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Reconstitution baking tests with defatted wheat flour are suitable for determining the functional effects of lipase-treated wheat lipids

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ABSTRACT

A microscale reconstitution baking test, using wheat flour defatted with 2-propanol at 20 °C, was established to determine the functional effects of lipids isolated from lipase-treated wheat dough. Proper selection of solvent and extraction temperature was of major importance to maintain the functionality of defatted flour. Dough and gluten from flour defatted with water-saturated 1-butanol (WSB; extracted at 20 °C) and 2-propanol (extracted at 75 °C) had inferior extensibility and loaf volume compared to control flour extracted with 2-propanol at 20 °C. Quantitation of gluten proteins showed that defatting with WSB (20 °C) or 2-propanol (75 °C) decreased the gliadin and increased the glutenin content. Possible reasons were thiol-disulfide interchange reactions, caused either by heat (2-propanol, 75 °C) or by the solvent WSB, which affected gluten proteins. Confocal laser scanning microscopy showed that regular, interconnected gluten structures were only present in dough from flour defatted with 2-propanol at 20 °C.

1. Introduction

Although lipids are present in wheat flour at considerably lower levels than starch or protein, they exhibit important functional properties in breadmaking. Selective modification of the wheat lipid composition by lipases drastically impacts the baking performance of the flour and the end-product quality (Moayedallaie, Mirzaei, & Paterson, 2010). Beside lipases, other exogenous enzymes are being used to improve the baking performance of wheat flours or to compensate for variations in quality of wheat flour. Currently available lipases for baking applications hydrolyze a number of lipid structures in flour (Gerits, Pareyt, & Delcour, 2013; Schaffarczyk, Oestdal, & Koehler, 2014) and lead to improved surface activity of endogenous lipids, thus resulting in significant increase in bread oven rise (Gerits, Pareyt, Masure, & Delcour, 2015) and specific volume (Gerits, Pareyt, & Delcour, 2014a). The understanding of lipase functionality is continuously being studied (Gerits, Pareyt, Decamps, & Delcour, 2014b), but is mostly based on the knowledge of the effects of added lipids in breadmaking. To investigate the contribution of flour components to baking performance, flour fractions can be added to native flour and the effects can be determined. For example, Selmair and Koehler (2009) analyzed the effects of individual glycolipid classes from commercial lecithins in breadmaking by means of microscale baking tests. It was shown that all isolated glycolipid classes had excellent baking performance. The disadvantage of such a method is that the composition of flour containing the additive, in particular the lipid content, is different from that of the control flour.

An important method for studying the contribution of lipid content or different lipid mixtures in breadmaking was fractionation and reconstitution (Pareyt, Finnie, Putseys, & Delcour, 2011). In this approach, flour is separated into fractions or components, which are then recombined to form reconstituted flour. With this method the reconstituted flour and the control flour contain the same amount of lipids. The extraction solvent is crucial because minimizing the effects of the solvent on flour functionality is a key for fractionation and reconstitution experiments. Especially for the complete extraction of the polar lipids, the proper selection of the extraction solvent and the extraction temperature is of

Abbreviations: AF, area fraction; ALGL, albumins/globulins; BI, branching index; BU, Brabender units; *C*, circularity; CLSM, confocal laser scanning microscopy; DF, Feret's diameter; EE, extensional energy; EX, extensibility; GLIA, gliadins; GLUT, glutenins; KLU, kilo-lipase-units; *P*, perimeter; RE, resistance to extension; RP-HPLC, reversed-phase high performance liquid chromatography; *S*, solidity; SD, standard deviation; TA, total area; WSB, water-saturated 1-butanol; ØA, average size; ΣP, particle count.

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major importance (Chung, Pomeranz, Finney, & Shogren, 1977; Chung, Pomeranz, Jacobs, & Howard, 1980). Water-saturated 1-butanol (WSB) is known to completely extract free and bound lipids of flour and dough. However, WSB-defatted flour is not suitable for studying the role of lipid mixtures because of its poor functional properties, possibly due to the formation of complexes between starch and WSB (Hoseney, Finney, Pomeranz, & Shogren, 1969; MacRitchie & Gras, 1973; Wieser, Antes, & Seilmeier, 1998).

To optimize lipase application in breadmaking, it is essential to understand functionality and the technological effects of reaction products at the molecular level. However, information about the functional effects of lipase-generated lipid classes is scarce. In particular, reconstitution baking tests would be a suitable approach to study the effects of lipases in breadmaking. To the best of our knowledge, no systematic fractionation–reconstitution studies have been done to unravel the functional effects of lipase-modified wheat lipids in breadmaking.

Therefore, the aim of this study was to develop a method for fractionation and reconstitution of wheat flour to investigate the functional effects of lipase-treated wheat lipids by means of microscale methods. The study was focussed on the production and characterization of fully functional defatted flour, suitable for recombination with lipase-treated dough lipids. The influence of the defatting method on the technological properties and the gluten network strength of doughs from defatted flours, as well as from recombined flours, should be determined by MixoLab tests and microscale extension tests, using the Kieffer rig. The results of such tests can be regarded as indirect quality parameters of the flour, which are correlated with the baking quality (Kieffer, Wieser, Henderson, & Graveland, 1998). The protein composition of flours should be quantitated by a modified Osborne fractionation to reveal changes in the protein compositions caused by treatment with different extraction solvents. The final aim was to visualize the gluten network structures of doughs from defatted and non-defatted flours by confocal scanning laser microscopy (CLSM). Once established, the new method would provide the ability to determine relationships between specific wheat lipid classes and their functional effects in wheat breadmaking.

2. Materials and methods

2.1. Materials

'Kolibri' flour, a commercial flour obtained from a mixture of wheat cultivars (Meneba, Rotterdam, The Netherlands, 2013 harvest, containing no additives), was characterized as follows: the moisture and ash contents of the flour were determined according to ICC-Standards 110/1 (ICC, 1976) and 104/1 (ICC, 1990), respectively. Nitrogen contents were determined by means of the method of Dumas on a TruSpec N nitrogen analyzer (Leco, Kirchheim, Germany). A conversion factor of 5.7 was used to calculate the crude protein content from the nitrogen content. Analytical characteristics of the flour were 14.3% moisture, 9.8% protein (dry mass), and 0.48% ash (dry mass).

Wheat starch (Sanostar) was obtained from Hermann Kröner GmbH (Ibbenbühren, Germany), wheat gluten (Amygluten 150) from Tereos Syral (Aalst, Belgium) and fresh baker's yeast from Wieninger GmbH (Passau, Germany). As in a previous study (Schaffarczyk et al., 2014), commercial enzyme granulate Lipopan F-BG (25.0 KLU/g) and commercial enzyme granulate Lipopan Xtra-BG (7.2 KLU/g) were from Novozymes A/S, Bagsvaerd, Denmark. Rhodamine B was from Sigma–Aldrich (Steinheim, Germany). All solvents used were of HPLC or LC–MS grade and from Sigma–Aldrich (Steinheim, Germany).

2.2. Lipid extraction

2.2.1. Lipid extraction from flour

Three different lipid extraction methods were compared regarding the functionality of the resulting defatted flour. Lipids were extracted from wheat flour, using WSB at room temperature (≈20 °C), 2-propanol at 75 °C, and 2-propanol at 20 °C, employing the procedure described by Schaffarczyk et al. (2014).

2.2.2. Lipid extraction from dough

Dough was prepared from 50 g of wheat flour as described recently (Schaffarczyk et al., 2014). The following ingredients were used: flour (50.2 g), water (29.9 ml), NaCl (1.0 g), and lipase (0–170 mg/kg flour; Lipopan F-BG or Lipopan Xtra-BG, Novozymes, Bagsvaerd, Denmark). The flour, NaCl, and lipase were premixed dry for 1 min in a Farinograph (50 g Z-blade mixer, Brabender, Duisburg, Germany) at 22 °C. Water (29.9 ml) was added within 25 s and mixing was continued until the optimum consistency of the dough was reached (550 Brabender units (BU) at 7 min). The dough was allowed to rest (20 min, 30 °C, water-saturated atmosphere). The dough was then reshaped on a dough rounder (Type 440, Brabender, Duisburg, Germany) for 10 cycles, and the resulting spherical dough piece was rolled (PTFE cylinder; diameter, 5 cm; length, 30 cm) to yield an oval dough piece of 5 mm thickness. The dough piece was folded twice to 1/4 of its original size and was reshaped on the dough rounder for 20 cycles. After resting (30 °C, water-saturated atmosphere, 38 min), the dough was frozen in liquid N_2, freeze-dried, and milled, using an ultracentrifugal mill ZM 200 (200 μm mesh size, Retsch, Haan, Germany), resulting in a powder with a mean particle size of 200 μm, which is larger than the particles of wheat flour (50–120 μm).

Lipids were extracted from the freeze-dried dough powder (about 44.5 g) by stirring with WSB (200 ml, 16 h, 20 °C). After centrifugation (5 min, 4 °C, 3550*g*), the supernatant was filtered (0.45 μm); the solvent was then evaporated to dryness under reduced pressure and stored under Ar atmosphere at −75 °C prior to further analysis.

2.3. Microbaking test (MBT)

2.3.1. General

The MBT, using 10 g of flour, was conducted as described by Schaffarczyk et al. (2014). To take dough losses during breadmaking into account, the loaf volumes were based on the dough weight (ml/g dough) before the second proofing session. The bread volume with modified lipid mixtures was expressed in relation to the respective mean value of the control breads in the individual test series, taking into account both climatic (temperature and relative humidity in the room) and technical fluctuations. All baking tests were done in triplicates.

2.3.2. MBT, using defatted flour

Recombined flour was prepared by adding different amounts of flour lipids, dough lipids or lipase-treated dough lipids to 10 g differently defatted flour (8.6 g dry mass) and blending with a mortar and a pestle. The recombined flour (10 g) was used for the MBT, as described recently (Schaffarczyk et al., 2014).

2.3.3. MBT using synthetic flour

Extracted untreated and lipase-treated dough lipids (0.15 g each) were each added to wheat starch (8.0 g) and wheat gluten (1.0 g) and blended with a mortar and a pestle. NaCl (0.2 g), glucose (0.2 g), and yeast (0.7 g) were added and premixed. After mixing for 1 min in a microfarinograph, gelatin-solution (4.6 ml, c = 43.5 g/l) and 0.54 ml water were added and the mixture was kneaded for 4 min to the dough optimum (550 BU). Dough