STUDIES WITH RADIOACTIVE TRACERS
IV. Degrees of Reduction of Br^{82}-Labeled Bromate to Bromide by Some Components of Flour[1]

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ABSTRACT

The treatment of dioxane solutions of fat from petroleum ether extraction of 100 g. of patent or clear flour with aqueous solutions of 1.5 mg. of Br^{82}-labeled potassium bromate at neutral pH or buffered to pH 4.7, in all cases did not give any measurable conversion of bromate to bromide after such mixtures were shaken for 4 hours. Similar treatment also caused no significant change in the fatty acid composition of the petroleum ether-extracted fat from patent or clear flour as measured by gas-liquid chromatography. Such results appear to indicate the absence of direct reactions between bromate and the petroleum ether-extracted fat, at least when treated in dioxane-water mixture. The previously reported pronounced effects of defatting on the degree of decomposition of bromate to bromide by water-flour doughs may possibly involve factors in the dough system besides the bromate and the fat. Water and flour slurry, water and freeze-dried gluten slurry, and the aqueous extract of flour, all can reduce some bromate to bromide. The results support the conclusion that bromate, a nonspecific oxidizing agent, may take part in reactions with a number of constitutents of flour.

In paper III of this series (6) it was reported that defatting of flours by extraction with petroleum ether markedly decreased the degree of reduction of bromate to bromide by water-flour doughs made from commercially milled, patent, intermediate and clear flours initially treated with 15 p.p.m. Br^{82}-labeled potassium bromate. The data indicated that the petroleum ether-extractable components of flour were involved, either directly or indirectly, in the decomposition of bromate by water-flour doughs. The present work includes attempts to ascertain whether the petroleum ether extract of flour can directly reduce bromate to bromide. The degrees of reduction of bromate to bromide by water slurries of flour and of freeze-dried gluten, and by aqueous extracts of flour, were also measured.

Materials and Methods

Untreated, patent and clear flours, commercially milled from Western Canadian hard red spring wheat, were used in these experiments. The contents of protein, ash, and petroleum ether-extracted fat in

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these flours, on a 14% moisture basis, are listed below:

	Protein (N × 5.7)	Ash	Fat
	%	%	%
Patent	12.8	0.35	0.79
Clear	16.8	0.80	1.80

The fat, obtained from petroleum ether extraction of 100 g. of flour in a series of Goldfisch fat extractors, was taken up in 15 ml. of purified dioxane. A solution of 1.5 mg. (15 p.p.m. based on the 100 g. of flour) of Br^{82}-labeled potassium bromate (7) in 15 ml. of distilled water was added. The mixture was shaken mechanically for 4 hours. Aliquots were withdrawn for the determination of any reduction to bromide by a method involving fractional precipitation of silver bromide described previously (7). Duplicate runs were carried out with fats from the patent and clear flours. Since aqueous extracts of water-flour doughs are slightly acidic, possible effects of acidity were also investigated by shaking the dioxane solution of fat with 1.5 mg. of labeled potassium bromate in 15 ml. of acetic acid-sodium acetate-buffered solution of pH 4.7.

The fatty acid composition in the petroleum ether-extracted fat, either as such or after shaking with 1.5 mg. of potassium bromate in dioxane-water for 4 hours and then re-extracted with petroleum ether, was analyzed by gas-liquid chromatography.[3] The detailed procedures have been reported elsewhere (3,4).

In the study with flour slurries, 10 g. of patent flour and 100 ml. of distilled water containing 1.5 mg. (150 p.p.m. based on the 10 g. of flour) of Br^{82}-labeled potassium bromate were shaken continuously by a mechanical shaker. At 1, 2, 3, or 4 hours after the beginning of the experiment, portions of the slurry were removed and centrifuged, and the bromide content in the aqueous supernatant determined by the fractional precipitation of silver bromide (7). Similar experiments were carried out with slurries of 1.5 g. freeze-dried gluten and 100 ml. of distilled water containing 1.5 mg. of labeled potassium bromate, or with 1.5 mg. of labeled potassium bromate dissolved in 100 ml. of aqueous extract of flour. The freeze-dried gluten was prepared from the patent flour according to the method of Lusena (8). The aqueous extract of flour used in this experiment was obtained from shaking patent flour with distilled water (100 ml. water per 10 g. flour) for 1 hour, followed by centrifugation.

Results and Discussion

Absence of Bromide in the Treatment of Fat with Bromate in Dioxane-water. The treatment of dioxane solutions of petroleum ether-

[3] Kindly carried out by B. M. Craig of the Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Sask.

extracted fat from either patent or clear flour with aqueous solutions of labeled potassium bromate at neutral pH or buffered to pH 4.7, as outlined in the previous section, gave, in all cases, no measurable conversion of the bromate to bromide. A typical set of experimental results is as follows:

A dioxane solution of fat from 100 g. of patent flour was shaken for 4 hours with an aqueous solution of 1.5 mg. of Br^{82}-labeled potassium bromate. The resulting mixture was centrifuged and 0.5 ml. of the dioxane-water layer was evaporated to dryness for counting. This sample represented the total Br^{82} activity.

To 5.0 ml. of the dioxane-water layer was added 1.0 ml. of solution containing 30.00 mg. of ordinary potassium bromide and 30.00 mg. of ordinary potassium bromate as carriers. Another 1.0 ml. of solution containing 42.82 mg. of silver nitrate, the molar equivalent of 30.00 mg. of potassium bromide, was introduced. The precipitate was centrifuged off and 0.5 ml. of the supernatant evaporated to dryness for counting. This sample represented the activity remaining after removal of all the bromide.

The sample representing total activity was counted immediately before and after the counting of the sample containing the activity after bromide removal. By means of this "sandwiching" technique, the activities of the two samples were directly comparable, thus eliminating the necessity of any correction for radioactive decays. The mean value for the two counts of the sample containing total activity was 1805 c.p.m., while the activity of the sample prepared after bromide removal was 1304 c.p.m. Since the precipitation of the silver bromide involved a dilution of the dioxane-water solution from 5.0 to 7.0 ml., taking this into account, the corrected activity in the sample after bromide removal was $1304 \times 7/5 = 1826$ c.p.m., essentially the same as the sample containing total activity, thus indicating no conversion of bromate to bromide.

The fatty acid composition of the petroleum ether-extracted fat, before and after treatment of the fat from 100 g. of flour with 1.5 mg. of potassium bromate in dioxane-water for 4 hours, as determined by gas-liquid chromatography, is given in Table I. The results clearly showed that the bromate treatment in dioxane-water caused no significant changes in the fatty acid composition, which supports the finding that such treatment also caused no reduction of bromate to bromide.

Possible Interaction between Bromate and Fat in the Dough. The results discussed so far indicated that there appears to be no direct reaction between bromate and petroleum ether-extracted fat when shaken in dioxane-water mixture. In the dough, the bromate and fat

TABLE I
FATTY ACID COMPOSITION OF PETROLEUM ETHER-EXTRACTED FAT FROM PATENT AND CLEAR FLOURS BEFORE AND AFTER TREATMENT WITH POTASSIUM BROMATE IN DIOXANE-WATER MIXTURE

Fatty Acid[a]	Fat from Patent Flour		Fat from Clear Flour	
	Before Bromate Treatment	After Bromate Treatment	Before Bromate Treatment	After Bromate Treatment
	%	%	%	%
Myristic	0.1	0.1	0.1	0.1
Pentadecanoic	0.1	0.1	0.1	0.1
Palmitic	19.0	18.3	16.7	17.3
Palmitoleic	0.4	0.4	0.4	0.4
Stearic	0.9	1.0	0.9	0.8
Oleic	13.7	14.0	18.2	17.6
Linoleic	61.3	61.6	58.2	58.3
Linolenic	3.2	3.2	3.8	3.6
Eicosenoic	1.3	1.3	1.6	1.8

[a] The presence of minor quantities of less than 0.1% of some fatty acids of lower molecular weight was not accurately measured in the current study.

are present in a much more complex system than in dioxane-water. It has been found that defatting of the flour greatly decreased the amount of bromate reduced to bromide by water-flour doughs (6). This finding, considered together with the absence of any direct reaction between bromate and fat outside of the dough system, pointed to some interaction between bromate and fat in the dough, though such interaction does not have to be a direct reaction but may involve other dough constituents besides the bromate and the fat. From a study on the rates of bromate loss in doughs from defatted and reconstituted flours, Cunningham and Hlynka (5) have reached a similar conclusion that lipid acts as an intermediate between bromate and reducing materials in the flour rather than reacting directly with bromate.

Fatty Acid Composition of the Petroleum Ether Extract of Flour. The quantitative determination of the fatty acid composition of the petroleum ether extract of flour is of interest in itself. Early work by classic methods, such as fractional distillation of the methyl esters and calculation for linoleic and linolenic acids from iodine and thiocyanogen numbers, gave only rough estimations of the most common C_{16} and C_{18} acids (1,9). More recently, Coppock *et al.* (2) reported a qualitative determination of the fatty acid composition of a carbon tetrachloride extract of flour by gas-liquid chromatography. Their findings, indicating that there are large proportions of oleic and linoleic acids relative to the small amount of stearic acid in the C_{18} fraction and that the C_{16} fraction consists mainly of palmitic acid, are in agreement with the present results. These workers (2), however, also reported the presence of a "large unsaturated C_{19} fraction" in their carbon tetrachloride-extracted fat. Such a fraction was not observed in the current studies.

Instead, eicosenoic acid (C_{20}, one double bond) was found to be present in amounts of the order of 1.5% in the petroleum ether-extracted fat of both patent and clear flours.

Reduction of Bromate to Bromide by Water-Flour Slurry, Water-Gluten Slurry, and Aqueous Extract of Flour. The extents of reduction of 1.5 mg. of labeled potassium bromate in slurries of 100 ml. of water and 10 g. of patent flour or of 100 ml. of water and 1.5 g. freeze-dried gluten (1.5 g. is the approximate amount of freeze-dried gluten obtainable from 10 g. of patent flour), as well as the reduction of 1.5 mg. of labeled potassium bromate dissolved in 100 ml. of aqueous extract of patent flour, are given in Table II. Some reduction of bromate to bromide was observed in all samples examined, though the bromide contents in the supernatants from the slurries or in the aqueous extract, expressed on a percentage basis, were relatively low. These low percentages of conversion are to be expected, as the use of 1.5 mg. of bromate per 10 g. of flour is equivalent to a treatment with 150 p.p.m. of bromate. Unfortunately, the specific activity of the Br^{82} available was not sufficiently high to permit the use of, for example, 0.15 mg. of labeled bromate per slurry containing 10 g. of flour. Nevertheless, the results given in Table II do indicate that flour slurry, freeze-dried gluten slurry, and aqueous extract of flour all can reduce some bromate to bromide. The fact that the degrees of reduction of bromate to bromide by the gluten slurry or by the aqueous extract of flour were lower than the corresponding reduction of bromate by the slurry of whole flour (Table II) may also suggest that whole flour contains materials other than gluten or other than the water-extractable components which could cause reduction of bromate to bromide. This is in

TABLE II

BROMIDE CONTENTS IN WATER-FLOUR SLURRY, WATER FREEZE-DRIED GLUTEN SLURRY, AND IN AQUEOUS EXTRACT OF FLOUR AFTER TREATMENT WITH BR^{82}-LABELED POTASSIUM BROMATE[a]

Reaction Time	Flour Slurry		Gluten Slurry		Aqueous Extract
	Recovery of Br^{82} in Supernatant	Bromide Content in Supernatant	Recovery of Br^{82} in Supernatant	Bromide Content in Supernatant	Bromide Content in Solution
hours	%	%	%	%	%
1	92	3	99	2	2
2	92	4	96	1	5
3	88	6	100	3	4
4	92	12	98	4	6

[a] Treatments were 1.5 mg. of labeled potassium bromate with 10 g. flour, 1.5 g. freeze-dried gluten or aqueous extract of 10 g. flour, total volume in each case being 100 ml. All data were calculated from mean activity values of duplicate experiments.

accord with a previous conclusion that bromate, a nonspecific oxidizing agent, may take part in reactions with a number of constituents of flour, rather than with any particular one (6).

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