

Effects of Reducing Agents and Baking Conditions on Potassium Bromate Residues in Bread

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ABSTRACT

Potassium bromate is a well known dough conditioner that has been used for many years in breadmaking around the world. Published results of animal tests, however, have linked potassium bromate with renal cancer in rats. Recently, an analytical method has been developed to provide accurate and precise measurement of potassium bromate residues in bread. This method, which utilizes ion-pairing reverse-phase chromatography and post-column derivatization, can detect potassium bromate residues at levels as low as 0.5 ppb. This HPLC method was used to determine the effects of reducing agents and baking conditions on potassium bromate residues in two types of bread. No residual potassium bromate was detected in pullman-type breads with potassium bromate added at ≤ 15 mg/kg of flour. Residual potassium bromate was detected in open top-type bread with potassium bromate added at ≥ 9 mg/kg of flour. The distribution of residual potassium bromate in open top-type bread was localized in the top region of crust that rises out of the baking pan. Residual potassium bromate was not detected in bread made from the same dough formula when baked with a cover on the baking pan. Adding reducing agents such as L-ascorbic acid and/or ferrous sulfate had a significant effect on potassium bromate residues, reducing or eliminating them.

Potassium bromate is a well known dough conditioner that has been used for many years in breadmaking around the world. It is used to improve dough-handling properties, oven rise, texture, and loaf volume and to control bread properties affected by changes in wheat qualities between crop years.

Potassium bromate is a slow-acting oxidizing agent. It reacts until the end of the make-up stage, moderately increasing dough viscoelasticity. The rate of the reaction then increases rapidly, following the rise in temperature during the final proofing and initial baking stage. Although L-ascorbic acid (AsA) exerts an effect on dough properties similar to that of potassium bromate, the mechanism of its oxidization properties in dough differs from that of potassium bromate. Unlike potassium bromate, AsA is a fast-acting agent that increases dough viscoelasticity during the mixing stage.

Potassium bromate was first introduced as a bread improver in the United States

during the 1910s. In Japan, potassium bromate has been registered as a food additive since 1953. In 1982 and 1983 Kurokawa and coworkers (11,12) published the results of animal tests that demonstrated that potassium bromate, when administered in drinking water, was associated with renal cancer in some rats. Furthermore, the development of analytical methods revealed the presence of potassium bromate residues in finished baked goods, which stimulated regulatory concerns in many countries (2,6). The UN Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) recommended in 1993 that potassium bromate should be removed from the list of approved flour treatment agents in the *Codex Alimentarius*. In Japan, following a request from the Ministry of Health and Welfare, the baking industry voluntarily stopped using potassium bromate. Recently, however, several new breads that use potassium bromate as a dough conditioner have been introduced to the Japanese market with the appropriate regulatory approval.

The baking industry in the United States has investigated both the optimization of baking parameters and effective agents that can be used to reduce residual bromate (1, 5). When used properly, potassium bromate turns into bromide in the finished product.

A recent risk assessment by the FDA estimated 20 ppb bromate as a one in 1 million upper-bound cancer risk level for consumers at the 90th percentile for intake of baked products, which often is used to indicate an insignificant risk level (4).

Due to health concerns and regulations, analytical methods that provide accurate and precise data on potassium bromate residues have been developed. Initially, high-performance liquid chromatography (HPLC) with a post-column reaction was developed to determine the amount of bromate in water (14). More recently, an HPLC method (8) that combines the clean-up procedure with a series of solid-phase extraction columns was developed to detect bromate residue levels as low as 3 ng/g in bread and has received approval with peer-verified status from AOAC International (9).

The current study was designed to measure the effects of bread type, ingredients, and processing conditions on potassium bromate residues in finished baked products as measured using the improved analytical method. This methodology, including the preconcentration procedure used in this study, has detection levels as low as 0.5 ppb potassium bromate in bread (10).

MATERIALS AND METHODS

Reagents

Chemicals were obtained from commercial manufacturers. Potassium bromate, prepared as a stock standard (1,000 $\mu\text{g/mL}$ as KBrO_3), was obtained from Kanto Chemical Co. (Tokyo). *o*-Dianisidine dihydrochloride was obtained from Sigma Chemical Co. (St. Louis). Tetrabutylammonium hydroxide (TBAH) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). These chemicals were reagent-grade. Methanol and water used in HPLC were from Kanto Chemical (Tokyo) and were HPLC-grade. Ultra-refined water (18 $\text{m}\Omega \cdot \text{cm}$ electrical resistance) was prepared using water-purification apparatus (Milli-Q Lab, Millipore Corporation, Billerica, MA).

Potassium bromate (Kishida Chemical Co., Ltd., Osaka, Japan) and AsA and ferrous sul-

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fate (Junsei Chemical Co., Ltd., Tokyo) used for baking were food additive-grade. Wheat flour, supplied by Nisshin Flour Milling Co., Ltd. (Tokyo), contained 12.3% protein (N \times 5.7) and 0.42% ash.

Determination of Residual Potassium Bromate in Bread

A high-performance liquid chromatograph with a post-column reaction system (Waters 616 LC, Millipore) equipped with a UV/VIS detector (Model SPD-10AV VP, Shimadzu Co., Ltd., Kyoto, Japan), autosampler (Model 717), and reaction coil (Waters RXN 1000 COIL, PN 030844), incubated

at 60°C by a column oven (Waters CHM), was used. An inert pump (Model 301, Flom Co., Ltd., Tokyo) was used to deliver a post-column reagent. Analytical columns used included the Zorbax SB C-18 (4.6 mm \times 25 cm) with Zorbax Guard column SB C-18 (Agilent Technologies, Inc., San Jose, CA). A chromatographic manager (Waters Millennium) was used for instrument control and data collection and processing.

HPLC conditions were set at the mobile phase (10% methanol solution containing 2.0 g of acetic acid and 45 g of 10% TBAH, adjusted to pH 5.0 with TBAH). The mobile phase was delivered isocratically at 0.9

mL/min, and injection volume was 100 μ L. The column was maintained at room temperature (25–28°C), and the UV/VIS detector was set at 450 nm.

Both the HPLC column eluent and the post-column reagent were directed into a reaction coil incubated in a column oven at 60°C. The eluent from the tubing was monitored at 450 nm with a UV/VIS detector. The flow rate of the reaction reagent was 0.3 mL/min.

The loaf of bread was sliced and homogenized using a blender. A 10-g bread sample was mixed for 30 min with 50 mL of ultra-refined water (18 m Ω · cm electrical resistance) using a magnetic stirrer. The sample solution was centrifuged at 12,000 rpm for 10 min. After centrifugation, the supernatant was filtered with filter paper (No. 5A, Toyo Roshi Kaisha, Ltd., Tokyo). A 10-mL sample solution was loaded in the clean-up columns connected, in order, to a 0.45- μ m membrane filter (Millex LH, Millipore), a desalting cartridge (Onguard Ag, Dionex Corporation, Sunnyvale, CA), and a reverse-phase and anion-exchange column (OASIS MAX, Waters Corporation, Milford, MA) to adsorb residual potassium bromate in the sample solution. Next, the clean-up columns were washed with 1 mL of acetic acid (20%, v/v) and 2 mL of ultra-refined water. Potassium bromate adsorbed onto the clean-up col-

Table I. Experimental pullman- and open top-type bread formulas used with sponge and dough method

Ingredient	Pullman		Open Top	
	Sponge (%)	Dough (%)	Sponge (%)	Dough (%)
Flour	70	30	70	30
Yeast	2	–	2	–
Yeast food ^a	0.1	–	0.1	–
Emulsifier	0.3	–	0.3	–
Salt	–	2	–	2
Nonfat dry milk	–	2	–	2
Sugar	–	7	–	5
Shortening	–	5	–	5
Egg	–	–	–	2
Water	40	28	40	25

^a Without oxidants.

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umns was eluted with 1 mL of 0.5% sodium nitrate.

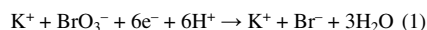
Preparation of Bread

Pullman- and open top-type breads were made using the sponge and dough method (Table I). The sponge was mixed in a mixer (SS-111, Kanto, Tokyo) for 3 min at low speed (100 rpm) and 2 min at medium speed (200 rpm) at 24°C and then fermented for 4 hr at 27°C. Dough ingredients were added to the sponge and mixed for 2 min at low speed and 6 min (pullman) or 4.5 min (open top) at medium speed at 27°C. After resting for 20 min, the dough was divided and scaled at 500 g (pullman) or 520 g (open top), rested for 20 min, sheeted, and molded. For the pullman- and open top-type breads, three and two pieces of dough, respectively, were panned into a bread pan (12.2 cm wide × 13.8 cm high × 36.3 cm long; 6,170.39-mL volume). The formula for the breads included untreated patent flour (12.3% protein [N×5.7] and 0.42% ash), compressed yeast (Oriental Yeast Co., Ltd., Tokyo), emulsifier (Emulgee MM100, Riken Vitamin Co., Ltd., Tokyo), granulated sugar (Dai-Nippon Meiji Sugar Co., Ltd., Tokyo), vegetable shortening (Miyoshi Oil & Fat Co., Ltd., Tokyo), nonfat dry milk (Meiji Dairies Corporation, Tokyo), and refined salt (Shinnihon Salt Co., Ltd., Tokyo). The reducing agents, potassium bromate, AsA, and ferrous sulfate, were food additive-grade.

The breads were analyzed by post-column HPLC as described above. Potassium bromate was added to pullman-type bread at levels of 13, 15, and 30 ppm (based on flour weight) and to open top-type bread at levels of 9, 13, and 30 ppm (based on flour weight).

Effect of Reducing Agents

The degradation of potassium bromate progresses by



From this equation it is apparent that the electron donor plays an important role in promoting the degradation of potassium bromate (1). Reducing agents, e.g., ferrous sulfate and AsA, act as electron donors and are known to have an accelerated degradative effect on potassium bromate (3,7,13).

To confirm the effect of reducing agents on the decrease in residual potassium bromate found in open top-type bread, ferrous sulfate and AsA were added to the sponge. Open top-type breads were produced by adding 11.7 ppm potassium bromate combined with ferrous sulfate (15, 30, or 45 ppm) and AsA (20, 30, 40, or 50 ppm). In addition, the effects of cysteine (CySH) and glutathione (GSH) on reduction of residual potassium bromate were evaluated. A concentration of CySH (11.7 ppm) or GSH (30 ppm) was added to the sponge in combination with 11.7 ppm potassium bromate, 15 ppm ferrous sulfate, and 20 ppm AsA.

Levels and Distribution of Residual Potassium Bromate

Dough and bread samples were collected to monitor residual potassium bromate levels during the breadmaking process. Both pullman- and open top-type breads were prepared by adding 11.7 ppm potassium bromate (in the form of powder or aqueous solution), with or without 15 ppm ferrous sulfate and 20 ppm AsA. Samples for analysis were collected 1) after sponge mixing, 2) after dough mixing, 3) before final proofing, 4) after proofing, 5) after baking for 10 min, 6) after baking for 20 min, and 7) after cooling. Samples 1–6 were freeze-dried and homogenized in a blender, and sample 7 was homogenized immediately after cooling. The potassium bromate in the samples was calculated based on a ratio to the concentration of added potassium bromate.

The distribution of residual potassium bromate in pullman- and open top-type breads with added potassium bromate was measured. The sampling areas are shown in Fig. 1. Po-

tassium bromate was added to the sponge in pullman- and open top-type breads at 11.7 ppm (aqueous solution), as well as 15 ppm ferrous sulfate and 20 ppm AsA.

Effects of Baking Conditions on Open Top-Type Bread

The effects of baking temperature, baking time, and use of a baking pan lid on potassium bromate residues were evaluated in open top-type bread. Open top-type bread, which is commonly baked at 170°C for 30 min, was baked at 210°C for various baking times (16, 22, 26, and 33 min) because pullman-type bread is usually baked at 210°C. The effect of baking an open top-type bread with a baking lid also was evaluated. Potassium bromate was added at 11.7 ppm (powder form).

Residues in Pullman-Type Bread Produced in Different Bread Plants

In bread plants equipped with large-scale bakery equipment, a large amount of dough (150–300 kg of flour) is prepared at one time, whereas only a small amount of flour (3–4 kg) is used in experimental baking. Furthermore, not every bakery machine and facility can expect to operate exactly the same. The aim of this study was to obtain information from actual processing settings rather than controlled laboratory experiments. Pullman-type bread was prepared by adding 12 ppm potassium bromate to flour in combination with 5 ppm AsA. The residual potassium bromate in the finished bread was measured.

RESULTS AND DISCUSSION

Residual Potassium Bromate in Experimental Breads

Residual potassium bromate measured in pullman- and open top-type breads is shown in Table II. No residues were detected in pullman-type bread with 13 or 15 ppm potassium bromate added to the flour, and the level was as low as 0.9 ppb for breads with 30 ppm potassium bromate added. Residual potassium bromate was detected in open top-type bread with 9 ppm potassium bromate added, and concentrations increased with increasing addition of potassium bromate. In pullman- and open top type-breads produced using the sponge and dough meth-

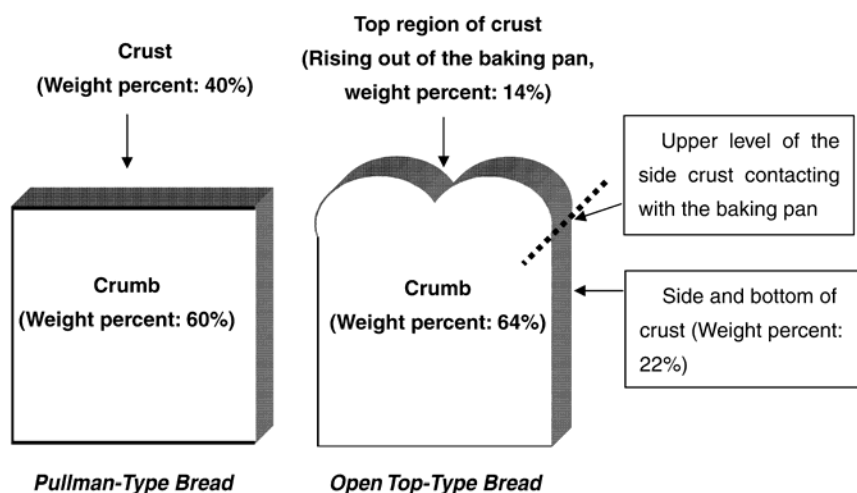


Fig. 1. Sampling for the distribution measurement of residual potassium bromate in two breads.

Table II. Potassium bromate (PB) residues in breads with added PB

Bread Type	Added PB (ppm, based on flour)	PB Residue (ppb, in bread)
Pullman	13	ND ^a
	15	ND
	30	0.9
Open top	9	7.1
	13	13.3
	30	48.0

^a Not detected; the detection limit of this method is 0.5 ppb.

od, 13 ppm potassium bromate added represented a compromise between improving effect and residue level. A significantly higher level of residual potassium bromate in open top-type bread was detected compared with that of pullman-type bread. Factors affecting potassium bromate residues in open top-type bread were studied further.

Effect of Reducing Agents in Open Top-Type Bread

The effect of reducing agents on potassium bromate residues was evaluated in open top-type bread. Potassium bromate was added at 11.7 ppm, based on 90% of the suggested addition of 13 ppm. Because the degradation of bromate begins after the dissociation of potassium bromate, the effects of adding potassium bromate as a powder or in aqueous solution were compared. As shown in Table III, when potassium bromate was added in powder form without AsA, the amount of residual potassium bromate in open top-type bread tended to decrease as the concentration of ferrous sulfate increased. However, increased levels of ferrous sulfate added led to decreased final

Table III. Effects of added ferrous sulfate (FS) and L-ascorbic acid (AsA) on reduction of potassium bromate (PB) residues in open top-type bread

PB Form ^a	Concentration (ppm, based on flour)		PB Residues (ppb)
	FS	AsA	
Powder	0	0	21.0
	15	0	11.5
	30	0	10.5
	45	0	5.5
	15	20	6.2
	15	30	2.8
	15	40	1.5
Aqueous solution	0	0	12.7
	15	20	2.1
	15	30	ND ^b
	15	40	ND
	15	50	0.8
	15	50	ND

^a Concentration of added potassium bromate was 11.7 ppm.

^b Not detected; the detection limit of this method is 0.5 ppb.

Table IV. Effects of baking conditions on potassium bromate (PB) residues in open top-type bread

Baking Condition			PB Residues (ppb)
Baking Pan	Temp. (°C)	Time (min)	
Uncovered	170	30	14.0
Covered	170	30	ND ^a
Uncovered	210	16	56.2
Uncovered	210	22	9.7
Uncovered	210	26	7.4
Uncovered	210	33	2.8

^a Not detected; the detection limit of this method is 0.5 ppb.

product quality. Sensory evaluation of these breads suggested that ferrous sulfate should be added at 15 ppm or less, even when added with potassium bromate. The amount of residual potassium bromate in open top-type bread with various concentrations of AsA (20, 30, 40, or 50 ppm) was determined in combination with 15 ppm ferrous sulfate. Potassium bromate residues tended to decrease as the concentration of AsA increased.

Adding potassium bromate in an aqueous solution resulted in much lower potassium bromate residues compared with the powder form. The levels of potassium bromate residues were below the detection limit when combined with more than 30 ppm AsA; however, more than 30 ppm AsA added to flour inhibited the benefits in baking quality obtained with the use of potassium bromate. Therefore, to obtain optimal baking quality and the lowest amount of residual potassium bromate, the recommendation for open top-type bread would be to add 11.7 ppm potassium bromate (powder form) in combination with 15 ppm ferrous sulfate and 20 ppm AsA. Using this formula, 6.2 ppb potassium bromate residue remained. When the reducing agent CySH or GSH was added in open top-type bread in combination with AsA (20 ppm) and ferrous sulfate (15 ppm), residual potassium bromate was reduced. The concentrations were almost equimolar with that of potassium bromate. Although both CySH and GSH showed an effect in reducing potassium bromate residues, 3–5 ppb potassium bromate residues remained in open top-type bread.

Changing Ratios of Residual Potassium Bromate

The degradation of residual potassium bromate throughout the baking process was evaluated to clarify in which stage of the process degradation was promoted. As shown in Fig. 2, approximately 50 and 30% of potassium bromate added to flour remained after mixing and final proofing, respectively, and then, a remarkable drop was observed during the baking stage. Although the remaining ratio of potassium bromate in the dough stage before baking when potassium bromate (powder or aqueous solution) was used in combination with ferrous sulfate and AsA followed a pattern similar to that of potassium bromate used alone before baking, degradation of potassium bromate began earlier during baking.

Subsequently, the cause of accelerating degradation during baking in pullman-type bread, in which no residues were observed, was compared with that in open top-type bread. Both pullman- and open top-type breads were prepared by adding 11.7 ppm potassium bromate (powder form) in combination with 15 ppm ferrous sulfate and 20 ppm AsA. The change in the remaining ratio during the dough stage of pullman-type bread was similar to that of open top-type bread. However, during baking, the remaining ratio in pullman-type bread decreased significantly compared with that of open top-type bread (data not shown). These results suggest that baking conditions affect the degradation of residual potassium bromate in bread and that it is accelerated in pullman-type bread.

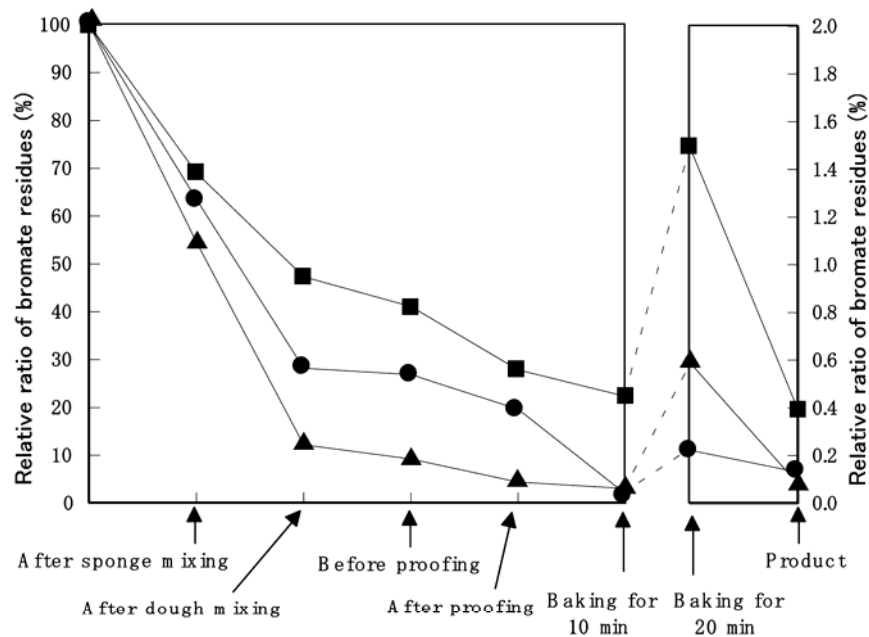


Fig. 2. Change in remaining ratios of potassium bromate residues during processing of open-top type breads. ■ = Potassium bromate added at 11.7 ppm (powder); ● = potassium bromate added at 11.7 ppm (powder), ferrous sulfate added at 15 ppm, and L-ascorbic acid added at 20 ppm; ▲ = potassium bromate added at 11.7 ppm (aqueous solution), ferrous sulfate added at 15 ppm, and L-ascorbic acid added at 20 ppm.

Distribution of Residual Potassium Bromate in Bread

The distribution of residual potassium bromate was measured in pullman- and open top-type breads prepared by adding 11.7 ppm potassium bromate (powder form) in combination with 15 ppm ferrous sulfate and 20 ppm AsA. Residual potassium bromate was not detected (the detection limit of this method is 0.5 ppb) in the crumb, crust, or whole bread sample of the pullman-type bread. In open top-type bread, residual potassium bromate was detected at levels up to 14.8 ppb in the top region of the crust that rises out of the baking pan and at 2.1 ppb in the whole bread sample but not in the crumb or side and bottom of the crust. These results indicate that the residual potassium bromate found in whole open top-type bread was localized in the top region of the crust.

When the dough was baked without a baking lid, potassium bromate remained in the top region of the crust in open top-type bread. It did not remain in pullman-type bread made from the same dough formula when baked with a baking lid. We also investigated baking conditions for efficiency of residual potassium bromate degradation in the region of the crust.

Effects of Baking Conditions on Residual Potassium Bromate in Bread

The effects of baking conditions, including the influence of a baking lid, baking temperature, and baking time, on the reduction of potassium bromate residues in open top-type bread were investigated (Table IV). Residual potassium bromate was not detected in bread baked with a baking lid but was detected in bread baked without a lid. As baking time increased from 16 to 33 min at 210°C, residual potassium bromate in the bread decreased, with 2.8 ppb detected in bread baked for 33 min. The data suggest that using extreme baking conditions, such as baking times longer than 33 min or temperatures higher than 210°C, will not totally eliminate residual potassium bromate. Based on the results, putting a lid on the baking pan during baking of open top-type bread dough is more effective in decreasing residual potassium bromate than changing the dough formula, baking temperature, or baking time.

It is presumed that the localization of residual potassium bromate in the top region of the crust that rises out of the baking pan is related to a decrease in the reactive effi-

ciency caused by drying of the crust or rapid thermal denaturation of the protein. However, further research will be needed to clarify the details of this mechanism and lower potassium bromate residues in open top-type bread baked without a baking lid to less than the detection limit (0.5 ppb).

Residues in Pullman-Type Bread Produced in Different Bread Plants

The results obtained in this study confirmed that potassium bromate residues do not remain in pullman-type bread at levels higher than the detection limit (0.5 ppb) when 15 ppm or less potassium bromate is added in experimental baking. Subsequently, we studied whether results obtained from a laboratory could accurately reflect the residual potassium bromate levels found in bread produced with actual process settings in a baking plant. Potassium bromate residues in pullman-type bread with 12 ppm potassium bromate added in combination with 5 ppm AsA and baked in six different plants, using large-scale bakery production equipment, were measured. No residual potassium bromate was detected in breads produced in different plants (150~300 kg of flour) or in the laboratory.

CONCLUSIONS

A post-column HPLC method developed to provide accurate and precise measurement of potassium bromate residues in bread was used to determine the effects of reducing agents and baking conditions on potassium bromate residues in two types of bread (pullman and open top). Using this method, we found that levels of residual potassium bromate were lower than the detection limit of 0.5 ppb in pullman-type bread to which potassium bromate was added at ≤ 15 mg/kg of flour. Residual potassium bromate levels were detectable in open top-type bread, however, when the optimum amount of potassium bromate was added (≥ 9 mg/kg of flour). Of the various formula specification and process condition parameters evaluated, 20 ppm AsA added in combination with 15 ppm ferrous sulfate accelerated the reduction of potassium bromate residues in both types of bread.

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