THE CONTROL OF FUNGI DURING THE MALTING OF WHEAT[1]

JAMES R. FLEMING, JOHN A. JOHNSON, AND BYRON S. MILLER[2]

ABSTRACT

Low levels of formaldehyde or 8-hydroxyquinoline sulfate completely inhibited growth of fungi on wheat during malting. Mercuric chloride, methylmercuricyandiamide, and *o*-phenylphenate (Dowicide) also were effective for control of fungi. Tetrachloro-*p*-benzoquinone, n-propyl vanillate, 2-thiobarbituric acid, thiourea, n-trichloromethylthiotetrahydrophthalimide, and several other substances were more effective than potassium permanganate and the hypochlorites. It is likely that many of these chemicals cannot be used in malting because of their toxicity to animals and humans.

Formaldehyde used in 0.05% concentration in the steep liquor during the last 1 to 6 hours of the steeping period completely controlled growth of fungi during germination. The amount of formaldehyde retained in the finished malt was as little as 0.003 p.p.m. This amount of formaldehyde did not inhibit yeast fermentation and would probably be insignificant so far as human consumption of bread is concerned.

The role of microorganisms during malting is unknown, but their development is generally not desired. They have been shown to be present both externally and internally (6). Reduction of germination (14,15), development of unusual flavors and of materials which inhibit yeast fermentation (14), water sensitivity of some barleys (2), and production of "gushing" beer (4) are among the undesirable conditions attributed to microorganisms which grow during malting of barley.

Comparatively little information concerning control of fungi during malting is available. Calcium and sodium hypochlorites, sodium hyposulfite, hydrogen peroxide, and potassium permanganate have been used commercially (13) to control fungi. Formaldehyde was one of the first chemicals used as a seed protectant. This treatment normally reduced seed germination, but Braun (3) found that presoaking of grains prior to formaldehyde treatment materially reduced the inhibitory effect on germination. Hurd (12) has shown that formaldehyde was absorbed very slowly by wheat and that it was dissipated gradually when wheat was stored moist. Recently

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[2] Instructor and Professor, respectively, Department of Flour and Feed Milling Industries, Kansas State University; and Chemist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture.

Piratzky (18) and Grimm and Baker (11) have advocated the use of formaldehyde to treat barley prior to malting.

The mold problem during the malting of wheat is thought to be greater than that with barley which may contain certain growth inhibitors in its husks (13). Difficulty in germinating some wheats for more than 6 days without excessive mold developing has been experienced by the writers. The object of this study was to investigate chemical means to control fungi during malting of wheat.

Materials and Methods

The malted wheats used were produced by using laboratory-scale malting equipment assembled for an earlier investigation (9). It consisted of a refrigerated steep tank, a germination chamber, and a forced-draft convection drying oven. All units were equipped with controls for close regulation of humidity and/or temperature. The malts were prepared by steeping wheats to 42% moisture, germinating at 62°F., and drying at 104°F. for 24 hours. Fifty-gram portions of wheat were used.

The seed treatments were of two types. The first consisted of steeping grain for various times in solutions of chemicals being examined. The second consisted of dusting the grain, after steeping, with powders in which wheat starch was used as the carrier for the chemicals to be applied. The powders were prepared by drying (in a rotary evaporator) a suspension of starch in an alcoholic solution of the chemicals. A 2.5-g. portion of powder was applied to each 50 g. of wheat. The substances and the concentrations employed in both techniques are given in Tables I and II.

Effectiveness of the chemical treatments was evaluated by an agar plate technique (6) to demonstrate the existence of molds in cereal grains. Incubation time was 3 days at 30°C.

Alpha-amylase activity was determined by the method of Sandstedt *et al.* (21) as modified by Redfern (19). Protease activity was measured by the method of Miller (16).

Residual formaldehyde in the malted wheats was determined by a colorimetric technique employing chromotropic acid (5). The effect of small amounts of formaldehyde added to flour on the rate of yeast fermentation was studied by measuring gas production (1).

Results and Discussion

Chemicals Ineffective or Only Partially Effective in Controlling Fungi. The following chemicals were investigated and found to be ineffective in controlling molds and, in addition, many reduced seed

TABLE I

EFFECT OF CERTAIN CHEMICALS ON THE DEVELOPMENT OF FUNGI AND GROWTH OF ACROSPIRES OF TRIUMPH WHEAT DURING MALTING

CHEMICAL[a]	CONCENTRATION	FUNGUS DEVELOPMENT[b]	ACROSPIRE DEVELOPMENT[c]
	%		
Control		+++++	+++++
1-acetyl-2-thiohydantoin	0.10	+++	+++++
	0.20	+++	+++++
Ethyl vanillate	0.20	++++	+++++
Calcium hypochlorite	0.10	++++	+++++
	0.20	++++	+++++
2-hydroxy-3-hexenoic acid delta-lactone, sodium salt	0.05	++++	+++++
(Dehydrochloroacetic acid, sodium salt)	0.10	+++	+++
Isobutyl vanillate	0.10	++++	+++++
	0.20	+++	+++++
2-mercaptobenzothiazole	0.10	++++	++++++
	0.20	+++	+++++
Paraformaldehyde	0.05	++++	+++
	0.10	+++	+++
n-Phenyl-2,4-thiazolidinedione	0.10	+++	+++++
	0.15	+++	+++
1-Phenyl-3,5-dimethyl-4-nitrosopyrazole	0.05	++++	+++++
	0.10	+++	+++
	0.15	++	+++
Potassium permanganate	0.01	++++	+++++
	0.03	++++	+++++
	0.05	+++	+++++
	0.10	+++	+++
Potassium sorbate	0.20	++++	+++
n-Propyl vanillate	0.10	+++	+++++
	0.20	++	+++
Sodium bisulfite	0.20	++++	+++
Sodium hypochlorite	0.10	++++	+++
	0.20	++++	+++++
Sorbic acid	0.07	++++	++
	0.10	++++	++
Tetrachloro-*p*-benzoquinone (Chloranil)	0.10	+++	+++++
	0.20	++	++++++
2-Thiobarbituric acid	0.05	++++	+++++
	0.10	+++	+++++
Thiourea	0.10	++++	+++++
	0.20	+++	++++++
n-Trichloromethylthio-tetrahydrophthalimide (Captan)	0.05	++++	+++++
	0.10	+++	+++++
Zinc trichlorophenate	0.025	++	+++++
	0.05	+	+++
Zinc ethylenebisthiocarbamate	0.025	+++	+++++
	0.05	++	+++++

[a] Applied as powder except for 0.10% potassium permanganate and 0.10% sodium hypochlorite which were added to the steepwater 6 hours prior to the end of the steeping period. The application of some of these chemicals in powder form was necessary because of their insolubility. Application in the steepwater is to be preferred because of its convenience and uniformity.

[b] Five plus signs indicate mold growth equivalent to that of the control. Fewer plus signs indicate progressively less fungal growth.

[c] Five plus signs indicate acrospire development equivalent to that of the control. Fewer plus signs indicate progressively less and more plus signs indicate progressively greater acrospire growth.

germination: acetaldehyde, acetic acid, acrolein, *p*-aminobenzoic acid, benzoxazolinone, caffeic acid, sodium *p*-toluene sulfone chloramide (chloramine T), crotonic acid, 3-chloropropionic acid, copper sulfate, 2-ethoxyethyl acetate, formic acid, furfural, diacetyl, lactic acid, phenothiazine, phenylacetic acid, potassium sorbate, propionic acid, propionaldehyde, propylene glycol, propylene glycol dipropionate, and Sucrodet D-608 (a sucrose dipalmitate).

Treatments partially effective in controlling fungi are given in Table I. The agar plate method (6) of evaluating these treatments is more sensitive than observations on the appearance, smell, or taste of the malt. With the agar plate technique, mold growth could be readily observed after 3 days of incubation, whereas in wheat malting, mold proliferation could not be discerned readily until after 4 to 5 days of germination.

The hypochlorites, sodium bisulfite, and potassium permanganate treatments were found to be among the least effective of the substances studied (Table I). They tended, however, to stimulate acrospire growth as indicated by Hopkins and Krause (13).

Ethyl vanillate, tetrachloro-*p*-benzoquinone, 2-mercaptobenzothiazole, and thiourea, when applied in powder form following the steep period, reduced mold growth slightly. The last two appeared to stimulate acrospire growth slightly, but both contributed unusual odors to the malt.

Paraformaldehyde reduced both mold and acrospire growth significantly when applied in dry form at 0.10% concentration. Crocker and Barton (7) have reported that seed treatment with formaldehyde may be injurious because of the formation of the paraformaldehyde by polymerization of formaldehyde.

n-Trichloromethylthio-tetrahydrophthalimide, sodium dehydroacetate, 2-thiobarbituric acid, n-phenyl-2,4-thiazolidinedione, tetrachloro-*p*-benzoquinone and 1-phenyl-3,5-dimethyl-4-nitrosopyrazole all significantly reduced mold growth without markedly reducing acrospire development. These substances, however (with the possible exception of sodium dehydroacetate), are of little practical value because of their toxicity. The esters of vanillic acid were more effective in controlling fungi than were the hypochlorites and have been found to be relatively innocuous (8). However, they possess strong aromas which may preclude their use. Sorbic acid and potassium sorbate, now being used as food preservatives, were found to be of no value in malting for they reduce the rate of germination markedly when used at concentrations high enough to affect the growth of fungi.

Chemicals Effective in Controlling Molds. Data concerning the

TABLE II

EFFECT OF CERTAIN CHEMICALS ON THE DEVELOPMENT OF FUNGI, GROWTH OF ACROSPIRES, AND ENZYME ACTIVITIES OF TRIUMPH WHEAT, DURING MALTING

CHEMICAL	TIME PRESENT IN STEEP-WATER	CONCEN-TRATION	FUNGUS DEVELOP-MENT [a]	ACRO-SPIRE DEVELOP-MENT [b]	ALPHA-AMYLASE ACTIVITY [c]	PROTEASE ACTIVITY [d]
	hours	%			*SKB units/g*	*HU/g*
Control			+++++	+++++	153	69
Formaldehyde	1	0.025	++	+++++	148	65
	1	0.05	+	+++++	144	65
	1	0.10	−	+++	138	60
	6	0.025	++	+++++	145	65
	6	0.05	+	+++	139	60
	6	0.10	−	+++	127	56
8-hydroxy-quinoline sulfate	1	0.025	++	+++	137	57
	1	0.05	−	+++	125	50
	1	0.10	−	+++	113	40
	6	0.025	+	+++	120	53
	6	0.05	−	+++	107	39
	6	0.10	−	++	78	34
Mercuric chloride	1	0.05	−	+++		
	1	0.10	−	++		
Methyl mercuri-cyandiamide (Panogen)	1	0.025	+	+++++		
	1	0.05	−	+++++		
	1	0.10	−	+++		
o-Phenyl-phenate (Dowicide A)	1	0.05	−	+++		
	1	0.10	−	+++		
	1	0.15	−	+		

a Five plus signs indicate mold growth equivalent to that of the control. Fewer plus signs indicate progressively less mold growth and a minus sign indicates the absence of mold.
b Five plus signs indicate acrospire development equivalent to that of the control. Fewer plus signs indicate progressively less acrospire growth.
c See ref. 21.
d See ref. 16.

effects of formaldehyde, 8-hydroxyquinoline sulfate, *o*-phenylphenate, methylmercuricyandiamide, and mercuric chloride on acrospire growth and enzyme development during malting are given in Table II. The 0.10% concentration of formaldehyde in steepwater during the final 1- and 6-hour periods completely prevented growth of fungi. The 0.05% concentration was almost completely effective, while 0.025% caused a pronounced reduction in mold growth. The formaldehyde treatments caused moderate reductions in enzyme activities. This effect may be countered by including gibberellic acid in the steepwater. Similar effects were reported by Grace (10), who indicated that the reduction in germination caused by formaldehyde could be reversed by 1-naphthylacetic acid or 3-indoleacetic acid.

At concentrations of 0.05% or more 8-hydroxyquinoline sulfate completely inhibited fungi, and it gave very good results at 0.025% concentration. This substance, however, severely reduced enzyme activities and may be of little importance from a practical standpoint because of its possible toxicity to animals and man (8).

Mercuric chloride, *o*-phenylphenate (Dowicide A),[3] and methyl mercuricyandiamide (Panogen) at concentrations of 0.05% or more of the active chemical completely controlled growth of fungi when present in the steepwater during the final hour of the steeping. Mercuric chloride and Dowicide A, however, materially reduced seed germination. Panogen, at concentrations of 0.025 to 0.1%, had little effect on acrospire growth. These results suggest that damp wheat is subject to damage by most mercurial and phenolic fungicides, and confirm observations of Roane and Starling (20) concerning the mercurial compounds. These materials would seem to be of no practical importance in malting, because of their highly toxic nature. For this reason data concerning their effect on enzyme activity were not determined.

Figure 1 illustrates the effect of formaldehyde, calcium and sodium hypochlorite, sorbic acid, potassium sorbate, and 8-hydroxyquinoline sulfate on the growth of molds during wheat germination. Treatment with either formaldehyde or 8-hydroxyquinoline sulfate was almost completely effective at the 0.025 and 0.05% levels. The hypochlorites, potassium sorbate, and sorbic acid had little or no effect when used at comparable concentrations. Treatment with 8-hydroxyquinoline sulfate reduced rootlet and shoot growth appreciably.

Conditions optimum for the development of fungi are not necessarily so for the development of the acrospire when the agar plate

[3] The mention in this publication of a trade product does not imply its endorsement by the U.S. Department of Agriculture over similar products not named.

Fig. 1. Effect of chemical treatments on the growth of fungi on wheat during malting. First row, L to R: Control, 0.025% formaldehyde, 0.050% formaldehyde. Second row, 0.10% calcium hypochlorite, 0.04% sorbic acid, 0.07% sorbic acid. Third row, 0.10% sodium hypochlorite, 0.2% potassium sorbate, 0.3% potassium sorbate. Fourth row, 0.10% 8-hydroxyquinoline sulfate, 0.05% 8-hydroxyquinoline sulfate, 0.025% 8-hydroxyquinoline sulfate.

technique is used. Hence, data for Fig. 1 concerning acrospire length may not agree with data in Tables I and II which were obtained by malting.

Residual Formaldehyde in the Malt. The results of assays of formaldehyde-treated malts for residual extractable formaldehyde are given in Table III. Amounts found in the finished malt were as little as 0.003 p.p.m. The study of the effect of formaldehyde on yeast fermentation which made use of pressuremeters (1) indicated that 10

TABLE III

FORMALDEHYDE REMAINING IN STEEPWATER AND IN FINISHED MALT[a]

ADDED TO STEEPWATER	REMAINING IN STEEPWATER	FOUND IN WHEAT AFTER STEEPING	FOUND IN MALT AFTER KILNING
mg	*mg*	*mg*	*mg*
Formaldehyde present for final hour of steep period			
49.2	47.4	2.0	0.07
25.2	24.1	1.2	0.05
11.9	10.7	1.3	0.03
Formaldehyde present for final 6 hours of steep period			
49.2	46.3	3.0	0.08
25.2	23.2	2.1	0.05
11.9	10.3	1.6	0.05

[a] Fifty grams of Triumph wheat steeped in 50 ml. of water with formaldehyde present for 1 or 6 hours.

or fewer p.p.m. of formaldehyde in doughs had no effect on gas production. Because the amount of malt which is added customarily to bread doughs is but 0.25–0.75%, based on flour weight, the amount of added formaldehyde would have no effect on gas production. The residual formaldehyde in bread, if 0.5% treated malt was used, would be 10–5 p.p.m. Ng, Reed, and Pence (17) have extracted formaldehyde from fresh bread. Presumably the formaldehyde is produced during yeast fermentation, since the authors have found formaldehyde in the gases evolved from pre-ferments.

High moisture content of germinating grain and the high humidity maintained during malting make possible the dissipation of formaldehyde rather than its conservation through polymerization to paraformaldehyde.

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