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Impact of thermostable amylases during bread making on wheat bread crumb structure and texture

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ABSTRACT

Thermostable amylases [*Bacillus* subtilis α-amylase (BSuA) and *B. stearothermophilus* maltogenic amylase (BStA)] with different modes of action and impacts on firming properties were added during straight-dough bread making. BSuA continuously degraded the starch fraction during bread making. Its action resulted in larger gas cells than in control bread, but did not change initial firmness. In contrast to BSuA, BStA mainly degraded starch at the end of the baking phase and during bread cooling, which caused little if any impact on bread crumb texture. However, it led to higher initial firmness readings than for the control breads. Neither BSuA nor BStA were inactivated during bread making. The results evidence that starch properties have a large impact on bread crumb structure and initial firmness and are highly influenced by the mode of action of the enzyme.

1. Introduction

Although mankind has practiced bread making for thousands of years, our understanding of the physical and molecular processes during the process is still limited. The baking phase is a key step in bread making, as it transforms the dough piece under the influence of heat into a light, porous, readily digestible and flavourful product. The most apparent changes during baking are volume expansion, crust formation, (in)activation of yeast and enzymatic activities, protein cross-linking (Lagrain, Thewissen, Brijs, & Delcour, 2007), and (partial) gelatinization of starch (Goesaert et al., 2005). Although enzymes are used in bread making (Goesaert et al., 2005; Gray & Bemiller, 2003), their application is, in many instances, based on empirical grounds. Currently, α-amylases are commonly employed in bread making. Their activity in dough systems and during baking impacts several product characteristics, including bread volume, firmness, and firming rate. However, little has been reported on their impact on crumb structure. Baking causes a pronounced temperature gradient in bread loafs (Wagner, Lucas, Le Ray, & Trystram, 2007). Thus, the transformations and changes in the starch and gluten protein fractions occur from the outside of the loaf toward the centre. The starch granules begin to swell at a temperature of about 45 °C. When the temperature reaches about 50–60 °C, the dough becomes more fluid. The accessibility of the amylases increases with increasing amorphous content. During NMR baking of bread, at ca. 55 °C and at ca. 80–90 °C significant shifts in the proton relaxation rates of water can be observed, corresponding to both onset and final starch gelatinization, respectively (Engelsen, Jensen, Pedersen, Norgaard, & Munck, 2001). According to Gan, Ellis, and Schofield (1995) the structure of bread crumb is set, when it becomes gas continuous. The latter has been attributed to the rupture of the matrix, as a result of the sharp increase in dough viscosity induced by starch gelatinization (Singh & Bhattacharya, 2005). Additionally, bread dough baked in an electrical resistance oven loses little carbon dioxide until the temperature reaches ca. 70–80 °C (Hoseney, 1994). It then loses its ability to retain carbon dioxide, which, as a consequence, is released from the partially baked bread. In spite of the above, little is known about the importance of the starch fraction on the crumb structure. It is widely accepted that intergranular amylose is an essential structuring element of fresh bread crumb (Eliasson & Larson, 1993; Hug-Iten, Escher, & Conde-Petit, 2003). Based on baking experiments with waxy starch, Ghiasi, Hoseney, Zeleznak, and Rogers (1984) concluded that amylose is necessary to obtain a good loaf of bread. According to Martin and Hoseney (1991), amylose leaching in bread is limited. In this respect, Singh, McCarthy, Singh, Moughan, and Kaur (2007) observed that a significant degree of swelling is necessary before amylose leaching occurs. In spite of

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the above, little is known about the importance of starch fraction on crumb structure.

The purpose of this study was to understand the role of the starch fraction during baking by using amylases as a tool to modify the starch properties. We reasoned that the use of amylolytic enzymes that preferably have little, if any impact on bread volume, still may well have an influence on that structure. The amylases used have some thermostability and differ in mode of action. The action mechanism of *Bacillus subtilis* α-amylase (BSuA) has been described as a multichain mechanism (Robyt & French, 1963). *B.* stearothermophilus maltogenic amylase (BStA) is widely used in bread making formulas. It operates via both exo and endo mechanisms, or via a high degree of multiple attack. With increasing temperature, its endo action becomes more pronounced (Bijttebier, Goesaert, & Delcour, 2007). In contrast to BSuA, BStA has a starch-binding domain (Dauter et al., 1999). The impact of BSuA and BStA on the properties of starch was evaluated and correlated with bread volume, crumb structure and initial firmness. Starch was analysed using high performance size exclusion chromatography (HPSEC), as well as using assays for soluble starch, reducing sugar and total sugar contents.

2. Experimental

2.1. Materials

Wheat flour [moisture content 13.8%; protein content (N × 5.7) 11.3% on dry basis; extraction rate 72%] was obtained by milling of Legat wheat on a Bühler MLU-202 laboratory mill (Uzwill, Switzerland) according to AACC Method 26-31 (AACC, 2000a). The level of damaged starch (Starch Damage Assay Kit, Megazyme, Bray, Ireland) determined in duplicate was 4.5% (on as is basis). The flour contained very low apparent xylanase (Xylazyme method; Megazyme), α-amylase (Amylazyme method; Megazyme) and protease activities.

B. subtilis α-amylase [BSuA, Fluka 10069, 380 U/mg protein (units as defined by the supplier)] was from Sigma–Aldrich Chemie (Bornem, Belgium). *B.* stearothermophilus maltogenic amylase (BStA) was from Novozymes (Novamyl® 10000BG, Bagsvaerd, Denmark). Amylase activities were assayed by quantifying the reducing sugars released from soluble starch [1.0% (w/v) solution] (Merck, Darmstadt, Germany) according to the Somogyi–Nelson method (Somogyi, 1952). One enzyme unit (1.0 EU) is the level of enzyme which releases 1.0 μmol maltose/min at 40 °C and pH 6.0 (100 mM sodium maleate + 5.0 mM $CaCl_2$) (Bijttebier et al., 2007; Leman, Goesaert, Vandeputte, Lagrain, & Delcour, 2005). In bread making applications, a BStA dosage of 5.06 EU/g flour was recommended earlier (Spendler, Nilsson, & Fuglsang, 1999). In a rapid visco analysis study (11.0% starch slurries), BStA addition (7.22 EU/g starch or 5.06 EU/g flour) resulted in a peak viscosity of ca. 3000 cP (Leman et al., 2005). The dosage of BSuA [0.170 EU/g starch or 0.119 EU/g flour] was chosen to have a similar effect on the peak viscosity (~3000 cP) in rapid visco analysis (Leman et al., 2005).

2.2. Bread making procedure

Bread was made according to the straight-dough bread making procedure of Finney (1984). Dough ingredients were flour (1000 parts), compressed yeast (Bruggeman, Ghent, Belgium; 53 parts), 5.0 mM calcium chloride solution (590 parts), sugar (60 parts) and salt (15 parts). The ingredients were mixed for 5.5 min at 20 °C in a spiral mixer (De Danieli, Legnaro, Italy) to obtain 4.0 kg dough. Dough was then divided into pieces of 450 g and punched during fermentation at 52 and 77 min. The fermentation was performed at 30 °C and a relative humidity of 95%. The third punch and molding were performed after 90 min of fermentation. Finally, dough was proofed (36 min) and baked at 210 °C for 40 min in a rotary oven (National Mfg., Lincoln, NE, USA). The temperatures at the centre of the crumb during baking and cooling to room temperature were measured using a thermocouple with a proportional integral derivative controller (Type K, model 2132, Eurotherm Controls, Leesburg, VA, USA). The temperature profiles were determined in triplicate. Samples were withdrawn from the centre of the dough or bread after fermentation and at different stages during heating in the oven (10, 20, 30 and 40 min) and immediately frozen in liquid nitrogen. Afterwards, dough, partially and fully baked bread samples were freeze-dried, milled and sieved (400 μm sieve) for further analysis. The loaves were weighed after 240 min cooling to ambient temperature. Their volumes were measured by rapeseed displacement. All analyses were done fivefold.

2.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed with a DSC Q1000 (TA Instruments, Newcastle, UK). Dough samples (18–22 mg) were collected after proofing and accurately weighed into coated aluminium pans without adding water. The pans were sealed, equilibrated at 30 °C and heated from 30 to 100 °C at a heating rate of 4 °C/min. The system was calibrated before analysis with indium and an empty pan was used as reference. The melting temperatures and enthalpies [expressed in J/g of sample (dry matter basis] corresponding to the gelatinization were evaluated from the thermograms.

2.4. Analysis of different starch fractions

A flour sample, freeze-dried dough, and bread crumb samples (10 mg) were dispersed in 1.0 ml of 1.0 M KOH for 5 h under mild magnetic stirring and then diluted to 10.0 ml with demineralized water. After filtration (0.45 μm; regenerated cellulose syringe filter), 5.0 ml of the filtrates were fractionated using a Sepharose CL-2B column (74 cm × 1.6 cm, GE Healthcare, Sweden) and 0.1 M KOH as eluent (Klucinec & Thompson, 1998; Leman et al., 2005). The amylose molecular weight (MW) distribution was visualized using post-fractionation complexation with KI/I_2-solution (0.38 mg I_2/ml and 0.90 mg KI/ml) and measurement at 620 nm as described earlier (Leman et al., 2005). The residual amylose fraction in the amylase supplemented bread samples was quantified by comparing the integrated KI/I_2 complexation values of the eluates with those of the control sample. The polydispersity (P) is the ratio of the weight average degree of polymerization (DP) (DP_w) to the number average DP (DP_n) (Gelders, Vanderstukken, Goesaert, & Delcour, 2004).

2.5. Scanning electron microscopy

Samples were withdrawn at the centre of the dough or crumb, freeze-dried and milled. They were mounted on a metal stub, coated in a vacuum evaporator (SPi-module, TM Sputter Coater, SPI Supplies, West Chester, PA, USA) with gold and viewed with a scanning electron microscope JSM-6360 SEM (JEOL, Peabody, MA, USA) at 10 kV.

2.6. Digital image analysis

To study the crumb structure, four slices (thickness 16 mm) from three breads of each type were cut and analysed. A single 40 × 40 mm field of view (FOV) was evaluated for each image. This