Effect of Gaseous Acetic Acid on Dough Rheological and Breadmaking Properties

MASAHARU SEGUCHI,[1,2] MACHIKO HAYASHI,[1] and HIROSHI MATSUMOTO[1]

Cereal Chem. 74(2):129–134

ABSTRACT

Breads baked from wheat flours (protein contents 14.1–16.5% at 14.0% mb) that were pretreated with 2–3 mL of gaseous acetic acid per kg of wheat flour, showed maximum bread height and specific volume (cm^3/g). Flour-water suspension and the crumb pH values were gradually decreased with increased amounts of acetic acid. Gas generation and dough expansion tests with bread dough showed that the addition of the same amount of acetic acid, which achieved maximum specific volume, also showed the highest rate of gas generation and dough expansion. However, increasing acetic acid decreased these values. Scanning electron microscope (Cryo-SEM) observation showed that the bread dough made from the same acetic acid-treated flour indicated continuum and no cracks in the dough matrix. Evaluation of mixograms showed the decrease of mixing stability with increased acetic acid levels. Viscosity and water binding capacity of flour-water suspensions were sharply increased by the addition of acetic acid at pH 5.0–3.5.

The improving effect by potassium bromate in breadmaking was first reported by Kohman et al (1915), and the usage of the bromate in breadmaking has been popular all over the world as an important improving method. Recently, bromate has been shown to be a carcinogen (Kurokawa et al 1983). Known replacements for the bromate, such as ascorbic acid, azodicarbonamide (ADA), and enzymes are used as flour improvers (Ranum 1992). We have searched for a more effective bread-improving method and arrived at a new method using a pH-dependent dough softness and higher gas production by yeast with a small amount of acetic acid. The rheological properties of dough are affected by changes in pH (Tsen 1966). Chlorination effects on baked volume improve the cake making properties of soft wheat flours (Bailey and Johnson 1924, Kulp 1972, Seguchi and Matsuki 1977). Chlorine gas reacted with sulfhydryl groups of protein and resulting HCl decreased the pH of dough (Eliasson and Larsson 1993). Bennett and Ewart (1962) reported that properties of wheat flour dough are sensitive to changes in pH, and acids react strongly with the protein of dough. Tanaka et al (1967) reported that farinograph dough consistency was decreased by lowering the pH with acetic acid. However, when salt (NaCl) was omitted from dough, the consistency increased with a decrease of pH. Galal et al (1978) summarized that lowering the pH will result in increasing net positive charge on the proteins. Furthermore, the study of mixograph test by Hoseney and Brown (1983) showed that decreasing the pH of flour-water dough gave mixograms with decreased mixing stability. Those data indicate that acids act as a dough softener as do L-cysteine hydrochloride and sodium metabisulphite, rather than as a dough strengtheners such as bromate, azodicarbonamide, and ascorbic acid. The effect of acid on the yeast in the leavening activity of baker's yeast is fairly constant at pH 4–6 (Pyler 1988). Most doughs have a pH value within this range. However, between pH 3 and 4, yeast activity gradually dropped; below pH 3, a sharp decrease was usually observed (van Dam 1988). Acetic acid shows low degree of dissociation and indicates strong inhibition of fermentation of yeast. This could be due to denaturation of proteins, inhibition of metabolic enzymes, inhibition of incorporation of amino acids to yeast cells from outside, and decrease of cell pH (Yamamoto et al 1989). However, it was also reported that yeast could assimilate the acetic acid at lower concentrations (10 m*M*) of acetic acid with pH 5 (Yamamoto et al 1990). In this article, we report the improved baking results obtained by acetic acid-treated wheat flour and discuss the improving effects on bread dough by acetic acid.

[1]Faculty of Home Economics, Laboratory of Food Technology, Kobe Women's University, Suma-ku, Kobe City, Japan 614.
[2]Corresponding author. E-mail: seguchi@suma.kobe-wu.ac.jp

Publication no. C-1997-0205-01R.

MATERIALS AND METHODS

Wheat Flours

Wheat flours used in this experiments were brand names, Red Knight (Nitto Flour Milling Co. LTD., Japan), Super King, Million, and Number One (Nissin Flour Milling Co. LTD., Japan), made from Hard Red Spring wheat flour. Protein conversion was N × 5.7, and ash was determined according to standard approved method 08–01 (AACC 1995) at 14.0% mb (Table I).

Acetic Acid Treatment of Wheat Flour

Wheat flour was treated with gaseous acetic acid at room temperature. Wheat flour (1.0 kg) was packed into a column (7 cm, dia.; 41 cm, height) and set (Fig. 1). Compressed air was introduced from the bottom of the column at 1.0 L/min. Variable volumes (mL) of 99.7% acetic acid solution were taken into a sample bottle, which was connected with tubes between compressor and column (Fig. 1 A–C). Gaseous acetic acid in compressed air was introduced into wheat flour overnight. Presence of acetic acid gas from the column outlet was qualitatively checked by litmus test paper. Control flour was also subjected to overnight aeration in the apparatus.

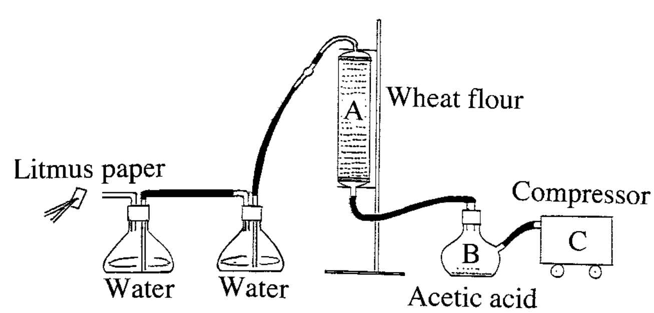

Fig. 1. Apparatus for treating wheat flour with acetic acid. A, column (diameter 7 cm and height 41 cm); B, sample bottle; C, compressor.

Determination of pH and Amount of Acetic Acid in Wheat Flour and Bread Crumb

The pH of wheat flour and bread crumb were determined by approved method 02–52 (AACC 1995). Acetic acid gas from the column outlet was dissolved into water and measured by titration (AACC approved method 04-20). The amount of "free" acetic acid in flour was first determined by measuring the amount of water soluble-acetic acid fraction; the amount of "bound" acetic acid in flour was obtained by calculating the difference between total amount of starting acetic acid and the amount of free acetic acid.

Brabender Farinograph and Mixograph Tests of Wheat Flour

Baking absorption was measured in farinograph with 300 g of flour (AACC approved method 54–21). Dough development was also measured in mixograph, which was performed with wheat flour (35 g) as described by Miller and Johnson (1954).

Scanning Electron Microscope Observations

Scanning electron microscope (SEM) observation of the dough was performed by Hitachi S-570 scanning electron microscope (20 kV) with cryo-system. Samples were prepared as follows. After second proof, bread dough was put on the sample stage and frozen by liquid nitrogen. The frozen dough was cracked, the temperature was raised to –60°C, and water was sublimated. Sample was coated with gold and observed by SEM.

TABLE I
Protein[a] and Ash Contents (%) of Wheat Flours[b,c]

Flour	Protein	Ash
Red Knight	14.1 ± 0.4	0.37 ± 0.02
Super King	16.5 ± 0.5	0.43 ± 0.03
Million	14.7 ± 0.4	0.41 ± 0.03
Number One	15.6 ± 0.3	0.38 ± 0.02

[a] N × 5.7.
[b] 14.0% mb.
[c] Values represent average and standard deviation of three replicates.

Gas Generation and Dough Expansion Tests of Bread Dough

Gas generation and dough expansion tests of bread dough were performed by the method of Hosomi et al (1992). After first proof, bread dough (100 g) was rounded and put into a glass cylinder (5 cm, dia.; 36 cm, height). The rate of gas generation (mL/10 min) was measured by the water displacement method. Dough expansion (mm/10 min) was measured with Cathetometer PCTM-300 (Pika Co. Ltd., Japan) in a constant-temperature waterbath (35°C) for 100 min.

Viscosity and Water Binding Capacity of Flour-Water Suspension

Wheat flour (50 g) was mixed with 300 mL of water and pH adjusted to 2.0–9.0 by addition of 17*N* acetic acid or 5*N* NaOH, and shaken vigorously for 60 min at room temperature. When the pH was acidic, 17*N* acetic acid was used; when the pH was alkaline, 5*N* NaOH was used. The viscosity (cP) of flour-water suspension was measured by Viscotester VT-03 and -04 (Rion Co. Ltd., Japan). The mixture was centrifuged at 1,700 × *g* for 10 min. Weight of the precipitate after 12 hr at 70°C was measured. Water binding capacity (WBC) was calculated as (wet wt. – dry wt.)g/dry wt. (g) × 100(%).

TABLE II
Bread Height (mm) of Flours Treated with Acetic Acid[a]

Acetic Acid (mL/kg)	Red Knight	Super King	Million	Number One
0.0	61.7 (0.4)a	85.4 (3.8)a	79.4 (2.9)a	83.4 (3.1)
1.0	68.8 (1.2)	90.9 (0.6)b	83.5 (2.3)a	93.8 (4.1)a–c
2.0	73.9 (0.6)	96.6 (1.5)	89.4 (6.1)b	93.4 (4.2)a–c
3.0	60.1 (0.6)a	87.9 (4.1)a–c	91.5 (0.2)b	98.2 (1.4)b,c
4.0	51.5 (2.3)	83.1 (2.6)a,c,d	66.7 (2.3)c	95.9 (2.1)c
5.0	· · ·	84.6 (2.8)a,c,d	64.6 (2.3)c	65.3 (1.4)

[a] Values represent means of four replicates and standard deviation in parenthesis. Means followed by the same letters in columns are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

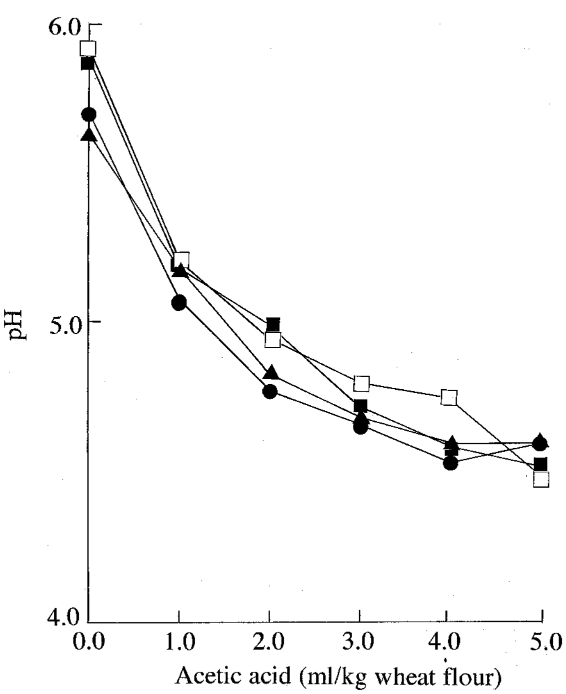

Fig. 2. Change of pH values in wheat flours treated with increasing levels of acetic acid. Flour samples: ● = Red Knight; ■ = Million; ▲ = Super King; □ = Number One.

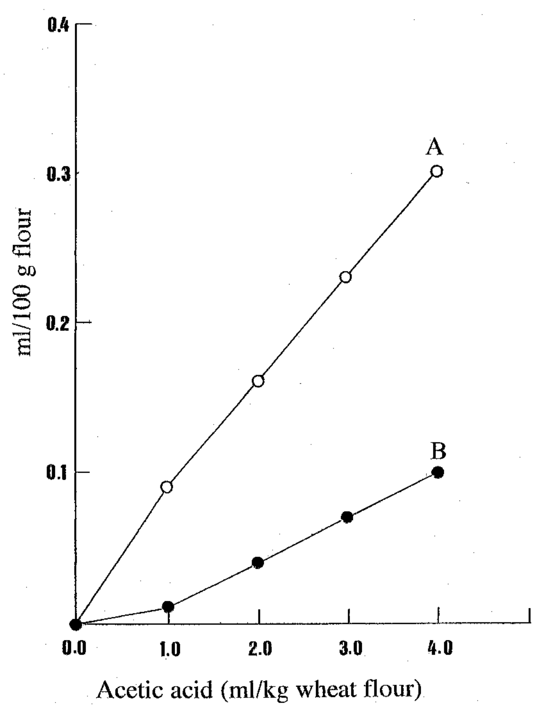

Fig. 3. Acetic acid form in Red Knight wheat flour. A, water-soluble ("free"); B, insoluble ("bound").

Breadmaking Method

Wheat flour (100%), compressed yeast (2.9%), sugar (5.0%), salt (1.0%), and water according to baking absorption estimated from farinograph absorption at 500 BU were mixed and put into a computer-controlled National Automatic Bread Maker (SD-BT6, Matsushita Electric Ind. Co. Ltd., Japan) and processed to first proof for 2 hr 20 min comprising 15 min for first mixing, 50 min of rest, 5 min for second mixing, and 70 min for fermentation. Dough was divided into 120-g pieces, rounded and molded, and placed in a baking pan (AACC approved method 10-10A). In this experiment, the AACC method was slightly modified and the dough was divided into 120-g pieces. The reason for the modification was that when larger dough pieces (160 g) were baked, the amount of bread outside the pan varied and a constant volume was not obtained. The dough was subjected to second proof for 22 min at 38°C, and baking was performed at constant temperature (210°C) (oven model DN-63 Yamato Scientific Co. Ltd., Japan) for 30 min. After baking, the bread was taken out of the pan and left for 1 hr at constant room temperature (26°C) and relative humidity (43%). Bread height (mm), weight (g), and volume (cm^3) were measured, and crumb grain was evaluated visually.

Statistical Analysis

Experiments were done in three or four replicates and the results averaged. Analytical determinations of individual samples were replicated four times. Analysis produced significant *F* values by analysis of variance, followed by Duncan's multiple range test for comparison of means.

TABLE III
Specific Volume (cm^3/g) of Flours Treated with Acetic Acid[a]

Acetic Acid (mL/kg)	Red Knight	Super King	Million	Number One
0.0	2.85 (0.08)a	4.09 (0.09)	3.47 (0.07)	3.67 (0.08)
1.0	3.19 (0.01)b	4.51 (0.04)	3.87 (0.05)a	4.09 (0.07)
2.0	3.33 (0.06)b	4.99 (0.09)	4.00 (0.11)a	4.32 (0.18)a
3.0	2.65 (0.07)a,c	4.30 (0.13)	4.23 (0.10)	4.71 (0.14)
4.0	2.58 (0.14)c	3.90 (0.08)a	2.74 (0.52)b	4.49 (0.10)a
5.0	· · ·	3.89 (0.09)a	2.73 (0.08)b	2.56 (0.13)

[a] Values represent means of four replicates and standard deviation in parenthesis. Means followed by the same letters in columns are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

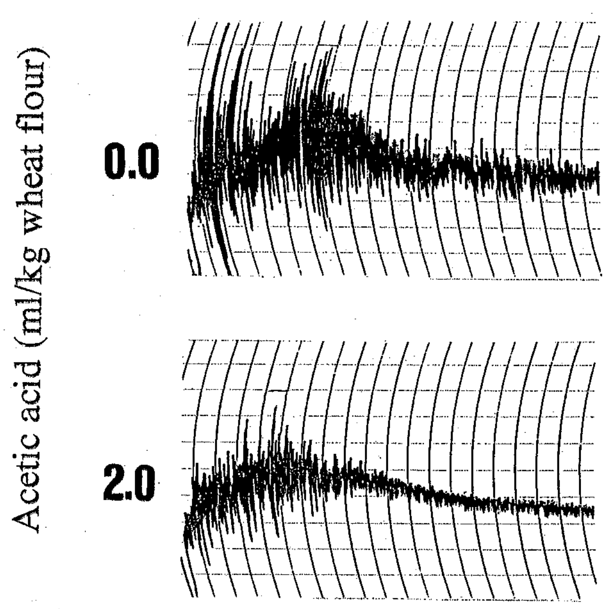

Fig. 4. Mixograms of Red Knight flour treated with acetic acid. Top and bottom: 0.0 and 2.0 mL of acetic acid/kg of flour, respectively.

RESULTS AND DISCUSSION

Acetic Acid Treatment of Wheat Flour

The pH of wheat flours (Red Knight, Million, Super King, and Number One flours) treated with acetic acid (Fig. 2) decreased from 5.7 to 4.5 as acetic acid increased from 0 to 5.0 mL/kg of flour. All flours showed the same pH decrease with increased acetic acid treatment. Just after treatment with 2.0 mL of acetic acid to 1 kg of Red Knight flour, which is the level for maximum height and specific volume of bread with the flour (Tables II and III), the nonabsorbed acetic acid from the outlet of the column (Fig. 1) was qualitatively checked by litmus test paper. Blue color of litmus test paper did not change to red, which indicated that acetic acid was not present in the compressed air from outlet of the column. Furthermore, the amount of acetic acid in water from outlet of the column (Fig. 1) was also measured by titration. However, the amount of acetic acid was so small (0.2%) that almost all of the acetic acid considered absorbed into the flour. It would be interesting to know whether the absorbed acetic acid is bound to flour components covalently or not. In this experiment, the mechanism of the binding to flour was not studied, although the amount of water-soluble acetic acid (free) and insoluble acetic acid (bound) in the flour was measured (Fig. 3). Line A and B shows the amount of free and bound form acetic acids respectively. The amount free form acetic acid may be 80% of total acetic acid.

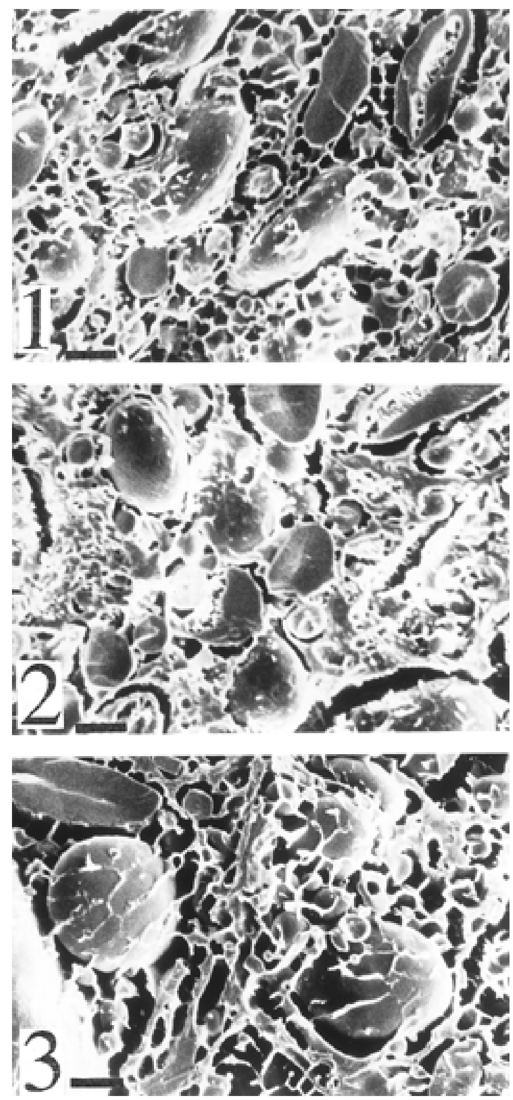

Fig. 5. Scanning electron microscopy of Red Knight bread dough. 1–3: 0.0, 2.0, and 4.0 mL of acetic acid/kg of flour, respectively.

Farinograph and Mixograph Tests of Treated Wheat Flour

Baking absorptions (%) for breadmaking of each flour were estimated from the farinograph absorption at 500 BU (Table IV). Mixogram of Red Knight flour showed that the control dough was rather strong and stable to overmixing. However, the addition of acetic acid (2.0 mL/kg of flour) gave mixograms with decreased mixing stability (Fig. 4). That effect was explained by the change in charge on the protein due to pH change (Hoseney and Brown 1983).

SEM Observation of Bread Dough

Ultrastructures of bread dough of Red Knight flour after second proof were observed by SEM using the cryo-system (Fig. 5). Dough matrix from flour treated with 2 mL of acetic acid was more continuous than that of the other doughs. Dough matrix of the 4.0 mL/kg treatment clearly had many cracks and was not continuous. It appears that the presence of discontinuities in dough is related to the lower bread height and specific volume (Tables II and III).

Gas Generation and Dough Expansion Tests of Bread Dough

To obtain additional information on the effects of acetic acid on bread dough, the rates of the gas generation and dough expansion were measured in each dough from the Red Knight flour (Fig. 6) by the method of Hosomi et al (1992). In the control dough, the rate of gas generation was about 50 mL/10 min at the beginning and was maintained for 50 min, and after it gradually decreased with time (Fig. 6). Over the same time, the rate of the dough expansion of the same dough was measured. The rate initially was 2.0 mm/10 min, maintained for 50 min, and then decreased (Fig. 6). The dough from flour treated with 2.0 mL of acetic acid/kg of flour, showing the maximum bread height and specific volume (Tables II and III), indicated that the highest rates of gas generation (60–70 mL/10 min) and dough expansion (3.0–3.3 mm/10 min) were maintained for the initial 20 min. Higher initial rates of gas generation and dough expansion, before the dough is baked into bread, would be necessary for finer crumb structure. When the flour treated with 4.0 mL of acetic acid/kg of flour was used, the initial rates of both gas generation and dough expansion were lower, but they were maintained for a long time. From these results it is obvious that yeast needed a small amount of acetic acid (2.0 mL/kg) for the highest speed of the gas generation. It was reported that in lower concentration, yeast could assimilate and ferment acetic acid (10 m*M* at pH 5.0) (Yamamoto et al 1990). It was further known that yeast could absorb acetic acid and maintain in the cell (3 mg/g of dry cells) (Maehashi et al 1996). But the rate decreased with increase of the acetic acid treatment level. Acetic acid acted as toxic material for yeast and inhibited the yeast fermentation with more than 0.17*M* acetic acid (Yamamoto et al 1990). It has been also known that glutathione is released from the dead yeast cells and damages breadmaking (Wolt and D'Appolonia 1984). There is a possibility that glutathione may be released from yeast when the flour is treated with higher level of acetic acid. This point was not investigated in the present study.

TABLE IV
Baking Absorption (%) of Flours Treated with Acetic Acid

Acetic Acid (mL/kg)	Red Knight	Super King	Million	Number One
0.0	69.0	65.4	62.6	69.6
1.0	70.0	62.7	57.7	66.4
2.0	70.0	60.9	65.0	65.0
3.0	72.0	64.2	66.0	61.2
4.0	72.9	62.3	66.3	65.5
5.0	· · ·	65.8	63.1	67.0

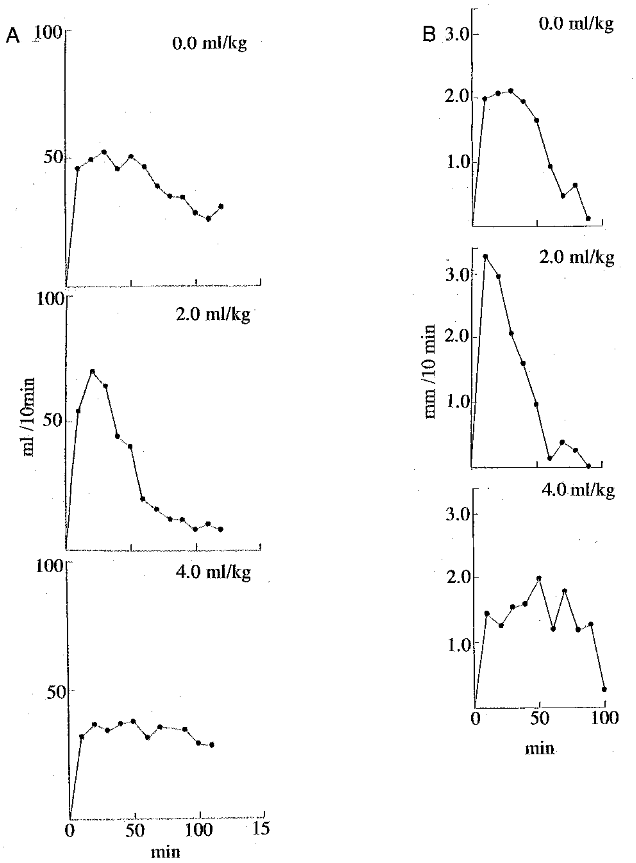

Fig. 6. Rates of gas generation (mL/10 min) (**A**) and dough expansion (mm/10 min) (**B**) in Red Knight bread dough treated with different levels of acetic acid.

Changes of Viscosity and WBC of Flour-Dough by pH Change

The viscosity and WBC of Red Knight flour dough were measured at various pH values. The results show that the viscosity of flour dough was drastically increased between pH 5.0 and 3.5 (Fig. 7), and, concomitantly, there was drastic increase of the WBC of the flour-water suspension (Figs. 8 and 9). The change of

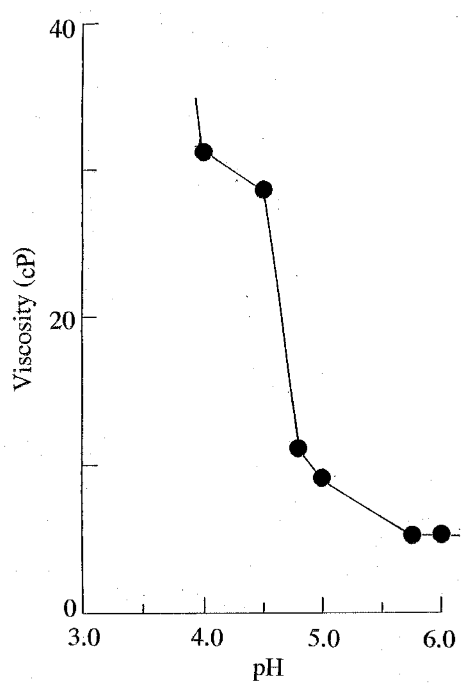

Fig. 7. Viscosity (cP) of Red Knight flour-water suspension with acetic acid at various pH levels.

pH from 5.0 to 3.5, turned the solid-like flour dough (pH 5.0) into a very soft jelly dough (pH 3.5) (Fig. 9). This change could contribute to the rheological properties that would facilitate the highest rate of expansion of the dough from flour treated with 2.0 mL of acetic acid (Fig. 6). However, because of lower gas generation by yeast in the lower pH dough, the rate of dough expansion did not increase to the same extent in the dough treated with 4.0 mL of acetic acid, in spite of higher viscosity and WBC.

Breadmaking Results

Tables II and III and Fig. 11 show the baking results for various flours treated with varying amount of acetic acid. Specific volume for the Super King flour was higher than that of other kinds of flour, and it followed by Number One, Million and Red Knight flour. This order of specific volume is the same as the order of

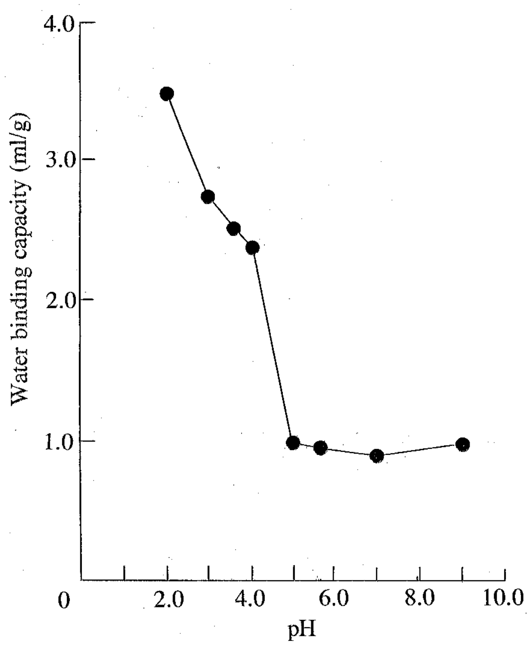

Fig. 8. Water binding capacity (WBC) of Red Knight flour with acetic acid at various pH levels.

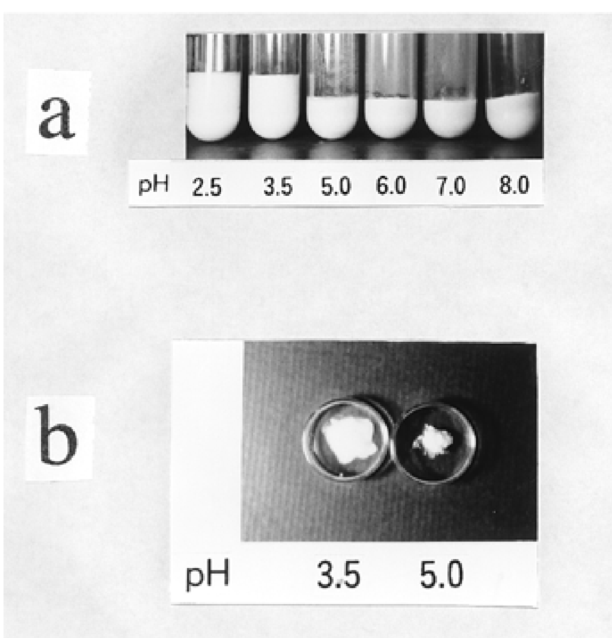

Fig. 9. Effects of pH level on flour dough after centrifugation (**a**) and on petri dish (**b**).

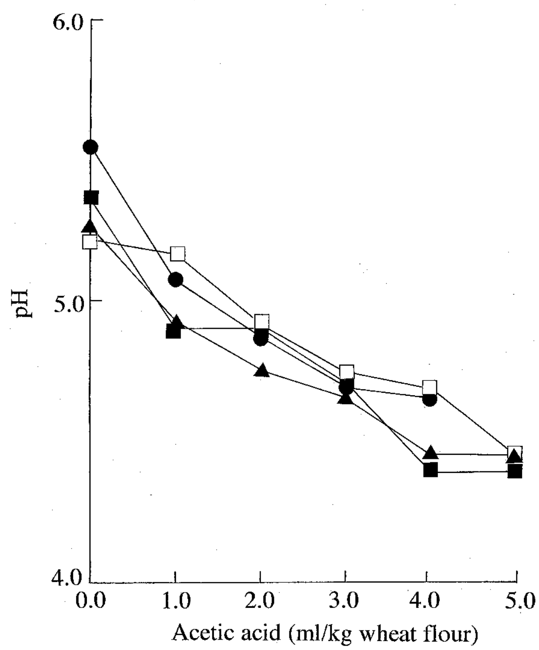

Fig. 10. Change of pH values in bread crumb treated with increasing levels of acetic acid. Flour samples: ● = Red Knight; ■ = Million; ▲ = Super King; □ = Number One.

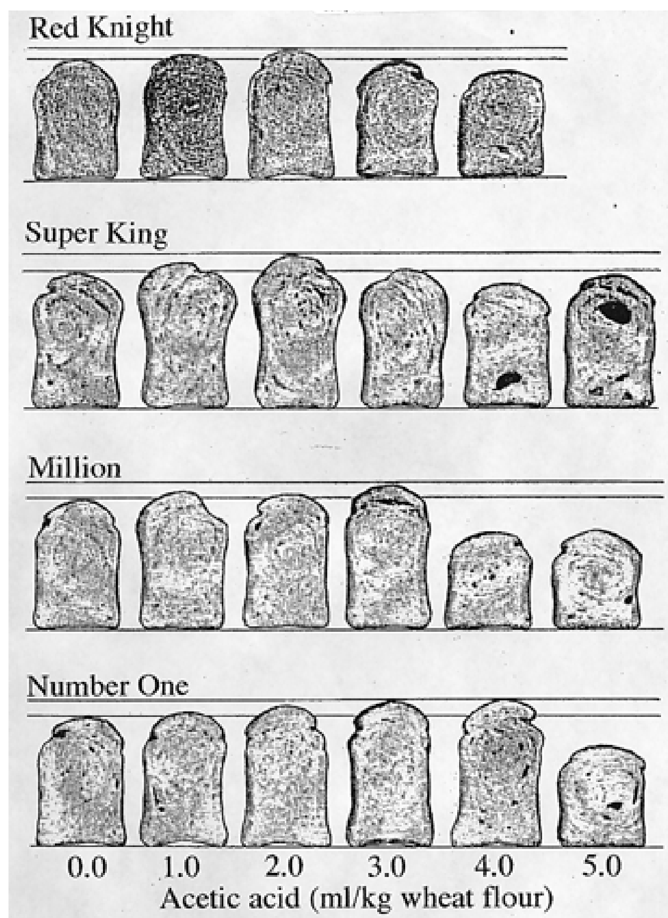

Fig. 11. Appearance of bread made with different wheat flours treated with different concentrations of acetic acid.

their flour's protein content (Table I). The level of acetic acid treatment of Super King and Red Knight flours for the maximum specific volume of bread was 2.0 mL/kg, whereas the equivalent level for Number One and Million flours was 3.0 mL/kg. The pH of bread crumb of all breads at the maximum specific volume pH 4.7– 4.9 (Fig. 10). The pH of the bread crumb showed the same decrease as the flours with increased acetic acid treatment (Fig. 10). Figure 11 shows the appearances of all bread crumbs. Finest crumb grain, better volume, and height for all flours were obtained from bread baked with flours treated with 2.0 or 3.0 mL of acetic acid/kg of flour.

CONCLUSIONS

Breadmaking tests were performed with four kinds of flour that were treated with gaseous acetic acid. Maximum bread height and specific volume were obtained with 2.0–3.0 mL of acetic acid/kg of flour. Gas generation and dough expansion tests for one flour (Red Knight) revealed that the highest rates of gas generation and dough expansion were obtained at the same treatment that gave the maximum bread results. The dough revealed drastically increased viscosity and WBC between pH 5.0–3.5 with acetic acid. We concluded that gaseous acetic acid treatment of flour improved the breadmaking properties and can be used as an alternate to bromate or ascorbic acid treatments.

ACKNOWLEDGMENTS

We thank W. Bushuk of the University of Manitoba for his helpful advice.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1995. Approved Methods of the AACC, 9th ed. Method 02-52, approved April 1961, reviewed October 1982 and October 1994; Method 04-20, approved April 1961, reviewed October 1982 and October 1994; Method 08-01, approved April 1961, revised October 1981 and October 1986; Method 10-10A; Method 54-21, approved April 1961, revised October 1994, final approval November 1995. The Association: St. Paul, MN.

BAILEY, C. H., and JOHNSON, A. H. 1924. Studies on wheat flour grades. IV. Changes in hydrogen ion concentration and electrolytic resistance of water extracts of natural and chlorine treated flour in storage. Cereal Chem. 1:133-137.

BENNETT, R., and EWART, J. A. D. 1962. The reaction of acids with dough proteins. J. Sci. Food Agric. 13:15-23.

ELIASSON, A. C., and LARSSON, K. 1993. Cereals in Breadmaking. Marcel Dekker: New York.

GALAL, A. M., VARRIANO-MARSTON, E., and JOHNSON, J. A. 1978. Rheological dough properties as affected by organic acids and salt. Cereal Chem. 55:683-691.

HOSENEY, R. C., and BROWN, R. A. 1983. Mixograph studies. V. Effect of pH. Cereal Chem. 60:124-126.

HOSOMI, K., UOZUMI. M., NISHIO, K., and MATSUMOTO, H. 1992. Studies on frozen dough baking—The effects of sugar esters with various HLB values. Nippon Shokuhin Kogyo Gakkaishi 39:806-812.

KOHMAN, H. A., HOFFMAN, C., and GODFREY, T. M. 1915. Manufacture of bread. U.S. patent 1,148,328.

KULP, K. 1972. Some effects of chlorine treatment of soft wheat flour. Baker's Dig. 46(3):26-32.

KUROKAWA, Y., HAYASHI, T., MAEKAWA, A., TAKAHASHI, T., KOKUBO, T., and ODASHIMA, S. 1983. Carcinogenicity of potassium bromate administered orally to F344 rats. J. Natl. Cancer Inst. 71:965.

MAEHASHI, K., YAMAMOTO, Y., HIGASHI, K., and YOSHII, H. 1996. Effects of acetic acid on respiration of *Debaryomyces hansenii*. Nippon Shokuhin Kogyo Gakkaishi 43:225-230.

MILLER, B. S., and JOHNSON, J. A. 1954. A review of methods for determining the quality of wheat and flour for breadmaking. Kans. Agric. Exp. Sta. Bull. No.76. Kansas State Univ.: Manhattan, KS.

PYLER, E. J. 1988. Baking Science and Technology, Vol. 2. E. J. Pyler, ed. Siebel: Chicago.

RANUM, P. 1992. Potassium bromate in bread baking. Cereal Foods World 37:253-258.

SEGUCHI, M., and MATSUKI, J. 1977. Studies on pan-cake baking. I. Effect of chlorination of flour on pan-cake qualities. Cereal Chem. 54:287-299.

TANAKA, K., FURUKAWA, K., and MATSUMOTO, H. 1967. The effect of acid and salt on the farinogram and extensigram of dough. Cereal Chem. 44:675-680.

TSEN, C. C. 1966. A note on effect of pH on sulfhydryl groups and rheological properties of dough and its implication with the sulfhydryl-disulfide interchange. Cereal Chem. 43:456-460.

van DAM, H.W. 1988. The biotechnology of baker's yeast: Old or new business? Pages 117-131 in: Chemistry and Physics of Baking. J. M. V. Blanshard, P. J. Frazier, and T. Galliard, eds. R. Soc. Chem.: London.

WOLT, M. J., and D'APPOLONIA, B. L. 1984. Factors involved in the stability of frozen dough. I. The influence of yeast reducing compounds on frozen-dough stability. Cereal Chem. 61:209-212.

YAMAMOTO, Y., HIRAIWA, T., HIGASHI, K., and YOSHII, H. 1989. Protective effect of liver extract on acetic acid inhibition against *Debaryomyces hansenii*. Nippon Shokuhin Kogyo Gakkaishi 36:1-6.

YAMAMOTO, Y., NAKAKOHARA, T., AZUMA, K., and YOSHII, H. 1990. Respiratory inhibition of *Debaryomyces hansenii* by acetic acid and the protection by liver extract. Nippon Shokuhin Kogyo Gakkaishi 37:178-183.

[Received May 28, 1996. Accepted December 4, 1996.]