SURVIVAL OF PATHOGENIC BACTERIA IN PRE-FERMENTS

by

GERALD LELAND GILARDI

B. A., University of California, 1955

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1959

TABLE OF CONTENTS

INTRODUCTION

One of the most significant developments in the baking industry during the past 50 years is the continuous, fully automatic bread production process devised by J. A. Baker (Barnard, 1954). This process utilizes a fermentation similar to the straight- and sponge-dough ferment (Pirrie, 1955; Jacobs, 1951) but conducted in tanks instead of in dough troughs and handled as a liquid instead of a dough.

The older straight-dough method consists of mixing all the ingredients in one step. The dough is allowed to ferment to double its volume and then is punched to release the gas. This process is repeated and the dough put into pans to bake. In the sponge-dough method, a sponge containing 50 to 75 percent of the ingredients is prepared. After three to five hours of fermentation, the sponge is mixed with the remaining ingredients and is baked in a manner similar to the straight-dough method (Miller and Johnson, 1957).

In the new, continuous, dough mixing method the process depends upon the production of a liquid pre-ferment, also called the yeast broth or "brew". The pre-ferment in the yeast fermentation tanks consists of water, yeast, yeast food, sugar, and possibly other materials intended to contribute flavor. By using sugars a rapidly progressing fermentation takes place resulting in a potently flavorful pre-ferment, which, after about six hours of fermentation, is ready for the doughing process. The flour plus the proportioned pre-ferment are continuously combined in the premixing machine and finally developed into a dough. This eliminates the conventional sponge-making operation so that without further fermentation the dough is put into the loaf pans and normal baking follows.

It appears that the pre-ferment method of dough making is practical, well adapted to bulk handling methods, and will produce bread of high quality without necessarily increasing the cost of ingredients. In addition, it saves time, simplifies mixing operations, may be easily controlled by automation, and presents an opportunity for introducing more accentuated "fermentation flavor" than in bread baked by conventional methods. Relatively little is required in the way of additional equipment, and considerable space may be saved (Barnard, 1954).

With the introduction of the new process for making bread, there is also introduced a new problem from the public health aspect. The problem is the possibility that the pre-ferment might serve as a source of food poisoning or food infection agents. This might occur through contamination of the pre-ferment by flies, rodents and workers in the bakery who are carriers of pathogenic bacteria.

There are two groups of individuals who might contract food poisoning or infection. One is the consumer of the bakery products. There is not much chance for food infection since the bread is baked at a temperature which normally would destroy the responsible bacteria. However, several of these organisms, e. g., Salmonella, Proteus, and certain strains of Escherichia coli, produce a heat-stable enterotoxin which will not be destroyed by the baking process and which will cause food poisoning. Certain Staphylococcus strains also produce heat-stable enterotoxin which causes food poisoning. The staphylococci may originate from the upper respiratory tract (nose, throat) of a person with a sore throat and cold or from the person's skin.

The second group of individuals possibly subject to risk is the

personnel who work in the bakery. These persons could pick up the organisms from the pre-ferment or baking equipment which was contaminated by carriers.

If bread or other bakery products were serving as a source of food poisoning, it would be a difficult problem to detect the source before the consumer used the product. This problem arises since there is no practicable way to check the bread or bakery products to see if they have enterotoxin present. The methods used in a diagnostic laboratory for detection of toxins would not be practical or economical for use in a bakery.

Since there is the possibility that the pre-ferment might serve as a source of food poisoning or food infection agents, this project was initiated to secure information on the survival of microbial populations of certain enteric microorganisms and staphylococci in the various preferments, i.e., the liquid phase of the dough.

REVIEW OF LITERATURE

Only one work on the survival of enteric organisms in pre-ferments has been published (Robinson, et al., 1958). Therefore, the following review of the literature consists of information on the survival of enteric organisms in the specific ingredients of the pre-ferment formulae such as salt, water, dried milk, flour, yeast, and sugar.

In an examination of 125 samples of salt, Yesair (1930) was unable to find Escherichia coli* but found Pseudomonas in a few samples. No other enteric organisms were found. Yesair mentioned that in the process of refining high grade table salt, practically all bacteria are destroyed.

---

*The nomenclature of organisms is that used by the original authors.

DeFreytag's (1890) experiments pointed out that Salmonella typhosa was viable after six months in concentrated salt solution. Rappin (1920) showed that E. coli survived in a solution of seven to eight percent salt for six weeks, Salmonella enteritidis for four and one-half weeks, and Proteus vulgaris for three weeks. The minimum salt concentration which inhibited growth of the test bacteria was 10 percent.

Other work by Rappin (1920) showed that salt may carry undesirable bacteria and if used in food preservation should be carefully handled and protected from undue contamination. He found that bacterial counts ranged from zero to 1,470 per gm in a series of 125 samples. In the process of refining, Rappin stated that practically all of the bacteria are destroyed in salt.

Ballentine (1930) stated that S. typhosa remained viable for from five to 32 months in 0.85 percent NaCl solution and 14 to 32 months in distilled water. Distilled water was more favorable for the survival of S. typhosa than was 0.85 percent NaCl solution and in either menstruum the organism survived five times as long at room temperature as at 37° C. Distilled water was also a more favorable medium for survival than 0.85 percent NaCl for E. coli, Streptococcus hemolyticus, and Streptococcus viridans. Prolonged survival of S. typhosa is associated with a late period of comparatively low death rate. Like S. typhosa, these other organisms survived longer at room temperature than at 37° C. in either menstruum.

Jordan, et al. (1906) noticed that there was variance in the results obtained when S. typhosa was suspended in collodion sacs in pure water. The organism died within three days in one experiment, seven days in another experiment, and 10 days in a third experiment. These experiments

were run on one ml. samples. When 100 ml. quantities were examined, it required nine weeks for the total disappearance of S. typhosa. Ninety-nine percent of the test bacteria disappeared in one week. The rate of death of S. typhosa and E. coli in pure, natural water has been reported to follow the monomolecular law (Jordan, et al. 1906). Under these conditions, the rate of death increased with the temperature.

Whipple (1922) found that 99 percent of the original number of S. typhosa in water died in one week and of the remainder some lived for five to eight weeks. When the effect of temperature was studied, the following storage periods were necessary for the different temperatures given to reduce S. typhosa organisms from 100,000 to three per ml.: five weeks at 32° F, four weeks at 40° F, three weeks at 50° F, two weeks at 64.4° F. It was recognized that temperature was a very important factor in the question of longevity of S. typhosa in water. Viability of Vibrio cholerae studied by Whipple (1922) in the same manner as S. typhosa showed that 99 percent of the cholera organisms perished in three days under the laboratory conditions of the test.

Kossowicz (1908) found that in dried milk samples Pseudomonas fluorescens was not destroyed during the drying of milk, and the survival of streptococci, S. enteritidis and E. coli was recorded also. In another experiment by Hunwicke and Jephcott (1925), milk which had been inoculated with E. coli, Aerobacter cloacae, and Staphylococcus albus was dried. These bacteria were recovered from the dried milk.

Boxby and Supplee (1930) inoculated cultures of hemolytic streptococci and Proteus morgani in very large concentrations into milk. The bacteria were destroyed when the milk was dried.

Nichols (1939), Macy (1928), and Higginbottom (1953) all stated there is a decrease in bacterial numbers in dried milk powder on continuous storage and a greater decrease with increase of the storage temperature up to 37° C. Maximum survival of bacteria was found to occur at about 10 percent relative humidity.

Higginbottom (1943) found viable coliform bacteria in 23 percent of dried milk samples tested. He stated that on storage there was either a decrease or no change in the numbers of coliform bacilli. Streptococcus faecalis and Micrococcus casei liquefaciens were the most common organisms found in reconstituted milk by Higginbottom (1948). When using pure cultures of a Streptococcus and Micrococcus originally isolated from dried milk, Higginbottom (1953) found maximum survival occurred at 5 to 15 percent relative humidity in the dried milk.

Escherichia coli of fecal origin has been found in flour. It was suggested by Kent-Jones and Amos (1930) that the organisms might originate in the water used for the purpose of washing the whole wheat. Schulz (1954) stated that E. coli is frequently encountered in flour and can cause serious defects in the finished baked product. Large splits in the texture, moisture strips at the bottom of the loaf, irregular porosity, as well as a bitter and stale taste are symptoms of a strong infection by these bacteria.

Gustafson and Parfitt (1933) reported that the total bacterial count of wheat was greater than that of the flour made from it. Gustafson and Parfitt (1933), Lyng and Aschehong (1935), and Kent-Jones and Amos (1930) claimed that the storage of flour usually results in a lowering of the bacterial count and that the rate of diminution depends upon the moisture content and the temperature.

Compressed yeast cake contains numerous bacteria according to Tanner (1944), but since the yeast cells are present in such large numbers they overgrow the bacteria. However, if the yeast is kept at a warm temperature, the bacteria spoil the yeast.

James (1928) found E. coli in only two of 197 samples of sugar he examined. He found no other enteric organisms.

EXPERIMENTAL

Cultures Used

The organisms used in this study were obtained from Dr. T. H. Lord, which included the following:

Shigella paradysenteriae Newcastle L-22

Shigella sonnei L-110

Shigella flexneri L-87

Shigella paradysenteriae Hiss L-86

Pseudomonas aeruginosa L-4

Proteus morgani L-150

Proteus rettgeri L-43

Proteus vulgaris L-6

Salmonella schottmülleri L-7

Salmonella choleraesuis L-2

Salmonella typhimurium L-88

Salmonella typhosa L-163

Aerobacter cloacae L-8

Escherichia coli L-145

Staphylococcus aureus L-47

Media Used

The media used in this study were:

Staphylococcus Medium 110 (Difco) - for *S. aureus*

Violet Red Bile Agar (Difco) - for coliforms

Bismuth Sulfite Agar (Difco) - for *Salmonella* species

S - S Agar (Difco) - for *Proteus* and *Shigella* species

2% Gelatin in Nutrient Agar - for *Ps. aeruginosa*

Preparation of Culture Inoculum

The cultures of the test organisms had been recently subjected to determinative varification. Prior to starting the experiment the cultures were checked for contamination by streaking onto an agar medium appropriate for each test organism and noting the uniformity of colony characteristics and the lack of contaminating bacteria. The cultures thus checked were inoculated onto nutrient agar slants to serve as stock cultures.

A tube containing 3.5 ml. of nutrient broth was inoculated the day before the pre-ferment was to be tested. The pre-ferment was then inoculated with a 24-hour broth culture diluted in such a way that there would be approximately 3,000 test bacteria per ml. of pre-ferment. This was obtained by the following procedure:

1. inoculating the organisms into nutrient broth and incubating for 24 hours,

2. making plate counts to find the number of organisms per ml. of broth of the stock culture,

3. calculating the dilution that would be necessary from a 24-hour broth culture to give approximately 1,000,000 bacteria per ml.,

4. making this dilution and adding one ml. of this dilution to approximately 350 ml. of the pre-ferment to give about 3,000 bacteria per ml. of pre-ferment, i.e., 1,000,000 bacteria per 350 ml. of pre-ferment.

Pre-ferments Used

The formulae used for the project consisted of the basic pre-ferment formulae, excluding malt (Table 1). Arkady yeast food was added to supply ammonium, calcium, and phosphate ions to stimulate yeast activity. Cerelose was used as the fermentable sugar source. The Fleischmann brew improver was added as the buffering agent in the Salts-buffered pre-ferment.

Procedure

Sterile quart jars were used as containers for the pre-ferments. For each batch of pre-ferment 100 ml. of tap water at 22° C was used to dissolve the yeast and the combined dry ingredients. The rest of the water was added to the mixture, and the jar was covered loosely with a mason type lid.

The calculated amount of test organism was added to each batch of pre-ferment at this time and the pre-ferment thoroughly mixed. The pre-ferments were incubated at 30° C until the end of the six-hour period. After the six-hour period, they were incubated at 8° C until the end of the 24-hour period. Samples were withdrawn after 0, 1, 2, 3, 4, 5, 6 and 24 hours of incubation.

For plate counts the pre-ferment was diluted 1:100 by adding one ml. of the pre-ferment to 99 ml. sterile distilled water. One ml. of this 1:100 dilution was plated using the appropriate medium previously mentioned. In

Table 1. Basic pre-ferment formulae.

Ingredients	Pre-ferment ADMI*	Pre-ferment Sugar	Pre-ferment Salts-buffered
Water	320 ml	320 ml	320 ml
**Cerelose	21 gm	21 gm	21 gm
***Arkady	3.5 gm	3.5 gm	3.5 gm
Salt	7 gm	7 gm	7 gm
Compressed yeast	14 gm	14 gm	14 gm
Dried skim milk	42 gm	—	—
****Fleischmann's Brew Improver	—	—	2.1 gm

*ADMI - American Dry Milk Institute formula.
**Cerelose - Commercial grade of dextrose manufactured by Corn Products Sales Company, New York.
***Arkady - A yeast food employed in bakery practice composed of ammonium, calcium and phosphorus compounds.
****Fleischmann's Brew Improver - Standard Brand, Inc. buffer salt mixture consisting of $CaSO_4$, $CaCl_2$, NH_4Cl, NaCl and flour.

those cases where it had been found that the count was less than 100 per ml., one ml. of undiluted pre-ferment was added directly to the plate for plating. All plates were incubated at 37° C, and counts were taken at the end of 48 to 72 hours of incubation.

RESULTS

Appendix 1 presents the results of the experiments on the survival of the various test organisms in the three pre-ferments. A statistical analysis of the counts on the survival of the various organisms is summarized in Table 2. The F value represents the probability of a true trend in the counts of survivors. If F is greater than 4.35, the probability that there

Table 2. Probability of a true trend in the counts (F) and the significance in the difference (b) of the counts on the survival of pathogenic microorganisms in the three pre-ferments.

Organism	Pre-ferment	F	b
Salmonella schottmülleri	ADMI	41.4052	-0.0617 ± 0.0632
	SUGAR	70.6814	-0.4550
	SALTS	61.6792	-0.7551
Salmonella choleraesuis	ADMI	19.9955	-0.0812 ± 0.0378
	SUGAR	111.3973	-0.5522
	SALTS	18.8938	-0.3052
Salmonella typhimurium	ADMI	12.1781	-0.1703 ± 0.1725
	SUGAR	105.8931	-0.5707
	SALTS	30.0279	-0.1915
Salmonella typhosa	ADMI	21.7640	-0.2319 ± 0.1773
	SUGAR	167.7732	-0.5514
	SALTS	210.6870	-0.3325
Escherichia coli	ADMI	4.3720	-0.0236 ± 0.0236
	SUGAR	11.3570	-0.5278
	SALTS	27.6084	-0.1329
Aerobacter cloacae	ADMI	12.7000	-0.0200 ± 0.0119
	SUGAR	80.6882	-0.4853
	SALTS	63.1652	-0.2189
Pseudomonas aeruginosa	ADMI	49.8544	-0.0638 ± 0.0597
	SUGAR	9.1588	-0.1817
	SALTS	18.8760	-0.0363
Proteus vulgaris	ADMI	18.2420	-0.0115 ± 0.0177
	SUGAR	67.0062	-0.4217
	SALTS	44.8991	-0.3103
Proteus morgani	ADMI	27.4878	-0.0299 ± 0.0119
	SUGAR	112.4852	-0.4789
	SALTS	5.0427	-0.1802
Proteus rettgeri	ADMI	9.3503	-0.1273 ± 0.1229
	SUGAR	77.4927	-0.4354
	SALTS	52.9559	-0.2913
Shigella paradysenteriae	ADMI	8.8269	-0.1359 ± 0.1606
	SUGAR	86.9612	-0.4992
	SALTS	25.7947	-0.2400

Table 2 (concl.)

Organism	Pre-ferment	F	b
Shigella sonnei	ADMI	12.4871	-0.1141 ± 0.0947
	SUGAR	84.7555	-0.6124
	SALTS	18.6771	-0.2687
Shigella flexneri	ADMI	8.3735	-0.0486 ± 0.0350
	SUGAR	53.1188	-0.4343
	SALTS	106.6210	-0.3374
Shigella paradysenteriae Hiss	ADMI	26.2172	-0.0983 ± 0.0401
	SUGAR	111.4207	-0.5466
	SALTS	114.7898	-0.3570
Staphylococcus aureus	ADMI	14.8536	-0.0727 ± 0.0405
	SUGAR	105.0037	-0.3700
	SALTS	4.5033	-0.0232

is no true trend in the counts is less than five percent. If F is greater than 8.10, the probability that there is no true trend is less than one percent. In other words the F value indicates the deviation from the trend in the counts of survivors. At the larger F value there is reasonable assurance that the counts reflect a true difference in population.

The b value represents the significance in the difference of the counts of the Sugar and Salts-buffered pre-ferments as compared to the ADMI pre-ferment. If the b value for the Sugar or Salts-buffered pre-ferments falls outside the range of the b value for ADMI, there was a significant difference in the count of the Sugar or Salts-buffered pre-ferment as compared to the ADMI pre-ferment. If the b value for the Sugar or Salts-buffered pre-ferment falls within the range of the b value for ADMI, there was no significant difference between the counts.

The b values also are used to indicate the rate of decrease in number of survivors of the micro-organisms in the pre-ferments. A large negative

value indicates a fast rate of decrease in number of survivors, whereas a small negative value indicates a slow rate. Table 3 presents the rate of decrease in number of survivors of the various organisms.

Table 2 indicates that for E. coli, in the ADMI pre-ferment, the probability that there is no true trend in the results of the counts was less than five percent but greater than one percent. For S. aureus and P. morgani in the Salts-buffered pre-ferment the probability that there is no true trend was less than five percent but greater than one percent. In all other cases the probability that there is no true trend in the results of the counts was less than one percent.

For Sh. paradysenteriae Newcastle, S. typhimurium, S. typhosa, and Ps. aeruginosa there were significant differences between the rates of survival in the Sugar pre-ferment as compared with the rate of survival in the ADMI pre-ferment, but in the Salts-buffered pre-ferment the differences between the rates of survival as compared with the ADMI pre-ferment were not significant. In all other cases there were significant differences in survival rates in both the Sugar pre-ferment and Salts-buffered pre-ferment as compared with the rates in the ADMI pre-ferment.

Table 3 shows that the species of Salmonella tested had the greatest rate of decrease in survivors, followed in order by the species of Shigella, Proteus, A. cloacae, E. coli, S. aureus, and Ps. aeruginosa, which had the slowest rate of decrease in survivors.

Table 3 indicates that the greatest rate of decrease in survivors occurred in the Sugar pre-ferment, followed by the Salts pre-ferment and ADMI pre-ferment which had the slowest rate of decrease in survivors.

The rapid rate of decrease in numbers of survivors in the Sugar

Table 3. Rate of decrease in number of survivors of pathogenic microorganisms in the three pre-ferments.

Organism	Rate of decrease in pre-ferment: ADMI b	ADMI Rate	Sugar b	Sugar Rate	Salts-buffered b	Salts-buffered Rate	Average rate of decrease of each organism b	Average rate of decrease of each organism Rate	Average rate of decrease of each genus or groups of organisms b	Average rate of decrease of each genus or groups of organisms Rate
S. schottmülleri	-0.0617	10*	-0.4550	10	-0.7551	1	-0.4239	1	-0.3548	1
S. typhosa	-0.2319	1	-0.5514	4	-0.3325	4	-0.3719	2		
S. choleraesuis	-0.0812	7	-0.5522	3	-0.3052	6	-0.3127	5		
S. typhimurium	-0.1703	2	-0.5707	2	-0.1915	11	-0.3108	6		
Sh. paradysenteriae Hiss	-0.0983	6	-0.5466	5	-0.3570	2	-0.3340	3	-0.3077	2
Sh. sonnei	-0.1141	5	-0.6124	1	-0.2687	8	-0.3317	4		
Sh. paradysenteriae Newcastle	-0.1359	3	-0.4992	7	-0.2400	9	-0.2917	7		
Sh. flexneri	-0.0486	11	-0.4343	12	-0.3374	3	-0.2734	9		
P. rettgeri	-0.1273	4	-0.4354	11	-0.2913	7	-0.2847	8	-0.2538	3
P. vulgaris	-0.0115	15	-0.4217	13	-0.3103	5	-0.2478	10		
P. morgani	-0.0299	12	-0.4789	9	-0.1802	12	-0.2290	12		
A. cloacae	-0.0200	14	-0.4853	8	-0.2189	10	-0.2414	11	-0.2347	4
E. coli	-0.0236	13	-0.5278	6	0.1329	13	-0.2281	13		

Table 3 (concl.)

Organism	Rate of decrease in pre-ferment: ADMI b	ADMI Rate	Sugar b	Sugar Rate	Salts-buffered b	Salts-buffered Rate	Average rate of decrease of each organism b	Average rate of decrease of each organism Rate	Average rate of decrease of each genus or groups of organisms b	Average rate of decrease of each genus or groups of organisms Rate
S. aureus	-0.0723	8	-0.3700	14	-0.0232	15	-0.1553	14	-0.1553	5
Ps. aeruginosa	-0.0638	9	-0.1817	15	-0.0363	14	-0.0939	15	-0.0939	6
Average rate of decrease for all organisms in each pre-ferment	-0.0860	3	-0.4748	1	-0.2663	2				

*The organism or group having the fastest rate of decrease in survivors would rank as number 1, the second fastest rate number 2, etc.

pre-ferment is indicated in Appendix 1. In most cases the count taken at the end of the second hour was less than 100 per ml. All the counts, except for Ps. aeruginosa and S. aureus, taken at the end of the fourth hour were zero per ml.

Table 4 shows that in the ADMI pre-ferment 18 percent of the 24-hour samples had a count of more than zero and less than 100 per ml. Sixty-four percent had a count greater than 100 and smaller than the count at zero time. Eighteen percent had a count greater or as large as the count at zero time.

Table 4. Percent of the final counts at the 24-hour period which fell within various ranges of magnitude.

Pre-ferment	Count zero	Count more than zero and less than 100.	Count more than 100 and smaller than count at zero time.	Count greater or as large as count at zero time.
	%	%	%	%
ADMI	0	18	64	18
SALTS	0	71	22	7
SUGAR	87	13	0	0

In the Salts-buffered pre-ferment 71 percent of the 24-hour samples had a count of more than zero and less than 100 per ml. Twenty-two percent had a count greater than 100 and smaller than the count at zero time. Seven percent had a count greater or as large as the count at zero time.

In the Sugar pre-ferment 13 percent of the 24-hour counts had a count of more than zero and less than 100 per ml. Eighty-seven percent of the tests had a count of zero per ml.

DISCUSSION

The experiments demonstrated that the organisms studied survived strikingly in the ADMI pre-ferment, possibly due to the buffering action of the milk offsetting a rapid increase in hydrogen-ion concentration. The organisms survived to a lesser degree in the Salts-buffered pre-ferment. Possibly the salts are not as good a buffering system as the milk protein. More than likely the organisms could not survive in the Sugar pre-ferment due to the lack of a buffering agent.

Besides the change in hydrogen-ion concentration, there are several other factors which might explain the different longevity of microorganisms in the pre-ferments. These include fermentation of the sugar with production of alcohol (Choi, et al., 1954), a gradual elimination of the bacterial food supply with accumulation of waste products, presence of bacteriophage, lowered oxygen tension, and more likely the sensitivity to the antibiotic effect of the yeast (Robinson, et al., 1958).

The enteric pathogens which cause typhoid and paratyphoid fevers, salmonellosis, bacillary dysentery, and shigellosis did not survive as long in the pre-ferments as did the other organisms used in this study. Of the test organisms, Ps. aeruginosa was the most hardy in the pre-ferments, followed by S. aureus which is a common cause of gastro-intestinal disorders. The Proteus species and E. coli which potentially might cause mild gastro-intestinal disorders, ranked between the two extremes as to survival in the pre-ferments.

Since S. aureus was found to survive the 24-hour incubation in large numbers, it is possible that after the normal six-hour fermentation of a grossly contaminated pre-ferment enough staphylococci would be present to

produce sufficient enterotoxin to cause food poisoning. This is likely to occur even though there is no apparent increase in the number of staphylococci present. The initially present organisms could be replaced by new organisms during the 24-hour period, and therefore cell division was going on with active metabolism. With the active metabolism enterotoxin could be produced. In a grossly contaminated pre-ferment there potentially could be enough enterotoxin produced, even without an apparent increase in numbers of bacteria, to produce food poisoning. The results indicate that the ADMI pre-ferment and possibly the Salts-buffered pre-ferment could serve as a source of food poisoning by S. aureus.

The number of survivors of the Salmonella species studied was low at the end of the normal six-hour fermentation. In some cases the number of survivors was reduced to zero. It is not likely that food poisoning would occur from pre-ferments contaminated by Salmonella species because there were not enough organisms surviving during the fermentation period to produce sufficient enterotoxin. The possibility of food infection can be ruled out since baking proceeds at a temperature which would destroy all food infection organisms which might be present in the pre-ferment.

It was concluded that there is a possibility that the pre-ferment might serve as a source of Staphylococcus poisoning agents thus causing a public health problem.

Since it has been established that food poisoning agents might occur in pre-ferments, it will be necessary to take preventive measures against such an occurrence in the bakery. Examination of food handlers for carriers of enteric microorganisms and staphylococci would serve as a preventive measure. Removal of personnel who are carriers would cut down on the

possibility of contamination of the pre-ferment or equipment in the bakery with the pathogenic bacteria. Educating the personnel handling pre-ferment ingredients concerning the role of colds and infections as a source of food poisoning would be important. Bakery workers with colds and infections could serve as a major source of staphylococci. Also the necessity of sanitation in the bakery as a preventive measure should be emphasized. Since staphylococci are ubiquitous, a dirty bakery would serve as another main source for staphylococci.

To minimize sampling errors, plate counts are theoretically derived from plates containing from 30 to 300 colonies. In these experiments the materials needed and time required per test period would have reached unwieldly proportions if an extended series of dilutions were plated per test. As a practical compromise the dilution of sample plated for most of the experiments was 1:100. As a result at the later time periods the plates contained only a few colonies thus possibly creating a recognized sampling error in the counts since less than 30 colonies were counted per plate. Yet, the statistical analysis showed that even with this potential error the majority of the experiments showed a true trend in the counts of survivors. This indicates that even though counts were recorded from plates with a higher possible sampling error than would be theoretically desired, the error did not affect the outcome of the trend analysis in the counts of survivors. This would justify the conclusions concerning the survival of the various organisms in the pre-ferments.

SUMMARY

The purpose of this project was to obtain information on the survival of microbial populations of enteric microorganisms and staphylococci in pre-ferments. Pre-ferments constitute the liquid phase of dough for making bread.

Selected organisms representing the genera Staphylococcus, Salmonella, Shigella, Proteus, Escherichia, Aerobacter, and Pseudomonas, were added to the pre-ferments, samples withdrawn after specified hours of incubation, and plate counts were made. Statistical analysis showed that in the great majority of cases, the probability that there was no true trend in the results of the counts was less than one percent.

The results of the experiments indicated that the Salmonella species had the greatest rate of decrease in survivors, followed in order by Shigella species, Proteus species, the coliforms, S. aureus, and Ps. aeruginosa which had the slowest rate of decrease in survivors. The greatest rate of decrease in survivors occurred in the Sugar pre-ferment, followed by the Salts-buffered pre-ferment and ADMI pre-ferment.

Change in hydrogen-ion concentration of the pre-ferments, production of alcohol, depletion of bacterial food supply with accumulation of waste products, presence of bacteriophage, lowered oxygen tension, and more likely the sensitivity to the antibiotic effect of the yeast were cited as possible factors which might have affected the longevity of the microorganisms in the pre-ferments.

It was concluded that there is a possibility that the pre-ferment might serve as a source of food poisoning by S. aureus, thus creating a potential public health problem.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Dr. Thomas H. Lord, Major Instructor, Department of Bacteriology, for his help and guidance; to Dr. Stanley Wearden, Department of Mathematics for assistance with the statistical analysis of data, and to the members of the Department of Flour and Feed Milling Industries for their assistance in this project.

LITERATURE CITED

Ballentine, E. N.
Certain factors influencing the survival of bacteria in water and in saline solutions. J. Bact. 19:303-20. 1930.

Barnard, T. H.
Using continuous doughing techniques in standard bread production. Bakers Digest. 27:60-79. 1954.

Boxby, E. M., and G. C. Supplee.
Bacteriology of dry milk. U. S. Pub. Health Eng. Abstracts. 10:11. 1930.

Choi, R. P., A. F. Koncus, and Staff.
ADMI stable ferment process (Chemical study-a progress report). American Dry Milk Institute: Chicago. April, 1954.

DeFreytag, C. J.
Ueber die Einwirkung concentrirter Kochsalzlösungen auf das Leben von Bacterien. Arch. f. Hyg. 11:60. 1890. (Original not seen. Cited by Tanner, F. W., Microbiology of Foods. 2nd Ed. p. 862.)

Gustafson, C. B., and E. H. Parfitt.
Effect of numbers of bacteria in the development of rancidity in soft wheat flour. Cereal Chemistry. 10:233-8. 1933.

Higginbottom, C.
Notes on bacteriology of roller-dried milk. Proc. Soc. Agr. Bacteriologists. 22: 1943. (Original not seen. Abstract in C. A. 38:3371. 1944.)

Higginbottom, C.
Bacterial growth in reconstituted spray-dried milk. J. Dairy Research. 15:285-91. 1948.

Higginbottom, C.
Effect of storage at different relative humidities on the survival of microorganisms in milk powder. J. Dairy Research. 20:65-75. 1953.

Hunwicke, R. F., and H. Jephcott.
Destruction of bacteria in the roller process of milk drying. J. Dairy Sci. 8:206-214. 1925.

Jacobs, M. B.
The chemistry and technology of food and food products. 2nd Ed. Vols. I and II. New York: Interscience Publishers, Inc., 1951.

James, L. H.
Bacterial content of sugars, Food Industries. 1:65-9. 1928.

Jordan, E. O., H. L. Russell, and F. R. Zeit.
The longevity of the typhoid bacillus in water. J. Infect. Dis. Supp. 2:40. 1906.

Kent-Jones, D. W., and A. J. Amos.
Preliminary studies in the bacteriology of wheat and flour. Analyst. 55:248-68. 1930.

Kossowicz, A.
Bacterial content of condensed milk. (Translated title) Ztschr. Landw. Versuchsw. Osterr. 11:719. 1908. (Original not seen. Cited by Tanner, F. W. Microbiology of Foods. 2nd Ed. p. 552.)

Lyng, J., and V. Aschehoug.
Bacteriological problems in connection with potato flour. (Translated title.) Tids. Hermetikind. 21: 1935. (Original not seen. Abstract in C. A. 32:4231. 1938.)

Macy, H.
The bacterial content of dry milk. J. Dairy Sci. 11:516-26. 1928.

Miller, B. S., and J. A. Johnson.
Present knowledge concerning baking processes employing pre-ferments. Wallerstein Communications. 20: No. 70. p. 297. 1957.

Nichols, A. A.
Bacteriology studies of dried milk powder. J. Dairy Research. 10:202-30. 1939.

Pirrie, P.
Practical aspects of the stable ferment process. Bakers Weekly. 164 (2):26. 1955.

Rappin, G.
Microbic flora of salt. J. Amer. Med. Assn. 75:618-619. 1920.

Robinson, R. J., T. H. Lord, J. A. Johnson, and B. S. Miller.
Studies on the decrease of the bacterial population in pre-ferments. Cereal Chem. 35:306-317. 1958.

Schulz, A.
An atlas of bread microbes. Stuttgart: Hugo Matthaes Verlag, 1954. p. 8-16.

Tanner, F. W.
Microbiology of foods. 2nd Ed. Champaign, Illinois: Garrard Press, 1944.

Whipple, J.
Survival of organisms in water. J. Amer. Water Wks. Assoc. 9:208-225. 1922.

Yesair, J.
Bacterial content of salt. Canning Trade. 52:112-5. 1930.

APPENDIX

Appendix 1. Bacterial counts on the survivors of pathogenic microorganisms in the pre-ferments.

ORGANISM	PRE-FERMENT	Hour 0	1	2	3	4	5	6	24
Salmonella schottmulleri	ADMI	4700	3600	3600	3500	4000	2000	3100	2100
	SUGAR	3500	< 100	< 100	< 100	0	0	0	0
	SALTS	3100	2900	2800	1000	< 100	200	100	< 100
	ADMI	4900	4000	2700	3200	2600	2600	1900	1600
	SUGAR	4700	< 100	< 100	< 100	0	0	0	0
	SALTS	4800	3500	2000	400	< 100	< 100	100	< 100
	ADMI	4800	2800	3100	1600	2000	800	1400	1400
	SUGAR	2300	< 100	< 100	< 100	0	0	0	0
	SALTS	5400	2600	2200	100	< 100	< 100	< 100	< 100
Salmonella choleraesuis	ADMI	4700	3300	3100	3000	2500	1800	1900	3100
	SUGAR	2700	1300	< 100	< 100	0	0	0	0
	SALTS	3400	3000	2800	1900	1200	900	< 100	< 100
	ADMI	4400	3600	2900	3200	3000	1600	1300	400
	SUGAR	3400	1300	< 100	< 100	0	0	0	0
	SALTS	4800	4200	2700	2000	1800	< 100	< 100	< 100
	ADMI	5100	3800	3600	3000	3100	2500	1000	1000
	SUGAR	4400	1400	< 100	< 100	0	0	0	0
	SALTS	5500	4200	2200	2100	1700	1200	< 100	< 100
Salmonella typhimurium	ADMI	4700	3900	4300	4200	3500	3000	2700	7300
	SUGAR	3200	3600	300	< 100	0	0	0	0
	SALTS	4700	4100	4300	3900	3400	2500	2000	7200
	ADMI	3300	2900	2900	2200	2300	2200	2200	< 100
	SUGAR	3500	3100	< 100	< 100	0	0	0	0
	SALTS	3500	3200	2800	2500	2400	1800	1200	< 100
	ADMI	6200	6200	5500	5100	3600	4500	< 100	< 100
	SUGAR	6700	100	100	< 100	0	0	0	0
	SALTS	6700	6300	2700	4700	3800	3200	< 100	< 100
Salmonella typhosa	ADMI	2500	2500	1500	1300	100	600	100	< 100
	SUGAR	1300	300	< 100	< 100	0	0	0	0
	SALTS	2600	2000	1300	1000	300	< 100	< 100	< 100
	ADMI	5100	3900	3100	4400	4200	< 100	< 100	< 100
	SUGAR	2200	600	100	100	0	0	0	0
	SALTS	6100	5900	3500	3100	4300	< 100	< 100	< 100
	ADMI	4600	2600	2200	2400	2400	2200	1600	1700
	SUGAR	4200	4300	< 100	< 100	0	0	0	0
	SALTS	4400	3800	2800	1300	1300	900	200	< 100

Appendix 1 (cont.)

ORGANISM	PRE-FERMENT	Hour 0	1	2	3	4	5	6	24
Escherichia coli	ADMI	3000	2400	3200	3200	2800	2600	2600	1400
	SUGAR	2800	2000	600	< 100	0	0	0	0
	SALTS	3000	3000	2900	4000	4200	5500	4500	1100
	ADMI	3600	2700	3000	3500	3600	3700	3700	3800
	SUGAR	2900	200	< 100	< 100	0	0	0	0
	SALTS	2300	1800	1400	900	600	700	600	500
	ADMI	2500	2100	1500	1400	2600	1900	700	2300
	SUGAR	2100	200	< 100	< 100	0	0	0	0
	SALTS	2300	1800	1300	300	300	300	< 100	< 100
Aerobacter cloacae	ADMI	3800	3800	4900	4100	4500	4900	4200	3000
	SUGAR	3800	< 100	< 100	< 100	0	0	0	0
	SALTS	3700	4000	1900	300	600	200	< 100	100
	ADMI	4800	4200	3800	3400	4100	4000	3900	4100
	SUGAR	2900	< 100	< 100	< 100	0	0	0	0
	SALTS	4300	3800	2100	1200	300	1500	900	300
	ADMI	4000	5400	8600	5700	8700	5700	6300	5900
	SUGAR	3000	1300	< 100	< 100	0	0	0	0
	SALTS	5300	4700	2400	900	700	300	400	500
Pseudomonas aeruginosa	ADMI	5500	5500	3700	4200	3900	3100	4100	2300
	SUGAR	2900	2500	1800	1000	800	800	800	< 100
	SALTS	4000	3400	3700	3300	2900	2900	4200	5700
	ADMI	4000	2500	2500	2700	3300	2300	1300	700
	SUGAR	6000	4100	1300	800	400	400	200	< 100
	SALTS	5300	4000	5200	2200	3700	2700	2900	1900
	ADMI	4700	4000	3100	3400	3600	2800	2700	1300
	SUGAR	4500	3300	1500	800	100	< 100	< 100	< 100
	SALTS	4600	3800	4400	2700	3500	2900	2900	2000
Staphylococcus aureus	ADMI	6000	4600	4700	3800	4600	4200	4300	400
	SUGAR	5100	6100	3800	100	< 100	< 100	< 100	< 100
	SALTS	4600	3500	3600	3900	4300	4100	4500	1300
	ADMI	4400	6600	5500	5300	5500	5100	1800	2300
	SUGAR	4500	8100	5900	1000	700	700	100	< 100
	SALTS	4200	7000	5000	5100	5600	5800	3600	3300
	ADMI	3300	5100	4000	4000	4100	3900	1200	1800
	SUGAR	3400	6000	4500	800	500	< 100	< 100	< 100
	SALTS	3000	5300	4000	4000	4400	4500	2900	3300

Appendix 1 (cont.)

ORGANISM	PRE-FERMENT	Hour 0	1	2	3	4	5	6	24
Shigella paradysenteriae (Newcastle)	ADMI	5900	6000	5500	4700	4800	4100	2800	< 100
	SUGAR	3000	< 100	< 100	< 100	0	0	0	0
	SALTS	5100	4900	4900	5000	3700	4200	2000	< 100
	ADMI	2800	2400	3900	4000	3800	3200	6000	4100
	SUGAR	2400	100	< 100	< 100	0	0	0	0
	SALTS	2200	2400	2900	2900	3000	2700	400	< 100
	ADMI	4100	4000	2800	2300	2100	1100	900	< 100
	SUGAR	3800	2700	< 100	< 100	0	0	0	0
	SALTS	4100	3700	3000	1600	1600	1500	< 100	< 100
Shigella sonnei	ADMI	3400	3400	3000	2600	2400	2000	3800	2000
	SUGAR	4000	3400	3000	2600	0	0	0	0
	SALTS	3600	2200	2400	2000	2200	700	< 100	< 100
	ADMI	2300	1900	1700	1000	800	800	< 100	< 100
	SUGAR	2700	2000	< 100	< 100	0	0	0	0
	SALTS	4200	2900	2200	1900	1600	1000	500	< 100
	ADMI	2000	1800	1600	1300	1200	900	1000	1100
	SUGAR	2300	2000	1500	< 100	0	0	0	0
	SALTS	2500	2000	2000	1600	1300	1300	< 100	< 100
Shigella flexneri	ADMI	2400	2000	1600	1200	2200	1400	1000	400
	SUGAR	1400	< 100	< 100	< 100	0	0	0	0
	SALTS	3400	2400	600	1000	< 100	< 100	< 100	< 100
	ADMI	2400	1200	1600	1800	2200	2400	1600	800
	SUGAR	1800	400	< 100	< 100	0	0	0	0
	SALTS	3700	1800	3000	1600	800	< 100	< 100	< 100
	ADMI	2800	2800	2200	4600	4200	3000	3000	800
	SUGAR	800	< 100	< 100	< 100	0	0	0	0
	SALTS	3000	1200	1200	400	200	< 100	< 100	< 100
Shigella paradysenteriae (Hiss)	ADMI	3900	3800	3300	2700	3200	2400	2400	800
	SUGAR	2900	1000	< 100	< 100	0	0	0	0
	SALTS	3900	3100	2500	2500	1800	< 100	< 100	< 100
	ADMI	3500	3000	2900	2000	3000	2600	600	200
	SUGAR	2500	800	300	< 100	0	0	0	0
	SALTS	3400	3000	2200	2000	600	< 100	< 100	< 100
	ADMI	4000	3900	3600	3000	3800	3200	1900	800
	SUGAR	2900	1000	< 100	< 100	0	0	0	0
	SALTS	3300	1800	600	100	< 100	< 100	< 100	< 100

Appendix 1 (concl.)

ORGANISM	PRE-FERMENT	Hour 0	1	2	3	4	5	6	24
Proteus morgani	ADMI	2900	2800	2000	2300	2600	2200	2100	4100
	SUGAR	1500	100	< 100	< 100	0	0	0	0
	SALTS	2200	2900	1600	2000	1500	2400	1200	1600
	ADMI	2000	2000	2100	2400	1900	2400	2600	6800
	SUGAR	1800	100	< 100	< 100	0	0	0	0
	SALTS	2700	3000	1300	2500	1900	2500	1500	< 100
	ADMI	3100	2700	2100	3000	2700	2400	2200	7700
	SUGAR	2800	500	< 100	< 100	0	0	0	0
	SALTS	3300	2600	2900	3900	2200	1600	< 100	< 100
Proteus rettgeri	ADMI	3500	3100	3800	3600	2600	1400	2800	1800
	SUGAR	2900	< 100	< 100	< 100	0	0	0	0
	SALTS	4600	4800	4300	4200	2500	2200	2200	< 100
	ADMI	3100	2700	2700	2000	1600	1100	< 100	900
	SUGAR	1600	< 100	< 100	< 100	0	0	0	0
	SALTS	3400	2400	2100	2000	1200	< 100	< 100	< 100
	ADMI	2900	2700	2700	2100	2800	3000	< 100	700
	SUGAR	1600	< 100	< 100	< 100	0	0	0	0
	SALTS	2900	1200	1000	1000	600	< 100	< 100	< 100
Proteus vulgaris	ADMI	3100	2400	2200	2900	2300	2400	1700	2200
	SUGAR	1400	< 100	< 100	< 100	0	0	0	0
	SALTS	3200	1600	200	200	< 100	< 100	< 100	< 100
	ADMI	3500	2400	2300	2200	3000	2300	1700	4000
	SUGAR	1400	< 100	< 100	< 100	0	0	0	0
	SALTS	3400	600	500	500	< 100	< 100	< 100	< 100
	ADMI	3300	2400	2200	2600	2600	2400	1700	3000
	SUGAR	2000	< 100	< 100	< 100	0	0	0	0
	SALTS	3200	2200	700	< 100	< 100	< 100	< 100	< 100

SURVIVAL OF PATHOGENIC BACTERIA IN PRE-FERMENTS

by

GERALD LELAND GILARDI

B. A., University of California, 1955

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1959

One of the most significant developments in the baking industry during the past fifty years, is the continuous, fully automatic bread production process devised by J. C. Baker. This process utilizes a fermentation similar to the straight- and sponge-dough ferment but depends upon the production of a liquid pre-ferment, also called yeast broth or "brew". The pre-ferment consists of water, yeast, yeast food, sugar, and possibly other materials intended to contribute flavor. The pre-ferment, after about six hours of fermentation, is ready for the doughing process. The flour plus the proportioned pre-ferment are continuously combined in the pre-mixing machine and finally developed into a dough. This eliminates the conventional sponge-making operation so that without further fermentation the dough is put into the loaf pans and normal baking follows.

With the introduction of this new process for making bread, there is also introduced a new public health problem: Is there the possibility that the pre-ferment might serve as a source of food poisoning or food infection agents? This might occur through contamination of the pre-ferment by workers in the bakery who are carriers of pathogenic bacteria.

The purpose of this project was to secure information on the survival of microbial populations of enteric micro-organisms and staphylococci in three different pre-ferments, i.e., the liquid phase of the dough.

The experimental portion consisted of making up batches of Sugar pre-ferment, ADMI (American Dry Milk Institute) pre-ferment, and Salts-buffered pre-ferment and adding the selected food poisoning or food infection organisms. The pre-ferments were incubated, samples withdrawn after specified hours of incubation and plate counts made.

The results of the counts on the survival of the various organisms

were analyzed statistically. The statistical analysis indicated that in the great majority of cases, the probability that there was no true trend in the results of the counts was less than one percent. In other words there is reasonable assurance that the counts reflect a true difference in population.

In most cases there were significant differences in survival rates in both the Sugar pre-ferment and Salts-buffered pre-ferment as compared with the rates in the ADMI pre-ferment.

The four species of Salmonella tested had the greatest rate of decrease in survivors, followed in order by the species of Shigella, Proteus, Aerobacter, Escherichia, Staphylococcus, and Pseudomonas.

The greatest rate of decrease in survivors occurred in the Sugar pre-ferment, followed by the Salts pre-ferment and ADMI pre-ferment.

The sensitivity to the antibiotic effect of the yeast and other factors were cited which might possibly have affected the longevity of the micro-organisms in the pre-ferments.

Since Staphylococcus aureus was found to survive the 24-hour incubation in large numbers, it is very likely that the number present after the normal six-hour fermentation of the pre-ferment would produce sufficient entero-toxin to cause food poisoning. The results indicate that the ADMI pre-ferment and possibly the Salts-buffered pre-ferment could serve as a source of food poisoning by S. aureus.

It was concluded that there is a possibility that the pre-ferment might serve as a source of food poisoning agents thus causing a potential public health problem.