Methoxyhydroquinone in Wheat Flour[1]

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ABSTRACT

Wheat flour dough, when mixed beyond its optimum mixing time, shows a rapid breakdown. This phenomenon is believed to be caused by something in the water-soluble fraction of flour becoming oxidized to form an activated double-bond compound (ADB) that reacts with gluten. Methoxyhydroquinone (MHQ) and related compounds were studied in relation to this rapid dough breakdown. The mixograph was used to determine their physical effects on dough mixing performance. MHQ accelerated dough breakdown when the dough was mixed in air. It was more effective than other activated double-bond compounds. No free MHQ was found in the flour. MHQ was released by acid or β-glucosidase hydrolysis of the water-soluble fraction of flour. The amount of MHQ was determined by high-performance liquid chromatography (HPLC) equipped with a fluorescence detector. MHQ endogenous in flour and bound by a β-glycosidic linkage was not effective in accelerating dough breakdown, presumably because it does not become oxidized as easily as the free hydroquinone.

Wheat flour dough, when mixed beyond optimum mixing time, shows a rapid breakdown. This phenomenon is believed to be caused by something in the water-soluble (WS) fraction of flour becoming oxidized and forming an activated double-bond compound (ADB) that reacts with gluten (Schroeder and Hoseney 1978, Sidhu et al 1980, Hoseney 1985, Jackson and Hoseney 1986). The native compound in flour responsible for this phenomenon is unknown.

Methoxyhydroquinone (MHQ), a phenolic compound, has merited our attention because of its structure, and the fact that it has been reported in flour. In the presence of oxygen or peroxidative enzymes, MHQ oxidizes to methoxybenzoquinone, an α-,β-unsaturated, rheologically active compound (Bungenberg de Jong et al 1953) that decreases mixing tolerance (Fig. 1). MHQ was detected in Manitoba wheat (4,700 ppm) and in the germ, bran, and endosperm of English wheat (Daniels 1959).

The earlier studies with MHQ focused on its presence in germ and its interaction with glutathione. Hullett (1941) reported that, if wheat germ was first fermented with yeast, it no longer reduced bread quality. The fermentation coincided with the disappearance of glutathione. The model proposed for this was a reaction of the glutathione with a product of fermentation. The active principle in the fermentation mixture was isolated and identified as a yellow crystalline compound, methoxybenzoquinone (mp 143–144°C) at 0.05% of the weight of germ. A second compound, 2:6-dimethoxy-benzoquinone (mp 259–260°C), was at 0.01% of the weight of germ (Vuataz 1950, Cosgrove et al 1952). Methoxybenzoquinone was effective in improving the volume and the crumb structure of bread. Dimethoxybenzoquinone displayed similar properties, but to a much lesser degree (Cosgrove et al 1952).

Burgenberg de Jong et al (1953) identified the precursor of methoxybenzoquinone in the germ as MHQ. The compound was isolated as the monoglucoside of MHQ (mp 202°C). Interestingly, neither the monoglucoside of methoxyhydroquinone nor dimethoxyhydroquinone showed any improving action on wheat flour dough.

Greer et al (1953) showed that methoxybenzoquinone, obtained by fermentation of wheat germ, improved bread volume. Dimethoxyhydroquinone was less effective. All quinones are unstable in flour, dough, and bread, and are toxic to yeast in concentrations not far removed from that required for optimum flour improvement.

Graveland et al (1984) identified endogenous MHQ present in flour as a hydroquinone glycoside 3-methoxy-hydroquinone-β-cellotrioside and reported it to be responsible for the depolymerization of glutenin. Kerr et al (1993) mixed dough with 100 ppm of hydroquinone both in the presence and absence of oxygen and found that it affected the mixograms only in the presence of oxygen. Lai et al (1989) showed that adding 60 ppm of MHQ to dough reduced the loaf volume. In the presence of KIO_3, MHQ was even more detrimental to loaf volume.

The objectives of this study were to determine the amount and form of MHQ in wheat flour and to examine the role of endogenous MHQ in rapid dough breakdown.

MATERIALS AND METHODS

Flour

Untreated, straight-grade flour milled from a blend of hard red winter wheat obtained from Cargill (Wichita, KS) was used. The ash and protein (N × 5.7) contents of the flour were 0.50 and 11.73%, respectively. The moisture content was 12.37%. Wheat germ was obtained from the Kansas State University mill.

Preparation of Standard

MHQ was obtained commercially from Fluka (Ronkonkoma, NY). Sublimation at 0.1 mm torr gave a colorless product, mp 88–89°C (Fodor and Mathelier 1988). The purity of the compound was verified by H^1-nuclear magnetic resonance.

HPLC Analysis

A Varian model 5000 liquid chromatograph (Walnut Creek, CA) was used with a Varian fluorochrome fluorescence detector. The excitation wave length was fixed at 280 nm, with a 330-nm cutoff interference filter combination on the emission side. Separation was via a C-18 reversed-phase hypersil ODS column (5-μm diameter particles), 4.6 mm (i.d.) × 150 mm, connected with a 5-cm guard column packed with a Hypersil ODS C18 cartridge (10 mm × 4.6 mm) from Alltech Associates (Deerfield, IL). The isocratic mobil phase was a mixture of methanol, glacial acetic acid, and water (15:5:80, v/v). Sample volume for injection was 20 μl, and flow rate was 1 ml/min. Minimum detectable level of MHQ was 1 ppm.

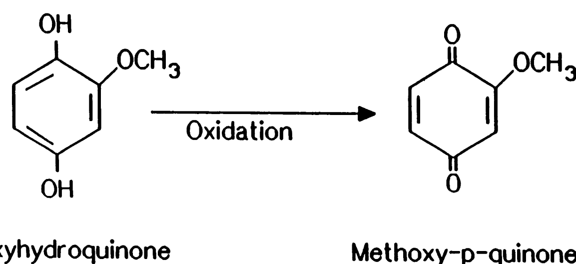

Fig. 1. Structure of methoxyhydroquinone and methoxy-p-quinone.

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Flour Fractionation

One part flour was suspended in 10 parts of distilled water and stirred continuously for 5 min. The suspension was centrifuged for 15 min at 1,000 × *g* (Damon/IEC, CU-5000). The insoluble residue after centrifugation was resuspended in water, and the extraction process repeated twice. The water-insoluble (WIS) fraction remaining in the centrifuge bottle after three extractions was lyophilized for later use. The three pooled supernatants (WS fractions) also were lyophilized. Dried WIS fraction was ground in a micro Wiley mill to pass through an 80-mesh sieve and rehydrated to about 14% moisture.

Mixograph Study (I)

AACC method 54-40, the procedure outlined for the 10-g mixograph (AACC 1983) was followed. A nitrogen atmosphere was produced by sealing the mixograph chamber, displacing air with nitrogen, and maintaining an atmosphere that would not support combustion. Before mixing, flour was purged with N_2 and allowed to stand for 2 hr under a N_2 atmosphere. Solutions used in the N_2 atmosphere studies were made from boiled (degassed) distilled water. The N_2 and air atmospheres were used to study the effect of MHQ on dough breakdown.

In the reconstitution study, the WS fraction was added back to the WIS fraction in an amount (14% mb) proportional to that recovered during fractionation. The WS fraction was added to the formula water and then mixed with the WIS fraction. Amounts of MHQ added are expressed in parts per million based on the flour weight at 14% moisture content.

Extraction of Free MHQ from Wheat Flour and Germ

Flour (10 g) or germ (10 g) was stirred with HPLC-grade ethyl acetate (30 ml) in the dark and under a N_2 atmosphere to minimize exposure to light and oxygen. The solvent and the residual flour were separated by filtration (Whatman filter paper 2), and the extraction process was repeated twice more. The three pooled solvents were removed by rotary evaporation in an aluminum foil-wrapped, round-bottom flask at 15°C. The residue was dissolved in 1 ml of HPLC-grade methanol. The MHQ content of the flour was analyzed using HPLC with fluorescence detection as described above. Other organic solvents, petroleum ether or methanol, were tested for extraction of free MHQ from flour or germ using the procedure described above.

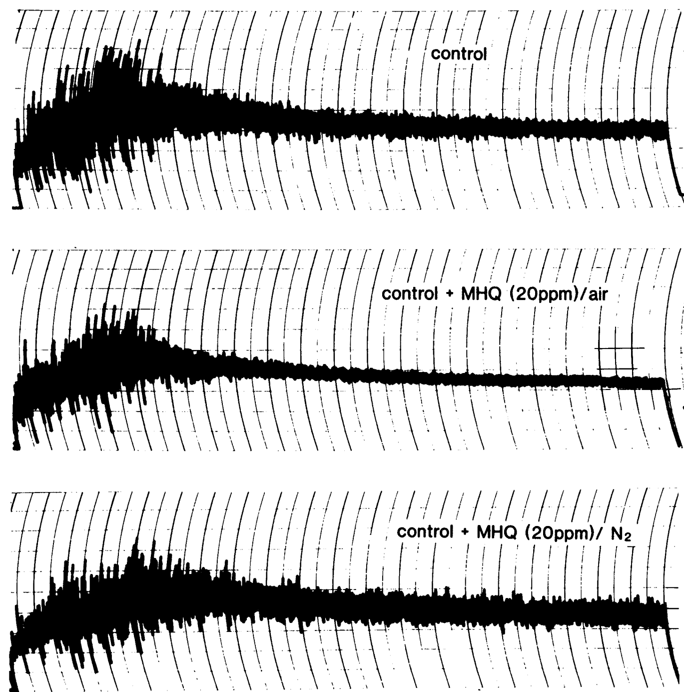

Fig. 2. Mixograms showing effects of methoxyhydroquinone (MHQ) on mixing characteristics in air and nitrogen.

Extraction of Bound MHQ from Wheat Flour

Acid hydrolysis. The WS (1 g) or WIS fraction (9 g) of flour was hydrolyzed separately under vacuum without light for 1 hr with 20 ml of 2*N* HCl in a boiling water bath. The hydrolysate was allowed to cool to room temperature and centrifuged (13,000 × *g*, 5 min) to remove the insoluble residue. The supernatant was extracted twice with 40 ml of a mixture of ethyl acetate and ethyl ether (1:1, v/v) to selectively remove MHQ. The extracts were pooled and evaporated by rotary evaporator at 30°C, then dissolved in 1 ml of HPLC-grade methanol. The MHQ content was analyzed using HPLC with fluorescence detection. During all of the above procedures, the sample was protected from oxygen and exposure to light.

Enzyme hydrolysis. The WS fraction of flour (1 g) was dissolved in 10 ml of 0.1*M* sodium acetate buffer (pH 5). β-Glucosidase (6.7 mg) from sweet almonds (Sigma Co., St Louis, MO) was added, and the mixture was incubated at 37°C for 0, 10, or 120 min. The resultant mixture was boiled at 100°C for 10 min, cooled to room temperature, and centrifuged (13,000 × *g*, 5 min). The supernatant was extracted with ethyl acetate three times. Then the ethyl acetate layer was removed, evaporated at 30°C with a rotary evaporator, and dissolved in 1 ml of HPLC-grade methanol. The MHQ content of the flour was analyzed using HPLC with fluorescence detection as described above.

One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μmol of glucose from salicin per minute at pH 5.0 at 37°C. Specific activity of enzyme was 30 units per gram of protein.

Dialysis of the WS Fraction of Flour

The WS fraction of flour (10 g) was fractionated by dialysis (MW cutoff 1,000; Spectrum, Houston, TX) against frequent changes of distilled water for 48 hr. Both fractions, the dialysate that passed through the membrane and the retentate that remained in the membrane, were lyophilized. Part of each lyophilized fraction was used for the reconstitution study in the mixograph and hydrolyzed with HCl by the same procedure described above to determine MHQ.

Mixograph Study (II)

To investigate the effects of endogenous MHQ on dough mixing properties, the dialysate or the retentate from the dialyzed WS fraction was added to the WIS fraction of flour in amounts proportional to those originally recovered. The reconstituted flour was mixed in the mixograph.

To determine the difference between effects of free hydroquinone (80 ppm) and β-glycosidic-bound hydroquinone (arbutin, 1,000 ppm) on mixing properties, solutions of each were added to flour, and a mixogram was produced. The amounts added are expressed in parts per million based on the flour weight at 14% moisture.

RESULTS AND DISCUSSION

Flour Fractionation

WS and WIS residues of the flour constituted 10 and 90%, respectively, of total flour weight. Fractionation of the WS by dialysis resulted in 5.6% retentate (WS_D) and 3.75% dialyzate (WS_{DZ}), based on total flour weight.

Reaction of MHQ in Wheat Flour During Mixing

Mixograms (Fig. 2) showed that MHQ added at 20 ppm accelerated dough breakdown in air compared to control flour. MHQ added in a N_2 atmosphere did not accelerate dough breakdown.

The effects of MHQ on dough mixing properties were similar to those of other ADB compounds. MHQ caused rapid dough breakdown, even in the absence of the WS fraction (Fig. 3). Similar results were obtained when ferulic acid or *N*-ethylmaleimide (NEMI) was added to WIS in the absence of WS fraction of flour (Danno and Hoseney 1982). However, MHQ was more effective: 20 ppm accelerated dough breakdown in flour, and 40

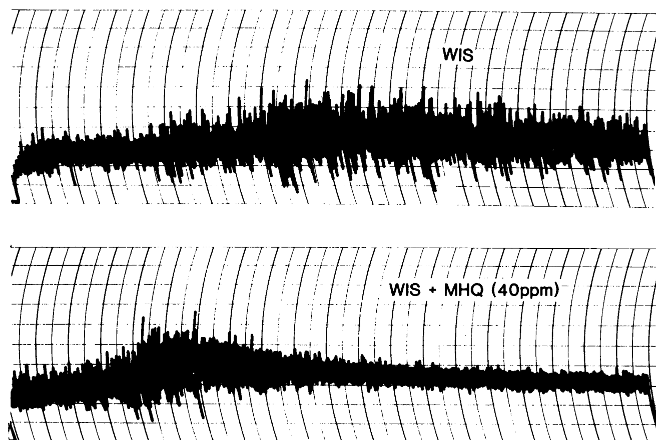

Fig. 3. Mixograms showing effect of methoxyhydroquinone (MHQ) on mixing characteristics on water insoluble fraction of flour (WIS).

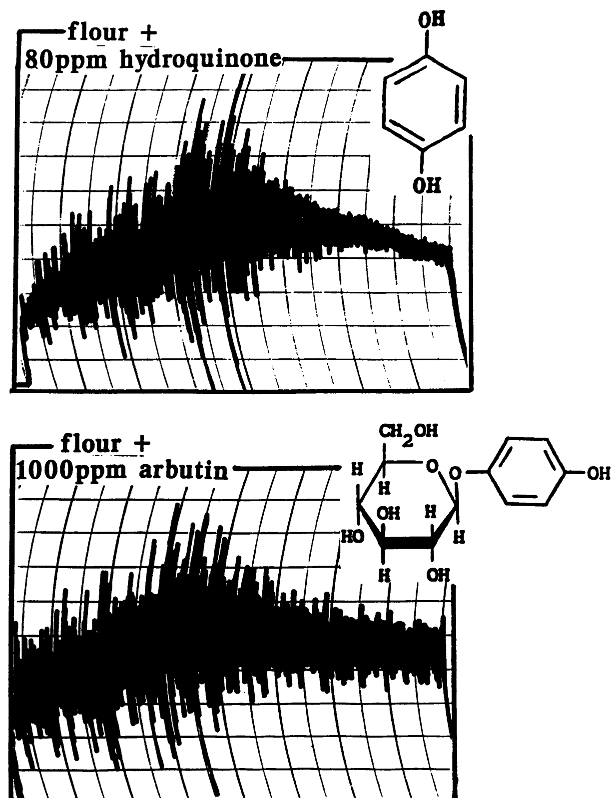

Fig. 4. Mixograms of flours with hydroquinone and arbutin.

ppm produced poor mixing tolerance with the WIS fraction of flour. This is much lower than the 2,000 ppm of fumaric acid or maleic acid (Weak et al 1977) and 250 ppm of ferulic acid (Danno and Hoseney 1982) reported to be required to accelerate dough breakdown.

Extraction of Free MHQ from Flour and Germ

Kerr et al (1993) found that several easily oxidized compounds could be extracted from petroleum ether-defatted flour with ethyl acetate. However, in this study, MHQ was not detected in ethyl acetate extracts of either flour or germ. Additional organic solvents, i.e., petroleum ether and methanol, also failed to extract any free MHQ. Therefore, MHQ apparently does not occur in its free form in either flour or germ.

Extraction of Bound MHQ from Flour

Either acid or β-glucosidase hydrolysis released MHQ from the WS fraction but not from the WIS fraction of flour. From

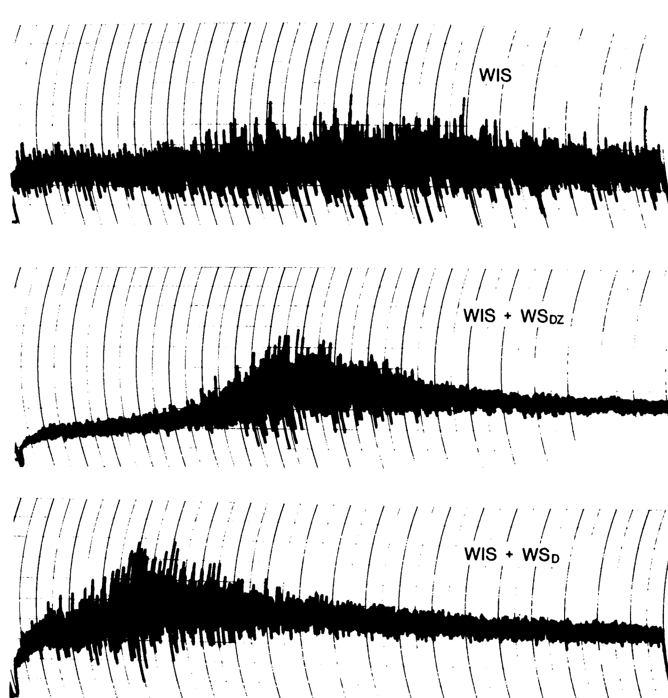

Fig. 5. Mixograms of reconstituted flour. WIS = water insoluble flour, WS_D = retentate of water soluble fraction of flour, WS_{DZ} = dialysate of water soluble fraction of flour.

the WS fraction of flour, more MHQ was released by the acid hydrolysis (34 ppm) method than by enzyme hydrolysis (5 ppm from a 5-min incubation and 25 ppm from a 2-hr incubation). Thus, MHQ apparently occurs only in the WS fraction of the flour and is bound by a β-glycosidic bond. This is reasonable in that phenolic substances in flour are frequently present as glycosides (Harborne, 1973) and, thus, tend to be water soluble. Graveland et al (1984) found a compound with such a structure in wheat with MHQ bound to glucose by a β-(1→4) bond.

Effect of Endogenous MHQ on Dough Breakdown

Clearly exogenous MHQ accelerated dough breakdown in air (Fig. 2). However, whether endogenous MHQ, which was bound with a β-glycosidic bond in flour, is a factor in the breakdown of dough was not yet known. Mixograms made with the addition of exogenous free hydroquinone or hydroquinone linked to glucose (arbutin) illustrate the difference between the two forms (Fig. 4). Free hydroquinone (80 ppm) accelerated dough breakdown, but arbutin was not effective in accelerating dough breakdown. Therefore, bound hydroquinone is not active in accelerating dough breakdown.

Analysis of the WS_D and WS_{DZ} fractions of flour water solubles showed that MHQ was found only in the WS_{DZ} (38 ppm). However, WIS flour dough mixed with the WS_D, neither containing MHQ, had poor mixing tolerance (Fig. 5). It is also shown in Fig. 5 that addition of WS_{DZ} to the WIS also gave poor mixing tolerance. This might suggest that there are two components in the water solubles that can cause poor mixing tolerance. One with a molecular weight larger than 1,000 and one with a molecular weight less than 1,000.

Although the mixing tolerance of the two fractions WS_D and WS_{DZ} are similar when they are added to WIS, the dough development time is quite different (Fig. 5). Low molecular weight sugars, presumably found in the WS_{DZ} fraction, are known to slow dough development.

SUMMARY

From this study, it is clear that endogenous MHQ bound by a β-glycosidic linkage in flour is not effective in accelerating dough breakdown. Presumably, this is because oxidization of the bound

MHQ is more difficult. Therefore, a quantitative analysis of MHQ in wheat flours did not yield an explanation for the breakdown process.

Thus, while MHQ is quite effective in decreasing mixing tolerance, it clearly is not the endogenous material in wheat flour that controls mixing tolerance. The endogenous material remains unknown.

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