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Assessment of amyloglucosidase activity during production and storage of laminated pie dough. Impact on raw dough properties and sweetness after baking

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ABSTRACT

Amyloglucosidase (AMG) is an enzyme that hydrolyzes starch into glucose units. AMG activity was tested in a model pie dough during the dough-making process (after mixing and sheeting) and during storage for 4 weeks at 4 °C. The activity was quantified by measuring the glucose content of dough and baked products using HPLC. The consequences of AMG activity on the sweet taste of the baked products (sensory ranking test) and on the rheological properties of the dough were studied and compared with a control dough formulated with sucrose. The results showed a significant production of glucose during the dough-making process and during baking when AMG was used. During the dough-making process, AMG activity was limited by the substrate. During baking, the substrate was no longer a limiting factor and the amount of glucose released was directly proportional to the amount of AMG used. The mixing time was increased and the elastic properties of the dough decreased when AMG was added. However, these impacts of AMG on dough properties were not as significant as those of sucrose addition. Addition of 0.75% AMG (flour basis) developed a sweet taste equivalent to that obtained by addition of 17% sucrose (flour basis).

1. Introduction

Amyloglucosidase (AMG) is a carbohydrase (EC 3.2.1.3, glucan 1,4-alpha-glucosidase) that hydrolyzes (1,4)-alpha-D-glucosidic and (1,6)-alpha-D-glucosidic linkages at the non-reducing ends of glucose polymers (Marin-Navarro and Polaina, 2011; Siddhartha Kumar et al., 2012). Its enzymatic action produces glucose mainly from amylose and amylopectin. AMG activity is often used in research to measure the degree of starch gelatinization (Shetty et al., 1974) or, more generally, the degree of starch accessibility (Eynard et al., 1995; Guerrieri et al., 1997). AMG is also used as a processing aid in many industries (brewing, alcoholic beverages, pastry-making) (Siddhartha Kumar et al., 2012). In brewing, AMG degrades dextrin in the wort to produce beer with a low sugar content and a higher alcohol content (Labat et al., 1988). In bread and Danish pastry industries, AMG is used to produce glucose from starch polymers (amylose and amylopectin) with the objective of enhancing CO_2 production from yeast (Pomeranz et al., 1964).

The optimum conditions for AMG activity have been widely studied and are generally in the range of 40–60 °C and a pH of 4.5–5 (Marin-Navarro and Polaina, 2011; Pandey et al., 2000; Siddhartha Kumar et al., 2012). The aim of the present investigation was first to evaluate this AMG activity during the dough-making process steps: mixing, sheeting and baking. The matrix studied was a non-yeasted laminated dough, a model system based on ready-to-bake refrigerated pie dough. Consequently, we were also interested in the AMG activity during prolonged refrigerated storage of the dough (4 weeks at 4 °C). Little has been reported on AMG activity at low temperature although studies have shown that some enzymes are active at low temperatures, as in Simsek et al.

Abbreviation: AMG, amyloglucosidase.

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(2010) where endoxylanases were active during 34 days of refrigerated storage, or in Gugerli et al. (2004) where amylases were still active but their activity was divided by 3 at 4 °C.

A second objective was to investigate the impact of AMG activity on dough rheological properties and the sweetness of baked products. Currently, α-amylases are commonly employed in breadmaking and they affect the texture properties of dough: a decrease in dough elasticity (G' modulus), a decrease in dough extensibility, and an increase in dough resistance have been described (Kim et al., 2006; Lindahl and Eliasson, 1992; Patel et al., 2012). However, little has been reported on the rheological consequences of added AMG. Industrial AMG applications use the glucose produced as a substrate to enhance CO_2 production from yeast. In such a context, the glucose released is consumed by yeast but, in our context of non-yeasted dough, the glucose released can affect the dough properties and the sweet taste of the product.

The objective of the present work was thus to study the glucose release by AMG activity during production and during storage in refrigerated conditions, and its consequences, on a non-yeasted laminated dough (pie dough as a model system) wherein the released glucose will not be consumed by yeast. These results were compared to the use of sucrose alone, and sucrose and AMG together in order to develop sweetness. Two amounts of AMG and two amounts of sucrose were tested.

2. Materials and methods

2.1. Sample preparation

The wheat flour used was a laminated flour T38 provided by Rettenmeier Mehl, Horb, Germany. The flour water content was 14.3% (Inframatic 8600, Perten Instruments, Sweden) and the protein content was 11.8% (Inframatic 8600, Perten Instruments, Sweden). The water absorption was 59.5% (Farinograph, Brabender, Germany, NF V 03 717-1) and the damaged starch content 25.9 UCD (unité Chopin Dubois) (SDmatic, Chopin Technologie, France, ISO 17715).

The dough recipe used for this work contained flour (Rettenmeier Mehl, Horb, Germany), water, sodium chloride (Sel raffiné fin 400–630 μm, Groupe Salins, Levallois-Perret, France) and different amounts of AMG (AMG 1100 BG, Novozymes, Bagsvaerd, Denmark) and sucrose (sucre cristal, CristalCo, Paris, France). The AMG provided by Novozymes was of food grade from *Aspergillus niger*, which presents an activity of 1100 AGU (amount of enzyme that forms 1 μmol of glucose per minute at pH 4.3 and 25 °C). The optimum conditions for AMG activity were a pH of 4 at 55 °C and a temperature of 70 °C at pH 4.5. Different amounts of AMG and sucrose were tested: 0 (E_0), 0.75 ($E_{0.75}$) and 1.5 ($E_{1.5}$) g of AMG per 100 g flour, and 0 (S_0), 8 (S_8) and 17 (S_{17}) g of sucrose per 100 g flour. The different recipes tested are detailed in Table 1. Each dough recipe was duplicated, except $E_{0.75}S_8$ that was triplicated. Ethyl alcohol (alcool ethylique surfin 85%, France Alcools, Paris, France) and lemon juice concentrate (Döhler, Darmstadt, Germany) were added to extend dough stability and shelf life. No yeast was added. In the dough containing sucrose, the added water was adjusted according to the sucrose level to maintain the free water content constant at 52% of total water (quantified by Differential Scanning Calorimetry, data not shown), as in the reference dough containing neither AMG nor sucrose, with the objective of not inducing any artifact in the dough rheology.

The dough was mixed in an SP11 spiral mixer (VMI, Montaigu, France). The liquid ingredients (water, alcohol and lemon juice) were put into the mixer first, and then flour, salt and, depending on the recipe, AMG and/or sucrose were added. The ingredients were mixed for 4 min at slow speed (spiral 50 rpm, bowl 6 rpm) and 8 min at fast speed (spiral 120 rpm, bowl 10 rpm). The temperature of ingredients was controlled to obtain a temperature of dough at around 25 °C at the end of mixing. The pH of the dough was 4.84 ± 0.07.

Table 1
Detailed dough recipes with the different AMG levels (0%, 0.75% and 1.5%) and sucrose levels (0%, 8% and 17%) used and water adjustment.

Ingredients (g)	Dough					
	E_0S_0[a]	E_0S_{17}[a]	$E_{0.75}S_8$[a]	$E_{1.5}S_{17}$[a]	$E_{1.5}S_0$[a]	$E_{0.75}S_0$[a]
Flour	100	100	100	100	100	100
Water	46.6	50.4	48	50.4	46.6	46.6
Salt	2.1	2.1	2.1	2.1	2.1	2.1
Alcohol	3.8	3.8	3.8	3.8	3.8	3.8
Lemon juice	0.5	0.5	0.5	0.5	0.5	0.5
AMG	0	0	0.75	1.5	1.5	0.75
Sucrose	0	17	8	17	0	0

[a] AMG levels are indicated after the "E" for enzyme and sucrose levels are indicated after the "S" for sucrose.

After mixing, each dough batch was divided into 3 pieces for analysis before and after 2 weeks and 4 weeks of storage at 4 °C. Each dough piece was laminated gradually to 5 mm with a dough sheeter (Rondo Doge, Burgdorf, Switzerland) and then cut into discs of 5 cm diameter. All samples were put into plastic bags. Samples for 2 and 4 weeks of storage were kept at 4 °C until analysis. Other samples were kept for 2 h at room temperature for analysis before storage.

Baking was conducted in a ventilated rack oven (Sirocco, Salva Industrial, Lezo, Spain) for 25 min at 200 °C, for analysis (color and HPLC) of baked products.

2.2. Instrumental analysis

2.2.1. Water activity (a_w) measurements

The a_w measurements were carried out on laminated dough samples using an Aqualab (Decagon Devices, USA) that measures water activity using the chilled-mirror dewpoint technique, with an accuracy of 0.003, with temperature control. Nine repetitions were done on each dough recipe (3 measurements/dough on 3 dough pieces).

2.2.2. Rheological measurements

Rheological measurements were performed with an AR 1000 Rheometer (TA Instruments, Guyancourt, France) equipped with parallel-plate geometry (40 mm diameter serrated plate) at 20 °C. Laminated dough discs, 40 mm in diameter and about 5 mm thick, were put on the lower plate, then the upper plate was lowered until it reached a gap of 2 mm. The excess dough was trimmed off.

Two different experiments were conducted. First, a strain sweep test was applied to validate the strain amplitude of 0.08% chosen for the frequency sweep test (to verify that, in this strain range, the viscoelastic behavior of dough is independent of strain). This test was performed at a constant frequency of 1 Hz and a relative strain range of 0.005–0.5% after 5 min equilibration. On the basis of the results obtained, a frequency sweep test was performed in triplicate for each sample, with a strain amplitude maintained at 0.08% and a frequency ranging from 0.05 Hz to 50 Hz.

2.2.3. Sugars separation and quantification with high performance liquid chromatography (HPLC), on dough and baked products

2.2.3.1. Extraction procedure. The HPLC procedure was derived from the Supelco recommended procedure. About 5 g of sample, weighed with a scale (Adventurer Pro AV264, Ohaus Corporation, Pine Brook, NJ USA) with a precision of 0.0001 g, was blended in 30 mL of water.