THE EFFECT OF PROOFING AND BAKING ON CONCENTRATIONS OF ORGANIC ACIDS, CARBONYL COMPOUNDS, AND ALCOHOLS IN BREAD DOUGHS PREPARED FROM PRE-FERMENTS[1]

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ABSTRACT

Bread doughs prepared from pre-ferments containing 7.0% yeast and 11.9% sucrose consistently contained greater quantities of organic acids, carbonyl compounds, and alcohol than those prepared from pre-ferments with 2.4% yeast and 3.2% sucrose. After pan proof and baking, the concentrations of acids and carbonyls in bread from the richer pre-ferment were about half again as large as from the lean pre-ferment. Significant increases in concentrations of acids and carbonyls occurred both during pan proof and baking. Alcohol increased substantially during pan proof, but most of it disappeared during baking.

As commercial bread-baking methods become increasingly mechanized, bread flavor and aroma characteristics desired by bakers and their customers tend to become more difficult to obtain and control. On the other hand, continuous mixing and other types of bread production employing pre-ferments provide an opportunity to manipulate flavor to an unprecedented degree. Before this can be accomplished, however, it is necessary to acquire a more intimate knowledge than now exists of the flavor compounds and precursors provided by fermentation and how their quantities can be changed by conditions used in handling the pre-ferments.

Production of possible flavor precursors such as organic acids, alcohols, and carbonyl compounds in pre-ferments with time of incubation and storage has been studied and reported in various papers (1,2,4,5,6,9). Some reports have been concerned with determination of concentrations of flavor substances in doughs prepared by conventional methods during the proofing and baking stages. Wiseblatt and Kohn (10,11,12) and Ng *et al.* (7), for example, have reported quantitative data on several volatile organic acids, carbonyl compounds, and alcohols in proofed doughs and bread.

However, little information has been reported concerning changes of total quantities of these compounds in pre-ferment doughs during

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proofing and baking. In an earlier paper (2), the present authors reported on changes in concentration of total organic acids, carbonyl compounds, and alcohol which occurred in pre-ferments when incubation and storage times were varied. The work reported here is an extension of the previous study and describes the changes which occur during proofing and baking.

Materials and Methods

Bread Formula and Extraction of Samples. The compositions of the two pre-ferments, Nos. 1 and 3, which were used in the present experiments are described in a previous paper (2). They contained, respectively, 3.2% sucrose, 2.4% yeast; and 11.9% sucrose, 7.0% yeast. Doughs were prepared according to the following formula:

	g.
Flour (14% moisture basis)	800
Shortening	24
Nonfat dry milk	32
Salt	16
Sucrose	32
	ml.
Water	104
Pre-ferment	435

After mixing, the dough was scaled into 500-g. pieces and fermented 30 min. at 86°F. The pieces were then punched and allowed to rest for 10 min. Sheeting for moulding was done in two stages, interspersed with another 10-min. rest period. Proofing was for 60 min. at 96°F. and baking for 25 min. at 425°F.

At the end of the mixing and the pan-proofing periods, some dough samples were frozen immediately for subsequent analyses. The baked bread, after cooling, was cut into small pieces and finely ground in a Waring Blendor.

For extraction, 50 g. of the dough or bread was added to 200 ml. of distilled water in an Osterizer. The mixture was adjusted to pH 8.3 with 2*N* sodium hydroxide; homogenized for 1 min., and centrifuged. The residue was extracted four more times in the same manner with 100-ml. portions of the alkaline solution each time. All of the supernatants were combined and centrifuged in a refrigerated centrifuge at 9,000 r.p.m. The resulting supernatant was analyzed for organic acids, alcohols, and carbonyl compounds.

Analysis for Organic Acids. One-half of the dough or bread extract was passed through a glass column containing Dowex 50 (20 ml. of bed resin in hydrogen form) to remove the cations. An estimate of total anions in the eluate was made by titrating a portion with 0.01*N* sodium hydroxide using phenol red indicator. The eluate was then passed through a column containing Permutit A-300 (20 ml. of bed resin in the hydroxyl form) (3) to adsorb the anions, after which the column was washed exhaustively with distilled water to remove contaminating proteins and carbohydrates. The anions were eluted with 10% ammonium hydroxide, and 150 ml. of eluate were collected. A calculated excess of sodium hydroxide was then added to the eluate to convert all anions to their sodium salts, and this mixture was evaporated to a small volume on a rotary evaporator at room temperature.

Two milliliters of the concentrate were mixed thoroughly with 8 g. of silicic acid along with 5 ml. of 0.5*N* sulfuric acid. This dry mixture was added to the top of a glass column packed with 8 g. of silicic acid impregnated with 5 ml. of 0.5*N* sulfuric acid. Elutions and titrations were carried out according to the procedure described in the previous paper (2).

Colorimetric Determination of Carbonyl Compounds. Two-tenths of a milliliter of the dough or bread extract was analyzed for total and residual carbonyl compounds according to the colorimetric procedure, using 2,4-dinitrophenylhydrazine, described in detail in the earlier paper (2).

Colorimetric Determination of Volatile Alcohols. Ten milliliters of the dough or bread extract were frozen and lyophilized *in vacuo* and the volatile substances captured in a dry ice-petroleum ether trap. The volatiles were then thawed and their alcohol concentration (percent v/v) determined by the colorimetric method of Reid and Truelove (8), using ceric ammonium nitrate.

Results and Discussion

The use of dilute sodium hydroxide for the extraction of organic acids, carbonyl compounds, and alcohols from bread doughs was found to be quite effective. Table I shows the extent to which total anions can be extracted from a sample of dough using successive portions of this solvent. After each extraction step, the mixture was centrifuged, the cations were removed from the supernatant with Dowex 50 (hydrogen form), and the supernatant was titrated. Table I shows that the total anions extracted were not appreciably increased when quantities of dilute sodium hydroxide in excess of 500 ml. were used.

The efficiency of the extraction and separation of the organic acids by means of the resins and silicic acid was determined by recovery experiments with a mixture of acetic, lactic, pyruvic, and citric acids added to bread doughs. A 92% recovery was obtained by the multistep

TABLE I

EFFECTIVENESS OF ANION REMOVAL FROM BREAD DOUGHS BY SUCCESSIVE EXTRACTION WITH DILUTE SODIUM HYDROXIDE

Extracting Solvent	Anions Extracted[a]	
	Dough, Not Proofed	Dough, Proofed
ml.	*meq.*	*meq.*
200	10.10	10.80
100	1.62	1.57
100	0.48	0.45
100	0.13	0.18
100	0.11	0.12

[a] The meq. of anions extracted from 50 g. of dough.

procedure, which indicates that most of the acid can be recovered without extensive decomposition and esterification.

Table II shows the concentration of organic acids found in doughs prepared with pre-ferments of high and low concentrations of sugar and yeast, as well as the amount found in the bread baked from these doughs. The values reported are the averages obtained from duplicate batches of doughs and breads. Average deviation among the sets of values varied from 20 to 50 parts per thousand.

TABLE II

TOTAL ORGANIC ACIDS IN DOUGHS AND BREAD PREPARED FROM PRE-FERMENTS[a]

Sample	Pre-Ferment No. 1	Pre-Ferment No. 3
	meq.	*meq.*
Dough, not proofed	10.1	14.4
Dough, proofed	11.8	16.8
Bread	13.1	18.9

[a] Meq. of organic acids in 500 g. of dough or bread baked from this amount of dough.

With pre-ferment No. 1, pan proofing did not lead to an appreciable increase of total organic acids. With the concentrated pre-ferment, No. 3, the increase during pan proof was also modest. The baking step caused an increase in organic acids in the bread made from both pre-ferments.

Table III shows results obtained for amounts of carbonyl compounds found in the alkaline extracts of doughs and breads[2]. Agreement between the runs on separate batches of dough and bread was quite good, with most of the values in the same set differing by only 0.2 or 0.3 of a millimole. A small increase in total carbonyl values

[2] Dilute sodium hydroxide as used in the present work was found by comparative runs to extract carbonyl compounds as efficiently as such organic solvents as isopropanol and methylene chloride, which are sometimes more effective under other circumstances.

TABLE III

TOTAL AND RESIDUAL CARBONYL COMPOUNDS IN DOUGHS AND BREADS PREPARED FROM PRE-FERMENTS

SAMPLE	TOTAL CARBONYL COMPOUNDS[a]		RESIDUAL CARBONYL COMPOUNDS[a]	
	Pre-Ferment No. 1	Pre-Ferment No. 3	Pre-Ferment No. 1	Pre-Ferment No. 3
	mmol.	*mmol.*	*mmol.*	*mmol.*
Dough, not proofed	2.3	3.7	1.7	3.0
Dough, proofed	2.8	4.5	2.3	4.2
Bread	3.8	5.9	3.3	5.4

[a] Carbonyl values are expressed as millimoles of acetophenone present in 500 g. of dough or in a loaf of bread baked from this amount of dough.

occurred as a result of fermentation during pan proofing and a little larger increase occurred during baking. The values for more concentrated pre-ferment are greater than those for the less concentrated by about 50%.

Residual carbonyl compounds are those remaining behind when an aliquot of the dough or bread extract is evaporated to dryness under vacuum at room temperature. They probably consist of high-boiling carbonyl compounds and salts of keto acids. As with total carbonyl compounds, the residual carbonyl concentration increased during proofing and baking. The bread was found to contain the same concentration of residual carbonyls as total carbonyls, indicating that the volatile carbonyl content of bread is rather low. Preliminary tests have shown that some of the acids in the acid fraction from doughs and bread, which were eluted from the silicic acid columns, contain carbonyl groups. This observation could account for part of the residual carbonyl fraction.

Table IV contains the values obtained for the volatile alcohol concentrations of dough and breads. Although other alcohols have been found in bread, there is no reason to suspect that any, other than ethanol, were measured here except in trace amounts. The alcohol

TABLE IV

TOTAL VOLATILE ALCOHOLS IN DOUGHS AND BREADS PREPARED FROM PRE-FERMENTS[a]

SAMPLE	PRE-FERMENT No. 1	PRE-FERMENT No. 3
	ml.	*ml.*
Dough, not proofed	1.74	3.19
Dough, proofed	2.80	7.57
Bread	1.00	0.94

[a] Volatile alcohols reported as ml. ethanol per 500 g. dough or a loaf of bread baked from this amount of dough.

increases during pan proof, and the quantities are greater for doughs made from the more concentrated pre-ferment.

Conclusions

From these studies it appears that total quantities of the several types of flavor and aroma compounds vary during bread production as a result of different levels of sugar and yeasts in the pre-ferments. In every instance, the amounts of organic acids, carbonyl compounds, and alcohol were greater in the doughs and breads prepared from the concentrated pre-ferment. Carbonyl values for the final breads were nearly doubled, and quantities of organic acids were about half again as large. Results of a preliminary organoleptic appraisal indicate that the bread from the richer pre-ferment had a more pronounced aroma and also a higher level of flavor. To determine whether the flavor and aroma of the bread from the richer pre-ferment is more pleasing than that from the less concentrated would require extensive preference determinations beyond the scope of the present study.

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