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Flour from wheat cultivars of varying hardness produces semi-sweet biscuits with varying textural and structural properties

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Semi-sweet biscuits were prepared from flour from wheat cultivars with Single Kernel Characterization System hardness values ranging from 22 to 92. The impact of flour protein level on biscuit properties was compensated for by diluting flour samples of higher protein level with their isolated prime starch fraction. Biscuits prepared from hard wheat flour showed higher fracture stress than their soft wheat flour counterparts. Softer texture mainly resulted from higher porosity, resulting from larger pores. Furthermore, biscuit matrix strength also contributed to biscuit texture. Biscuit texture is strongly affected by wheat hardness and results from the interplay between different hardness-associated flour properties (damaged starch, protein quality, …) as well as from their effect on both biscuit structure and matrix properties.

1. Introduction

Flour from soft wheat (*Triticum aestivum* L.) is preferred for cookie and biscuit making. During sugar-snap cookie baking, dough from soft wheat flour spreads faster and longer and yields larger cookies than that from hard wheat flour (Doescher, Hoseney, Milliken, & Rubenthaler, 1987). This is due to lower protein and damaged starch levels in soft than in hard wheat flour. Both protein and damaged starch absorb high levels of water, thereby increasing dough viscosity (Gaines & Finney, 1989). Furthermore, cookies made from soft wheat flour have a better appearance and eating quality (Wade, 1988) and a more tender bite than cookies made from hard wheat flour (Delcour & Hoseney, 2010). Food textural properties strongly contribute to consumer acceptance. They depend *inter alia* on its microstructure (Aguilera, 2005). However, little information is available on how cookie and biscuit texture relate to their microstructural properties. Pareyt et al. (2009) reported that decreasing dough fat levels increase sugar-snap cookie hardness, which was partially related to decreased porosity. Frisullo, Conte, and Del Nobile (2010) found a correlation between microstructure of commercially available biscuits and consumer perception of crunchiness. However, to the best of our knowledge, no data are available on the relation between flour quality and biscuit structure.

Against this background, we investigated the impact of wheat hardness and associated flour properties on biscuit texture and structure. Five wheat cultivars (cvs.) were selected based on varying kernel hardness. A semi-sweet biscuit recipe was used. It contains higher flour and lower sugar and fat levels than sugar-snap cookies, which makes product quality more dependent on flour quality than in the case of the latter cookie type. The impact of flour quality was studied by measuring biscuit fracture stress using a three-point-bending test. Further, internal biscuit structure was studied with X-ray microfocus computer tomography (μCT). Biscuit fracture stress could then be related to internal structural parameters such as porosity, pore size and matrix thickness.

2. Materials and methods

2.1. Materials

Wheat cvs. Claire and Apache were from Limagrain (Rilland, The Netherlands), cv. Julius from Aveve (Leuven, Belgium), while durum wheat (*Triticum turgidum* L. var. *durum*) cv. Svevo was kindly donated by Dr. R. Ranieri (Open Fields, Collecchio, Italy). Wheat

Abbreviations: AX, arabinoxylans; cvs., cultivars; dm, dry matter; DSC, differential scanning calorimetry; ELISA, enzyme-linked immunosorbent assay; FS, flour-starch; FS-sSv, FS-blend of soft Svevo wheat flour and starch; FS-Sv, FS-blend of Svevo wheat flour and starch; $\Delta H_{biscuit}$, biscuit enthalpy of gelatinization; ΔH_{flour}, flour enthalpy of gelatinization; LA-SRC, lactic acid solvent retention capacity; μCT, microfocus computer tomography; PBS, phosphate buffered saline; PBS-T, PBS containing 100 μL/L Tween 20; PIN, Apuroindoline a; PIN, Bpuroindoline b; PIN, puroindoline; SKCS, Single Kernel Characterization System; 3D, three-dimensional; 2D, two-dimensional.

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grains were conditioned (to 16.0 g/100 g moisture for cvs. Claire, Apache and Julius; to 16.5 g/100 g moisture for cv. Svevo) and subsequently milled with a Bühler MLU-202 laboratory mill (Bühler AG, Uzwil, Switzerland), which yielded three break and three reduction fractions (Delcour, Vanhamel, & Degeest, 1989). First break flour from cv. soft Svevo, developed by back-crossing durum wheat cv. Svevo and a homoeologous translocation line involving Langdon durum and soft wheat cv. Chinese Spring (Morris, Simeone, King, & Lafiandra, 2011), was kindly provided by Dr. C. F. Morris (USDA-ARS, Western Wheat Quality Laboratory, Pullman, WA, USA). Sugar was from Iscal Sugar (Moerbeke-Waas, Belgium), sodium bicarbonate (BICAR®) from Solvay Chemicals International (Brussels, Belgium) and shortening from Vandemoortele (Izegem, Belgium). 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. Moisture content

Moisture contents of kernels, flour and starch were determined according to Approved Method 44-19.01 (AACCI, 1999) with slight modifications. Kernels were dried at 130 °C for 16 h, while flour and starch were dried at 130 °C for 2 h.

2.2.2. Single Kernel Characterization System

Single Kernel Characterization System (SKCS) hardness values of the wheat cvs. were determined according to Approved Method 55-31.01 (AACCI, 1999) and kindly supplied by Dr. C. F. Morris (Western Wheat Quality Laboratory).

2.2.3. Physicochemical characterization of flour samples

Protein levels (N $\times$ 5.7) were determined using the Dumas combustion method (Pauly, Pareyt, De Brier, Fierens, & Delcour, 2012), and AX levels by gas chromatography (Courtin, Roelants, & Delcour, 1999). Ash and damaged starch levels were measured according to Approved Methods 08-01.01 and 76-31.01 (AACCI, 1999), respectively. Particle size distribution was analyzed according to Pareyt, Wilderjans, Goesaert, Brijs, and Delcour (2008). Lactic acid solvent retention capacity (LA-SRC), an indication of gluten quality (Slade & Levine, 1994), was determined according to Duyvejonck, Lagrain, Pareyt, Courtin, and Delcour (2011).

Flour puroindoline (PIN) levels were quantified using an indirect enzyme-linked immunosorbent assay (ELISA). PINs were extracted with 200 mmol/L KCl in 50 mmol/L Tris–HCl (pH 7.8) containing 2.0 mL/100 mL Triton X-114 (1/10 w/v; in duplicate) (Turnbull, Gaborit, Marion, & Rahman, 2000). After shaking (60 min; 150 rpm; room temperature) and centrifuging (11,400$\times$ g; 5.0 min), the supernatant was diluted 5000 times with phosphate buffered saline (PBS; 1.8 mmol/L KH_2PO_4, 10.0 mmol/L Na_2HPO_4, 2.7 mmol/L KCl, 137.0 mmol/L NaCl, pH 7.4). An aliquot (100 μL; in duplicate) of the diluted extract was used to coat the wells of a microtiterplate (Maxisorp, Nunc, Roskilde, Denmark). After overnight incubation at 4 °C, unbound material was removed by washing with 300 μL PBS containing 100 μL/L Tween-20 (PBS-T; 3 $\times$ 5 min). Free bindings sites were blocked with 300 μL of 3.0 g/100 mL casein in PBS-T (60 min; room temperature). The wells were washed with PBS-T (300 μL; 3 $\times$ 5 min) and 100 μL of the Durotest-P primary antibody solution (dilution 1:4000 in PBS-T) was added (120 min, room temperature). Unbound antibodies were removed by washing with PBS-T (300 μL; 3 $\times$ 5 min). Then, 100 μL of the secondary goat anti-mouse antibody (coupled to horseradish peroxidase) solution (dilution 1:2500 in PBS-T) was added (120 min, room temperature). After three final washes with PBS-T (300 μL; 5 min each), 100 μL of 3,3′,5,5′-tetramethylbenzidine solution (1-Step™ Turbo TMB-ELISA, Thermo Scientific, Sunnyvale, CA, USA) was added. Exactly 20 min later, the reaction was stopped by adding 100 μL 2.0 mol/L H_2SO_4 and the extinction was measured at 450 nm. PINs were quantified based on a standard curve of PINs purified as described elsewhere (Blochet et al., 1993). To estimate the non-specific binding of the antibodies, a Triton X-114 extract from durum wheat cv. Svevo (containing no PINs) was included. At the extract dilution used (5000 times), no non-specific binding was detected.

2.2.4. Starch isolation and preparation of flour-starch blends

Prime starch was isolated from flour from cvs. Svevo and soft Svevo using a dough ball method as in Pauly et al. (2012) and subsequently air-dried (final moisture content *ca.* 10 g/100 g). Flour from cvs. Svevo and soft Svevo was blended overnight by end over end shaking with their respective isolated prime starch fraction to obtain flour-starch (FS) blends with a protein level of 10.0 g/100 g [dry matter (dm) basis].

2.2.5. Biscuit making

Dough was prepared from 100.0 g flour (14.0 g/100 g moisture), 30.0 g sucrose, 15.0 g shortening and 1.0 g sodium bicarbonate, and water to yield moisture contents of 23.7 g/100 g (on dough basis) for dough prepared from Claire, Apache and soft Svevo and FS-sSv, 25.1 g/100 g for dough prepared from Julius, 25.8 g/100 g for dough prepared from FS-Sv and 26.9 g/100 g for dough prepared from Svevo. The water contents were chosen based on Mixograph (National Manufacturing, Lincoln, NE, USA) [Approved Method 54-40.02 (AACCI, 1999)] analyses. Flour, sugar and sodium bicarbonate were mixed for 1.0 min using a KitchenAid Professional KPM5 mixer (KitchenAid, St. Joseph, MI, USA). Shortening was added and after 1.0 min of mixing, deionized water was added and mixing was continued for 8.0 min with intermediate scraping (every 60 s). Dough mixing was performed at 29 °C. Dough pieces were sheeted (gap width 3.17 mm), cut (circular dough pieces of 63.5 mm diameter) and weighed. Dough density was the ratio of dough weight to its volume, which was calculated based on its cylindrical shape. At least ten biscuits were baked (15.5 min at 220 °C) from each flour sample. Biscuits were weighed after cooling for 30 min. Their length (in the sheeting direction), width (perpendicular to length) and centre height were determined with a digital caliper (Mitutoyo Belgium, Kruibeke, Belgium). Biscuit density was the ratio of biscuit weight to volume. The latter was calculated based on the elliptical cylindrical biscuit shape [(width/2) $\times$ (length/2) $\times$ π $\times$ height]. Batch-to-batch and day-to-day variability was tested for cv. Claire, and coefficients of variation within and between batches were less than 7.0%.

2.2.6. Biscuit mechanical properties

Break strength of at least five biscuits was determined at room temperature with an Instron 3342 (Instron, Norwood, MA, USA), equipped with a 50 N load cell. The peak force in a three-point-bending test was determined. Each biscuit was centered on a base consisting of two support beams positioned at a distance of 41.2 mm (span width), with top side up and with biscuit width in direction of span width. A third beam (70.0 mm wide; 3.0 mm thick), positioned at a point equidistant from the two support beams, moved downwards until the biscuit broke. Pre-test, test and post-test speeds were respectively 2.5, 2.0 and 10.0 m/s. Biscuit break strength was measured 60 min after baking to minimize the impact of checking on mechanical properties. Checking can result from moisture gradients in the baked product during cooling and storage (Wade, 1988). Fracture stress was calculated as: