Effect of Type and Amount of Mixing and Quantity of Water on Inhibitors of Yeast Activity in Wheat Flour Doughs and Slurries

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ABSTRACT

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The effect of the type and amount of mixing and the quantity of water differentially influenced the effect of antimicrobial and antimycotic agents on the activity of yeast in dough and on loaf volume of bread. Sorbic acid, an antimycotic agent, greatly inhibited gas production in doughs or slurries of bread ingredients, regardless of the amount or type of mixing and the amount of water. Monolaurin and monolaurin-plus, antimicrobial agents, had small stimulating rather than depressing effects on yeast activities when diluted in the slurries containing the dough ingredients and 150% water. Even when doughs were manually mixed for 2 min, monolaurin and monolaurin-plus inhibited yeast activities only slightly. Yeast activities were materially inhibited by the two microbicides only after the doughs received appreciable mechanical mixing (an important phase of breadmaking). To determine whether a chemical will impair yeast activity in breadmaking, gas production tests should be made on doughs that contain the bread-making ingredients and are mechanically mixed to optimum.

In a study of the antimicrobial activity of more than 40 natural or synthetic lipophilic compounds, Kabara et al (1977) found that monolaurin was highly active and the most active of the monoglycerol esters (Conley and Kabara 1973). We found that 1% of monolaurin had an extremely adverse effect on bread volume but little or no adverse effect on yeast activity as determined by the gas production method described by Rubenthaler et al (1980). However, dough size during punching and proofing indicated that gas production was far below normal. Even the loaf crumb grain suggested that the dough had been grossly underfermented and that gas retention of the gluten protein probably had not been adversely affected. We present the results of studies to explain why the gas production values did not correlate with impaired loaf volumes and what changes in techniques were necessary for gas production to indicate the extent to which loaf volume was impaired.

MATERIALS AND METHODS

Dough Ingredients and Special Chemicals

Ingredients used were sugar (grocery cane sucrose); chemically pure salt; Crisco shortening (melting point, 41°C, Procter and Gamble); compressed yeast (a 50/50 blend of weekly shipments from Anheuser-Busch, Inc., and Standard Brands, Inc.); barley malt (52 DU/g) was from Ross Industries of Cargill, Inc.; and L-ascorbic acid (ICN Pharmaceuticals, Inc.). Monolaurin and monolaurin-plus were supplied by J. J. Kabara, Department of Biomechanics, College of Osteopathic Medicine, Michigan State University, East Lansing. Monolaurin-plus was composed of equal parts of monolaurin, a mixture of 2- and 3-tertiary butyl-4-methoxyphenol, and calcium disodium ethylenediaminetetraacetate. Chemically pure sorbic acid was from Nutritional Biochemicals Corp., and the coated 77% sorbic acid was from Union Carbide Corp. Calcium propionate was from Anheuser-Busch, Inc. Calcium sulfate dihydrate was Fisher certified ACS grade.

Wheat Flours

Straight grade flours BCS-77 and CS-79 were milled from two composites of many hard winter wheat varieties harvested at several locations throughout the Great Plains in 1977 and 1979. BCS-77 flour contained 11.8% moisture and 12.2% protein (14% mb). CS-79 flour contained 12.5% moisture and 12.6% protein (14% mb). Each had excellent loaf volume potential. Mixing times of BCS-77 and CS-79 were 4⅛ and 5 min, respectively, except as otherwise indicated.

BCS-77 was used in the first two studies on manual mixing at absorption levels of 60 and 150% water and on mechanical mixing (optimum) at 60% water. CS-79 was used in the remaining four studies with various amounts of mechanical mixing at 60% water, hand mixing and various amounts of water, mechanical mixing (optimum) and various amounts of water, and monolaurin added after various amounts of mechanical mixing.

Dough Mixing Requirements

During the first 30 sec of mixing, water was absorbed by the flour, and all dough ingredients were incorporated into a rough, lumpy, wet-appearing dough. As mixing continued, the gluten protein was gradually developed and the dough gradually lost the rough, lumpy, and wet appearance until optimum mixing (point of minimum mobility) was attained, at which point the dough had a smooth and satiny appearance, was not sticky from overmixing, and had optimum handling properties. Replicate subjective determinations of mixing requirements usually agreed within ⅛ min and were corroborated by mixogram mixing time to the peak (Finney and Barmore 1945b).

The three bowl pins of the 10-g mixograph form an equilateral triangle. Two of the pins are at the back, in a plane parallel to the front of the mixograph. In our 10- and 100-g nonrecording dough mixers, the right back pin was eliminated, so that the dough was less restricted than with three pins and thereby facilitated pickup of flour and dough on inside bowl surfaces. Also, the point of minimum mobility of dough was sharper and easier to see with two pins than with three pins in the bowl.

Analytical Methods

Protein and moisture were determined by AACC methods 44-15A and 46-11, except that catalysts were titanium dioxide and cupric sulfate.

The bread-making method included mixing to minimum mobility (optimum), 60.0 ml (optimum) of water, and 50 ppm of ascorbic acid, an excess in the absence of nonfat dry milk. Additional formula ingredients were 100 g of flour (14% mb), 6.0 g of sugar, 1.5 g of salt, 3.0 g of shortening, 3.5 g of compressed yeast, and 0.25 g of barley malt (52 DU/g). Ascorbic acid is its own buffer against overoxidation (Shogren and Finney 1974). Straight doughs were fermented 69 min to first punch, 103 min to second punch, and 120 min to pan, and proofed 48 ± 1 min (the time to proof controls to 7.8 cm) at 30°C. Baking time was 24 min at 215°C. Loaf volume was determined immediately by dwarf rape seed displacement. Loaf volumes (average of triplicates) that differed by 25 cc were statistically significant at $P = 0.05$. Additional related details are

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given by Finney (1945), Finney and Barmore (1943, 1945a, 1945b), and Finney et al (1976).

Gasograph gas production was determined on a dough (Finney et al 1949) and a slurry (Rubenthaler et al 1980). The dough (6.0 ml of water) and slurry (15.0 ml of water) contained 10 g of flour (14% mb), 0.6 g of sugar, 0.15 g of salt, 0.35 g of compressed yeast, 0.3 g of shortening, 0.025 g of barley malt (52 DU/g), and 0.5 mg of ascorbic acid. Doughs were mixed to minimum mobility (Finney and Shogren 1972) and also to less than and more than minimum mobility and fermented 180 min. Slurries and some doughs were manually mixed 2 min with a stirring rod. Gas production in 180 min was expressed as gasograph units (GU). The 10-g nonrecording dough mixer differed from that of the mixograph described by Finney and Shogren (1972) in that it was nonrecording, contained two instead of three pins in the bowl, and was operated at 100 instead of 88 rpm. Gas production values (average of duplicates) that differed by 0.7 GU (for slurries) to 1 GU (manually and mechanically mixed) were statistically significant at $P = 0.05$.

RESULTS AND DISCUSSION

Chemicals That Impaired Volume and Crumb Grain of Bread

Loaf volume varied from 1,077 cc for the treatment of 0.1% calcium propionate to 410 cc for 1% monolaurin (Table I). The volume of 1,077 cc was 64 cc greater than that of the control. Inorganic calcium sulfate was included as a common denominator to the organic calcium propionate. Volume of bread that contained 0.4% monolaurin-plus was affected more adversely than was that containing 0.4% monolaurin. Sorbic acid (0.15%) reduced loaf volume (to 485 cc) about as much as did 0.8% monolaurin (to 503 cc). Coated sorbic acid greatly minimized the adverse effect of sorbic acid (908 instead of 485 cc). The desirability of crumb grains generally decreased as loaf volume decreased.

Loaf Volume and Gas Production of All Chemical Treatments

Hand Mixing, 60 and 150% Water. When the seven bread dough ingredients and each of the treatments in Table I were manually slurried in 150% water, loaf volume decreased from 1,077 cc to 410 cc with increasing gas production, except for the treatment of 0.15% sorbic acid (Fig. 1A, Table I). The other chemical treatments generally had a relatively small stimulating effect on yeast activity.

By manually mixing the seven bread dough ingredients with 60% water (baking absorption) instead of 150%, loaf volume decreased as gas production decreased over a range of only 6.7 GU, except for the treatment with 0.15% sorbic acid. Sorbic acid materially inhibited yeast activity even when the system was diluted with 150% water. Thus, the data on manual mixing indicate that a low concentration of monolaurin stimulated yeast activity when a large amount of free water was present and inhibited activity when the water was decreased from 150 to 60%.

TABLE I
Bread Volume and Crumb Grain When Wheat Flour (100 g, BCS-77) was Treated with Antimicrobial Chemicals

Chemical and Amount Added (%)	Bread	
	Crumb Grain[a]	Volume (cc)
Control	S	1,013
Calcium propionate		
0.100	S	1,077
$CaSO_4 \cdot 2H_2O$		
0.0843	S	1,020
Monolaurin		
0.400	Q-S	900
0.600	Q-U	765
0.800	UU	503
1.000	UU	410
Monolaurin-plus		
0.050	S	992
0.150	S	965
0.400	Q	850
Sorbic acid		
0.025	Q-S	923
0.150	UU	485
Sorbic acid, coated		
0.150	Q-S	908

[a] S = satisfactory, Q = questionable, U = unsatisfactory, UU = poorer than unsatisfactory.

Optimum Mechanical Mixing, 60% Water. However, when th seven dough ingredients, each of the treatments, and 60% water were mechanically mixed to optimum (the point of minimum mobility), loaf volume decreased as gas production decreased from 62.8 to 8.2 GU (Fig. 1B). A decrease in yeast activity of 1 GU was equivalent to a decrease in loaf volume of 12.2 cc (1,077 − 410 cc)/(62.8 − 8.2).

Monolaurin, a monoglyceride of glycerol and lauric acid with 12 carbon atoms in the chain, is similar to the monoglycerides of palmitic and stearic acids with 16 and 18 carbons, respectively. Yet, the monoglycerides of palmitic and stearic acids are dough improvers similar to shortening and certainly do not inhibit yeast activity.

With baking absorption, an appreciable amount of free water was still present after the dough ingredients were manually mixed for only 2 min with a stirring rod. Appreciable free water could permit protective layers of water to build up around each molecule of monolaurin and thereby reduce or eliminate its inhibition of yeast activity. When mechanically mixed to optimum, however, the gluten protein was thoroughly hydrated, so that the dough was relatively dry. Optimum mechanical mixing also may facilitate the interaction of monolaurin and yeast or produce a yeast-inhibiting material from the interaction of monolaurin with another dough ingredient, a flour component, or a degradation or interaction product of mixing.

Gas Production of Monolaurin Only

Varying Amounts of Mechanical Mixing, 60% Water. When 1% monolaurin and the seven bread dough ingredients were mechanically mixed for 20 sec to 5 min (optimum), gas production decreased from 56.3 GU to 6.3 GU (Fig. 2). Overmixing 1.5 min beyond the optimum (point of minimum mobility) further decreased gas production by only 1.8 GU. The 56.3 GU of gas production for 1% monolaurin mechanically mixed for 20 sec agreed with 57.1 GU for 1% monolaurin manually mixed 2 min (equivalent to about 12 sec of mechanical mixing). Thus, mechanically mixing to optimum apparently created physical and chemical changes that greatly increased the effectiveness of monolaurin to inhibit yeast activity.

Hand Mixing, Varying Amounts of Water. Gas production of 1% monolaurin in the dough formula manually mixed for 2 min was materially increased 28.6 GU (from 48.2 GU to 76.8 GU) as water was increased from 55% (5% less than bread-dough

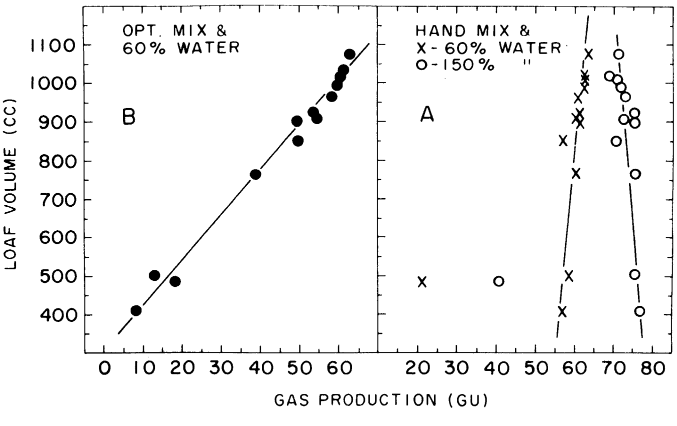

Fig. 1. Loaf volume and gas production when the seven bread dough ingredients plus treatments of antimicrobial and antimycotic agents were **(A)** manually (hand) mixed with 60 and 150% water and were **(B)** mixed to optimum (point of minimum mobility) with 60% water. Optimum mixing and 60.0 ml water were used in breadmaking.

absorption) to 150% (Fig. 3). Most of the 28.6 GU increase can be attributed to the increased availability of fermentable sugar with increasing absorption of water; gas production was 70.8 GU when monolaurin was omitted in 150% water (Table II); and gas production was 62 GU when monolaurin was omitted in the formula manually mixed for 2 min in 60% water (H in Figs. 2 and 3).

When all ingredients and 60% water were mixed to optimum (5 min) and then manually slurried for 2 min in an additional 90% water that contained 1% monolaurin, gas production was 69.9 GU (O in Fig. 3), only 6.9 GU less than that when all ingredients and 1% monolaurin were manually slurried for 2 min in 150% water (h in Fig. 3). Those data indicate that the chemical products of optimum mixing did not belatedly interact with monolaurin when manually mixed 2 min in 150% water.

Optimum Mechanical Mixing, Varying Amounts of Water. When all dough ingredients, 1% monolaurin, and 50.5–70.5% water were mixed to optimum, gas production increased only from 5.2 to 9.3 GU (Fig. 3). The increase probably is attributable to the increased availability of sugar with increasing dough water. Optimum absorption was 60.5%. The stiff dough (50.5% water) mixed to minimum mobility (optimum) in 3¾ min, the dough containing 60.5% water mixed to optimum in 4¾ min, and that containing 70.5% water mixed to optimum in 6⅞ min.

Added Before and After Varying Amounts of Mixing, 60 and 150% Water. When 1% monolaurin was added to dough after mixing 1.5, 3, and 4.5 min, followed by mixing each dough a total of 5 min (optimum), gas production increased from 8.3 GU to 32.1 GU (Table II). Dough mixed 3.5 min after adding monolaurin had essentially the same gas production as that mixed 5 min. The amount of mechanical mixing after adding monolaurin is important, but so is the mixing before adding monolaurin. For example, dough mechanically mixed 20 sec after adding monolaurin produced 56.3 GU of gas. From the gas production values of treatments 5 and 6, we would expect a gas production of about 55.3 GU if the dough of treatment 6 had been mixed 30 instead of 20 sec. Thus, 4.5 min of mixing before adding monolaurin reduced gas production 23.2 GU (55.3−32.1 GU). Gas

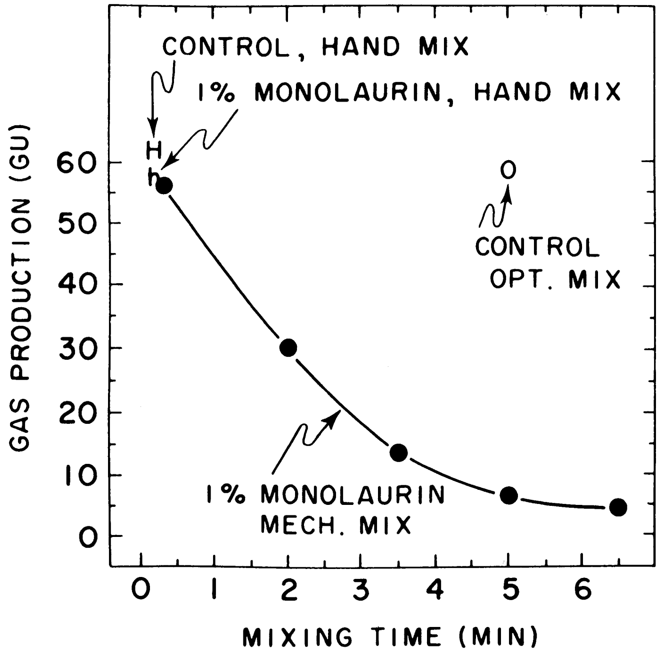

Fig. 2. Gas productions and various amounts of water when the seven dough ingredients plus 1% monolaurin were manually (hand) mixed 2 min and mixed to optimum (point of minimum mobility), and gas productions of related controls.

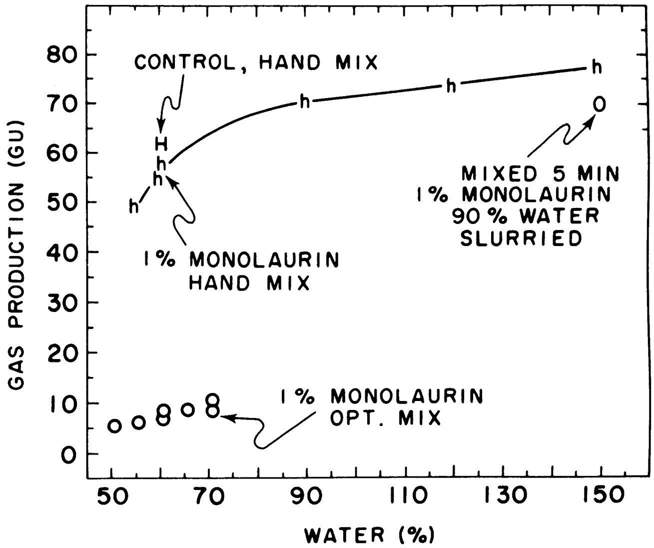

Fig. 3. Gas productions of bread doughs that contained 60% absorption water and 1% monolaurin and were mechanically mixed for 20 sec to 6.5 min, and gas productions of controls.

TABLE II
Yeast Activity Expressed as Gas Production of 10 G CS-79 Wheat Flour When 1% Monolaurin was Added in 60 and 150% Absorption Water and Before and After Various Amounts of Mixing

	Treament								
No.	Water (%)	Monolaurin (%)	Slurried (min)	Manually Mixed (min)	Mechanically Mixed (min)	Monolaurin (%)	Mechanically Mixed (min)	90% Water Slurried (min)	Gas Produced (GU)[a]
1	150	···	2	···	···	···	···	···	70.8
2	60	···	···	2	···	···	···	···	62.7
3	60	···	···	···	5 (Opt)[b]	···	···	···	59.0
4	150	1	2	···	···	···	···	···	76.8
5	60	1	···	2	0.2	···	···	···	57.1
6	60	1	···	···	0.33	···	···	···	56.3
7	60	1	···	···	5 (Opt)	···	···	···	8.2
8	60	···	···	···	1.5	1	3.5	···	8.3
9	60	···	···	···	3	1	2	···	18.6
10	60	···	···	···	4.5	1	0.5	···	32.1
11	60	···	···	···	5	1	0.33	···	34.9
12	60	···	···	···	6.5	1	0.33	···	30.4
13	60	1	···	···	5	···	···	2	13.3
14	60	···	···	···	5	1	···	2	69.9

[a] GU = gasograph units.
[b] Opt = optimum.

production data for treatments 6 and 11 demonstrate a similar reduction in gas production by mixing 5 min prior to adding monolaurin. Mixing 6.5 min before adding monolaurin further reduced gas production from 34.9 to 30.4 GU.

When the dough ingredients were mechanically mixed 5 min with 60% absorption water and 1% monolaurin and the dough was disintegrated and slurried 2 min in 90% water, gas production was only 13.3 GU. Thus, the damage done by 5 min of mechanical mixing was only slightly reduced by diluting with 90% water in the slurry. However, when monolaurin was added after 5 min of mechanical mixing and the dough was disintegrated in a slurry, gas production was 69.9 GU, essentially equal to that of the control. Thus, mechanical mixing before adding 1% monolaurin materially reduced gas production only when followed by more mechanical mixing of the dough and had little or no effect on gas production when followed by a disintegration of the dough in a slurry with 90% water. Apparently, monolaurin may inhibit yeast activity by interacting with yeast or another dough ingredient, a flour component, or a degradation or interaction product of mechanical mixing. Ascorbic acid, malt, and shortening are not involved, because when they were omitted from the formula, gas production data were essentially identical to those plotted in Fig. 1 (data not given).

Doughs that contained monolaurin and were mechanically mixed to optimum contained about the same number of dead yeast cells (stained blue with methylene blue, Fink and Kuhles 1933) as that of the control. Thus, monolaurin apparently inhibited or inactivated but did not kill the yeast cells.

CONCLUSIONS

Sorbic acid, an antimycotic agent, greatly inhibited gas production in doughs or slurries of bread ingredients regardless of the amount or type of mixing and the amount of water. Monolaurin and monolaurin-plus, antimicrobial agents, had small stimulating effects on yeast activities when diluted in the slurries containing the dough ingredients and 150% water. Even when in doughs manually mixed for 2 min, monolaurin and monolaurin-plus inhibited yeast activities only slightly. Yeast activities were materially inhibited by the two microbicides only after the doughs received appreciable mechanical mixing, an important phase of breadmaking. The inhibiting and stimulating effects of certain metals and their salts on gas production were studied on mechanically mixed doughs (Finney et al 1949).

When studying the effect of varying concentrations of most baking ingredients on yeast activity, we found that gas production by AACC method 22-11 (1976), and gas production in slurries of bread-making ingredients in 100–150% water described by Shogren et al (1977) and Rubenthaler et al (1980) are entirely satisfactory for relating gas production to functional bread-making properties. However, to determine whether a chemical will impair yeast activity in breadmaking, gas production tests should be made on doughs that contain the bread-making ingredients and are mechanically mixed to optimum. Only in that way can one establish whether impaired bread-making properties are attributable to impaired gas production or gas retention.

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