Optimization of Aspergillus oryzae S2 α-amylase, ascorbic acid, and glucose oxidase combination for improved French and composite Ukrainian wheat dough properties and bread quality using a mixture design approach

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Introduce

Bread is an important element in human diets in both developed and developing countries worldwide. Dough quality is mainly influenced by the presence of disulfide cross-linking generated by the oxidation of gluten sulfhydryl residues, amylase amount, and starch particle size distribution and digestibility (1). Selecting the type and levels of mixed additives is a crucial step in the baking process. Alpha-amylases (EC 3.2.1.1) are endo-acting amylases that hydrolyse α-(1-4) glycosidic bonds of starch polymers, thereby resulting in oligosaccharides with varying lengths and α-limit dextrins. Note that α-amylase increases the amount of fermentable sugar and therefore enhances the yeast fermentation and the Maillard reaction products, which, in turn, strengthen the flavor and color of bread (2). Additionally, α-amylase increases the resistance, elasticity, and softness of the dough (3) and decreases crumb firmness and hardness (4-6). However, it decreases dough extensibility and stability; this may be ascribed to the high amounts of amylase-reducing oligosaccharides (2). These amylase-reducing products cleave the gluten disulfide bonds and therefore involve the alteration of such wheat proteins. Consequently, incorporation of oxidizing improvers would be required (2). Compared with other cereal proteins, wheat gluten proteins have high levels of water-binding capacity, distinctive amino acid composition, and distinct particle size distributions; this contributes to their unique viscoelastic properties (7). Wheat flour contains glutathione (GSH), which is capable of opening the disulfide bonds of gluten proteins. It can, therefore, cause depolymerization of its network (8).

Ascorbic acid (Asc) added to flour is firstly oxidized by the endogenous Asc oxidase (E.C. 1.10.3.3) to its major stable oxidized product, i.e., dehydroascorbic acid (DAsc) (8). Consequently, GSH is oxidized to oxidized glutathione (GSSG) using DAsc as a cosubstrate. Additionally, the GSSG produced is able to block free protein-associated thiol groups. Hence, the addition of Asc allows an increase in dough strength, hardness, and bread volume (9-10) and a decrease

Abstract

A simplex-centroid experimental design was used for the optimization of both reducing and oxidizing improvers, namely Aspergillus oryzae S2 α-amylase (Amy), ascorbic acid (Asc), and glucose oxidase (GOD). This optimization was performed to enhance the dough and breadmaking qualities of soft French wheat flour and a composite counterpart that contained 30% Ukrainian wheat flour. Statistically significant correlations were calculated between the W index and textural parameters (e.g., dough chewiness and bread cohesiveness). The findings revealed that while the best mixture for French flour comprised 21.8% of Amy, 41.2% of Asc, and 37% of GOD, for the composite counterpart, it comprised 2.3% of Amy, 66% of Asc, and 31.7% of GOD. These optimized mixtures rearranged soft French wheat flour and its composite counterpart to a good quality and an improved flour texture, respectively. Additionally, they increased the loaf specific volumes of the breads made from soft French wheat flour and its counterpart by 25.8 and 45.43%, respectively, significantly decreased the breads’ susceptibility to microbial contamination, and reclassified the breads as “good” in terms of sensory attributes.

Keywords: α-amylase, ascorbic acid, glucose oxidase, dough properties, bread quality
in crumb firmness (10) and crust thickness (3). It also amplifies extensibility when combined to the alpha amylase (3).

The use of oxidative enzymes instead of chemical oxidants is a very interesting option because they are inactivated early in the baking process prior to the distinct gelatinization of starch (11). The glucose oxidase (GOD) enzyme (EC 1.1.3.4) has generally been used as an oxidizing agent in the baking industry. In the presence of oxygen, it catalyzes the oxidation of \( \alpha \)-\( \alpha \)-glucose to gluconic acid and hydrogen peroxide (\( \text{H}_2\text{O}_2 \)). \( \text{H}_2\text{O}_2 \) oxidizes the free thiol groups of gluten proteins and lead to the formation of arabinoxylan cross-linkings, phenolic linkages, and oxidative gelation of water-soluble pentosans, thereby modifying the rheological characteristics of the dough (12). Several authors have, therefore, suggested that GOD could be used as a partial substitute for Asc (13). The addition of 0.001% GOD has, for instance, been reported to increase dough tenacity, elasticity, and extensibility (12-14).

The present study was conducted to improve the breadmaking functionalities of French wheat flour and a composite counterpart that contains 30% Ukrainian wheat flour. The choice of a 30/70 mixture of Ukrainian and French wheat flour is justified by an economic reason. A mixture containing a high proportion of weak, low-cost flour and a small amount of strong, expensive flour is generally used in baking, especially in less developed countries. A simplex-centroid mixture design (15) was adopted to find optimum mixture proportions of \( \alpha \)-amylase (Amy), Asc, and GOD for enhancing the dough properties and bread quality of soft French wheat flour and a composite counterpart.

## Materials and Methods

### Materials

The mixtures used in the experimental design were prepared using GOD (Bakenzyme® GOP 10000BG; DSM, Delft, Netherlands) produced by *Aspergillus niger* 10,000 U/g, a crude extract of *Aspergillus oryzae* S2 118148.14 U/g, which did not contain aflatoxins (16) formulated according to the method described in a previous study (2), and Asc (Asc, Cargill Food Ingredients, São Paulo, Brazil). Two wheat flour samples were used. The first sample was French soft whole wheat flour containing 0.66% ash, 9.65% protein (N*5.70), 21.5% wet gluten, and 14.5% moisture. The second sample was a composite wheat flour containing 70% soft French wheat flour and 30% Ukrainian hard wheat flour with 0.64% ash, 10.24% protein (N*5.70), 24% wet gluten, and 14% moisture.

### Methods

#### Wheat flour characterization:

The dry matter and protein content (N*5.7) of the wheat flour samples were determined using the standard methods 44-15A and 46-10A, respectively (17). The ash content was evaluated via combustion at 550°C in a muffle furnace for 12 h. The amount of wet gluten was determined according to a method described in a previous study (18). Tests were performed in triplicates.

#### Evaluation of dough and bread texture:

The textural parameters of the dough and bread were determined using a texture analyzer (Texture analyzer; Lloyd Instruments, Fareham, UK). It was equipped with a 19-mm-diameter cylindrical aluminum probe and a 1000 (N) load cell. The detection range was 0.05 (N). The samples (2 cm in thickness) of dough portions or bread slices were compressed to 50% of their initial height at a speed of 10 mm/s, with a 30 s interruption between the first and second compression. The sample was cut by an electronic knife to obtain a regular shape. Primary parameters [springiness, hardness, and cohesion] and secondary parameters [chewiness and adhesion] were determined from the curves using the well-known texture profile analysis (TPA) method (19). Hardness was defined as the peak force of the primary compression cycle; adhesion was defined as the essential work required for pulling the compressing plunger away from the sample; and cohesiveness was defined as the quotient of the positive areas of the subsequent cycle to the initial cycle area. The height during the moment that elapses between the end of the first compression cycle and start of the second compression cycle is established as springiness. Chewiness is calculated as the product of cohesiveness, hardness, and springiness (20). Assays were performed after cooling for 2 h at room temperature. The values are the averages of three different determinations.

#### Alveograph testing:

The alveograph measurements were obtained under conditions of constant dough water content and mixing times using the standard AACC method 54-30 (17). The dough samples were prepared using 100 g of flour, 58.5 mL of distilled water, and 2.2 g of salt (NaCl). They were then mixed in an alveograph mixer for 6 min at 60 rpm, laminated, and left to rest for 20 min in the alveograph compartment. The following alveograph parameters were automatically recorded by the computer software R-Design (Pullman, WA, USA): maximum over-pressure needed to blow the dough bubble (P; index of resistance to extension), average abscissa at bubble rupture (L; index of dough extensibility), dough elasticity (Elastic index, \( P_{200}/P_{200} \): pressure at 4 cm from the beginning of dough bubble), deformation energy (W) index (dough strength), and P/L ratio, which indicates dough quality. Two curves were plotted for each sample, and the analysis was realized using the Chopin S.A. (Chopin, Triplette et Renaud, Villenuve La Garenne, French) at 25°C.

#### Baking test:

The bread was prepared using the following formula (percentages on a flour weight basis): 100 g of flour, 3% of dry baker’s yeast, 2.2% of salt, and 58.5% of water, Amy, Asc, and GOD. A separate control was set up with the same protocol and without the supplementation of enhancers for the two wheat flours. All the ingredients were mixed, and the dough was left to rest for 15 min at 30°C. The dough was then divided into 100-g portions, hand-molded, kept at 30°C and 96% of moisture for 30 min, and baked in an electric oven at 200°C for 18 min.

#### Sensory evaluation:

The breads were chosen for sensory assessment on Day 8 of storage. The evaluation was conducted using the scoring system reported by Gomes–Ruffi et al. as the reference (6). The

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