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Effect of an enzyme preparation on wheat flour and dough color, mixing, and test baking☆

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ABSTRACT

Bleaching flours with natural rather than chemical extracts is attractive because it reduces risks upon exposure and appeals to natural food consumers. This paper reports effects of a commercial proprietary blend of 'natural' bleaching enzymes on wheat flour and dough color, mixing behavior and test bake performance. Enzyme preparation did not improve whiteness (L^*) and yellowness (b^*) of flour system, but benzoyl peroxide sharply reduced b^*. For whole wheat flour dough, L^* increased for enzyme-treated dough after 2-h resting, ending higher than benzoyl peroxide and control dough. Yellowness increased in enzyme-treated dough. When enzyme application was during milling, resulting whole wheat flour dough had much higher L^*. Mixograms of flours with increasing enzyme concentration showed severe dough weakness and rapid breakdown. Dialysis of the enzyme preparation in 3500 molecular weight cutoff (MWCO) tubes and freeze-drying prior to flour application recovered dough strength. L-cysteine in the enzyme mix was thought to adversely affect dough strength, and dialysis helped recover dough strength. However, pup loaf baking with dialyzed enzyme showed some loss of baking characteristics (loaf volume, loaf weight, and proof height) over the control. Although the natural enzyme extract addition enhanced whiteness for whole wheat dough, its effects on dough and baking properties were not favorable.

1. Introduction

Natural yellowish pigments present in wheat flour affect resulting bread crumb color. These pigments, also termed carotenoids, include β-carotene, xanthophyll, and flavones (Gelinas, Poitras, McKinnon, & Morin, 1998). Freshly milled wheat flour has a pale yellow tint, with pigmentation in amounts of 1.5–4 mg/kg expressed as carotenoid (Pyler, 1988a). During flour storage, reactions occur between flour components, especially unsaturated lipids, and oxygen in a process known as aging or maturing (Saiz, Manrique, & Fritz, 2001). This process gradually whitens the flour over time and renders dough with improved rheological and baking qualities. This improvement and whitening effect are direct consequences of oxidative reactions (Saiz et al., 2001). Whiter flour is preferred in pan white bread baking because it results in better crumb color. However, the natural oxidation process for aging/maturing wheat flour is time consuming; the process requires several weeks to months, making natural bleaching unsuitable for modern milling and baking practices.

Using chemicals can hasten the flour-bleaching process. Benzoyl peroxide, a free-radical initiator that produces carotenoid oxidation by a free-radical mechanism, is the most common flour-bleaching agent used by the industry. Disrupting the conjugated double-bond system of carotenoid to a less conjugated colorless system produces the bleaching effect (Saiz et al., 2001). Presence of highly conjugated double bonds makes carotenoids extremely efficient quenchers of singlet molecular oxygen (Yamauchi, Miyake, Inoue, & Kato, 1993), and carotenoids also participate in free-radical reactions. Benzoic acid is the reaction product when wheat flour is bleached with benzoyl peroxide, although complete conversion of benzoyl peroxide may not occur. In one study, benzoyl peroxide concentration in treated wheat flour decreased from 150 mg/kg at time 0 to 11 mg/kg after 9 days of contact (Saiz et al., 2001). The researchers also reported that benzoic acid, the product of bleaching reaction, was at concentration of 11 mg/kg in flour after 30 h of reaction, which was a drop from 16 mg/kg after 12 h of reaction.

☆ Mention of any company name or product does not constitute endorsement by associated departments and universities.

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Residual levels of both benzoyl peroxide and its reaction product benzoic acid have prompted health concerns. Benzoyl peroxide is a known tumor promoter and progression agent in mouse skin, though it is not an initiator or complete carcinogen (Hazlewood & Davies, 1996; Swauger, Dolan, Zweier, Kuppusamy, & Kensler, 1991). Such activity was attributed to generation of DNA strand breaks in cells exposed to the compound as a result of free-radical generation, especially the benzoyloxyl radical. Although epidemiological studies have found a lack of association between this specific use of benzoyl peroxide and human skin cancer (Kraus et al., 1995), consumers are becoming wary of the presence of various chemicals in food.

Alternative means of bleaching wheat flour have not been successfully adopted by the industry. Two alternative bleaching methods are heat treatment of flour (Brabender Thermo Process) and the use of enzyme-active soy flour, but only the latter is applied in American baking practices (Pyler, 1988a). Lipoxygenase in enzyme-active soy flour catalyzes polyunsaturated fatty acid (PUFA) oxidation, yielding hydroperoxides with intermediate free radicals that are able to cooxidize lipophilic pigments and thiol groups (Drapron & Godon, 1987). The main purpose for adding enzyme-active soy flour (lipoxygenase) to wheat flour is to improve dough properties.

Bleaching wheat flour with natural rather than chemical extracts is attractive because it reduces risks upon exposure and appeals to natural food consumers. Although preferable to chemical treatment, effects of a natural or enzymatic treatment on other important properties (i.e., mixing, baking quality, flavor, and color) need to be minimal or nonexistent. A major drawback of lipoxygenase full-fat enzyme-active flours is their potential negative effect on bread flavor. One consequence of PUFA oxidation by lipoxygenase is production of oxidative rancid products. This has prompted lipoxygenase usage at lower than recommended levels of 0.5–1 g/100 g (flour basis) enzyme-active soy flour in bread formulations (Gelinas et al., 1998). This limits lipoxygenase's role and usage as an effective flour-bleaching agent in bread formulations. A screening of oxidoreductases and lipases as dough-bleaching agents showed that peroxidases possess better bleaching activity based on carotene degradation in a liquid system (Gelinas et al., 1998). The same researchers reported that some catalases show bleaching potential in liquid systems but not dough systems. A combination of peroxidase, lipase, and linoleic acid was reported to satisfactorily bleach bread dough.

The general objective of this research was to evaluate a proprietary bioenhancer (enzyme) mix for use as a flour and/or dough-bleaching agent during or after milling of wheat kernels. The specific objective was to evaluate the effect of bioenhancer enzyme mix on color, mixing and bake characteristics of flour and dough systems of mixed-variety hard red winter wheat.

2. Materials and methods

2.1. Wheat flour

The straight grade flour and whole wheat flour (100 g/100 g extraction) used in the study was freshly milled from mixed-variety hard red winter wheat from the local elevator (Manhattan, KS, USA). The milling was done in the Kansas State University (KSU) Grain Science and Industry Department's pilot flour mills. For the preparation of straight grade flour, cleaning and milling procedures for the KSU cleaning house and the KSU pilot flour mill, as described in Gwirtz, Eustace, and Curran (1996), were followed. The wheat kernels were tempered to 16 g/100 g moisture and held for 24 h prior to milling, the feed rate for which was maintained at 45.6 kg/min. The whole wheat flour produced on the micro bud grinder in KSU's new Hal Ross pilot flour mill. Unfortunately, no one has published anything relative to the Hal Ross Mill Flow that can be referenced (personal communication with the Mill operator/supervisor). After flour preparation, approximately 22.5 kg of flour was set aside and stored in sealed bags at 4 °C. Flours were not fortified nor were any other additives included. The straight grade flour had an average moisture content of 13.5 g/100 g wet basis (Method 44-15A, AACC, 1983), and average protein content of 10.5 g/100 g using the Dumas method (Method 993.13, AOAC, 2005) in a Leco combustion-type nitrogen analyzer (FP-2000, Leco Corp., St. Joseph, MI, USA). The whole wheat flour had a moisture content of 11.3 g/100 g and protein content of 12.4 g/100 g. Flour samples were stored at 4 °C for a maximum period of 2 weeks in sealed bags. Flour produced on the KSU pilot flour mills passed through flour cloths with aperture size no larger than 150 μm, which is typical for the industry. Flour had a particle size profile by volume as follows; $D_{10} = 12.6$ μm, $D_{50} = 62.3$ μm and $D_{90} = 145.8$ μm, with a range of ±10 μm for each measure. For test baking study, fresh, non-fortified mixed-variety straight grade wheat flour was obtained from the Archer Daniel Midland (ADM) flour mill in Salina, KS, USA. There was a time lapse of about 2 months between the first milling in our pilot mills, and test baking. So, even though the flour samples were kept at 4° C in closed plastic bags throughout, we decided to obtain a fresh batch of flour, discounting the possibility of flour bleaching with natural oxidation.

2.2. Enzyme and chemicals

The enzyme extract used in the study 'EXTRACT SEB WB' was obtained from Specialty Enzymes and Biochemicals, Chino, CA, USA. The enzyme extract was composed of specific pentosanases, proteolytic enzymes, redox enzymes, and reducing agents (Rathi, Pradhan, Giri, & Iyer, 2007). Benzoyl peroxide was donated by Research Products Co., Salina, KS, USA. All other chemicals used in the laboratory were of analytical grade.

2.3. Enzyme application in flour and color measurement

A portion of flour to be treated was taken out of the cold room the day before the study and equilibrated at room temperature. Thirty grams of flour was mixed with extract WB, hereafter called the enzyme, or with benzoyl peroxide. The application rates were 0.4 g/100 g flour for the bioenhancer and 0.016 g/100 g flour for benzoyl peroxide, the dose used in the industry. Change in flour color (white/black, yellow/blue, and red/green) was followed over 72 h using Hunter LAB color system (Chromameter CR410, Konica Minolta, Japan; $L^* = 100/0$ white/black, $\pm a^*$ red/green, and $\pm b^*$ = yellow/blue). Three replicate samples were measured, with three color measurements for each sample.

2.4. Enzyme application during flour dough preparation

Non-yeasted dough was prepared from treated and control flours at optimum water absorption level. Optimum water absorption levels via the Farinograph (Method 54-21, AACC, 1983) were 61.5 g/100 g and 64.5 g/100 g for straight grade flour and whole wheat flour, respectively. Enzyme extract WB (0.4 g/100 g) and benzoyl peroxide treatment (0.016 g/100 g) of flour was done at least 3–4 days prior to dough making. Mixing was done in a Hobart laboratory mixer (A200 model, with McDuffie bowl; Hobart, Troy, OH, USA) at low speed for 1 min and then at high speed until dough developed. Fully mixed dough was sheeted to 0.01 m thickness (Reversible sheeter, Rondo, Switzerland). Dough