CEREAL CHEMISTRY

VOL. 37 JULY, 1960 No. 4

DETERMINATION OF YEAST GROWTH IN DOUGHS[1]

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ABSTRACT

Yeast cells were quantitatively recovered from dough by a process in which the dough was blended, the starch was gelatinized by heat-treatment, and the major part of the starch and protein was solubilized by digestion with fungal amylase and protease. After removal of lipids by an acid extraction with ethanol-ether, a further enzymatic digestion provided a suspension containing the yeast cells but very little extraneous material. The cells were readily counted in a Petroff-Hausser chamber, and buds were easily distinguished. The method was used to determine the amount of growth undergone by compressed and active dry yeasts in various types of commercial doughs. In sponges, both yeasts grew about 50–60% in 4 hours, but no further increase occurred in the sponge doughs. The yeasts grew about 35% in straight doughs fermented 3–3.5 hours. Most of the bud formation occurred during pan proof. No growth was found in sweet dough sponges or in flour brews fermented 3.5–4 hours. Because recovery varied during the course of dough fermentation, the nitrogen content of the isolated yeast could not be used as an indication of growth.

The growth of yeast in doughs has been investigated thoroughly only in the case of straight dough fermentations. In 1941, Hoffman, Schweitzer, and Dalby (3) described a method for counting yeast cells in dough in which the cells were washed out of the dough with salt solution and were subsequently counted in a hemocytometer. In an accompanying publication (4), the effects on yeast growth of fermentation time, yeast concentration, and various inorganic salts were described. In calculating the amount of growth, yeast buds were counted as single cells. In view of the large size which buds attain in fermenting doughs, this practice would appear well founded since the buds must contribute significantly to the gassing activity of the yeast.

In their studies, Hoffman *et al.* found that compressed yeast multi-

[1] Manuscript received October 21, 1959. Contribution from Red Star Yeast and Products Co., Milwaukee, Wis.

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plied from 29 to 88% in 6 hours when used at levels ranging from 2.0 to 0.5%. These results compared well with those of Simpson who, in 1936, observed yeast growth of 9–131% in straight doughs containing from 2.0 to 0.25% compressed yeast (6). More recently, Carlin (1) reported that no multiplication occurred in either straight or sponge doughs when commercial levels of yeast were employed. The method of analysis was not mentioned, but probably involved a plating technique which of course would not measure bud formation.

Because of the prevalent use of sponge doughs for the commercial production of various baked goods, and the increasing use of other fermentation systems such as brew doughs, it was of interest to us to study the growth of yeast in some of these systems. Also, no comparative data on the growth of compressed and active dry yeasts have appeared in the literature. In this paper, a method for isolating yeast cells from dough by enzymatic degradation of the insoluble starch and protein will be described, together with the results of its application to sponge, straight, sweet, and brew doughs made with compressed and active dry yeasts.

Materials and Methods

Degradation of Dough Samples. The method used in isolating yeast cells from dough samples was analogous to that of Simpson (5) in that it employed enzymatic degradation of the insoluble dough constituents (other than yeast). The suspensions of cells so obtained were practically devoid of extraneous material, and were well suited for cell count determinations with a hemocytometer.

A suitable sample of dough (20–40 g., depending upon the yeast concentration) was macerated with 80 ml. of water for 30 seconds in a Waring Blendor. The sides of the jar were scraped down with a rubber policeman and a minimum amount of water, and the suspension was blended for 60 seconds additionally. It was then transferred to a 250-ml. graduated cylinder and made up to volume. After thorough mixing, duplicate 25-ml. aliquots were placed in 50-ml. centrifuge tubes.

The aliquots were then heated in a boiling-water bath for 2.5 minutes with constant stirring. This killed the yeast cells and gelatinized the starch. The suspensions were then cooled, and to each were added 5 ml. of a fungal protease solution. This was prepared by extracting 3.5 g. of Rhozyme A-44 with 30 ml. of water for 30 minutes at room temperature, then removing insoluble materials by centrifuging

[4] Rohm and Haas Co., Philadelphia, Pa.

for 10 minutes (all centrifugations in this work were made with an International Equipment Co. clinical centrifuge running at top speed). The enzyme-dough mixtures were then adjusted to pH 4.8 with 0.3*N* hydrochloric acid, and were incubated in a 50°C. water bath for 60 minutes with occasional stirring.

This preliminary digestion solubilized the major portion of the protein and starch of the dough, but sufficient remained to interfere with the counting of the yeast cells. The residual material was resistant to further enzymatic hydrolysis, but was readily hydrolyzed after lipids were removed by an acidic ethanol-ether extraction.

Therefore, at the end of the first digestion period, the samples were cooled and each was transferred to a 250-ml. Erlenmeyer flask with the aid of 30 ml. of ethanol. To each flask were added 2 ml. of concentrated hydrochloric acid and 50 ml. of ether. The mixtures were shaken vigorously and, when phase separation occurred, the ether layers were siphoned off and discarded. Another 30 ml. of ethanol and 50 ml. of ether were added to each flask with shaking, followed by 22 ml. of water and further shaking. The upper phases were again discarded and the aqueous suspensions were centrifuged. The supernates were discarded, and the residues washed twice with centrifugation.

The washed residues were then suspended in about 25 ml. of water, 7.5 ml. of protease solution were added to each, and after being adjusted to pH 4.8 with hydrochloric acid, they were incubated at 50°C. for 90 minutes. The digested suspensions were centrifuged, and the solids washed twice with centrifugation; they were then made up to 50 ml.

Cell Counts. Twenty-five milliliters of the final suspension were

TABLE I

FERMENTATION SYSTEMS

	Sponge Process		Sweet Dough Sponge	Straight Dough	Brew Process	
	Sponge	Dough			Brew	Dough
	%	%	%	%	%	%
Flour	66.7	33.3	65	100	8.3	91.7
Water	42	25	43	70	33.6	32.1
Yeast food[a]	0.5	..	0.5	..	0.5	0.17
Yeast, active dry	0.9	..	2.4	0.675	1.2	..
Or yeast, compressed	2.25	..	6.0	2.0	3.0	..
Sugar	..	4.0	..	4.0	1.0	5.0
Salt	..	2.0	..	2.0	1.0	1.0
Nonfat dry milk	..	4.0	..	4.0	..	4.0
Shortening	..	3.0	..	3.0	..	3.0
Calcium carbonate	..	..	..	..	0.04	..

[a] A product of Red Star Yeast and Products Co. containing 0.3% potassium bromate and 9.7% ammonium chloride.

treated with 5 ml. of methylene blue solution (200 mg. methylene blue and 54 g. of potassium dihydrogen phosphate dissolved in 200 ml. of water). Cell counts were obtained with a Petroff-Hausser counting chamber and a microscope with 600× magnification. At least 10 fields of 90 smallest squares were counted for each sample, each field normally containing 50 to 150 yeast cells. Every bud large enough to be recognizable was also counted.

Dough Formulas. Yeast growth was determined in the fermentation systems shown in Table I.

The sponges were set at 26°–27°C. and fermented 4 hours. The sponge doughs received about 40 minutes of combined floor time and intermediate proof, followed by 50 to 60 minutes of pan proof at 35.6°C.

Sweet dough sponges were fermented 3.5 hours. Growth of yeast in the doughs was not determined.

The straight doughs were allowed three rises, followed by pan proof of 50–60 minutes. Total fermentation time was 3.0–3.5 hours.

Brews were fermented 3.5 hours at 30°C., and the doughs received about 50 minutes of combined floor time and intermediate proof and 55–65 minutes of pan proof.

Results and Discussion

The recovery of yeast cells from doughs was quite satisfactory, as shown in Table II. The data are for recoveries of compressed yeast from straight doughs and sponges sampled immediately after mixing.

TABLE II

RECOVERY OF YEAST CELLS FROM DOUGHS

DOUGH	YEAST ADDED	YEAST FOUND	RECOVERY
	millions/g	*millions/g*	%
Straight dough	239	243	102
	239	231	97
	239	231	97
Sponge	450	452	100
	450	428	95

Direct counts of yeast samples showed that compressed yeast contained about 22.1 × 10^9 cells per g., and active dry yeast (ADY) 38.7 × 10^9 per g. (both on an "as-is" basis). The ADY cells are larger and somewhat more active than compressed yeast cells, and to provide the same fermentation rate fewer ADY cells are needed than CY cells.

Some other factors affecting fermentation rate, including the relative amounts of the two types of yeast needed for equal rates, have been discussed elsewhere (7).

In this paper, growth of yeast is defined as cell increase, as evidenced either by an increase in the number of single cells or by the formation of buds. In reality, very little division occurred and nearly all growth consisted of bud formation. Toward the end of most of the dough fermentations, the majority of the buds were very large and often equal in size to the mother cells. It seemed reasonable, therefore, that the buds played a significant role in the over-all gassing activity of the yeast.

Table III shows the growth of yeast in straight doughs. Three different lots of compressed yeast were compared with three lots of ADY. It is apparent that the two types of yeast grew to the same extent.

TABLE III
GROWTH OF YEAST IN STRAIGHT DOUGHS

YEAST	CELLS (Millions per g. of Dough)						OVER-ALL INCREASE
	ZERO-TIME		END OF THIRD RISE		END OF PROOF		
	Cells	Buds	Cells	Buds	Cells	Buds	
							%
CY-A	239	4	225	16	229	85	
CY-B	226	5	222	35	241	92	
CY-C	224	7	226	31	225	92	
Av.	230	5	224	27	232	90	37
ADY-A	163	1	147	13	167	56	
ADY-B	145	3	134	22	133	51	
ADY-C	143	4	139	32	137	63	
Av.	150	3	140	22	146	57	33

The proportion of buds to mother cells at the end of proof was the same for both yeasts (39%). The data also show that most of the budding occurred during the proof stage, i.e., in the last hour of fermentation.

Growth characteristics of compressed yeast and ADY in sponge doughs are illustrated in Table IV. All or nearly all of the growth occurred during the sponge fermentation, the cell counts changing but little during the dough fermentation. As in the case of straight doughs, little difference was noted between compressed yeast and ADY. In general, it was found that both types of yeast form 50 to 60% buds in the 4-hour sponge fermentation. Most of the buds formed

in the first 3 hours. For example, in one set of experiments in which both yeasts grew 59% in 4 hours, the proportion of buds at 3 hours was 50% in the case of compressed yeast, and 51% in the case of ADY. The almost complete cessation of cell growth when the sponges were converted to doughs may have represented a lag phase engendered by the rather drastic change in the yeasts' environment.

TABLE IV

GROWTH OF YEAST IN SPONGE DOUGHS

YEAST	CELLS (Millions per g. of Dough)								OVER-ALL INCREASE
	SPONGE				DOUGH				
	Zero-Time		4 Hours		Zero-Time		End Proof		
	Cells	Buds	Cells	Buds	Cells	Buds	Cells	Buds	
									%
ADY-A	344	2	316	124	212	82	213	74	
ADY-B	313	6	330	114	215	79	233	93	
Av.	329	4	323	119	214	81	223	84	50[b]
			expected[a]		197	73			
CY-A	446	6	474	236	296	148	316	127	
CY-B	421	7	437	223	250	130	274	117	
Av.	434	7	456	230	273	139	295	122	55[b]
			expected[a]		277	140			

[a] Calculated from counts found for 4-hour sponges.
[b] Based on total population (cells + buds) in dough at end of proof compared to that initially in sponge.

TABLE V

YEAST POPULATIONS IN SWEET DOUGH SPONGES

YEAST	CELLS (Millions per g.)			
	ZERO-HOUR		3.5 HOURS	
	Cells	Buds	Cells	Buds
ADY	800	27	790	21
CY	1090	33	1060	47

Neither compressed yeast nor ADY grew in the sweet-dough sponges, as shown in Table V. These sponges were fermented 3.5 hours, and contained typically high levels of yeast, the yeast:flour ratio being about 2.7 times as great as that in regular or pan bread sponges. The reason for lack of growth is not known, but the results agreed qualitatively at least with those of Simpson (6) and Hoffman *et al.* (4), who found growth to decrease as yeast concentration was raised.

Examination of flour brews at the end of the 3.5-hour fermentation period showed that growth was obtained with neither compressed yeast nor ADY in this system. With compressed yeast, for example,

TABLE VI
GROWTH OF YEAST IN BREW DOUGHS

YEAST	CELLS (Millions per g.)				OVER-ALL INCREASE
	ZERO-TIME		END PROOF		
	Cells	Buds	Cells	Buds	
ADY	284	6	293	55	20%
CY	386	5	388	63	16%

the proportion of buds at the end of the fermentation was 3% as compared to 4% at the start. There was no change in the number of single cells. These results agreed with those of Choi (2), who reported that compressed yeast did not bud or multiply in milk-buffered brews. Growth did occur in the doughs made with flour brews, however, as Table VI shows. While the amount of budding was relatively small, its occurrence was unexpected in view of the lack of growth found in sponge doughs.

When these studies were initiated, it was hoped that changes in the nitrogen content of the isolated cells could be used as a second index of growth. Analyses of dough samples taken immediately after mixing showed that the degradation procedure eliminated about 99.5% of the flour nitrogen but only 30% of the yeast nitrogen. Further experiments, however, showed that the recovery of yeast nitrogen varied considerably during straight dough fermentations, presumably because of the changes in the types and distribution of nitrogenous compounds in the actively growing yeast. Because of this inconstancy, the nitrogen values could not be used as a criterion of cell growth.

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