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Fermentation affects the composition and foaming properties of the aqueous phase of dough from soft wheat flour

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ABSTRACT

A quality characteristic of bread is that it has a homogeneous and fine crumb structure. The latter depends on the number and size of gas cells present in dough as well as their stabilization during fermentation and baking. Discontinuities in the gluten–starch matrix result in liquid films formed from the dough aqueous phase taking over gas cell stabilization. This is especially important in late fermentation. How fermentation affects (the composition and properties of) the dough aqueous phase is unclear. In the present study, the dough's aqueous phase was isolated as dough liquor (DL) from unfermented and fermented dough made from flour and water (with or without added sugar and salt). Fermentation increased DL yield and changed its composition. With regards to surface active proteins, especially the extractability of α-amylase/trypsin inhibitors and puroindolines (PINs) in DL increased. More foam of higher stability was formed from DL from (defatted) fermented dough than from that from (defatted) flour-water dough. PINs were specifically enriched in foam from DL from fermented dough. Finally, supplementation of dough with the latter DL sample yielded bread with finer gas cells than those of control bread. Thus, fermentation enhances the foaming properties of DL, which is also translated in improved bread quality.

1. Introduction

Bread making starts with mixing (wheat) flour, water, yeast, salt and (optionally) other (minor) ingredients into dough, which then undergoes fermentation, proofing and baking. Major bread quality characteristics include its volume and crumb structure, which depend on air incorporation during dough mixing and gas cell stabilization during further stages of bread making (Gan, Ellis, & Schofield, 1995). The gluten–starch matrix has been thought to enclose and stabilize the expanding gas cells during fermentation and early baking. However, Gan et al. (1990), using scanning electron microscopy, showed that discontinuities appear in the gluten–starch matrix surrounding the gas cells already after about 15 min of fermentation. Since their work, an increasing number of studies support the hypothesis of a liquid film surrounding the expanding gas cells and stabilizing them when discontinuities appear in the gluten–starch matrix (Gan et al., 1995; Sroan, Bean, & MacRitchie, 2009; Sroan & MacRitchie, 2009). Surface active proteins, present in the liquid layer, can form two-dimensional (2D) continuous films at the air/water interface which supports the gas cells during expansion (Gan et al., 1995). Polar lipids, on the other hand, act by the Gibbs–Marangoni mechanism (Mills, Wilde, Salt, & Skeggs, 2003) which relies on the stabilization of the air–water interface by highly fluid lipid layers. Deformation of such layers decreases the (local) lipid concentration, which is counteracted by lipid molecules migrating to the depleted area to restore the concentration gradient (Gan et al., 1995; Mills et al., 2003). Non-starch polysaccharides can stabilize the interfacial layer by cross-linking proteins (Sarker, Wilde, & Clark, 1998) and increase the viscosity of the aqueous phase (Courtin & Delcour, 2002; Mills et al., 2003).

The liquid film in bread dough is believed to originate from the dough aqueous phase. A fraction representing this dough aqueous phase, the so-called dough liquor (DL), can be isolated from dough by ultracentrifugation. While the supernatant obtained after ultracentrifugation is defined as DL, it also contains a lipid pellicle on top of a viscous, straw-colored water phase (Sahi, 1994; Salt et al., 2006). Using two-dimensional gel electrophoresis and mass spectrometry, Salt, Robertson, Jenkins, Mulholland, and Mills (2005) identified the proteins in DL from dough from flour from a hard

Abbreviations: 2D, two-dimensional; DL, dough liquor; dm, dry matter; ELISA, enzyme-linked immunosorbent assay; LPC, lysophosphatidylcholine; PBS, phosphate buffered saline; PINs, puroindolines; Tris, tris-(hydroxymethyl)-aminomethane.

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Triticum aestivum wheat as mainly β-amylases, tritins and serpins and members of the α-amylase/trypsin inhibitor family. They found no gliadins nor puroindolines (PINs). This is somewhat in contrast to reports by several authors (Gan et al., 1995; Mills et al., 2003; Sahi, 2003) suggesting a role for PINs in gas cell stabilization because of their high surface activity (Biswas, Dubreil, & Marion, 2001) and exceptional foam stabilization properties (Dubreil et al., 1998). Furthermore, adding purified PINs to a dough recipe yields fermented dough with a fine and uniform gas cell distribution (Rouillé, Bonny, Della Valle, Devaux, & Renou, 2005) which, after baking, yields bread loaves with small and fine gas cells and a thin gluten–starch matrix (Dubreil et al., 1998; Rouillé, Della Valle, Devaux, Marion & Dubreil, 2005).

Although the liquid film takes over the stabilizing role of the gluten–starch matrix during late fermentation (*cfr. supra*), to date, the impact of fermentation on DL properties has not been explored. Bakers' yeast (*Saccharomyces cerevisiae*) not only produces carbon dioxide, but also small quantities of alcohols and organic acids (Delcour & Hoseney, 2010; Jayaram et al., 2013). The latter causes a pH drop to *ca.* 5 in fermenting dough (Jayaram et al., 2013). pH can affect protein solubility in the dough aqueous phase, protein conformation, surface activity and foaming properties (Kinsella, 1981). Against this background, this work aimed at studying how fermentation impacts DL yield, composition (with a focus on proteins) and functional (*i.e.* foaming) properties.

2. Materials and methods

2.1. Materials

Soft wheat cultivar Claire was from Limagrain (Rilland, The Netherlands). Wheat grains were conditioned to 16.0% moisture and subsequently milled with a Bühler MLU-202 laboratory mill (Bühler AG, Uzwil, Switzerland) with the milling flow scheme depicted in Delcour, Vanhamel, and De Geest (1989). Compressed fresh yeast (*S. cerevisiae*) was from Algist Bruggeman (Ghent, Belgium). Sugar and salt were commercial food grade products. Durotest-P antibodies were from R-Biopharm (Darmstadt, Germany). All other chemicals, solvents and reagents were from Sigma–Aldrich (Bornem, Belgium) and were analytical grade, unless specified otherwise.

2.2. Experimental

2.2.1. Flour characterization and defatting

Moisture content of flour was determined according to Approved Method 44-19.01 (AACCI, 1999) and protein level using the Dumas combustion method, an adaptation of the AOAC Official Method (AOAC, 1995) to an automated Dumas protein analysis system (EAS Vario Max CN, Elt, Gouda, The Netherlands) with 5.70 as nitrogen to protein conversion factor. Carbohydrate level and composition were determined with gas chromatography following acid hydrolysis and conversion to alditol peracetates (Courtin, Roelants, & Delcour, 1999). Arabinoxylan level was calculated as 0.88 times the sum of xylose and arabinose levels after correcting for the arabinose level present in arabinogalactan peptide based on Loosveld, Grobet, and Delcour (1997). PIN level was determined with an indirect enzyme-linked immunosorbent assay (ELISA) as in Pauly, Pareyt, Lambrecht, Fierens, and Delcour (2013). Moisture content, protein and arabinoxylan level were determined in triplicate, PIN level in fourfold.

For flour defatting, chloroform (2/1, v/w) was added and the mixture stirred during 30 min at room temperature and then filtered through a MN 615 filter (Macherey-Nagel, Düren, Germany). This procedure was repeated four times until the filtrate was colorless. Residual chloroform in the extracted flour was removed by air-drying.

2.2.2. DL isolation

Flour optimum hydration level and dough optimum mixing time were determined by Mixograph (National Manufacturing, Lincoln, NE, USA) analysis based on AACCI Approved Method 54-40.02 (AACCI, 1999). Flour-water dough pieces of optimal consistency were formed by mixing flour (10.0 g on 14.0% moisture basis) and deionized water (4.8 mL) in a 10 g pin mixer (National Manufacturing) during 165 s. Deionized water was used instead of tap water to avoid extra mineral addition. Yeasted dough samples were prepared similarly but also contained 0.60 g sugar, 0.15 g NaCl and 0.53 g compressed fresh yeast. As fermented dough differs in several aspects from flour-water dough (*i.e.* presence of yeast, salt and sugar, and resting time), additional dough samples (with only added salt or with added salt and sugar or with different resting times) were included in the experiments. These dough samples without yeast were prepared including both 0.60 g sugar and 0.15 g NaCl or only 0.15 g NaCl in the recipes. Dough pieces were placed in a fermentation cabinet (National Manufacturing) for 120 min, *i.e.* the fermentation time applied in the bread making procedure, at 30 °C (90% relative humidity). They were then sheeted (3.17 mm), rolled into a cylinder to fit into ultracentrifuge tubes (38 mL thick-walled polycarbonate tubes, Beckman Coulter, Brea, CA, USA), centrifuged at 165 000 *g* (60 min, 20 °C) (L7 Ultracentrifuge, Beckman Coulter) and immediately placed on ice to minimize enzymatic activities. The obtained supernatants, further referred to as DL, were collected in tared dark vials, weighed, and freeze-dried (Sahi, 1994) prior to determining dry matter (dm) contents and compositions. Total DL yield and yield on dry basis were calculated as follows:

$$\text{Total yield (\%)} = \frac{\text{DL weight (wet basis)}}{\text{dough weight (wet basis)}} \times 100$$

$$\text{Yield on dry basis (\%)} = \frac{\text{DL weight (dry basis)}}{\text{dough weight (dry basis)}} \times 100$$

For bread making experiments (Section 2.2.6), isolated DL was kept frozen until tested. Each dough treatment and, hence, DL isolation, was performed at least in triplicate, starting from three separate dough preparations.

2.2.3. Determination of DL composition

Protein level of DL was determined with the Coomassie Brilliant Blue method (Bradford, 1976). This method was chosen above other (small-scale) colorimetric methods (*e.g.* Lowry or bicinchoninic acid method) because of its lower sensitivity to reducing sugars, which are abundantly present in DL. DL from flour-water dough, of which the protein content was determined with the Dumas combustion method (Section 2.2.1) with 6.25 as nitrogen to protein conversion factor, was used as a standard. Protein composition was analyzed by capillary electrophoresis with an Agilent (Palo Alto, CA, USA) 2100 Bioanalyzer. The Protein 80 kit was used according to the manufacturer's instructions. To each sample (3.0 mg protein suspended in 1.0 mL deionized water), an internal standard comprising both a lower (1.6 kDa) and an upper (95 kDa) marker were added, against which protein mobilities were compared. Each chip included molecular mass markers of 6.5, 15, 28, 46, 63 and 95 kDa. PIN levels were determined with the indirect ELISA method described in Section 2.2.1. Freeze-dried DL samples were suspended in 200 mM KCl in 50 mM tris-(hydroxymethyl)-aminomethane (Tris)-HCl (pH 7.8) containing 2.0% (v/v) Triton X-114 (1/20 DL/buffer; in duplicate) (Turnbull, Gaborit, Marion, & Rahman, 2000).