Nature of the San Francisco Sour Dough French Bread Process

II. Microbiological Aspects

By T. F. Sugihara, Leo Kline, and Linda Bele McCready
Western Regional Research Laboratory,
Albany, Calif.

In investigating the nature of the microorganisms of the sour dough process, we examined the starter or mother sponges from five different major producers of sour dough French bread in the San Francisco Bay area for both bacteria and yeasts. Initially all attempts to isolate bacteria in appreciable numbers, using several dozen agar media designed for isolation of total, lactic, acetic, anaerobic, coliform, etc. bacteria, were unsuccessful. However, all five starter sponges were found to contain a rather unusual species of yeast readily identified in part by its *inability* to ferment or grow on *maltose* and by its surprising ability to grow out well in the presence of Actidione (cycloheximide), an antibiotic inhibiting the growth of most yeasts (**Table I**). With the aid of Professor Martin Miller, University of California at Davis, this yeast was classified as *Saccharomyces exiguus* (spore former) or its non-spore-forming equivalent, *Torulopsis holmii*. In four of these starter sponges this was the only yeast strain present in the approximately 160 isolates examined. The fifth starter sponge also contained *S. exiguus* but, in addition, contained a second yeast in various proportions which did ferment maltose but not galactose and was identified as *Saccharomyces inusitatus*. No baker's yeast types (*Saccharomyces cerevisiae*) were found in any of the approximately 200 isolates made from the five sources. For the purpose of this paper, the principal yeast isolate, *S. exiguus*, will be considered as the "sour dough yeast."

The inability of this sour dough yeast to ferment or grow on maltose was surprising since maltose is generally considered to be the principal fermentable sugar available in doughs prepared without addition of sugars which, of course, is the case here. However, this inability to use maltose was found to be of special significance in the sour dough system as will shortly become clear.

The mere isolation of a microorganism from a fermented food product is not sufficient to establish its role in the making of that product. That this sour dough yeast isolate has a key role in the sour dough process was established in several ways. First, of course, the finding of the same yeast strain in all five sources to the virtual exclusion of other yeasts suggests that it may be of some significance. Secondly, the numbers (yeast counts) found under various handling conditions correlated quite well with the *leavening* action observed in the sour dough. Thus, a count in the starter sponge of 10 to 25 million yeast cells per gram gave normal proofing power in the bread dough made from that sponge; less than four million gave poor proofing power and loaf volume; counts in execss of 40 million gave undesirable ballooning effects. In the bread dough itself, where the starter sponge is diluted by a factor of 7 to 10, the initial yeast count on make-up ranged from two to four million per gram, or roughly 1/50th to 1/100th the level at which baker's yeast is used in conventional bread dough – hence the long proof time necessary for sour dough.

Studies with pure cultures of the isolated sour dough yeast lent further support for its probable role as the leavening agent in sour dough. In these studies the yeast isolates were grown on sterile broth media (yeast extract + tryptone + glucose), the cells separated out and washed, and then added back to a *simulated* sour bread dough in numbers comparable to those encountered normally. The *simulated* bread dough was made in the usual manner but with a "dead" starter sponge in place of the live starter so that the bread dough by itself possessed no *leavening* or *souring* power, permitting the contribution of the added sour dough yeast to be appraised. The "dead" starter was obtained by freezing and storing a fully developed live starter for several months after which time it loses all of

Table I
Sour Dough Yeast vs. Bakers Yeast

Yeast	Fermentation ±					Actidione ±
	Glucose	Sucrose	Raffinose	Galactose	Maltose	
Bakers	+	+	+	+	+	−
Sour Dough	+	+	+	+	−	+

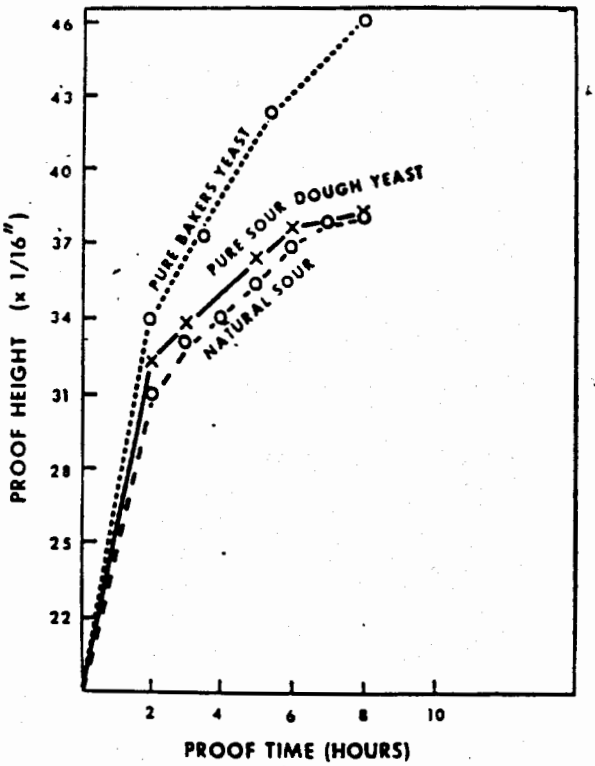

Figure 1: *Proofing ability of pure yeasts in simulated sour bread doughs vs. normal proof with live starter sponge (1 lb. loaves). Initial pH = 5.3*

Initial yeast counts/g. of bread dough { *1. live starter: 4 × 10⁶*; *2. pure sour dough yeast: 5 × 10⁶*; *3. pure baker's yeast: 6 × 10⁶* }

(Note: This study is atypical in that bread doughs were held overnight at 55°F. before being proofed at 86°F.)

its activities. Using the "dead" starter in the normal proportion, however, provided a typical beginning acidic environment and also was essential in providing strength and structure to the bread dough.

One such study is illustrated in **Figure 1** where the proof height increase with time observed for a bread dough made in the usual manner with a live starter sponge is compared with those observed for pure sour dough yeast and a pure baker's yeast strain used in the "dead" starter formulation.

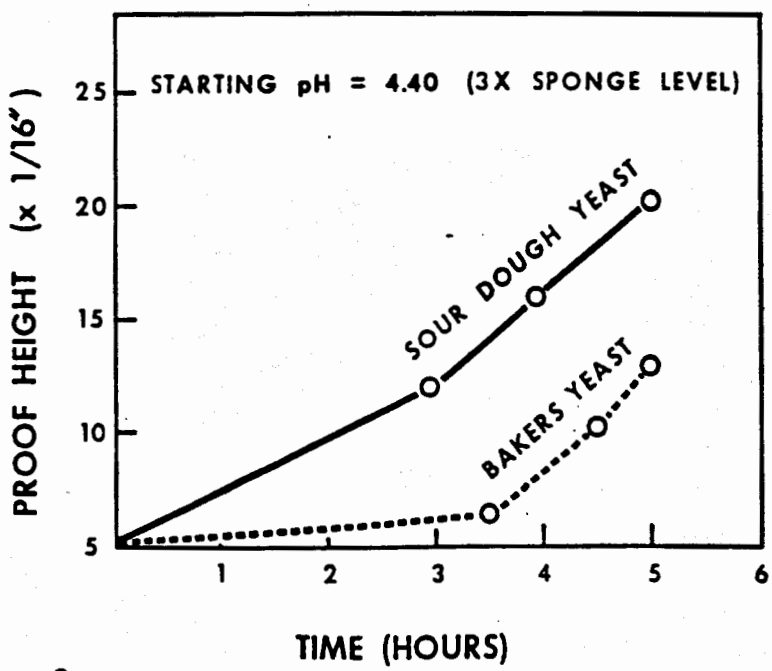

Figure 2: *Proofing ability of pure yeasts in simulated sour bread doughs (pup loaves). 3 × normal level of "dead" starter sponge used to achieve initial pH of 4.4.*

Initial yeast counts/g. of bread dough { *1. sour dough yeast: 9 × 10⁶*; *2. baker's yeast: 9 × 10⁶* }

(The pure baker's yeast strain was an isolate from commercial compressed yeast and was grown, etc. in the same manner as that used for the sour dough yeast.) It is significant that the curves are virtually identical for the live starter and pure sour dough yeast + "dead" starter formulation. In both cases the early tapering off of the curves can be accounted for by their inability to ferment maltose (which, of course, is developed in the dough) and their exhaustion of whatever fermentable carbohydrate are contributed by the flour. Thus, the type of leavening action exhibited by the natural starter sponge is more or less duplicated by the pure sour dough yeast suggesting their identity. The proof height vs. time curve for the *simulated* sour dough made with the baker's yeast strain continues to much higher levels since it can utilize the maltose as well as the flour carbohydrates.

Another significant result of the pure culture yeast studies was the demonstration of the unusual vigor and growth of the sour dough yeast, as compared to baker's yeast, in the particular acidic environment of sour dough starter sponge (pH 3.8 – 4.5, approximately 30 to 50 per cent of acidity = acetic). **Figure 2** illustrates the leavening superiority of the sour dough yeast over that of the baker's yeast in a system where the low pH of 4.4 at make-up was obtained by using a higher than normal proportion of "dead" starter sponge (the normal proportion gives a pH at make-up of 5.2 to 5.3). In **Figure 3** the normal proportion of "dead" starter sponge was used but the pH at make-up was decreased to 4.15 by addition of a blend of lactic:acetic acids (7:3) and the superior leavening ability of the sour dough yeast was even more evident. In both of these studies the numbers of viable baker's yeast cells *decreased* during the six hour proof period to about one-third of their original count, while the numbers of sour dough yeast cells *increased* about one and one-half times. At higher pH's, such as 5.3, representing the usual starting pH of sour bread dough at make-up, baker's yeast is, not surprisingly, superior in proofing power to the sour dough yeast (**Figure 4**). This, of course, explains the benefits achievable by addition of low levels of baker's yeast at make-up as described in the first paper. In view of the above comparisons, however, one would expect that baker's yeast would become increasingly inactive in the sour dough environment as the pH drops below 4.5, probably due to its intolerance to the acetic acid produced in the sour dough fermentation.

The superior vigor of the sour dough yeast in an acetic acid containing environment was also observed in the flour suspension studies as illustrated in **Table II.** A 10 per cent flour suspension was adjusted to pH 4.15 with acetic acid and separate portions inoculated with sour dough yeast, the pure baker's yeast strain, and commercial compressed baker's yeast. As shown, the viable counts of both baker's yeasts decreased in six hours to less than 0.1 per cent of their original numbers, whereas the sour dough yeast eventually multiplied over-all.

Sour Dough Bacteria

At the time these pure culture studies with the isolated sour dough yeast were carried out, all our attempts, as mentioned earlier, to isolate bacteria of any type, including lactics, in appreciable numbers from the sour dough starter had been unsuccessful and the possibility, therefore, existed that the isolated yeasts might be responsible for the souring as well as the leavening action. However, the above studies with the pure sour dough yeasts made it very clear that these yeasts were *not* directly involved in the souring mechanism. For in none of these studies using the pure yeast and the *simulated* sour dough prepared with "dead" starter sponge was any significant pH drop, i.e., souring action, observed, regardless of the starting pH. Furthermore, in other studies it was found that under some conditions there appeared to be a separation of the leavening and souring functions. For example, it was observed that freezing a fully developed starter sponge for a limited time (three to four days at 0°F.) destroyed virtually all of the leavening power (and yeast count) without greatly reducing the souring power, i.e., the bread dough formulated from it would not rise appreciably but soured in the usual proof time all the way down to pH 3.9.

At this point in our studies renewed attempts were made to isolate bacteria from the sour dough system. After many abortive attempts, an artificial isolation medium was developed on which the sour dough bacteria could readily be isolated and, accordingly, their numbers in the sour dough enumerated. This isolation medium called for an unusual combination of nutritional requirements many of which are supplied in its natural environment either from the flour or the sour dough yeast. First of all, the key finding was that these sour dough bacteria would grow on or ferment only [illegible]ose and not on any of the

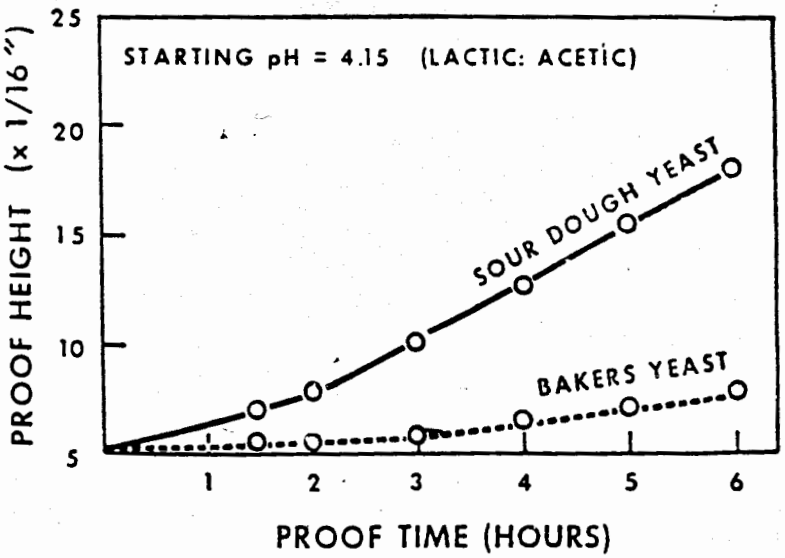

Figure 3: ***Proofing ability of pure yeasts in simulated sour bread doughs (pup loaves). Normal level of "dead" starter sponge used, but initial pH adjusted to 4.15 with lactic:acetic acid (7:3).***

Initial yeast counts/g. of bread dough { ***1. sour dough yeast: 10 × 10⁶*** ***2. baker's yeast: 9 × 10⁶***

Table II

Comparison of Yeasts in Flour Suspension Studies

Formula: 100 g. flour Starting pH = 4.5 (acetic)
1000 ml. water
1.5 g. salt
1.0 g. yeast cake

	pH			Yeast count × 10^3/ml*		
	0	6 hr	24 hr	0	6 hr	24 hr
Control	4.4	4.4	4.5	0	0	0
Pure Bakers Yeast	4.5	4.5	4.6	70	0	0
Commercial Bakers Yeast**	4.5	4.5	3.7	80	0	0
Sour Dough Yeast	4.4	4.5	4.7	40	60	200

*0 Count = $<1 \times 10^4$/ml

**Compressed Bakers Yeast: Yeast count = 17 billion/g., bacterial count = 1 billion/g. Growth of these bacteria responsible for pH drop in 24 hrs. in this sample.

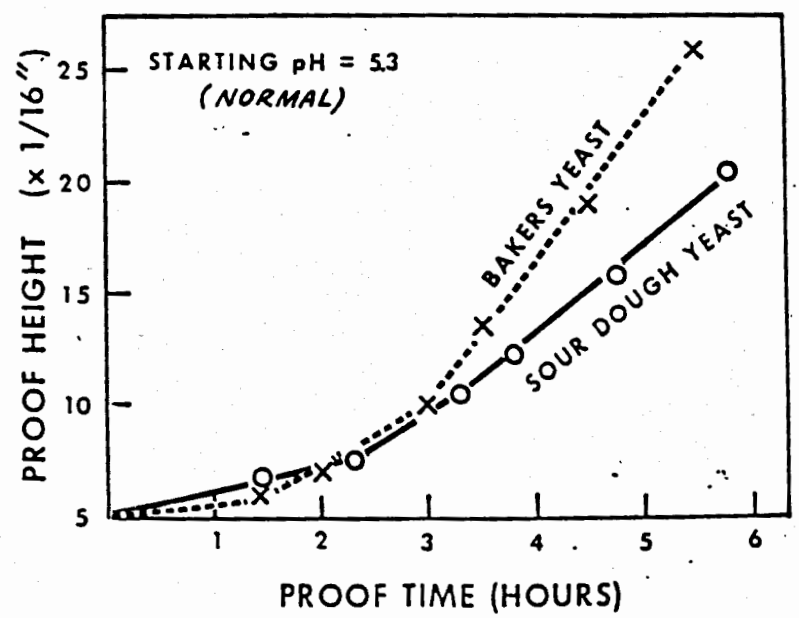

Figure 4: ***Proofing ability of pure yeasts in simulated sour bread doughs (pup loaves), using normal level of "dead" starter sponge. Initial pH = 5.3***

Initial yeast counts/g. of bread dough { ***1. sour dough yeast: 10 × 10⁶*** ***2. baker's yeast: 9 × 10⁶***

Table III

Sour Dough French Bacteria vs. Sour Rye Starters (Commercial)

	Fermentation Patterns		
Sugar	Sour dough French	Rye #1	Rye #2
Xylose	−	+	−
Arabinose	−	+	−
Glucose	−	+	+
Galactose	−	+	+
Lactose	−	+	−
Sucrose	−	+	±*
Maltose	+	+	+
Raffinose	−	+	−
Rhamnose	−	+	−

* + only in presence of CO_2

usual sugars such as glucose, sucrose or lactose. This absolute requirement for maltose is illustrated in **Table III** where comparison is made with the markedly different carbohydrate fermentation patterns of two commercial rye bread sours (starters). Rye sour No. 1, by its carbohydrate fermentation patterns, would be judged to be a heterofermentative type *Lactobacillus* or one that produces substantial amounts of acetic acid (and CO_2) from sugars as well as lactic acid. Obviously it is not identical with the sour dough French bacteria, although these too produce large amounts of acetic acid as discussed above.

It is significant that the absolute requirement of the sour dough bacteria for maltose dovetails with the earlier mentioned observation that the sour dough yeast does not utilize maltose. Thus, the two microorganisms of the sour dough system (no others have been found to date) are not competitive for the same carbohydrate source which, doubtless, has contributed to their ability to jointly survive in this system over such an extended period.

Other properties of these sour dough bacteria might place them with the *Lactobacilli* were it not for their absolute requirement for maltose. They are gram-positive when young, catalase-negative, non-motile, appear variously as short rods as well as in many unusual forms (involuted, pleomorphic and filamentous) and produce substantial amounts of lactic acid, all of which might group them with the *Lactobacilli*. Their nutritional requirements include, in addition to maltose, an absolute requirement for unsaturated fatty acids (which we supply as Tween-80), a critical need for fresh yeast extractives (supplied either by freshly prepared yeast autolysate or autoclaving a suspension of compressed baker's yeast and using the clarified filtrate), a critical need for CO_2, and a requirement for an acidic environment (pH <6.0). They are indifferent to the presence or absence of oxygen. Casein hydrolysate solids are stimulating. None of these requirements or properties are particularly foreign to some of the lactic acid bacteria although they are more rigorous. There are other differences, such as the complete inhibition of these sour dough bacteria by 0.1 per cent sorbic acid. Further work is progressing to determine if these sour dough bacteria are mutants derived from a known species of *Lactobacillus* which has lost its ability to utilize any carbohydrate except maltose or are, indeed, new species.

Development of an artificial growth medium for these bacteria combining the above-mentioned nutritional requirements has permitted us to count their numbers in the starter sponges and bread dough. Generally they occurred at levels of about one billion per gram of dough or roughly 50 times as numerous as the sour dough yeast cells. This is illustrated in **Table IV.**

The ability to enumerate these bacteria enables us to understand many of the effects we have observed. For example, it has been found that excessive amounts of added baker's yeast may inhibit the souring action somewhat. In **Table V** it is noted that at the higher levels of baker's yeast the growth of the souring bacteria is indeed inhibited. This is not surprising since they would be competing for the maltose. **Table V** also illustrates the superior vigor of the sour dough yeast in competing with baker's yeast in the actual bread dough. Thus, at a level of 0.1 per cent added baker's yeast, it is observed after a seven-hour proof that the numbers of viable baker's yeast cells are reduced to about 1 per cent of their original value, while the sour dough yeast has multiplied four-fold. It is no great trick to count these yeasts separately: a medium containing Actidione, which completely inhibits baker's yeast, is used to enumerate the sour dough yeast, while a medium containing maltose as the only fermentable carbohydrate is used to enumerate baker's yeast.

It was mentioned earlier that a short freezing period would virtually destroy all the leavening power in a fully developed starter sponge without greatly reducing its souring power. This has been found to correlate excellently with the retention of 60 to 80 per cent of the viable bacterial count after frozen storage and a reduction of the yeast count to less than 0.05 per cent of the original numbers **(Table VI).**

The evidence correlating the occurrence and growth of these bacteria with the souring action in the sour dough is not as complete as we would like as we haven't, as yet, carried out the ultimate proof which will depend on growing these bacteria out in pure culture and adding the cells back to the *simulated* sour bread dough along with pure cultures of the sour dough yeast. However, the circumstantial evidence for their role is pretty convincing, as follows:

1. The enormous numbers at which they occur. Several billion per gram of starter sponge is too high a level not to have a profound effect in the dough.

2. They are the only bacteria isolated in appreciable numbers from any of the four different sources (manufacturers) examined and the bacteria from each source have similar or identical properties.

3. Their numbers correlate very well with souring power as illustrated by the abbreviated frozen storage study described above and in other circumstances.

4. Finally, quite recent studies have demonstrated the striking souring ability of the sour dough bacteria when grown in pure broth culture on synthetic media.

Table IV

Microbial Changes During Sour Dough Bread Proofing

	Millions of microorganisms per gram of bread dough	
	"0" Time	After 7 hr. proof
Sour dough yeast	3	18
Sour dough bacteria	86	1650

Note: Composition of starter sponge { Yeast = 15 to 23 million per gram; Bacteria = 600 to 2000 million per gram }

General Comments

Some light has been thrown on the nature of the San Francisco sour dough system by characterizing its

particular acidic environment as one containing substantial levels of acetic acid and by isolation and characterization of two unusual microorganisms which play a role in the process and which thrive in this environment. The bacterium responsible for the souring activity remains unidentified although it resembles *Lactobacilli* in many respects. However, its absolute requirement for maltose as the only carbohydrate it can utilize sets it apart, at least for the moment. The yeast, *S. exiguus*, shown to be responsible for the leavening activity in the sour dough is, perhaps not fortuitously, unable to utilize maltose, thereby not competing with the sour dough bacteria for this principal source of carbohydrate. Instead, the yeast must rely on the fermentable carbohydrates, other than maltose, contributed by the flour and these, of course, are not competed for by the bacteria. According to Dr. Robin Saunders of our laboratory, these fermentable carbohydrates are somewhat in excess of 2 per cent (of the flour) and consist of small amounts of glucose and fructose and larger amounts of low molecular weight glucofructosans.

Other aspects of the process which contribute to its self-protective and sustaining nature include the finding that the sour dough yeast shows an unusual vigor and tolerance in this acetic acid containing environment in which most microorganisms, including baker's yeast, die off. Both the yeast and the flour contribute to the unusual combination of nutritional requirements of the sour dough bacteria for growth which includes, in addition to the absolute requirement for maltose, such factors as fresh yeast extractives, unsaturated fatty acids, CO_2 and an acidic environment. It seems probable that the absence of previous reports on these particular bacteria may have been due to the lack of success others have encountered in attempting to isolate them from the doughs.

Another perhaps significant contribution of the sour dough yeast may result "inadvertently" from its inability to utilize maltose and thereby leave a residue of reducing sugar to contribute to proper crust browning. We have found, for example, that addition of baker's yeast at higher levels interferes with this browning.

It seems apparent that this system would not work, e.g., if baker's yeast were teamed with the sour dough bacteria. Not only would they be competing for the maltose but the baker's yeast could not tolerate the type of acidity developed. Similarly, ordinary lactic acid bacteria would probably not work well if teamed with the sour dough yeast, for they too would be competing for some of the same fermentable carbohydrates.

The development of this knowledge on the sour dough microorganisms should provide a basis for development of starter cultures either on flour or grain, or in dried or frozen concentrated form. However, we feel that the present commercial process would probably have to be modified to utilize these effectively and along those lines we have had some fairly promising preliminary results in making sour dough French bread from liquid sponges. One batch of the latter might replace 50 to 100 starter sponges as presently made and could, of course, be directly derived from the concentrated starter cultures.

Other applications of these microorganisms include the possibility of using them to enhance the flavor of white bread made by any of the abbreviated fermentation methods, in sour ryes or possibly in soda cracker sponges.

THE END

Table V
Added Bakers Yeast: Effect on Microbial Changes During Proofing

Added Bakers yeast % (of flour)	Cell Counts Per Gram Of Bread Dough*					
	Bakers Yeast		Sour Dough Yeast		Sour Dough Bacteria	
	0	7 Hr.	0	7 Hr.	0	7 Hr.
0	0	0	26	156	64	1027
0.10	86	1	28	118	73	1132
0.25	202	46	25	88	59	700
0.50	395	178	25	83	62	708

* × 100,000 for yeasts; × 1,000,000 for bacteria

Table VI
Effect of Short Freezing Time on Retention of Yeast and Bacterial Activities in Starter Sponges

Sample Frozen	Sour Dough Yeast Count/g.		Sour Dough Bacteria Count/g.	
	At Make-Up	After 4 Days at 0°F.	At Make-Up	After 4 Days at 0°F.
Fully developed starter, pH 3.9	15 million	Less than 10,000	1,200 million	700 million
Slightly developed starter, pH 4.5	8 million	500,000	700 million	600 million

Dough Rheology

(Continued from page 46)

There is also a transition in the oven in which the initial structure of dough of individual gas cells is transformed into a porous structure permeable to air. This structure is brought about likely by the action of amylases on gelatinized starch in the walls of the gas cells. In addition there may also be some physical rupture as the total pressure of carbon dioxide, water and ethanol vapor increases rapidly.

Eventually the total structure is set in the baking process. As the loaf is cooled the process of expansion is changed to one of contraction. Here the rigidity of the final structure and its porosity are important. Otherwise one can expect a contraction of the loaf leading to a variety of faults such as keyholing or cave-in.

In summary, it will be readily appreciated that the quality of a loaf of bread is the sum total of a great many factors. There is a delicate balance in their interaction with one another, and a critical timing when the influence of one factor is taken over by another which becomes dominant. This interplay of various forces continues throughout until the loaf of bread is removed from the oven and cooled. The baker or the bakery engineer soon learns by trial and error the adjustments that he must make in order to obtain optimum quality in the bread he produces. The rheologist attempts to systematize general experience into established cause and effect relationships. It is hoped that this analytical survey has contributed to an increased awareness and a better understanding of the significance of physical and rheological properties in the breadmaking process.

THE END