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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.

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 et al., 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 (Primo-Martín, Hamer, & De Jongh, 2006; Sarker, Wilde, & Clark,

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 polysaccharide; PC, phosphatidylcholine; SG, steryl glycosides; TAG, triacylglycerols

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1998).

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 (Gerits, Pareyt, Masure, & Delcour, 2015; Pauly, Pareyt, Fierens, & Delcour, 2014; Salt, Robertson, Jenkins, Mulholland, & Mills, 2005; Salt et al., 2018), the foaming, and/or the surface-active properties (MacRitchie, 1976; MacRitchie & Gras, 1973; Pauly et al., 2014; Primo-Martín et al., 2006; Sahi, 2003; Salt et al., 2006, 2018; Turbin-Orger et al., 2015) 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 et al., 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, 2018) 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, 2018) 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 et al., 1946; MacRitchie, 1976). Wheat dough optimum water level and mixing time were determined using (AACCI, 2018) 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