THE EFFECT OF PROCESSING VARIATIONS ON THE ALCOHOL, CARBONYL, AND ORGANIC ACID CONTENTS OF PRE-FERMENTS FOR BREAD BAKING[1]

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ABSTRACT

Pre-ferments containing different amounts of yeast and sugar were fermented at 35°C. for 6 hours and then maintained at 4°C. for 17 hours. Samples were removed hourly during the first 6 hours and at greater intervals thereafter. Production of total organic acids and alcohol was proportional to the starting sugar concentration, but total carbonyl compounds were less responsive. Alcohol and acid production tended to reach maximum values within 3 to 5 hours, whereas total volatile carbonyls continued to increase during the entire 6-hour period. Nonvolatile or residual carbonyls reached a peak within 2 to 4 hours and then declined substantially. Concentrations of all of the substances remained essentially constant during the 17-hour period at 4°C. Substitution of glucose for sucrose in the pre-ferments caused no significant effect. Fermentation at 25° or 30°C. caused a drop in total carbonyl production but no significant change in the quantities of alcohol and total organic acids. The major compound among the nonvolatile carbonyls was pyruvic acid.

Pre-ferments have gained importance in the bread-baking industry today in combination with the continuous method of bread production, in which they are virtually obligatory (11). A factor which restricts the wider adoption of pre-ferments in other bread-making methods appears to be the difficulty in obtaining as intense a flavor and aroma as desired (11). The composition and manner of handling of the liquid pre-ferment has a noticeable effect on the flavor of the finished loaf of bread (12). Basic information is needed that will help to provide guidance for control of conditions under which pre-ferments are prepared so as to develop maximum desirable flavor. Such information can be obtained from a study of the flavor substances or precursors produced in the pre-ferment and from the study of the effect of variation in handling and composition on the concentration of these flavor constituents.

Compounds which may be flavor precursors or flavor compounds of bread and which are formed in pre-ferments during fermentation have been investigated by several workers (3,9,11), among whom Johnson *et al.* (5,6) should be mentioned particularly. The latter authors described changes in gas production and in such chemical constituents as organic acids, alcohol, and esters which occurred during production of pre-ferments of various compositions. The present

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research extends the work of these investigators by providing additional quantitative information on volatile and nonvolatile carbonyl compounds, ethanol, and organic acids produced in pre-ferments. The effect of variations in pre-ferment incubation time and temperature on the production of these chemical constituents is also described.

Materials and Methods

Preparation of Pre-Ferments. The compositions of the three pre-ferments used are shown below. They are modifications of the formulas of Manewal (8), Johnson *et al.* (5), and Swortfiguer (15).

Basic Pre-Ferment Ingredients	*Pre-Ferment*		
	1	2	3
	g/100 ml water	*g/100 ml water*	*g/100 ml water*
Sucrose	3.2	6.6	11.9
Sodium chloride	1.9	2.2	2.9
Bakers yeast	2.4	4.4	7.0

To each 100 ml. of pre-ferment were added 0.65 g. of a brew improver[3] having the following composition:

Salt	%
Ammonium phosphate, dibasic	61.5
Potassium sulfate	15.4
Magnesium sulfate	7.7
Calcium carbonate	15.4

Two extra mixtures of pre-ferment No. 2 were prepared in which the type and concentration of sugar used were varied. In one sample, glucose was substituted for sucrose; the other sample contained a twofold starting concentration of sucrose. Five-liter quantities of the pre-ferments were prepared at a time and were stirred continuously during fermentation at 35°C. Samples of pre-ferment No. 2 were also incubated at 30° and 25°C. At the end of each hour 100-ml. samples of pre-ferment were withdrawn, the yeast cells were removed by centrifugation, and the supernatant was adjusted to pH 8.3 with concentrated sodium hydroxide. Before the pre-ferment supernatant was adjusted to pH 8.3, it was tested qualitatively for esters using the hydroxamic acid method of Hestrin (4). At the end of 6 hours of fermentation the pre-ferments were stored in a cold room at 4°C. for 17 additional hours, after which time the final sample was withdrawn for analysis.

Analysis for Organic Acids. Ten milliliters of the pre-ferment super-

[3] Garibaldi, J. A. Private communication.

natant were evaporated to dryness at room temperature on a rotary evaporator attached to a vacuum pump through a dry ice-petroleum ether trap. The dried residue was analyzed for total organic acid content using the procedure of Bulen *et al.* (2), with the following modifications. The residue was acidified with 0.7 ml. of 0.5*N* sulfuric acid, mixed with 1.5 g. of silicic acid, and transferred to the top of a silicic acid chromatographic column. Organic acids were eluted with chloroform:butanol (50:50 v/v) equilibrated against carbon dioxide-free distilled water. The solvent was allowed to flow through the column by gravity at the rate of about 90 ml. per hour. Usually, the total organic acids were eluted from the column in the first 300 ml. of solvent. Fractions of 25 ml. each of the eluate were mixed with 10 ml. of carbon dioxide-free distilled water and 4 drops of a 2% Duponol solution (13) to aid in the emulsification. This mixture was titrated with 0.01*N* sodium hydroxide to the phenol red end point. A blank column containing no pre-ferment was run, and the titration value obtained on the eluate was subtracted from the sample value. This procedure provided a means whereby the pre-ferment sample containing a mixture of organic and inorganic anions (phosphate and chloride) could be applied directly to the silicic acid column without a preliminary separation of the two types of anions. During the elution, only organic anions appeared in the eluate. Inorganic anions were held on the silicic acid. Known quantities of organic acids which were added to the pre-ferment could be recovered from the column in the order of 95–98%.

Determination of Ethanol. Ten milliliters of the pre-ferment supernatant were evaporated on a rotary evaporator and the volatile substances were captured in a dry ice-petroleum ether trap. These volatiles were then thawed, and their ethanol content (percent v/v) was determined using a refractometer (1).

Determination of Carbonyl Compounds. One milliliter of the supernatant was diluted to 50 ml. with carbon dioxide-free distilled water, and 0.5 ml. of this mixture was analyzed for approximate total carbonyl compounds colorimetrically (7). Ten milliliters of the diluted mixture were also evaporated to dryness on a rotary evaporator, and the quantity of carbonyl compounds in the residue was determined. The approximate concentration of volatile carbonyl compounds was determined by subtracting the residual carbonyl value from the total carbonyl value. The analytical values obtained must be considered approximate, because different carbonyl compounds are known to give different colors and different color intensities (7). In the present study values were expressed in terms of color intensities produced by

the 2,4-dinitrophenylhydrazone of acetophenone.

Separation of Nonvolatile Carbonyl 2,4-Dinitrophenylhydrazones. In order to acquire information on the identity of the pre-ferment carbonyls, the yeast cells were filtered from 5 l. of a pre-ferment which had been incubated for 3 hours at 35°C. The supernatant was then evaporated to dryness at room temperature. The residue was reacted with 2,4-dinitrophenylhydrazine in methyl alcohol to form the hydrazone derivatives. The bulk of the hydrazones which precipitated from this mixture was filtered off and dissolved in chloroform. These hydrazones were then chromatographed on a column containing 40 g. of silicic acid impregnated with 28 ml. of 0.5*N* sulfuric acid. Most of the derivative was eluted with chloroform and the remainder with chloroform:butanol (50:50 v/v) equilibrated against distilled water. Pyruvic acid 2,4-dinitrophenylhydrazone was identified by paper chromatography (10) and by comparison of the melting point of the unknown hydrazone derivative with the authentic sample.

Detection of Pyruvic Acid Enzymatically. Fifty milliliters of a 2-hour pre-ferment supernatant were boiled for 1 minute and cooled to room temperature. This mixture was then analyzed qualitatively for pyruvic acid using the lactic dehydrogenase procedure of Schwimmer and Weston (14).

Results and Discussion

The pre-ferments containing low, intermediate, and high concentrations of yeast and sugar produced very active fermentation at 35°C. in all instances. Without a buffer the pH of a pre-ferment would change from about 6.5 at the beginning of fermentation to pH 3.5 after 6 hours. When calcium carbonate was added, the pH approached a limit value of about 5.0 and remained there throughout the rest of the experimental period. Before the pre-ferment supernatant was adjusted to pH 8.3, it was tested qualitatively for esters. None were detected, so this class of compounds was not considered further in this study.

Ethanol and Organic Acid Production. Figures 1 and 2 show the amounts of ethanol and organic acids produced in the three types of pre-ferment during fermentation. Both constituents reached a maximum within 3 to 5 hours at 35°C. and then essentially leveled off (see also Table I). The pre-ferment which contained high concentrations of yeast and sugar (No. 3, p. 115) produced the most acid and ethanol. Pre-ferment No. 2, which contained a twofold starting concentration of sucrose, produced more ethanol than pre-ferments containing less sugar. No significant increase in the production of total

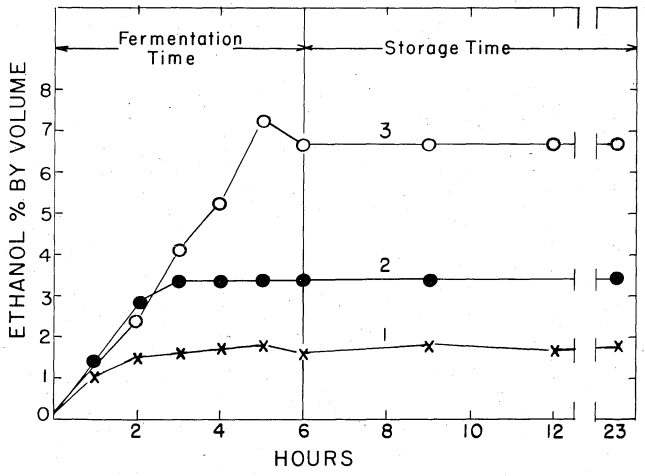

Fig. 1. Effect of fermentation and holding time on ethanol production in pre-ferments. No. 1: 3.2% sucrose, 2.4% yeast; No. 2: 6.6% sucrose, 4.4% yeast; No. 3: 11.9% sucrose, 7.0% yeast.

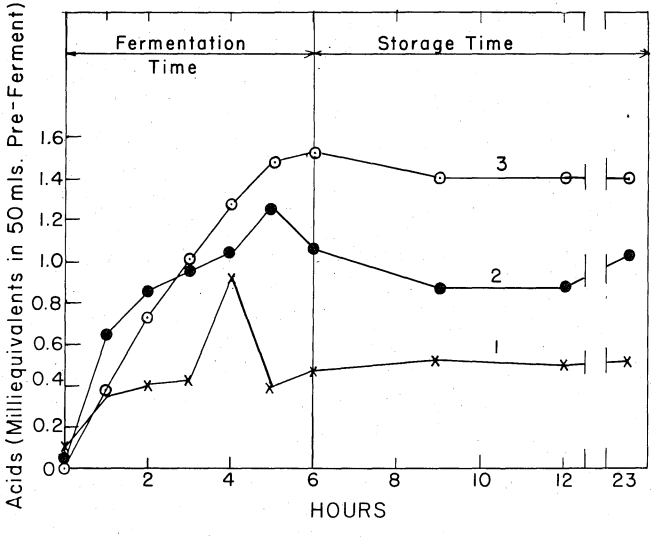

Fig. 2. Effect of fermentation and holding time on the production of total organic acids in pre-ferments. No. 1: 3.2% sucrose, 2.4% yeast; No. 2: 6.6% sucrose, 4.4% yeast; No. 3: 11.9% sucrose, 7.0% yeast.

organic acids occurred, however, owing to the presence of extra sugar. When glucose was substituted for sucrose in this pre-ferment, there was no difference in the concentration or rate of production of ethanol

and acids. Decreases of 5° and 10°C. in the fermentation temperature of the mixture had no significant effect on total alcohol and acid production. Presumably, factors other than the temperature used were rate-controlling for these substances.

Carbonyl Compound Production. Volatile carbonyl compounds had to be determined indirectly by subtracting residual carbonyl from the total carbonyls, because the carbonyl values obtained directly on the volatiles trapped during evaporation of the pre-ferment were found to be inaccurate. When an aqueous solution of acetaldehyde was volatilized on a rotary evaporator and condensed in a dry ice-petroleum ether trap, none of the acetaldehyde was recovered. This experiment showed that low boiling carbonyl compounds, such as acetaldehyde, which occur in pre-ferments, could not be determined in this manner.

Table I shows the reproducibility of carbonyl values obtained

TABLE I

ORGANIC ACID AND CARBONYL VALUES OBTAINED FROM TWO DIFFERENT FERMENTATION RUNS OF PRE-FERMENT NO. 2 CONTAINING TWOFOLD SUCROSE STARTING CONCENTRATION

FERMENTATION TIME	TOTAL ORGANIC ACIDS[a]		TOTAL CARBONYL COMPOUND[b]	
	RUN NO. 1	RUN NO. 2	RUN NO. 1	RUN NO. 2
hours				
0	0.00	0.14	2.8	2.4
1	0.04	0.33	11.0	8.8
2	0.69	0.71	20.0	15.5
3	0.91	1.07	24.0	20.4
4	0.91	0.88	23.0	20.3
5	1.10	0.98	21.7	19.6
6	1.25	1.34	19.3	18.8

[a] Milliequivalents of NaOH required to neutralize the organic acids in 50 ml. of pre-ferment supernatant.
[b] Each carbonyl value is an average of two determinations on aliquots of pre-ferment. Values are expressed as millimoles of acetophenone present in 1 liter of pre-ferment.

from duplicate pre-ferment runs made on different days.

Figure 3 shows the production of total carbonyls as fermentation (at 35°C.) progressed. In general, the carbonyl concentration reached a maximum within 2 to 4 hours and then leveled off. When fermentation temperature was lowered 5° and 10°C., there was a corresponding decrease in carbonyl production. Figure 4 shows that even at the end of the 6-hour fermentation period at 25°C., the total carbonyl concentration in pre-ferment No. 2 did not reach or equal that at 35°C. The substitution of glucose for sucrose in the pre-ferment did not alter the production of carbonyls.

Figure 5 shows changes with incubation time in volatile and residual

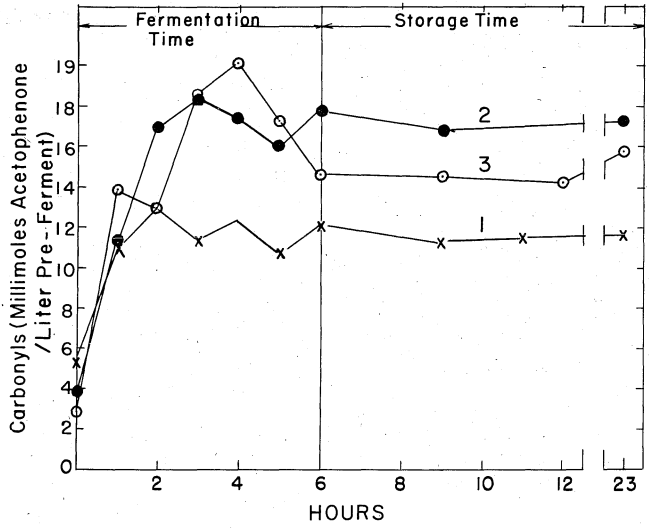

Fig. 3. Effect of fermentation and holding time on the production of total carbonyls in pre-ferments. No. 1: 3.2% sucrose, 2.4% yeast; No. 2: 6.6% sucrose, 4.4% yeast; No. 3: 11.9% sucrose, 7.0% yeast.

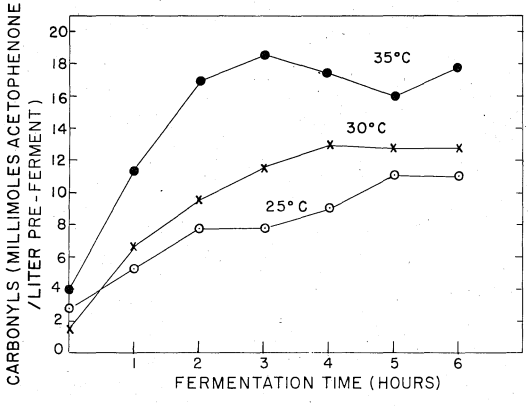

Fig. 4. Effect of temperature production of carbonyls in pre-ferment No. 2: 6.6% sucrose, 4.4% yeast.

carbonyl compounds in pre-ferment No. 3. In general, volatile carbonyls tended to increase throughout a 6-hour period. Residual carbonyls, however, increased within 2 to 4 hours to a maximum and decreased thereafter. In every pre-ferment examined, residual car-

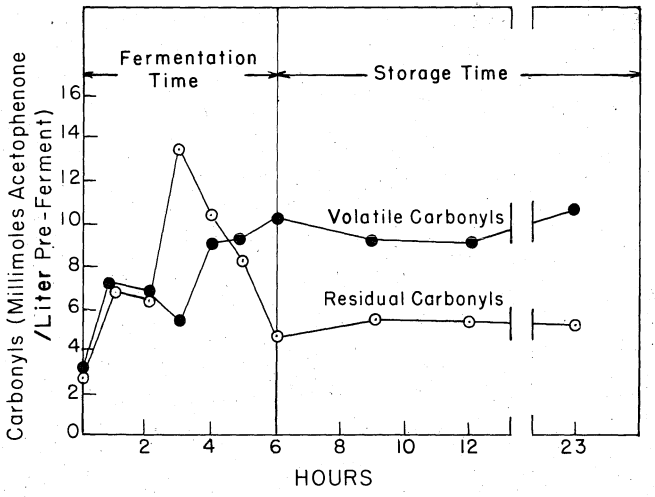

Fig. 5. Effect of fermentation time and storage on the production of volatile and residual carbonyls in pre-ferment No. 2: 6.6% sucrose, 4.4% yeast.

bonyls showed this behavior. The effect was most prominent in pre-ferments containing high starting concentrations of sucrose. At maximum concentration (after 2 to 4 hours of fermentation), the residual carbonyl fraction comprised well over one-half of the total carbonyl compounds.

Identification of Pyruvic Acid in Pre-Ferments. The 2,4-dinitrophenylhydrazone derivatives of the residual carbonyl fraction were partially separated by the column chromatographic procedure described earlier. Most of the hydrazone fraction could be eluted with chloroform. This fraction when chromatographed on paper (10) resolved into two spots having R_f values of 0.49 and 0.69. These fractions were identified as the syn- and anti-forms of pyruvic acid 2,4-dinitrophenylhydrazone when chromatographed with the authentic derivative. The melting point of the hydrazone was 218°–219°C. Mixed melting point of the hydrazone with authentic pyruvic acid 2,4-dinitrophenylhydrazone was 216°–218°C. Pyruvic acid in the boiled pre-ferment supernatant was also detected enzymatically. The oxidation of diphosphopyridine nucleotide (reduced form) in the presence of lactic dehydrogenase confirmed the presence of pyruvic acid.

The remaining hydrazone fraction on the column was eluted with chloroform:butanol (50:50 v/v) and chromatographed on paper (10). One component, which occurred in the greatest amount, moved in a distinct spot ($R_f = 0.1$). The identity of this derivative was not es-

tablished. It did not appear to be the osazone or hydrazone of glucose or fructose, because these compounds are not extractable from ether solution by dilute solutions of sodium bicarbonate as the unknown substance was found to be.

Pyruvic acid has been found in bread by Wiseblatt and Kohn (16); this indicates that some of the acid may be carried unchanged from the pre-ferment through the baking process. Additional amounts probably are produced during the pan-proof period, however. Miller and Johnson (11) have attempted to relate the presence of such pre-ferment organic acids as lactic and acetic to the flavor of bread, but found no clear-cut relationship. They made no mention of the flavor effect of pyruvic acid. Further studies may show that such carbonyl compounds as the ones which are formed in pre-ferments play an important part in the production of flavor in bread.

Literature Cited

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. (9th ed.). Sec. 9.016, p. 106. The Association: Washington, D.C. (1960).
2. BULEN, W. A., VARNER, J. E., and BURRELL, R. C. Separation of organic acids from plant tissue. Anal. Chem. **24:** 187-190 (1952).
3. CHOI, R. C. Factors influencing brew fermentation. Proc. 31st Ann. Meeting, Am. Soc. Bakery Eng., Chicago, Ill. pp. 44-52 (1955).
4. HESTRIN, S. The reaction of acetyl choline and other carboxylic acid derivatives with hydroxylamine and its analytical application. J. Biol. Chem. **180:** 249–261 (1949).
5. JOHNSON, J. A., MILLER, B. S., REFAI, F. Y., and MILLER, D. Effect of fermentation time on certain chemical constituents of pre-ferments used in breadmaking. J. Agr. Food Chem. **4:** 82-84 (1956).
6. JOHNSON, J. A., MILLER, B. S., and CURNUTTE, B. Organic acids and esters produced in pre-ferments. J. Agr. Food Chem. **6:** 384-387 (1958).
7. LAPPIN, G. R., and CLARK, L. C. Colorimetric method for determination of traces of carbonyl compounds. Anal. Chem. **23:** 541-542 (1951).
8. MANEWAL, R. Practical applications of the brew process. Baking Industry **104:** 43-45 (Oct. 8, 1955).
9. MASELLI, J. Theoretical and practical aspects of brew fermentation. Symposium on yeast, its characteristics, growth, and function in baked products, ed. by C. S. McWilliams and M. S. Peterson; pp. 70-84. U. S. Quartermaster Food and Container Institute for the Armed Forces: Chicago, Ill. (1957).
10. MCARDLE, B. The quantitative estimation of pyruvic and α-oxoglutaric acids by paper chromatography in blood, urine and cerebrospinal fluid. Biochem. J. **66:** 144-148 (1957).
11. MILLER, B. S., and JOHNSON, J. A. Present knowledge concerning baking processes employing pre-ferments. Wallerstein Labs. Commun. **21:** 115-132 (1958).
12. PIRRIE, P. G., and GLABAU, C. A. Are sponge doughs on the way out? Bakers Weekly **163**(9): 25-28 (Aug. 30, 1954).
13. RICE, A. C., and PEDERSON, C. S. Chromatographic analysis of organic acids in canned tomato juice, including the identification of pyrrolidonecarboxylic acid. Food Research **19:** 106-114 (1954).
14. SCHWIMMER, S., and WESTON, W. J. Enzymatic development of pyruvic acid in onion as a measure of pungency. J. Agr. Food Chem. **9:** 301-304 (1961).
15. SWORTFIGUER, M. J. Liquid ferment system. Bakers Digest **29:** 120-124, 168-171 (1955).
16. WISEBLATT, L., and KOHN, F. E. Some volatile aromatic compounds in fresh bread. Cereal Chem. **37:** 55-66 (1960).