The Release of Hydrogen Sulfide during Dough Mixing[1]

D. K. MECHAM and **MAURA M. BEAN**, Western Regional Research Laboratory, USDA, Albany, Calif.

ABSTRACT

Doughs from 50 g. flour were mixed under nitrogen in a farinograph at 189 r.p.m. (3 × normal speed). In 30 min. at 30°C., hydrogen sulfide was released in amounts from 10 to 65 γ when 16 flours of widely varied source and baking properties were compared. Four flours yielded less than 30 γ and had very poor stability to mixing; but in the remaining flours the amounts of hydrogen sulfide released were not closely correlated with dough or baking properties. When temperature was increased to 44°C., the amounts of hydrogen sulfide released from two commercial flours (a hard red spring and a hard red winter) increased; when dough pH was lowered to about 5.0, less hydrogen sulfide was released. When 6% high-heat nonfat dry milk (NFDM) was added, doughs released somewhat more hydrogen sulfide; with low-heat NFDM, less hydrogen sulfide was released than with flour alone. Evidence was obtained that the hydrogen sulfide is not formed by microbiological activity.

In earlier work, hydrogen sulfide was detected over wheat flour doughs being mixed in a nitrogen atmosphere (1). The amount produced was small, but clearly was affected by speed of mixing, addition of oxidants, and the presence of yeast. The observations suggested that the release of hydrogen sulfide might be related to the development of doughs by mixing and the response of doughs to oxidizing agents, neither of which is well understood.

The influences of some additional factors on the quantity of hydrogen sulfide released from a dough have now been determined. Wide variations occur among flours, and temperature, pH, and nonfat dry milk have definite effects. These observations are reported herein.

MATERIALS AND METHODS

Materials

The flours, with descriptive information, are listed in Table I. The commercial HRW and HRS and the Lemhi flours were long patents, commercially milled respectively from 100% Kansas HRW wheat, 100% HRS wheat, and an intermountain white wheat mix made up predominantly (about 90%) of Lemhi variety. The durum flour was prepared from commercial 100% durum semolina by passage through the reduction stand of a Quadrumat Senior mill. The HRW and HRS variety samples were milled on a Miag Multimat and are described elsewhere (2,3). The Nebraska Q-J and Pawnee Q-J flours were milled on a Quadrumat Junior mill; the Cheyenne and Wasatch, on a Buhler laboratory mill. All the flours were unbleached and unbromated.

The nonfat dry milk (NFDM) designated "high-heat" was a commercial product intended for bakery use; the "low-heat" product was a commercial agglomerated product sold at retail for home use; and the "freeze-dried"

[1]Presented at the 52nd annual meeting, Los Angeles, Calif., April 1967. Contribution from the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif. 94710.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

TABLE I

HYDROGEN SULFIDE RELEASED BY DOUGHS MIXED UNDER NITROGEN FROM FLOURS OF VARIOUS SOURCES AND CHARACTERISTICS

Wheat			Mill	Flour Protein[a]	Flour Ash[a]	Dough Stability[b]	H_2S Released[c]
Class	Area	Variety		%	%	*min.*	γ
HRW	Nebraska	(Mixed)	Q-J	15.3	0.67	16.5	58
		Pawnee	Q-J	12.4	0.55	9.5	44
	Kansas	Bison	Multimat	12.6	0.49	26.0	55
		Comanche	Multimat	15.0	0.47	24.5	65
		Triumph	Multimat	12.3	0.44	4.0	43
		Wichita	Multimat	12.8	0.45	5.0	43
HRS	N. Dakota	Conley	Multimat	15.2	0.45	18.0	53
		Thatcher	Multimat	13.6	0.51	14.0	41
		Selkirk	Multimat	14.8	0.48	15.5	51
		Lee	Multimat	16.0	0.50	9.5	41
HRW		(Mixed)	Commercial	12.8	0.52	14.0	54
HRS		(Mixed)	Commercial	17.1	0.52	12.0	52
HRW	Montana	Cheyenne	Buhler	16.5	0.45	3.0	10
		Wasatch	Buhler	13.6	0.44	3.5	26
Durum	N. Dakota	(Mixed)	Commercial	15.9	0.81	2.0	19
White	Idaho	Lemhi	Commercial	9.2	0.63	1.5	28

[a]Dry basis.
[b]By the conventional procedure, i.e., in air at 63 r.p.m.
[c]Flour-water dough of 50 g. flour, 14% m.b., mixed 30 min. at 30°C. in farinograph at 189 r.p.m.

solids were prepared in the laboratory by freeze-drying pasteurized nonfat milk.

Mixing Bowl

Doughs were mixed in a 50-g. stainless-steel farinograph bowl fitted with a transparent plastic cover, with inlet and outlet holes, and gaskets to make the bowl gas-tight. The gasket between the front and rear sections was cut from Vellumoid 1/64-in. sheet packing (Vellumoid Co., Worcester, Mass.). The top surfaces of the front and rear sections of the bowl were machined to the same level, and a Neoprene foam gasket, 1/8 in. thick, was used between the bowl and the transparent plastic cover. Some leakage of gas occurred around the mixer-blade shafts when dry flour was mixed, but dough formed an effective seal around the shafts.

Dough Mixing

Flour samples were deaerated by dry mixing in the bowl for 5 min. at 63 r.p.m. while nitrogen was passed through. (Longer deaeration treatments did not increase the amount of hydrogen sulfide subsequently released from the dough.) The mixer then was stopped, water (distilled, deionized, boiled, and flushed with nitrogen) added, the mixer restarted, and the speed gradually increased to 189 r.p.m. (within 30 sec.). Other reagents were dissolved in the water before it was added to the flour; NFDM was mixed dry in the flour before deaeration. The nitrogen stream (500 ml./min.) was humidified by being bubbled through water before it was passed over the

dough. Dough temperatures were checked with a thermometer at the completion of runs; with water at 30°C. being circulated through the bowl jacket, dough temperatures did not exceed 32°C. at the highest speed of mixing (189 r.p.m.) after 50 min., and differences in temperature between dough and the circulating water were smaller at higher temperatures. To determine the pH of doughs, glass and calomel electrodes were inserted directly into a portion of dough. The electrodes were standardized with commercial reference buffer, pH 6.86 and pH 4.01 at the temperature of the dough to be examined.

Water absorption of the flours was determined by the standard farinograph method (500 B.U. maximum, 63 r.p.m., in air) (4). Unless otherwise indicated, this absorption was used throughout the studies for all variations, with no attempt to standardize maximum resistances; e.g., at 189 r.p.m. the mixing curve peaked at much higher than 500 B.U.

Hydrogen Sulfide Determinations

The gas stream leaving the mixing bowl was passed through 25 ml. of 2% (w./v.) zinc acetate in a gas-washing bottle to trap hydrogen sulfide. By use of a 3-way stopcock, traps were changed at 5-min. intervals. The determination then followed the procedures of Mecchi *et al.* (5) and Prince (6), methylene blue being formed by addition of *p*-aminodimethylaniline.

The method is sensitive and precise. Occasional obviously low values were discarded. These occurred infrequently; and if the first two runs on a sample disagreed by more than $\pm$ 5% from their average, a third was made. The third value almost always supported the higher of the first two values. The low values could not be consistently related to an obvious cause such as leaks in the bowl enclosure, nor were the mixing curves different when low values were obtained. However, it was found that the first dough each day usually gave a low value. This apparently resulted from retention of some hydrogen sulfide in water droplets collecting in the tubing leading to the zinc acetate trap and perhaps absorption on bowl and tubing surfaces. Consequently, a "conditioner" dough was mixed under nitrogen each day before doughs were mixed for analysis.

Microbial Flora

The viable microbial populations of certain flours were determined by total plate count with a serial dilution method using plate count agar. Total counts were made after plates were incubated 3 days at 31°C.

RESULTS

Flours

The amounts of hydrogen sulfide released by various flours (from flour-water doughs mixed 30 min. at 189 r.p.m.) are shown in Table I and Fig. 1. The choice of mixing time and speed was an arbitrary one, and other conditions would show about the same relationships among the flours except for very short mixing times. The samples include both commercially and experimentally milled flours from a variety of sources with a wide range in baking characteristics.

The amounts released range from 10 to 65 γ hydrogen sulfide. With the

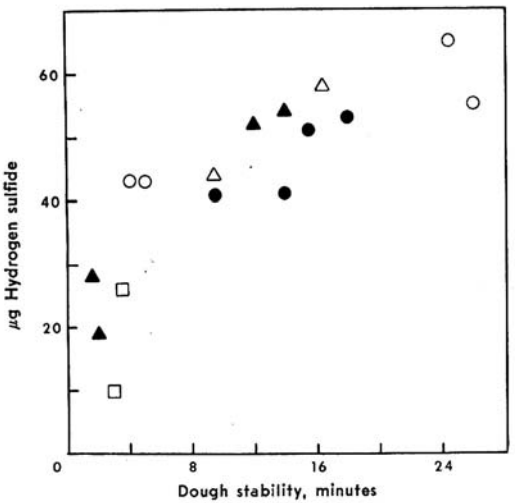


Fig. 1. Scatter diagram, dough stability in farinograph *vs.* hydrogen sulfide released from flours of various sources and characteristics. △, HRW Nebraska Q-J samples; ○, HRW Kansas Multimat samples; □, HRW Montana Buhler samples; ▲, Commercial samples; ●, HRS North Dakota Multimat samples. For further description see Table I.

exception of four flours, however, the range is from 41 to 65 γ. The flours in this upper range differ appreciably in baking characteristics, from fair to excellent, and in farinograph mixing stability. The four flours in the 10- to 28-γ range, however, have very low stabilities in the farinograph. The low protein content of the Lemhi flour may be partially responsible, but the proteins of the other three flours lack the proper characteristics to give a typical curve.

Within other subgroups of the flours, correlations with dough or baking properties are not so evident, but in general some tendency appears for less hydrogen sulfide to be released from the doughs of poorer stability (Fig. 1). For example, the Nebraska Q-J dough was more stable and released more hydrogen sulfide than the Pawnee Q-J dough. In the HRW group milled on a Multimat, the two least stable flours (Triumph and Wichita) released 43 γ hydrogen sulfide compared to 55 and 65 for the more stable flours (Bison and Comanche). In the HRS group, the extremes (Conley and Lee) follow the same pattern, but the Thatcher and Selkirk samples differ in hydrogen sulfide release despite having about the same stability.

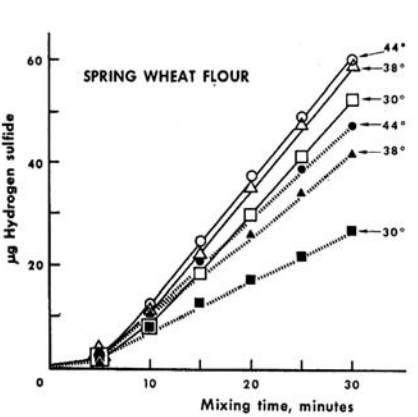
The two commercially milled flours gave very nearly equal amounts of hydrogen sulfide and are of similar stability. The protein content of the spring wheat flour was much the higher, however, so per unit of protein the spring wheat flour released appreciably less hydrogen sulfide than the winter wheat flour.

Wide variations in release of hydrogen sulfide certainly occur among different flours, and the amount largely reflects factors other than protein content. A relation between release of hydrogen sulfide and stability to mixing

may exist, but it appeared more important to determine the effect of several other factors on release of hydrogen sulfide than to extend the work to additional flours.

Temperature and pH

Dough temperatures are higher in continuous-mix processes than in conventional methods of breadmaking. The use of a liquid pre-ferment also gives a dough of lower pH at the mixing step. The release of hydrogen sulfide at three temperatures and two pH levels therefore was measured with the two commercial flours. The results are shown in Figs. 2 and 3.



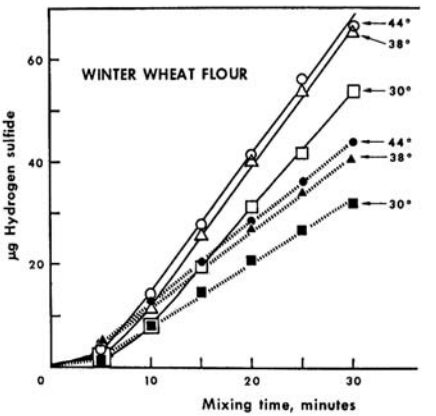


Fig. 2 (left). Effect of temperature and pH changes on amount of H_2S released from doughs of a commercial spring wheat flour: pH 6.1 (continuous lines, open symbols), flour-water doughs; pH 5.1 (broken lines, solid symbols), acetic acid added; 50 g. flour, 30-min. mixing under nitrogen; farinograph, 189 r.p.m. Points shown are averages from two to six doughs.

Fig. 3 (right). As Fig. 2 except that a commercial winter wheat flour was used and pH values were 5.9, flour-water doughs (open symbols), and 5.0, acetic acid added (solid symbols).

Total hydrogen sulfide increased with temperature at both pH values and with both flours. The increase with the spring wheat flour at pH 6.1 (flour-water dough) was small and at pH 5.1 (flour-water-acetic acid) relatively large, in contrast to the winter wheat flour doughs for which the increase was about equal at the two pH levels.

Conversely, when pH was lowered, the amount of hydrogen sulfide released was lowered, more consistently with the winter than with the spring flour doughs. It was noted that the change in pH did not cause a significant change in release of hydrogen sulfide during the first 10 min. of dough mixing with either flour or at any temperature. Although exact values cannot be read from the figures, in every pair of samples the hydrogen sulfide released at the two pH levels in 10 min. did not differ by more than 1 γ. For example, with spring wheat flour at 30°C., amounts released were 8 and 9 γ at pH 6.1 and 5.1, respectively, in the first 10 min.; with winter wheat flour at 44°C., 14 and 13 γ at pH 5.9 and 5.0, respectively. With continued mixing, how-

ever, the rate of release declined at the lower pH, the difference due to pH being easily observed in the next 5 min. Thus with spring wheat flour at 30°C., 19 and 13 γ were released at pH 6.1 and 5.1, respectively, in 15 min.; with the winter wheat flour at 44°C., 28 and 21 γ at pH 5.9 and 5.0, respectively.

When hydrochloric acid was used in place of acetic acid, the effect on hydrogen sulfide release was almost identical. For example, at 44°C. the spring wheat flour gave 44 γ hydrogen sulfide with acetic acid added to pH 5.1, and 43 γ with hydrochloric acid added; and the winter wheat flour gave 47 and 49 γ with acetic and hydrochloric, respectively.

The dough-mixing curves recorded by the farinograph in obtaining the hydrogen sulfide values given above changed with temperature in a consistent way with both flours. Maximum values decreased with temperature and the curves became flatter, with a less pronounced peak. The lowering of pH tended to give a slightly higher peak value, a shorter time to peak, and in general somewhat more rapid decline with long mixing.

Nonfat Dry Milk

The effects of the different nonfat dry milks (NFDM) with two flours are shown in Table II. Doughs containing 6% high-heat NFDM released more hydrogen sulfide than doughs containing freeze-dried and low-heat dry milk. The effects were about equal on both flours.

TABLE II

EFFECT OF NONFAT DRY MILKS ON HYDROGEN SULFIDE RELEASED FROM DOUGHS[a]

Flour	Control	6% Nonfat Dry Milk			6% Added Flour
		High-Heat	Low-Heat	Freeze-Dried	
	γ				γ
Commercial spring	52	57	40	43	60
Commercial winter	54	63	45	49	66

[a]Spring flour control, 50 g. flour (14% m.b.) and 30.7 ml. water mixed under nitrogen in farinograph at 189 r.p.m. for 30 min. Added water was maintained at 30.7 ml. in spring flour doughs containing 3 g. NFDM or 3 g. additional flour. In winter flour doughs, 29.7 ml. water was used.

In attempting to interpret the effects of the milk solids, it is not clear what part the water-absorbing properties of the dry milks may have had. The amount of work done on a dough affects the amount of hydrogen sulfide released, as shown by runs at two mixing speeds (1). In the same way, at constant mixing speed a stiff dough will have more work performed on it than a slack dough, and the doughs containing milk solids were stiffer than their controls, since no additional water was added. Therefore, an attempt to gage the influence of this factor was made by including 6% additional flour without increasing the water added to a dough. About 15% increase in the release of hydrogen sulfide was observed with both flours[2]. The effect of 6% high-heat NFDM did not quite equal that of 6% added flour. In marked contrast, the other NFDM's not only decreased hydrogen sulfide

[2]The same flour-water ratio also was obtained with the winter wheat flour by a 3.6% decrease in water absorption instead of a 6% addition of flour. This increased the hydrogen sulfide released to 63 γ, compared to the 66 γ with added flour.

release below that given by the high-heat milk but also below that obtained with control doughs of only 50 g. flour, despite the lower resistance to mixing of the control dough.

Microbial Flora

Hydrogen sulfide might be produced by the microbial flora of flour. The number of viable organisms found in four flours differing widely in hydrogen sulfide release indicate that they are not a factor in the present observations, however (Table III, control values). The four flours all gave relatively low total plate counts. (For comparison, Vojnovich and Pfeifer (7) used flours with counts to 75,000/g. in studies aimed at reducing the total microbial population to less than 5,000/g. as required for some "convenience" foods.) Also, the microbial counts bear no relation to the amounts of hydrogen sulfide released.

TABLE III

MICROBIAL POPULATION, HYDROGEN SULFIDE RELEASED, AND DOUGH STABILITY OF CONTROL AND STORED FLOURS

FLOUR	TOTAL PLATE COUNT			HYDROGEN SULFIDE RELEASED[a]		
	Control	7 Weeks at 100°F.	5 Days at 115°F.	Control	7 Weeks at 100°F.	5 Days at 115°F.
	per g.	*per g.*	*per g.*	γ	γ	γ
Commercial spring	1,300	200	200	52	50	50
Cheyenne	5,500	300	400	10	15	17
Comanche	200	.....	.....	65	....	....
Wasatch	500	.....	.....	26	....	....

[a]Flour-water dough containing 50 g. flour (14% m.b.), mixed under nitrogen in farinograph for 30 min. at 189 r.p.m.

In addition, the two flours with the highest counts were stored at elevated temperatures (100° and 115°F.) to reduce microbial population. Storage at 115°F. was limited to 5 days to minimize changes in farinograph characteristics. (The Cheyenne doughs increased 1 min. in stability; an increase of 4 min. was found for the commercial HRS flour.) The heat-treatments effectively reduced the microbial counts in both flours, but the hydrogen sulfide results did not show any trend related to the number of viable or dead organisms (Table III). No significant change occurred in the hydrogen sulfide released from the spring wheat flour. The small increase for the poor-mixing Cheyenne is not understood at present but does not appear to be related to the presence of microorganisms.

In going from a resting state as in dry flours to an active state as in dough, the lag time before the microbial population becomes active is usually considered to be a matter of hours, whereas release of hydrogen sulfide starts after a few minutes of dough mixing. Also, tripling the mixing speed was shown (1) to about triple the hydrogen sulfide production from doughs of two flours. Such an increase in speed should have no effect on production of hydrogen sulfide by microorganisms. These considerations, together with the lack of correlation between the microbial population and amount of hydrogen sulfide released, indicate that the presence or activity of microorganisms is not a significant factor in the formation of the hydrogen sulfide observed in this work.

DISCUSSION

The observations reported here show that the amounts of hydrogen sulfide released from a dough can vary over a considerable range. Several factors have a marked influence, but for most it is not possible to distinguish the extent to which a change in hydrogen sulfide released reflects a primary or a secondary effect. Thus, lowering the water content of a dough increased its resistance to mixing and thereby the work performed on the dough and the amount of hydrogen sulfide released. When high-heat milk solids were added, the dough became stiffer, and the additional hydrogen sulfide released may be due only to the greater resistance to mixing. The low-heat milk samples also increased resistance, although to a lesser extent, but the release of hydrogen sulfide actually decreased. Clearly the heat-treatment of milk affects the release of hydrogen sulfide through some unidentified changes as well as by increasing water absorption. In somewhat the same way, the effects of a rise in temperature must to some extent counter one another; i.e., a rise in temperature lowers the mechanical energy required to mix a dough, but it may also decrease the additional energy input needed from the mechanical treatment to degrade certain sulfur-containing groups in the dough proteins.

Actually very few groups are required to produce the amounts of hydrogen sulfide released. Thus if a sulfhydryl content of about 1 μeq./g. flour is assumed, then only about 3% of these groups need be degraded to give the average amounts of hydrogen sulfide released in a 30-min. mixing period. We have noted also that other volatile sulfur-containing compounds probably are given off during mixing under nitrogen. This is indicated by a cabbagelike aroma in the nitrogen stream leaving the zinc acetate trap, after removal of hydrogen sulfide[3]. It is hoped that the components and the reactions responsible for their presence also can be investigated.

Finally, perhaps it should be repeated that the dough conditions for these studies (doughs mixed under nitrogen, increased r.p.m. in the farinograph) are not far removed from the conditions of dough mixing in a continuous-mix unit. This, together with the variations among flours and the effects of oxidizing agents noted earlier (1), suggests that further investigations of the formation and release of hydrogen sulfide should help to give a better understanding of the behavior of doughs in baking despite the very small amounts that are released.

Acknowledgments

The authors are indebted to Frank Sugihara and Leo Kline for the microbial counts and to E. P. Mecchi for instruction and assistance in the determination of hydrogen sulfide.

[3]A reviewer has pointed out that objectionable odors can be given off by products baked from flour that has been treated with methyl bromide, and that volatile sulfur compounds, probably dimethyl sulfide, are responsible (Hermitte and Shellenberger, Cereal Chem. 24: 449–464 (1947); Winteringham *et al.*, J. Sci. Food Agr. 6: 251–261 (1955)). Of the flours used in our work, however, we have been assured that none of the experimentally milled samples were exposed at any time to methyl bromide. Furthermore, we treated portions of the commercial HRS flour with methyl bromide at two levels, following in general the procedures of Winteringham *et al.* At both levels, a decrease in hydrogen sulfide released was found rather than an increase. When 200 g. flour was exposed to 5 ml. methyl bromide vapor in 2 liters air for 48 hr., the treated flour doughs released 44 γ hydrogen sulfide (*vs.* 52 γ for the untreated control). When the much heavier treatment of 100 ml. methyl bromide vapor in 2 liters air was used under otherwise the same conditions, doughs of the treated flour released 39 γ hydrogen sulfide.

Literature Cited

1. MECHAM, D. K., BEAN, MAURA M., and KNAPP, CHERYL. Hydrogen sulfide from wheat flour doughs. Chem. Ind. (London) 1966: pp. 989–990.
2. STRINGFELLOW, A. C., and PEPLINSKI, A. J. Air classification of Kansas hard red winter wheat flours. Northwestern Miller 270 (6): 19, 20, 22 (1964).
3. PEPLINSKI, A. J., STRINGFELLOW, A. C., and GRIFFIN, E. L., JR. Air classification response of durum and hard red spring wheat flours. Northwestern Miller 272 (5): 34, 36, 37 (1965).
4. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minn. (1962).
5. MECCHI, E. P., PIPPEN, E. L., and LINEWEAVER, H. Origin of hydrogen sulfide in heated chicken muscle. J. Food Sci. 29: 393–399 (1964).
6. PRINCE, C. G. T. The determination of very low concentrations of hydrogen sulfide in gas. J. Appl. Chem. 5: 364–374 (1955).
7. VOJNOVICH, C., and PFEIFER, V. F. Reducing the microbial population of flour by warm storage. Northwestern Miller 273 (7): 12–14 (1966).

[Received July 5, 1967. Accepted January 5, 1968]