

Fermentation of Water Ferments and Bread Quality¹

K. KULP,² H. CHUNG,² M. A. MARTINEZ-ANAYA,³ and W. DOERRY²

ABSTRACT

Cereal Chem. 62(1):55-59

Fermentation of water liquid ferments of formulated white pan bread with 0-0.5% (total flour basis) buffer was investigated in regard to rates, effects on yeast activity, and bread quality. Titratable acidity, pH, glucose, fructose, and ethanol were determined, of which fructose, glucose, and ethanol were good indices of yeast metabolism. Breads produced using the

liquid ferments were of good quality regardless of the buffer level present in the ferment. Experiments with compressed yeast subjected to various treatments (soaking, fermentation at different acidities) demonstrated benefits of fermentation for yeast activity. Levels of residual sugars were higher in liquid ferment breads than in conventional sponge/dough breads.

The long-established method of bread production has been the sponge and dough process, but modified procedures have been used in recent years to take advantage of developments in equipment design for handling liquid or semiliquid forms of liquid ferments (Euverard 1967, Borthwick 1971). The basic difference between the conventional sponge and dough and the liquid ferment processes is the higher ratio of flour to water in the sponge stage (Pylar 1970). Basic biochemical information on application of liquid ferments is needed to optimize white pan bread manufacturing by this method.

This paper is the first part of a systematic investigation covering various types of liquid ferments (e.g., water and flour ferments) technology. This report, which is limited to water ferments, focuses on acidity conditions (pH, total titratable acidity) and their effects on fermentation rates of sucrose, yeast activity, and final bread quality.

MATERIALS AND METHODS

Bread Preparation

White pan breads (one-pound loaves) were prepared according to formulas given in Table I by liquid ferment, sponge and dough, or straight dough processes. The flour used (11.3% protein [N × 5.7], 0.45% ash at 14% mb) was of patent grade, commercially milled from a blend of spring and winter red hard wheats.

Liquid ferments consisted of water, compressed yeast, sucrose, and various levels (0, 0.2, and 0.5%, flour basis) of a commercial ferment buffer containing 36% calcium carbonate, 20% ammonium chloride, 14% calcium sulfate, 10% sodium chloride, and 20% flour. Flour, additional sucrose, salt, nonfat dry milk, shortening, and an optimum amount of oxidant (KBrO₃) were added at the dough stage. Fermentation temperature was maintained at 30°C.

Bread Quality Evaluation

Breads were scored for external and internal properties. The maximum score was 100, composed of volume 10, symmetry 5, crust color 10, break and shred 5, grain quality 10, crumb texture 15, crumb color 10, aroma 10, taste 15, mouthfeel 10. The loaf volume was determined by rapeseed displacement.

Yeast Activity

Gassing power was estimated by the AACC method 22-11 (AACC 1976) using Gasograph model 12B (D&S Instrument Ltd.,

¹Presented in part at the AACC 68th Annual Meeting, Kansas City, MO, Oct. 30-Nov. 3, 1983.

²American Institute of Baking, Manhattan, KS 66502.

³Visiting postdoctoral scientist from Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Valencia, Spain.

TABLE I
Formulas of Bread Processes

Ingredient	Liquid Ferment		Sponge and Dough		Straight
	Brew	Dough	Sponge	Dough	
Flour	...	100.0	70.0	30.0	100.0
Water	66.0	...	42.0	22.0	65.2
Yeast, compressed	3.5	...	2.0	...	3.0
Sucrose	3.0	6.0	...	6.0	6.0
Salt	0.5	1.5	...	2.0	2.0
Nonfat dry milk	...	2.0	...	2.0	2.0
Shortening	...	3.0	...	3.0	3.0
NH ₄ Cl	0.04	0.04
KBrO ₃ , ppm	...	5.0	...	5.0	5.0
Buffer	Variable	5.0

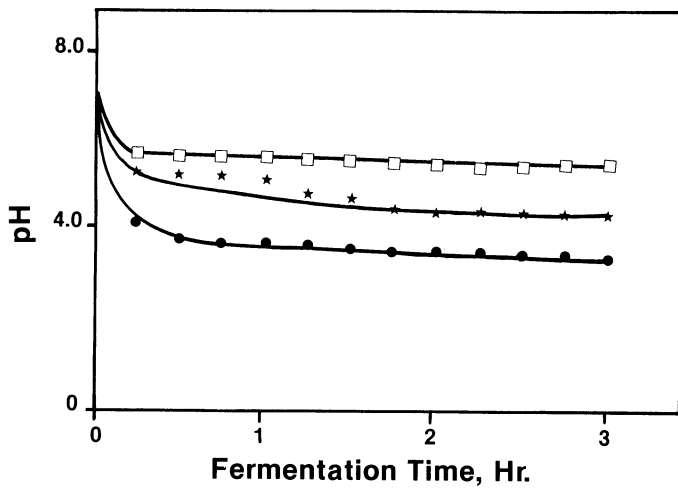


Fig. 1. Change of pH in liquid ferments during fermentation: ● = 0% buffer, ★ = 0.2% buffer, □ = 0.5% buffer.

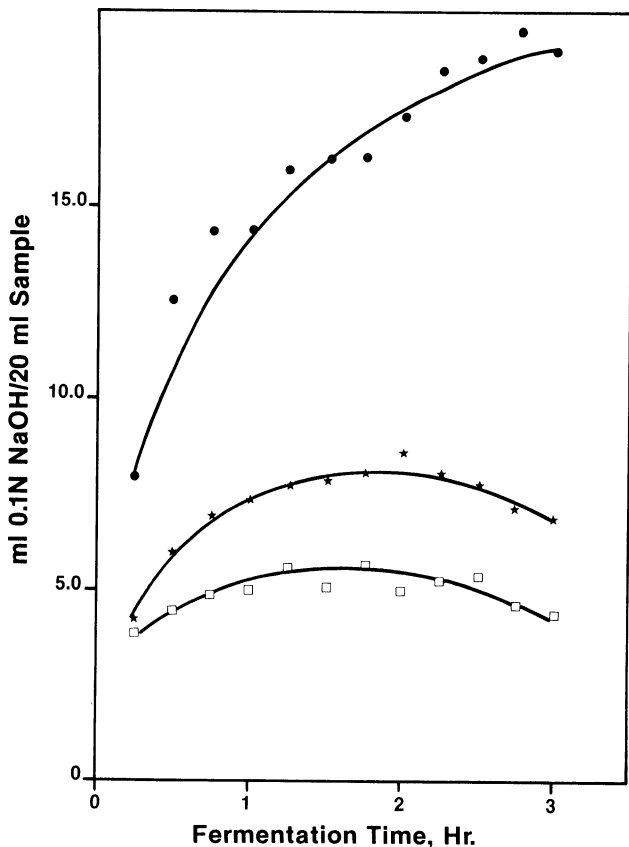


Fig. 2. Change of total titratable acidity in liquid ferments during fermentation: ● = 0% buffer, ★ = 0.2% buffer, □ = 0.5% buffer.

Pullman, WA). A straight-dough procedure (Table I) was used to determine fermentation activity in doughs, taking proof time as an index of yeast activity.

Yeast Treatments

Samples of compressed yeast were examined for fermentation activity in fresh state, after 3-hr hydration in water at 30°C, and after undergoing liquid ferment fermentation (3 hr at 30°C). In the latter two cases, the yeast was recovered by centrifugation at 5,000

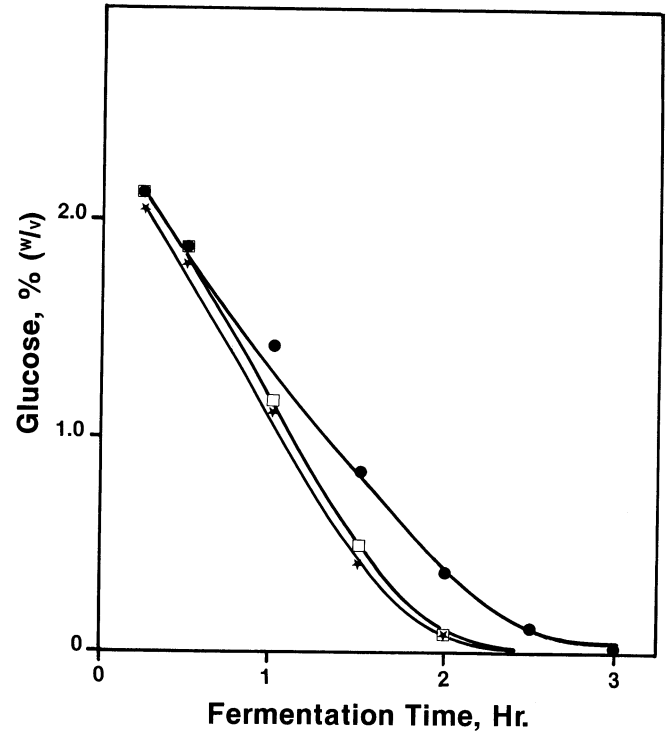


Fig. 3. Change of glucose concentration in liquid ferments during fermentation: ● = 0% buffer, ★ = 0.2% buffer, □ = 0.5% buffer.

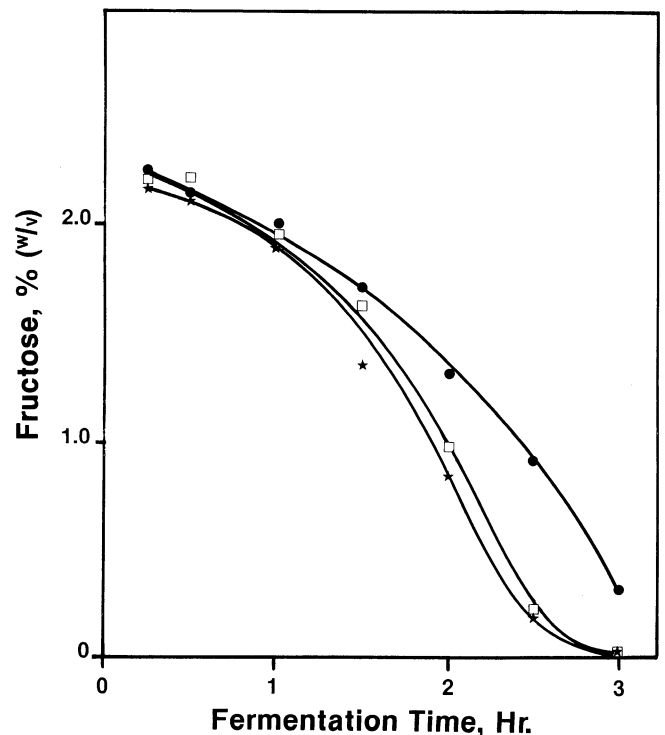


Fig. 4. Change of fructose concentration in liquid ferments: ● = 0% buffer, ★ = 0.2% buffer, □ = 0.5% buffer.

rpm for 30 min and washed by suspending in water and recentrifuging three times.

Acidity Determinations

For total titratable acidity (TTA), a sample of 20 ml of liquid ferment was taken, the yeast activity arrested by addition of 1 ml of formaldehyde (37% w/w), and the sample titrated with 0.1N sodium hydroxide to the end point of pH 6.6. The TTAs of breads were established for a 15-g sample using AACC method 02-31 (1976). The TTA values are reported as milliliters of 0.1N hydroxide required to neutralize 20 ml of liquid ferment or 15 g of bread samples.

The pH measurements were made with a Beckman pH meter model 3560 (Beckman Instruments, Inc., Fullerton, CA) according to AACC method 02-52 (1976).

Sugar Determinations

Glucose, fructose, and maltose were measured by gas-liquid chromatography using Hewlett-Packard GC model 5840A with model 5840A GC terminal (Hewlett-Packard, Avondale, PA). The unit was equipped with a flame-ionization detector and stainless steel column (6 ft × 1/8 in.) packed with 3% SP-2250 on 80-100 mesh Supelcoport (Supelco, Inc., Bellefonte, PA). Nitrogen at a flow rate of 20 ml/min was the carrier gas. Operating temperature

conditions: injection port 270°C; detector 280°C; and column temperature interval 170-280°C, programmed for a 10°C/min rise.

Liquid ferment samples were heated for 8 min at 98°C (water bath) to stop yeast activity and centrifuged at 5,000 rpm for 20 min. Aliquots of the supernatant were diluted 100-500 times and 1-μl volumes injected for analysis. Air-dried breads were ground in a meat grinder, and 0.5-g samples suspended in 50% (v/v) aqueous methanol for 1 hr, then centrifuged. One milliliter of supernatant was derived (Li and Schuhman 1980) and used for gas-liquid chromatography.

Glucose was estimated by using a Yellow Springs Instrument (YSI) industrial analyzer, model 27 (Yellow Springs Instrument Co., Yellow Springs, OH) with an immobilized glucose oxidase membrane. Heat-treated ferments were diluted with 0.44M phosphate buffer (pH 6.71), kept at room temperature for 30 min to permit equilibration of α and β forms of glucose; then glucose was measured by YSI test procedure no. 107.

TABLE II
Analysis of Glucose in Brew (% w/v)^a

Time (min)	Buffer Levels (flour basis)					
	0%		0.2%		0.5%	
	GLC ^b	YSI ^c	GLC ^b	YSI ^c	GLC ^b	YSI ^c
15	2.12 a	2.14 a	2.05 a	2.00 a	2.10 a	2.01 a
30	1.87 b	1.80 b	1.80 b	1.65 c	1.86 b	1.69 c
60	1.42 c	1.46 c	1.11 d	0.93 d	1.16 d	1.00 d
90	0.84 d	0.80 d	0.42 e	0.35 e	0.49 e	0.40 e
120	0.38 e	0.28 f	0.07 g	0.03 g	0.07 f	0.04 f
150	0.10 g	0.11 g	...	0.005
180	0.03 h	0.11 i

^a Each value is the mean of three samples. Means in each time row and buffer level column followed by the same letter are not significantly different ($P < 0.05$).

^b Gas liquid chromatographic procedure.

^c Yellow Springs industrial analyzer procedure.

TABLE III
Sugar Fermentation Losses

Process	Sugar	
	Glucose, % Used	Fructose, % Used
0% Buffer brew		
Brew stage	-61.1	-21.3
Dough stage	-39.0	- 0.5
Overall total	-51.4	- 7.5
0.2% Buffer brew		
Brew stage	-80.56	-37.0
Dough stage	-44.4	-11.7
Overall total	-59.3	-22.5
Plastic sponge/dough		
Overall total	-40.0	-10.1

TABLE IV
Gassing Power

Treatment Time (min)	Gas Production (mm Hg) ^a				
	Fresh	Soaked	0%	0.2%	0.5%
30	56.2	55.0	63.8	65.7	64.6
60	134.5	124.1	148.4	147.5	149.7
120	281.4	268.2	291.5	290.9	297.1
180	402.05	388.9	413.2	407.2	410.6

^a Each value is the mean of four samples.

TABLE V
Yeast Activity^a

Yeast Treatment	Dough Proof Time (min)	Bread	
		Sp. Vol. (cc/g)	Total Score
Fresh	60 a	5.13 a	78.1 a
Soaked in Water (3 hr)	57 b	5.21 a	81.5 b
From brew (0% buffer)	52 c	5.22 a	84.2 c
From brew (0.2% buffer)	47 d	5.27 a	85.7 c
From brew (0.5% buffer)	45 d	5.26 a	83.7 c

^a Each value is the mean of three samples. Means in each column followed by the same letter are not significantly different ($P < 0.05$).

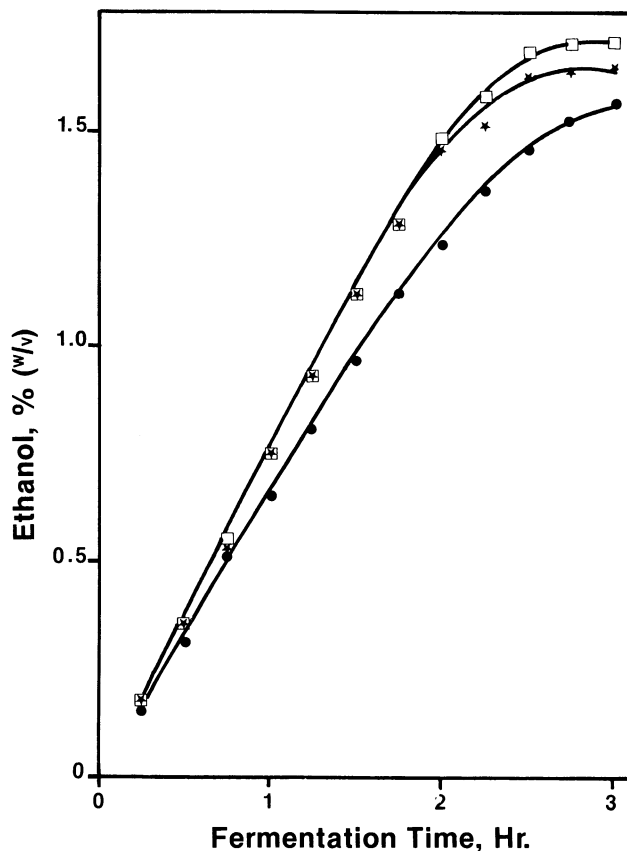


Fig. 5. Ethanol production in liquid ferments during fermentation: ● = 0% buffer, ★ = 0.2% buffer, □ = 0.5% buffer.

Ethanol

The content of ethanol during fermentation of liquid ferments was estimated with a YSI analyzer equipped with immobilized alcohol dehydrogenase membrane according to YSI test procedure no. 110. Ferment samples were diluted two to 10 times with distilled water.

Statistical Treatment

Data were analyzed by analysis of variance and *F* test with $\alpha = 0.05$.

RESULTS AND DISCUSSION

As evident from the formula in Table I (liquid ferments), the brew consists of water, yeast, sucrose, salt, and various amounts of a commercial buffer. It is believed that acidity conditions must be maintained within an optimal acidity range for speedy and efficient yeast metabolism and high quality of bread (Thorn 1963, Bayfield

and Lannuir 1962, Bayfield et al 1963). On the other hand, yeast functions well over a broad pH range (4.0–6.0) (Reed and Peppler 1973). Consequently, the level of acidity may not be as critical as generally assumed, since the fermented brew is added to flour and other ingredients at the dough stage, which neutralizes the acids and buffers the system. Under these conditions, the brew acidity does not exert adverse effects on flour functionality. It may, however, affect the yeast activity with concomitant changes in bread quality and flavor due to formation of varied metabolites at different pH values.

The fermentation of the brew was conducted with 0, 0.2, and 0.5% buffer (flour basis). The active component of commercial buffer is calcium carbonate, which, with carbonic acid generated by fermentation, forms a buffer system. The effect of the buffer level on acidity development in the brew is indicated by pH and TTA values shown in Figures 1 and 2, respectively.

Significant differences ($P < 0.0001$) in the pH changes were observed among the buffer treatments. In each case, pH dropped quickly within 30 min, then dropped slowly (Fig. 1). The pH of the unbuffered brew reached the 3.4 value with 120-min fermentation. This value was close to that for saturated carbon dioxide water solution (pH 3.8), indicating that the acidity was predominantly due to carbonic acid. The brew with 0.2% buffer was 4.4 at 135 min. This condition is considered optimal for bread production in baking practice. The 0.5% buffer kept the pH relatively high (5.5) after an initial drop.

TTA values (Fig. 2) increased sharply in 0% buffer brew but only moderately with 0.2 and 0.5% buffer. These brews reached their maxima (8.6 and 5.6 ml, respectively) between 105 and 120 min fermentation, then decreased slightly. Here again, 0.2% buffer approximated an optimal commercial fermentation. The TTA differed significantly ($P < 0.0001$) at all buffer levels tested.

The data indicate that if controls of pH and TTA are essential in water brew processes, a suitable level of chemical buffer or flour must be used.

Sugar Fermentation

Figures 3 and 4 represent the fate of glucose and fructose during fermentation in brews with 0, 0.2, and 0.5% buffer. In all cases, glucose was utilized by yeast at a significantly faster rate ($P < 0.05$) than fructose. These data are in agreement with those Koch et al (1954) observed in straight dough fermentation. Sucrose, the sugar ingredient used, was not detectable by either analytical method

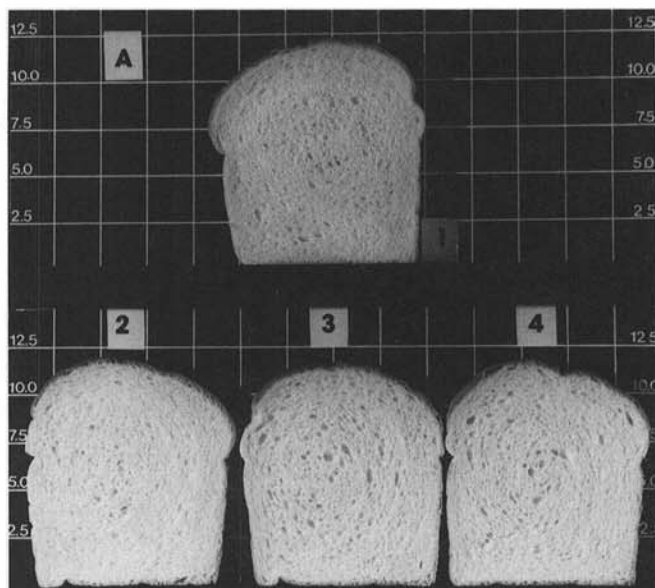


Fig. 6. Breads made from liquid ferments with 90-min fermentation and: 1, Sponge-dough (control); 2, 0% buffer; 3, 0.2% buffer; 4, 0.5% buffer.

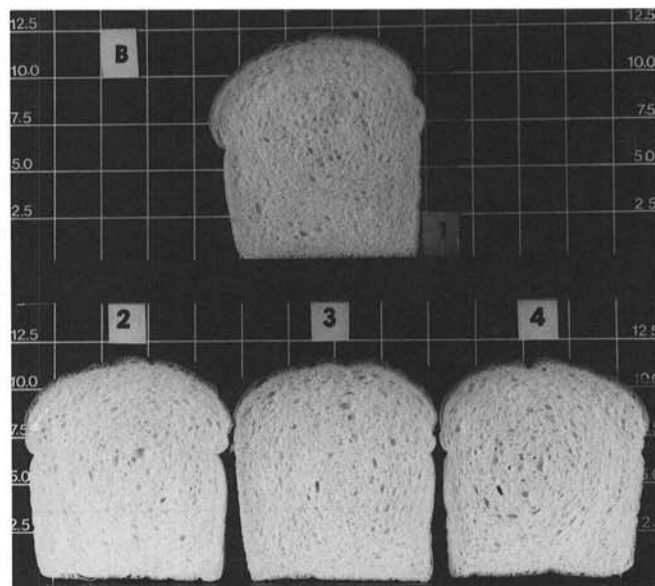


Fig. 7. Breads made with liquid ferments with 150-min fermentation time and: 1, Sponge-dough (control); 2, 0% buffer; 3, 0.2% buffer; 4, 0.5% buffer.

TABLE VI
Bread Characteristics^a

Buffer (%)	Fermentation Time (hr)	Proof Time (min)	Spec. Vol. (cc/g)	pH	TTA	Total Score
0	1.5	55 a	5.24 a	5.36 a	2.41 a	80.5 ab
	2.5	57 a	5.19 a	5.63 b	2.52 b	83.5 c
0.2	1.5	49 c	5.22 a	5.65 b	2.24 c	82.9 c
	2.5	52 b	5.24 a	5.66 b	2.34 d	84.6 d
0.5	1.5	48 c	5.22 a	5.82 c	1.64 e	79.5 a
	2.5	49 c	5.26 a	5.78 d	1.64 e	82.0 b

^a Each value is the mean of four replicates. Means in each column followed by the same letter are not significantly different ($P < 0.05$).

TABLE VII
Sugar Composition of Breads (38% mb)

Process	Sugar (%) ^a			
	Sucrose	Glucose	Fructose	Maltose
0% Buffer brew	ND ^b	1.35 a	2.57 a	2.10 a
0.2% Buffer brew	ND	1.13 a	2.15 b	1.71 a
Plastic sponge dough	ND	1.18 a	1.75 c	0.34 b

^a Each value is the mean of three samples. Means in each column followed by the same letter are not significantly different ($P < 0.05$).

^b Not detectable.

after 5-min fermentation due to quick hydrolysis by yeast invertase. Similar observations were reported for straight doughs by Koch et al (1954). In general, the control of brew pH affects the sugar utilization causing a higher fermentation rate. Fructose is more sensitive than glucose to pH changes.

Analytical Methodology

Glucose was determined in brews by gas-liquid chromatography and YSI analyzer (Table II). The results obtained by both methods were not significantly different ($P < 0.096$), although the immobilized enzyme method (YSI glucose oxidase) gave generally lower values than the GLC procedure.

Ethanol Generation

The rates of ethanol formation were significantly increased ($P < 0.029$) when buffer was used in the water liquid ferment (Fig. 5). A linear relationship between fermentation time and ethanol formation was observed for a period as long as 1.5–2.0 hr. Unbuffered brews showed lower ethanol production rates due to less favorable pH conditions. This effect was in conformance with the utilization of sugars.

The baking industry generally ferments the brews for 1.5–2.0 hr, essentially to a point where the alcohol production ceases to be linear. At the same time the levels of glucose and fructose become critically low (Figs. 1, 2). Consequently, the measurements of ethanol or glucose may be useful indices for monitoring the progress and end point of fermentation in commercial practice.

Sugar Requirements

Table III shows sugar fermentation losses during preparation of white pan bread by the water liquid ferment and conventional sponge/dough procedures (formulas given in Table I). Sucrose, 6% flour basis, was added at the dough stage in the sponge/dough process. The brew in this case was fermented for 1.5 hr, the general commercial practice. Sucrose, 3% flour basis, was supplied at the brew and, 6% flour basis, at the dough stages. The data show that 1) yeast metabolizes sugar more rapidly in brew/dough fermentation than in the plastic sponge/dough system, 2) utilization of fructose is less rapid than that of glucose in both brew and dough stages, and 3) level of buffer affects the fermentation rate. Fructose is more affected than glucose by the acidity conditions. When the sponge and dough process is compared with the liquid ferment method, the latter requires more fermentable sugars for bread production. The difference is mainly due to the higher utilization of fructose in liquid ferments than in the conventional process.

Yeast Treatments

One of the functions of the sponge or brew stages is to bring the yeast into a fully active state. To define this effect, yeast activity was tested with no treatment (fresh compressed yeast); after hydration (3 hr soaking at 30°C); and after subsequent fermentation at different acidities (0, 0.2, and 0.5% buffer for 3 hr followed by separation of yeast from fermented brews). Yeast activities of these preparations were determined by means of gasograph. Table IV demonstrates that yeast preparations that had been subjected to fermentation were more active than fresh or soaked ones. The gas production values shown were significantly different ($P < 0.015$),

but the rates of gas evolution (slopes) did not differ significantly ($P < 0.9906$). Further, baking tests were conducted using a straight-dough procedure (Table I) to assess the yeast performance in the dough system. The results (Table V) indicate that the yeast treatment did not affect specific bread volumes. The differences in loaf volumes were not significant ($P < 0.49$), but proofing times were shortened ($P < 0.0001$) and total bread scores significantly improved ($P < 0.001$) in experiments with yeast samples that had been subjected to fermentation.

Bread Quality

Characteristics of breads produced by the liquid ferment process are summarized in Table VI and Figures 6 and 7. Adjustment of pH with 0.2 and 0.5% buffer reduced the proof times significantly ($P < 0.001$); specific volumes were not affected ($P < 0.9367$). No substantial differences were observed between breads with 1.5 and 2.5 hr fermentation periods. Other characteristics of bread quality e.g., grain and texture, were similar in all breads (Table VI). These data demonstrate that a good quality bread, substantially comparable to the sponge and dough bread (Figs. 6 and 7), can be prepared by the liquid water ferment process.

The composition of residual sugars is given in Table VII. The level of sugar components varied with the production procedure. The unbuffered ferments produced breads with higher glucose and fructose contents than those made by the buffered ferments or the sponge/dough process. The levels of monosaccharides in breads prepared with buffered and sponge/dough processes were similar. Maltose fermentation was less effective in liquid ferment processes than in the sponge/dough method, which produced breads with low maltose values.

ACKNOWLEDGMENT

We thank the Yellow Springs Instrument Company for donating the Yellow Springs industrial analyzer and the enzyme membranes.

LITERATURE CITED

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved Methods of the AACC. Methods 02-52, 02-31, and 22-11, approved April 1961. The Association: St. Paul, MN.
- BAYFIELD, E. G., and LANNUIR, G. L. 1962. The effects of acids, pH and oxidants upon brew fermentation on the resulting bread. *Bakers Dig.* 36(1):34.
- BAYFIELD, E. G., LANNUIR, G. L., and YOUNG, W. E. 1963. The importance of correct pH. *Bakers Dig.* 37(2):55.
- BORTHWICK, J. T. 1971. Automatic liquid sponge production. *Bakers Dig.* 45(1):50.
- EUVERARD, M. R. 1967. Liquid ferment systems for conventional dough processing. *Bakers Dig.* 41(5):124.
- KOCH, R. B., SMITH, F., and GEDDES, W. F. 1954. The fate of sugars in bread doughs and synthetic nutrient solutions undergoing fermentation with baker's yeast. *Cereal Chem.* 31:55.
- LI, B. W., and SCHUHMAN, P. J. 1980. Analysis of sugar content of ready-to-eat cereals. *Cereal Foods World* 25:150.
- PYLER, E. J. 1970. Liquid ferment processes. *Bakers Dig.* 44(1):34.
- REED, G., and PEPLER, H. J. 1973. Use of yeast in baking. Page 113 in: *Yeast Technology*. The Avi Publishing Co.: West Port, CO.
- THORN, J. A. 1963. Yeast performance in liquid ferments. *Bakers Dig.* 49(2):43.

[Received July 2, 1984. Accepted August 29, 1984.]