphatidylethanolamines; NAPEs, *N*-acyl phosphatidylethanolamines.

(B)								
	Bligh and Dyer (1959)				Hexane			
Lipid class	Wheat DL		Rye DL		Oat DL		Oat DL	
TLs	3,357 ± 638	A	10,135 ± 801	A	206,484 ± 12,303	B, a	181,775 ± 15,823	a
PLs	1,219 ± 393	A	4,764 ± 209	A	39,882 ± 6,425	B, a	33,477 ± 7,303	a
GLs	166 ± 79	A	1,572 ± 243	A	7,233 ± 1441	B, a	4,008 ± 354	b
MGMGs	50 ± 36	A	124 ± 42	B	76 ± 15	AB, a	51 ± 7	b
DGMGs	3 ± 1	A	4 ± 2	A	12 ± 4	B, a	9 ± 5	a
MGDGs	48 ± 20	A	412 ± 178	B	1,087 ± 203	C, a	669 ± 89	b
DGDGs	64 ± 23	A	1,032 ± 140	B	6,059 ± 1328	C, a	3,278 ± 298	b
PhLs	1,053 ± 318	A	3,192 ± 226	A	32,649 ± 5,937	B, a	29,470 ± 7,154	a
LPCs	120 ± 86	A	238 ± 209	A	1,392 ± 91	B, a	855 ± 151	b
PCs	131 ± 44	A	242 ± 27	A	2,507 ± 146	B, a	1,494 ± 104	b
LPEs	4 ± 2	A	27 ± 8	A	395 ± 52	B, a	240 ± 23	b
PEs	209 ± 29	A	407 ± 91	A	4,069 ± 355	B, a	2,424 ± 180	b
PGs	12 ± 5	A	55 ± 8	B	113 ± 9	C, a	96 ± 6	a
Pls	171 ± 39	A	319 ± 59	B	450 ± 50	C, a	251 ± 21	b
NALPEs	244 ± 168	A	1,047 ± 233	A	11,671 ± 2,357	B, a	11,329 ± 3,425	a
NAPEs	164 ± 44	A	858 ± 91	A	12,051 ± 3,636	B, a	12,807 ± 3,924	a
NPLs	2,138 ± 314	A	3,703 ± 704	A	166,602 ± 10,371	B, a	148,298 ± 10,634	a
TAGs	501 ± 47	A	1,835 ± 678	A	101,145 ± 11,266	B, a	89,926 ± 11,618	a
DAGs	1,438 ± 258	A	1,306 ± 184	A	52,924 ± 9,391	B, a	46,481 ± 8,327	a
MAGs	199 ± 73	A	561 ± 48	A	12,532 ± 3,575	B, a	11,891 ± 2,785	a

3.2.1. Wheat, rye, and oat dough liquor total lipid extracts obtained with the protocol of Bligh and Dyer (1959)

The TL levels in wheat, rye, and oat DL extracts were 4.86, 15.36, and 297.46 mg/g dm DL, respectively. Although DL is an aqueous phase, PLs constituted only 19, 26, and 8% of the TLs in wheat, rye, and oat DL extracts, respectively (Tables 2A and 2B). Thus, the majority of the lipids present in wheat, rye, and oat DLs were nonpolar. The relative amount of extractable GLs decreased in the order rye, oat, and wheat (35, 27, and 13% of the total PLs, respectively) DLs (Tables 2A and 2B). However, PhLs still accounted for most of the PLs in each of the DL extracts (Tables 2A and 2B). The remaining fraction,

which largely consisted of FFAs, made up 54, 45, and 50% of the TLs in wheat, rye, and oat DL extracts, respectively (Tables 2A and 2B).

MGMGs, MGDGs, and DGDGs were the predominant GLs in all three DLs (Tables 2A and 2B). The relative amount of MGMGs in wheat DL extracts (30% of the GLs) was much higher than that in extracts of rye (8% of the GLs) and oat (1% of the GLs) DLs. In contrast, rye DL extracts contained significantly ($P < 0.05$) higher MGMG, MGDG, and DGDG levels than did wheat DL extracts, while oat DL contained significantly ($P < 0.05$) more extractable DGMGs, MGDGs, and DGDGs than did wheat and rye DLs.

The most common PhLs in wheat DL extracts were PEs (20% of the PhLs) and NALPEs (23% of the PhLs). In rye and oat DL extracts,

the most common PhLs were NApEs and NALPEs amounting to 27 and 33% of the PhLs in rye DL extracts and 37 and 36% of the PhLs in oat DL extracts, respectively (Tables 2A and 2B). All DL extracts also contained LPCs, PCs, LPEs, PGs, and PIs. The level of each reported PhL class in oat DL extracts was significantly ($P < 0.05$) higher than that in rye and wheat DL extracts (Tables 2A and 2B). Of note here is that Salt et al. (2018) did not include NApE and NALPE species in their DL lipid analyses. However, the fact that these species made up 40, 60, and 73% of the total extractable PhLs in wheat, rye, and oat DLs, respectively, demonstrates their relevance.

With respect to the NPLs, DAGs were the most abundant lipid class in wheat DL extracts and TAGs in rye and oat DL extracts (Tables 2A and 2B).

3.2.2. Oat dough liquor hexane extracts

Tables 2A and 2B also lists the levels of the different lipid classes in the hexane extract of oat DL. Comparison of these levels with those of the Bligh & Dyer oat DL extract allowed assessing the lipid distribution in defatted oat DL. Hexane extracts contained significantly ($P < 0.05$) lower levels of most GL and PhL classes, as well as of the remaining fraction than did oat DL total lipid extracts obtained with the protocol of Bligh and Dyer (1959) (Tables 2A and 2B). In contrast, the levels of DGMGs, NApEs, NALPEs, PGs, TAGs, DAGs, and MAGs in hexane extracts were not significantly ($P > 0.05$) different from those in Bligh & Dyer extracts (Tables 2A and 2B). Thus, oat DL defatted with hexane still contained GLs, PhLs, and FFAs.

3.3. Dough liquor air-water interfacial properties

As mentioned, both lipids and proteins can diffuse to and adsorb at A-W interfaces and thus contribute to gas cell stability in dough systems. Their contribution as well as the influence of their mutual interplay on the stability of control, treated, and defatted DL stabilized A-W interfaces is discussed below. Because proteins likely play an important role in stabilizing dough A-W interfaces and because their relative level in DL increased as a result of defatting, control, treated, and defatted DLs were dissolved at a constant protein concentration prior to studying their A-W interfacial properties. As wheat, rye, and oat DL powders had different protein levels (Table 1), this impacted the concentrations of the other DL constituents. A table listing the lipid and protein concentrations and protein/lipid ratio (on a mass basis) of the different control, treated and defatted DLs at 5.00 mg protein/mL is given as supplementary information.

3.3.1. Maximum bubble pressure tensiometry

The decrease of σ_s as a function of the surface age of control, treated, and defatted wheat, rye, and oat DLs (all at 1.50 mg protein/mL) is shown in Fig. 2. It is important to note that σ_s measurements via bubble pressure tensiometry at very low surface ages to some extent are influenced by hydrostatic effects which in turn depend on, amongst others, the bulk viscosity. Hence, bubble pressure tensiometry σ_s measurements in a highly viscous medium may not be fully reliable at low surface ages (Fainerman & Miller, 1998). Here, wheat, rye, and oat DLs powders were dissolved at 1.50 mg protein/mL to ensure sufficiently low bulk viscosity.

The σ_s of DL solutions decreased when the surface age increased as surface-active molecules had more time to diffuse to and adsorb at the A-W interface. As described in section 2.2.4.1, two parameters were derived from a typical maximum bubble pressure measurement: (i) the lag time, a measure for the rate of diffusion and early stage adsorption of surface-active constituents to the bubble A-W interface, and (ii) the absolute value of the slope of the decrease in σ_s as function of the logarithm of the magnitude of the surface age (IS_{ST-I}) as an indication of the rate of continuous adsorption and rearrangement of surface-active

constituents at the bubble A-W interface (see supplementary information).

First, it is important to note that the solvent treatment associated with defatting had no impact on the diffusion and adsorption kinetics of the constituents in the DL samples. The σ_s versus surface age plots of treated wheat, rye, and oat DLs mimicked those of their control counterparts (Fig. 2). Indeed, no significant ($P > 0.05$) differences in lag time or IS_{ST-I} values between control and treated samples were observed (see supplementary information) for any of the DLs.

Lipids decrease σ of A-W interfaces more rapidly and to a lower value than proteins (Bos & van Vliet, 2001). Thus, if the rate at which control DL constituents decrease σ is more pronounced than that at which defatted DL constituents do, lipids are evidently present at control DL stabilized A-W interfaces. In line with this reasoning, there were some differences between the σ_s versus time plots of defatted DLs and those of their respective control DLs. Indeed, the observed lag times noted for defatted wheat and rye DLs were not significantly ($P > 0.05$) different from those of the respective control samples (see supplementary information). However, the IS_{ST-I} readings of control wheat and rye DLs (4.8 and 6.4, respectively) were significantly higher ($P < 0.05$) than those of their defatted counterparts (4.1 and 5.5, respectively) (see supplementary information). This implies that the constituents in control wheat and rye DLs decreased σ_s more rapidly than those in their respective defatted DLs. This shows that both wheat and rye DL lipids to an extent adsorbed at the A-W interface in the early stages of A-W interface development (at the low surface ages characteristic for the bubble pressure method) for the control samples. Nevertheless, the defatting procedure had only a limited impact on the rate of diffusion to and adsorption at the A-W interface of defatted wheat and rye DL constituents. Thus, constituents other than lipids, most likely proteins, also adsorbed at the A-W interface at the concentrations tested here, even at these very low surface ages. In contrast to what was the case for control wheat and rye DLs, no lag time was recorded for control oat DL samples, even at very low surface ages. This implies that even at the lowest surface ages measurable σ_s had already decreased and thus that control oat DL constituents had adsorbed at the A-W interface. Most likely, these were lipids considering their high concentration in oat DL (Table 1). Indeed, in contrast to what was observed for the σ_s versus surface age plot of control oat DL, the plots of defatted oat DL did have a lag time of 81 ms. Thus, lipids clearly adsorbed at control oat DL stabilized A-W interfaces.

3.3.2. Pendant drop tensiometry

The decrease of σ_s over time of A-W interfaces stabilized by constituents in control, treated, and defatted wheat, rye, and oat DLs, all at 5.00 mg protein/mL (Table 1), is shown in Fig. 2. It should be noted that in pendant drop tensiometry measurements the ratio between bulk viscous and σ_s forces needs to be low. As the bulk viscosity increases, the shape of the pendant droplet may deviate from its equilibrium state. This would render the Young-Laplace equation and thus the σ_s values derived from it invalid (Jaensson & Vermant, 2018; Leser, Acquistapace, Cagna, Makievski, & Miller, 2005). Here, wheat, rye, and oat DLs powders were dissolved at 5.00 mg protein/mL to sufficiently reduce their bulk viscosities.

There was no mentionable impact of the solvent defatting treatment of wheat and rye DLs on the decrease of σ_s over time. However, the hexane treatment associated with defatting of oat DL seemed to result in an increased rate at which σ_s decreased over time. As the compositions of the control and treated oat DLs were identical, this suggests that the hexane treatment itself resulted in increased mobility of oat DL surface-active constituents and thus allowed them to adsorb more rapidly at the A-W interface. We indeed speculate that the hexane treatment liberated some lipid molecules that were previously present in supramolecular structures such as micelles or bilayers (Melis,

Fig. 2. Left: Evolution of the surface stress (σ_s) as function of surface age, which is indicative for the adsorption kinetics at the air–water (A–W) interface of control (green circles), treated (blue squares), and defatted (yellow triangles) wheat, rye, and oat dough liquors (DLs) (all at 1.50 mg protein/mL), as measured with a maximum bubble pressure tensiometer. Right: Evolution of σ_s as function of time at the A–W interface of a pendant drop of diluted control (green circles), treated (blue squares), and defatted (yellow triangles) wheat, rye, and oat DLs (all at 5.00 mg protein/mL), as measured by pendant drop tensiometer. Error values represent the deviation from the mean for duplicate measurements of two separate DL isolation experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Meza Morales, & Delcour, 2019; Selmaier, 2010), leading to an increased rate of continuous adsorption of these lipids at the A–W interface. Alternatively, the hexane treatment may have increased the surface activity of oat DL constituents by weakening some of the originally present hydrophobic lipid–protein and/or lipid– β -D-glucan interactions. However, that there were no differences in σ_s 15 min after drop formation of A–W interfaces stabilized by either treated or control oat DL samples suggests that there was an equilibrium A–W interface composition at that point in time.

The σ_s values of defatted wheat, rye, and oat DL stabilized A–W interfaces after 15 min (48, 43, and 43 mN/m, respectively) were significantly ($P < 0.05$) higher than those of the respective control samples (43, 40, and 32 mN/m). This confirms that their lipids at least to some extent adsorbed at A–W interfaces stabilized by the control DL constituents, an observation which is in line with the results of the bubble pressure measurements (section 3.3.1). Nonetheless, since 8% of the dm of defatted oat DL were PLs as well as NPLs (see section 3.2), we suspect that even for A–W interfaces stabilized by defatted oat DL constituents, lipids still play a substantial if not predominant role.

3.3.3. Stress-controlled interfacial shear rheometry

Interfacial shear rheology measurements are most valuable for studying interactions between constituents adsorbed at A–W inter-

faces such as proteins and lipids (Dickinson, 2011; Krägel, Derkatch, & Miller, 2008) as the interfacial area is constant and shear viscosities are sensitive to lateral interactions between surface-active constituents. Important to mention is that the accuracy of interfacial shear rheology measurements largely depends on the contribution of the bulk phase viscosity (Jaensson and Vermant, 2018) (). The relative importance of the interfacial and bulk phase contributions is defined by the Bq number (see section 2.2.4.3). When this number would become too low, the measured shear response would be dominated by the bulk phase viscosity and not by that of the A–W interface. In the present case, wheat, rye, and oat DLs powders were dissolved at 1.50 mg protein/mL to obtain sufficiently low bulk viscosities. Moreover, as wheat, rye, and oat DLs at 1.50 mg protein/mL had different bulk viscosities (Janssen et al., 2020), the contribution of the bulk phase viscosity was subtracted from that of the A–W interfacial film by means of an iterative solver (see section 2.2.4.3) (Renggli et al., 2020).

Although the solvent treatment associated with defatting wheat and rye DLs changed neither their diffusion nor adsorption behavior (section 3.3.1) nor the evolution of σ_s over time (section 3.3.2), it had a strong impact on the shear rheology of the adsorbed film. Indeed, whereas measurable moduli were obtained for control and defatted wheat and rye DLs at 1.50 mg protein/mL (Fig. 3), the shear moduli of the films formed at A–W interfaces stabilized by treated wheat and

Fig. 3. Air-water (A-W) interfacial elastic (η''_s), viscous (η'_s), and magnitude of the complex ($|\eta^*_s|$) shear viscosities as a function of time for control and defatted wheat and rye dough liquors (DLs) (all at 1.50 mg protein/mL), as measured with a stress-controlled rheometer coupled to a DWR geometry. A-W Interfacial shear rheology experiments were duplicate (control DLs) or single (defatted DLs) measurements on DLs from each of two separate isolations.

rye DLs (at the same protein concentration) did not exceed the detection limit (i.e. about 5.0×10^{-6} Pa.m.s) of the DWR geometry (Vandebriel et al., 2010), even after 126 min. At 0.50 mg protein/mL, reliable shear moduli were obtained for treated wheat and rye DL stabilized A-W interfaces (see supplementary information). This seems counter-intuitive as higher bulk concentrations of surface-active material would be expected to result in increased amounts of adsorbed material at the A-W interface and thus in higher interfacial shear moduli. However, the shear moduli of an A-W interfacial film also depend on the film composition and thus on the ratio of lipid to protein molecules thereat. We hypothesize that increasing bulk concentrations, which of course result in proportionate increases in the bulk protein and lipid concentrations, can nevertheless result in altering ratios of lipid to protein at the interface. Due to faster adsorption of lipids at the A-W interface than proteins (Bos & van Vliet, 2001), the former would in a more concentrated system occupy a relatively larger interfacial area than in a more diluted system. As lipids interfere with the formation of a coherent protein film, the above effect would explain why lower interfacial shear moduli were observed at higher than at lower bulk concentrations.

This implies that the solvent treatment associated with the lipid extraction procedure itself might have resulted in an A-W interfacial film with a higher ratio of lipid to protein molecules and thus with a different microstructure.

Fig. 3 shows the evolution of $|\eta^*_s|$, η''_s , and η'_s over time of A-W interfaces stabilized by constituents in control and defatted wheat and rye DLs at 1.50 mg protein/mL. The film formed at the A-W interface by control wheat DL constituents was viscoelastic over the entire measurement time frame (Fig. 3). At 0.50 Hz, the response of the film when deformed by shear was predominantly viscous ($\eta''_s > \eta'_s$) during the first 33 min and predominantly elastic ($\eta''_s < \eta'_s$) between 33 and 126 min. This was also reflected in δ_s , which varied between 60 and 45° during the first 33 min and between 45 and 28° between 33 and 126 min (see supplementary information). Frequency sweeps immediately after the time sweep showed that control wheat DL stabilized A-W interfaces were predominantly elastic ($\eta''_s < \eta'_s$) between 0.50 and 5.00 Hz (data not shown). Thus, no shift of the dominant relaxation time was observed. Defatted wheat DL resulted in a lower initial $|\eta^*_s|$ of the adsorbed film (Fig. 3). This resulted from adsorption of pro-

teins at the A-W interface, which occurs more slowly than that of low molecular weight surfactants such as lipids (Bos & van Vliet, 2001). The above confirm the results obtained by maximum bubble pressure (section 3.3.1) and pendant drop (section 3.3.2) tensiometry and again show that lipids adsorb at control wheat DL stabilized A-W interfaces. However, after 17 min the $|\eta^*_s|$ values of the film formed by defatted wheat DL constituents were higher than that of the film formed by control wheat DL constituents (Fig. 3). Indeed, after 126 min, the former and latter films had $|\eta^*_s|$ values of 6.41×10^{-4} and 4.81×10^{-4} Pa.s.m, respectively. In contrast to what was observed for control wheat DLs, at 0.50 Hz defatted wheat DL stabilized A-W interfaces evolved from being predominantly viscous ($\eta'_s > \eta''_s$) during the first 58 min (compared to 33 min in the case of control DLs) to being predominantly elastic ($\eta'_s > \eta''_s$) later on. Frequency sweeps following the time sweep revealed that defatted wheat DL stabilized A-W interfaces were also predominantly elastic ($\eta'_s > \eta''_s$) at frequencies between 0.50 and 5.00 Hz (data not shown). Defatted wheat DL stabilized A-W interfacial films had much higher η''_s and η'_s values (5.52×10^{-4} and 3.26×10^{-4} Pa.s.m, respectively, after 126 min) than those of adsorbed A-W interfacial films stabilized by control wheat DL constituents (η''_s and η'_s values of 4.25×10^{-4} and 2.38×10^{-4} Pa.s.m, respectively, after 126 min). That proteins adsorb more slowly at A-W interfaces than lipids (Bos & van Vliet, 2001) explains why in the present case during the first 83 min δ_s of defatted wheat DL stabilized films was higher (a strong decrease from 90 to 36° was observed) than that of control wheat DL stabilized films (see supplementary information). After 83 min, the δ_s values of both films were more or less equal to 30°. This means that the relative changes of the elastic (η''_s) and viscous (η'_s) contributions to the adsorbed film as a result of defatting wheat DL were similar. Several researchers (MacRitchie & Gras, 1973; MacRitchie, 1976; Pauly et al., 2014; Primo-Martín et al., 2006; Sahi, 2003; Salt et al., 2006, 2018; Sloan & MacRitchie, 2009; Turbin-Orger et al., 2015) have proposed that control wheat DL stabilized A-W interfaces are unstable due to the co-occurrence of proteins and lipids. The current observations confirm this and even show that the lipids in wheat DL prevent the development of a strong viscoelastic film at the A-W interface by wheat DL proteins.

In line with what was observed for control wheat DLs, the film formed at the A-W interface by control rye DL constituents was also viscoelastic (Fig. 3). Unlike what was the case for a wheat DL film, the adsorbed A-W interfacial film at 0.50 Hz was predominantly viscous ($\eta'_s > \eta''_s$) over the entire measurement time frame. This was also reflected in the evolution of δ_s over time, which was constant at 66° (see supplementary information). This predominantly viscous ($\eta'_s > \eta''_s$) response of control rye DL stabilized A-W interfaces was also observed at frequencies between 0.50 and 5.00 Hz (data not shown). Hence, no shift of the dominant relaxation time occurred. In contrast to what was observed for wheat DL, defatting rye DL did not result in a lower initial $|\eta^*_s|$ value (Fig. 3). This implies that rye DL proteins adsorbed more rapidly at the A-W interface than did wheat DL proteins. This agrees well with the average IS_{ST-I} value (see Supplementary information), which was much higher for defatted rye (5.5) than for defatted wheat (4.1) DLs. Furthermore, the $|\eta^*_s|$ values of A-W interfacial films formed by defatted rye DL constituents (1.13×10^{-3} Pa.s.m after 126 min) were almost one order of magnitude larger than that of control rye DL stabilized films (2.11×10^{-4} Pa.s.m after 126 min). Similar to what was observed for control rye DLs, defatted rye DL stabilized A-W interfaces at 0.50 Hz were predominantly viscous ($\eta'_s > \eta''_s$) over the 126 min time frame. Frequency sweeps directly after the time sweep showed a predominantly viscous ($\eta'_s > \eta''_s$) shear response of defatted rye DL stabilized A-W interfaces between 0.50 and 5.00 Hz. Additionally, after 126 min, defatted rye DL stabilized films had much higher η''_s and η'_s values (6.83×10^{-4} and 9.02×10^{-4} Pa.s.m, respectively) than control rye DL stabilized films (1.08×10^{-4} and 2.09×10^{-4}

Pa.s.m, respectively). Furthermore, A-W interfaces stabilized by defatted rye DL constituents had a much lower δ_s , which decreased from 61 to 52° after 126 min. This means that the relative increase of η''_s as a result of defatting rye DL was more pronounced than that of η'_s (see supplementary information). In a former paper by our research group (Janssen et al., 2018) the hypothesis was put forward that A-W interfaces in rye dough are quite stable as a result of the adsorbed films being dominated by proteins. That defatting of rye DL resulted in a strong increase of $|\eta^*_s|$, η''_s , and η'_s contradicts this. Moreover, similar to what was observed for wheat DL constituents it seems that rye DL lipids hamper the ability of its DL proteins to form strong viscoelastic films. Thus, proteins and lipids clearly co-exist at rye DL stabilized A-W interfaces.

In spite of the presence of lipids, control wheat DL stabilized films were predominantly elastic ($\eta''_s > \eta'_s$) after 33 min, whereas control rye DL stabilized films were predominantly viscous ($\eta'_s > \eta''_s$) over the entire measurement time frame. This evidently indicates that the microstructure of and thus the interactions between adsorbed constituents in both films are different. The ability of globular proteins such as β -lactoglobulin to form an interfacial viscoelastic layer depends on the protein concentration in the bulk phase (Krägel et al., 2008; Wüstneck, Krägel, Miller, Wilde, & Clark, 1996). At sufficiently low protein concentrations (0.04 and 0.09 mg protein/mL in case of β -lactoglobulin), adsorbed protein molecules can unfold, mutually interact, and form a viscoelastic film that is predominantly elastic ($\eta''_s > \eta'_s$). At higher protein concentrations (0.18 mg protein/mL in case of β -lactoglobulin), the rate of protein adsorption naturally increases. This can partially prevent adsorbed proteins from unfolding and may result in a viscoelastic layer with similar viscous and elastic contributions (Krägel et al., 2008; Wüstneck et al., 1996). Additional shear rheology experiments at 0.05 mg protein/mL (data not shown) showed that the protein concentration used here (1.50 mg protein/mL) was not too high for rye DL proteins to form a predominantly elastic ($\eta''_s > \eta'_s$) viscoelastic film. Indeed, even at 0.05 mg protein/mL was the adsorbed interfacial film predominantly viscous ($\eta'_s > \eta''_s$) over the entire measurement time frame (data not shown). It thus seems that the predominantly viscous nature of rye DL stabilized films is related to the inherent structure of adsorbed rye DL proteins. Such behavior has also been reported for adsorbed β -casein films (Bantchev & Schwartz, 2003; Krägel et al., 2008; Wüstneck et al., 1996).

Finally, reliable interfacial shear moduli were obtained neither for control nor for defatted oat DL stabilized A-W interfaces, because the measured values did not exceed the detection limit of the DWR geometry (Vandebril et al., 2010). Spontaneous adsorption of low concentrations of surfactants generates a very low surface shear response (Zell et al., 2014). This agrees well with the present maximum bubble pressure and pendant drop tensiometry results indicating that both in control and in defatted oat DL lipids are the predominant DL constituents present at the A-W interface.

4. Conclusions

The role of wheat, rye, and oat DL proteins and lipids and their mutual interplay in determining the stability of DL stabilized A-W interfaces was identified. A graphical overview of the A-W interfacial composition of films stabilized by the constituents in control and defatted wheat, rye, and oat DLs is provided in Fig. 4. Despite DL being an aqueous system, PLs only accounted for 19, 26, and 8%, of the TLs in wheat, rye, and oat DL extracts, respectively. Thus, mainly NPLs were retrieved in wheat, rye, and oat DLs. PhLs were the predominant PLs and made up at least 65% of the total PL level in all DLs. Proteins and lipids were found to compete for adsorption at wheat as well as at rye DL stabilized A-W interfaces. Additionally, defatting wheat and rye DLs in both cases led to a pronounced increase of the magnitude of $|\eta^*_s|$ of the A-W interfacial film. Thus, the lipids at the A-W interface impact

Fig. 4. Graphical overview of the A-W interfacial composition of films stabilized by the constituents in control and defatted wheat, rye, and oat DLs. Proteins and lipids compete for adsorption at control wheat and rye DL stabilized A-W interfaces, whereas lipids dominate control oat DL stabilized A-W interfaces.

on the ability of wheat and rye DL proteins to form a strong viscoelastic film thereat. Finally, removing 80% of the lipids from oat DL resulted in a pronounced increase of σ_s . In addition, shear moduli of oat DL stabilized A-W interfaces did not exceed the detection limit of the DWR geometry over the entire measurement time frame. Thus, lipids dominate oat DL stabilized A-W interfaces.

Uncited reference

Jaensson and Vermant (2018).

CRediT authorship contribution statement

Frederik Janssen: Conceptualization, Methodology, Investigation, Writing - original draft. **Arno G.B. Wouters:** Conceptualization, Writing - review & editing. **Lize Linclau:** Investigation, Methodology. **Etiënne Waelkens:** Investigation, Methodology. **Rita Derua:** Investigation, Methodology, Writing - review & editing. **Jonas Dehaire:** Investigation, Methodology. **Paula Moldenaers:** Writing - review & editing. **Jan Vermant:** Writing - review & editing. **Jan A. Delcour:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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