THE APPLICATION OF ENZYMES IN THE STABLE FERMENT PROCESS FOR COMMERCIAL BREAD PRODUCTION

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1952

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

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INTRODUCTION

The adaptability of a preferment to bulk handling methods and the economy and control that such a system may offer for bread production has been of considerable recent interest. Advantages of a preferment process include the elimination of the setting of individual sponges, saving of floor space, reduction of processing time, reduction in labor costs (Choi, 1), and possibly greater control during fermentation. The American Dry Milk Institute (ADMI) preferment has attracted much attention and reportedly produces a satisfactory loaf of bread provided proper control is maintained throughout the operation (Pirrie and Glabau, 14). Other preferments utilize a formulated salt mixture to stabilize the acidity of the preferment during fermentation (Manewal, 8). A few bakeries have employed a sugar, yeast, and water preferment. It seems reasonable that some preferment system eventually may be used widely in commercial baking.

Both alpha-amylase and protease are generally accepted as beneficial adjuncts in the sponge baking process. The amylases are able to attack only damaged or gelatinized starch granules. Normal bread flour contains only about four percent of starch that has been damaged in the milling process, therefore, the action of the amylases in sponge fermentation is limited.

The effect of protease action in dough is proportional to the amount of enzyme that is added (Miller and Johnson, 11).

Tight, non-elastic or "bucky" doughs that cannot be processed without deleterious effects can be improved by proper protease supplementation. Although "bucky" characteristics also can be alleviated by changes in fermentation time, mixing or floor time, such changes are not feasible in commercial production.

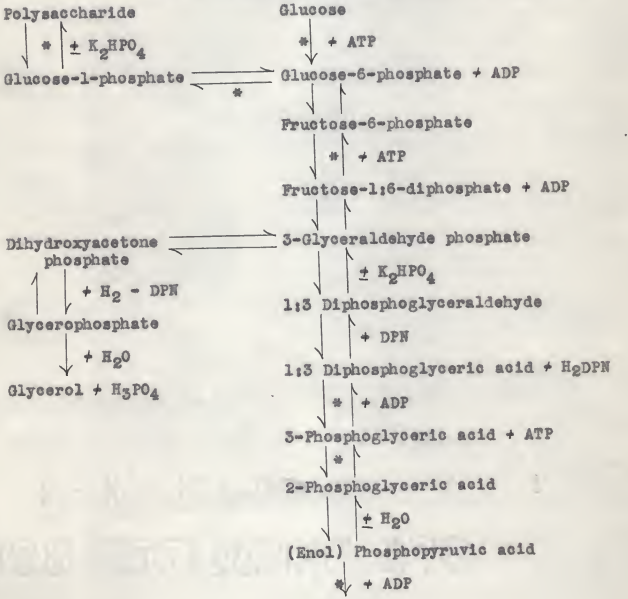
In addition to their beneficial effect on dough properties, the effects of amylase and protease on bread quality also are beneficial. The baking test is the best method of determining proper enzyme supplementation.

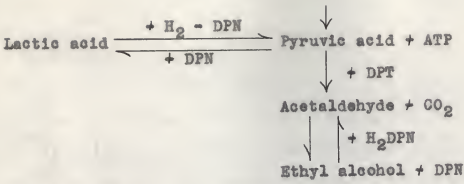
Since enzymes play such an important role in the sponge process, it was felt that they also would be beneficial in the preferment process. No information is available concerning their stability or utility in preferments for bread production.

The purpose of this study was to determine the stability of alpha-amylase and protease in different preferment formulations. In addition, the optimum relative protease supplementation for preferment systems was compared with that used in the sponge method of breadmaking.

An additional study was made to determine if constituents produced in preferments improved bread quality and whether such constituents were present in the yeast cells or in the preferment liquor. To gain preliminary information as to what possible constituents might be present in preferments to enhance bread quality, the author has referred to the process and mechanism of alcoholic fermentation. Studies (Krebs, 7) pertaining to yeast technology and alcoholic fermentation reactions have shown that

succinic, pyruvic, and fumaric acids are produced, although the primary action of the yeast is to convert sugars into carbon dioxide and alcohol. Sugar fermentation, however, is an involved chain of reactions which forms many intermediate compounds and by-products. The Embden-Meyerhof-Parnas scheme (Porter, 15) for sugar fermentation shows the compounds that result and may be expected to be found in fermentation cultures and is as follows:

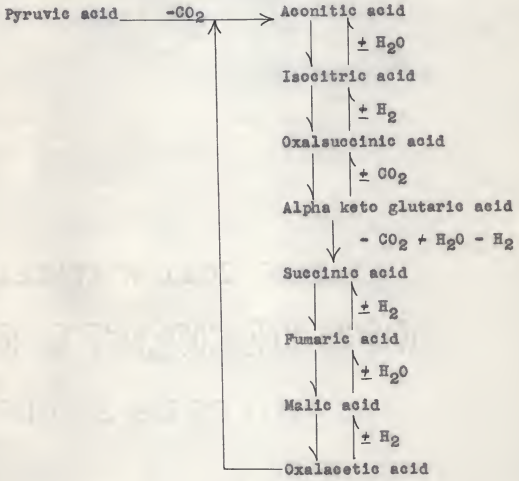




* Mg^{++} or Mn^{++} ion required

Acetaldehyde is formed in the above scheme by the action of the enzyme carboxylase which initiates the dicarboxylation of the pyruvic acid to form acetaldehyde and carbon dioxide.

The Krebs cycle is as follows:



Krebs tricarboxylic acid cycle theory indicates that the breakdown of pyruvic acid is a complicated series of reactions. These reactions take place predominately under aerobic conditions (Gortner and Gortner, 2). Bacteria normally present in the preferments may cause aerobic changes to occur.

The Krebs cycle and the Embden-Meyerhof-Parnas scheme, account for almost all of the products of yeast fermentation. It was reported by Joslyn (5) that acetic acid 0.05 to 0.25 percent, lactic acid 0.0 to 0.2 percent, and succinic acid 0.5 to 0.7 percent of the total ferment are produced as intermediate or by-products during industrial fermentation. Neuberg suggests that formic and acetic acids may be produced by yeast action in the dismutation of acetaldehyde or by the breakdown of pyruvic acid (Porter, 15).

It has been shown that the addition of carbon dioxide to some bacterial systems will increase the fermentation rate. Any intermediate in the Krebs cycle tends to produce the same effect as carbon dioxide. Indeed, one important part of the evidence for the Krebs cycle is that the addition of any of the intermediates will increase respiration when added to the proper tissue. The glycolytic scheme and Krebs cycle are the pathways of carbohydrate metabolism, the former being anaerobic, the latter aerobic. Both are important in that they release energy which is retained in high energy phosphate bonds. These high energy phosphate bonds supply the energy for the living cells.

The addition of adenosine triphosphate (ATP), which contains two high energy phosphate bonds, might be beneficial in preferments. It was thought that ATP might speed the rate of fermentation and improve the quality of bread produced from preferments that were given no fermentation time. These are known as "no time" preferments. Perhaps the use of ATP might permit bread production without the use of preferments.

MATERIALS AND METHODS

Enzyme Sources

A commercial fungal enzyme concentrate, Rhozyme A-4[1] and malted wheat flour[2] were used as sources of alpha-amylase and protease. Diastase 33[1] was used as a source of excess alpha-amylase in the baking experiments. The alpha-amylase activities of the three preparations were 5322, 54 and 5136 SKB units (Sandstedt, et al., 17) per gram, respectively, and the protease activities were 33,000, 44, and 650 hemoglobin units (Miller and Johnson, 12) per gram, respectively.

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[1]Obtained from Rohm and Haas Co., Philadelphia, Pa.
[2]Obtained from Kansas Milling Co., Wichita, Kans.

Enzyme Preparation

The preparation of the enzymes for use in baking was similar in all cases. The malted wheat flour was extracted with distilled water for 30 minutes, centrifuged, and filtered through glass wool. The fungal preparations were extracted for 15 minutes and filtered through S and S #597 filter paper[1]. For the alpha-amylase determination the concentration of the malted wheat flour and Rhozyme A-4 water extracts were 0.05 g. and 0.002 g. per ml., respectively. For the protease determination, the concentration of the malted wheat flour and Rhozyme A-4 water extracts were 0.666 g. and 0.001 g. per ml., respectively. In the baking experiments the concentrations of the water extracts of both Rhozyme A-4 and Diastase 33 was 0.001 g. per ml. Suitable quantities of alpha-amylase were used for the determination by the modified Wohlgemuth procedure of Sandstedt, et al. (17). The amount of protease activity was determined by the modified Ayre-Anderson technic (Miller, 9) with bacto-hemoglobin as a substrate.

Flours Used

Ten hard red winter wheat flours were used in the baking experiments. They ranged from 10.4 percent to 13.2 percent protein. The ash ranged from 0.39 percent to 0.48 percent. All

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[1]Carl Schleicher and Schuell Co., Keene, N. H.

flours were unmalted. Six were of good baking quality and four were of poor baking quality. The flour analyses are shown in Table 1.

Preferment Formulae

The ADMI, sugar and Fleischmann preferment formulae used in determining the stability of alpha-amylase and protease are shown in Table 2. The enzymes were added to the preferments in the form of water extracts. The ingredients of each preferment were mixed in a Waring Blendor for one minute, poured into Erlenmeyer flasks and allowed to ferment at 30° C. (86° F.). They were shaken gently and continuously on a mechanical shaker. Samples were withdrawn at suitable intervals for analysis.

Table 1. Analytical data of flours (14% moisture basis).

Flour No.	Protein %	Ash %	Absorption %	Mixing time min.
		Poor Quality Flours		
1	11.1	0.44	63	2.5
2	10.4	0.48	62	2.5
3	12.3	0.48	65	3.0
4	11.4	0.42	63	2.5
		Good Quality Flours		
5	11.9	0.41	65	3.5
6	13.2	0.39	70	4.0
7	11.4	0.44	65	3.5
8	12.2	0.43	67	4.0
9	12.9	0.39	68	4.5
10	12.5	0.42	67	4.5

Table 2. Preferment formulae.

Ingredients	Type of Preferment		
	ADMI (1)	Sugar (4)	Fleischmann (8)
	g.	g.	g.
Water	320	320	320
Dextrose	21	21	21
Yeast food[1]	3.5	--	--
Enzyme preparation	Malted wheat flour[2] and Rhozyme A-4		
Diammonium hydrogen phosphate	--	0.3	--
Brew improver[3]	--	--	2.1
Salt	7	7	7
Dry milk solids	42	--	--
Compressed yeast	14	14	14

1 Arkady type.
2 Kansas Milling Co., Wichita, Kans.
3 Standard Brands, Inc., New York, N. Y.

Determination of Alpha-Amylase Activity

Reagents. Stock iodine solution: 5.5 g. of iodine crystals and 11 g. of potassium iodide were made up to 250 ml. with water. The solution was stored in the dark and fresh solution made monthly.

Dilute iodine solution: Two ml. of stock iodine solution and 20 g. of potassium iodide were made up to 500 ml. with water. Color comparisons were made at 30° C. using a No. 17 Hellige glass varnish color standard (Redfern, 16).

Buffer solution: 120 ml. of glacial acetic acid and 164 g. of anhydrous sodium acetate were made up to 1000 ml. with water.

Buffered limit-dextrin substrate: A suspension of 5.5 g. of Merck's soluble starch was prepared and poured slowly into boiling

water, boiled for two minutes, cooled and 25 ml. of buffer solution and 125 mg. of "special" B-amylase powder added. The solution was made up to 500 ml. volume, saturated with toluene and stored at 30° C. for between 24 and 72 hours before use.

Procedure. Twenty ml. of the buffered limit-dextrin substrate was transferred to a 50 ml. Erlenmeyer flask and placed in the 30° C. water bath. After two minutes, 10 ml. of the diluted enzyme extract was added. The enzyme extract was blown from the pipette to minimize the errors inherent in slow mixing of the enzyme and substrate. The enzyme solution was adjusted in each case so that the dextrinization time was between 15 and 60 minutes.

At appropriate time intervals, one ml. of the hydrolyzing mixture was pipetted into 5 ml. of the dilute iodine solution in a test tube 1/2 inch by 4 inches, shook and compared with a No. 17 Hellige glass varnish color standard (Redfern, 16). Color comparisons were made before a lightly screened 100 watt daylight bulb.

From the time interval necessary for dextrinization and the weight of enzyme represented by the extract aliquot taken, alpha-amylase units were calculated. Alpha-amylase units are the number of grams of soluble starch, which in the presence of an excess of beta-amylase, are dextrinized by one gram of enzyme in one hour at 30° C. Units of enzyme were calculated with the following equation:

$$\frac{(0.4 \text{ g. of starch})\ 60}{(\text{grams of enzyme})(\text{dextrinization time in minutes})} = \text{alpha-amylase units per gram}$$

For the determination of alpha-amylase activity in the preferments, aliquots were withdrawn at intervals and centrifuged at 2000 rpm for five minutes. Aliquots of the supernatant liquid were used to determine alpha-amylase activity. The loss of enzyme activity was calculated on the basis of the activity determined immediately after mixing the preferments.

The pH of each preferment was measured each time an aliquot was centrifuged prior to determining the alpha-amylase activity. A Beckman Model H-2 pH meter was used. The pH was determined to establish whether there was a correlation between loss of alpha-amylase or protease activity and decrease of pH of the preferments.

Determination of Protease Activity

Protease activity was determined by the modified Ayre-Anderson technic (Miller, 9) with bacto-hemoglobin as a substrate. For each determination 2.67 g. of bacto-hemoglobin were weighed into each of two 125 ml. Erlenmeyer flasks and 6 g. pumice added. Solutions of the enzyme and acetate buffer (pH 4.7) to make a total of 50 ml. volume were added and the mixture shaken thoroughly. A 10 ml. portion of trichloroacetic acid containing 1.125 g. per ml. was added to one flask immediately and to the second flask after 5 hours digestion at 40° C. After the addition of the trichloroacetic acid the flasks were shaken for 30

minutes and filtered through S and S #597 filter paper. Ten ml. aliquots of the filtrate were used for the determination of soluble nitrogen by the Kjeldahl method. Activity was expressed as increase in titration value of 0.0714 N sodium hydroxide.

Aliquots were withdrawn from the preferments at regular intervals, centrifuged and used to determine protease activity. The loss of activity was calculated on the basis of an original activity of 100 percent.

The gelatin viscosity technic of Koch and Ferrari (6) also was employed for determination of protease activity. This method consisted of measuring the decrease in viscosity of a standard gelatin solution when subjected to proteolytic enzymes. The decrease in viscosity of the gelatin with time was used as a measure of protease activity.

Determination of the Protease Requirements of Preferments

Both sponge-dough and preferment baking procedures were employed (Johnson and Miller, 3). Rhozyme A-4 was used as a source of protease. Excess alpha-amylase (11 SKB units per 100 g. of flour) provided by Diastase-33 was used in all cases. The ADMI preferment was used.

Six different hard red winter wheat flours were used (No. 1, 2, 3, 5, 6, and 7 of Table 1). These ranged from a very strong bread flour to a comparatively weak bread flour. The ingredients of the preferments were mixed in a Waring Blendor for

one minute, poured into Erlenmeyer flasks, and allowed to ferment four hours at 30° C. (86° F.). After four hours of fermentation both the sponges and preferments were mixed into a dough. The doughs were handled similarly except for a longer mixing time given the preferments doughs. The preferment doughs, also were given 10 minutes and 5 minutes additional intermediate and final proof, respectively. Both the sponge and preferment doughs were proofed to a constant height before being placed into the oven. The formulae for the sponge and preferments are shown in Table 3.

Studies of Factors Produced in Preferments that Enhance Bread Quality

Each baking experiment involved both the Fleischmann and ADMI preferments and consisted of the following:

(1) A control (sponge-dough).

(2) A control preferment in which the ingredients were mixed one minute in a Waring Blendor and allowed to ferment four hours in a fermentation cabinet at 30° C. (86° F.).

(3) A preferment handled similarly to No. 2 except that at the end of four hours of fermentation the yeast cells were removed by centrifuging and new yeast cells added in making a bread dough.

(4) A fresh preferment using the yeast cells removed from No. 3.

(5) A fresh preferment.

The formulae used are shown in Table 3.

Six hard red winter wheat flours were used (No. 1, 2, 4, 8, 9, and 10 of Table 1). These consisted of three good and three poor baking quality flours. The preferment and sponge doughs were handled similarly after being mixed into a dough. The preferments required a longer optimum mixing time and they were given 10 minutes additional floor time and 5 minutes additional proof time. Both the sponge and preferment doughs were proofed to a definite height.

The Use of ATP in Preferments

To study the effect of ATP a good baking quality flour was used (No. 8, Table 1). The preferments were handled similarly to the control preferment except that no fermentation time was allowed. The ATP was added in aqueous solution and mixed for one minute in the Waring Blendor with the other ingredients of the preferment. The ATP series included preferments with 0 mg., 25 mg., 50 mg., 75 mg., 100 mg., 125 mg., and 150 mg. of ATP per preferment. After mixing into a dough, the doughs containing ATP were handled similarly to the regular preferment doughs.

Table 3. Standard sponge and preferment baking formulae.

Ingredients	Quantity of ingredients in grams		
		Preferments	
	Sponge	Fleischmann	ADMI
Water	314	320	320
Dextrose	--	14	14
Dry milk solids	--	--	42
Yeast food[1]	3.5	--	3.5
Rhozyme A-4	See Table 4	See Table 4	See Table 4
Rhozyme-33	0.015	0.015	0.015
Brew improver[2]	--	1.14	--
Salt	--	9	14
Compressed yeast	14	11	14
Flour	490	--	--
		Dough	
Flour	210	700	700
Dextrose	42	42	42
Shortening	21	21	21
Salt	14	5	--
Dry milk solids	42	42	--
Water	155	149	149
Rhozyme A-4	--	See Table 4	See Table 4
Compressed yeast	--	14	7
Yeast food[1]	--	3.5	--

[1]Arkady type, Standard Brands, Inc., New York 22, N. Y.
[2]Standard Brands, Inc., New York 22, N. Y.

EXPERIMENTAL RESULTS AND DISCUSSION

Stability of Alpha-Amylase and Protease in Preferments

The changes in the alpha-amylase activity with time in the different preferments are shown in Fig. 1. The alpha-amylase activity of the Rhozyme A-4 and malted wheat flour preparations remained essentially constant in the ADMI and Fleischmann preferments but declined rapidly in the sugar preferment. The rapid

decline in the alpha-amylase activity in the sugar preferment apparently was due to the low pH developed during fermentation.

The changes in pH with time for the different preferments are shown in Fig. 2. These data are similar to those reported by Johnson and co-workers (4). The milk served as a buffer in the ADMI preferment and salts present in the brew improver provided the buffering effect in the Fleischmann preferment. Since there was no buffer present in the sugar preferment, the pH decreased rapidly. The changes in pH with time for the ADMI and Fleischmann preferments were similar to those reported previously for the ADMI process (Choi, 1). The pH for the Fleischmann preferment, however, tended to be 0.4 to 1.0 pH unit lower than that for the ADMI preferment.

The losses of protease activity in the different preferments are shown in Fig. 3. The decrease in activity of Rhozyme A-4 and malted wheat flour protease in the Fleischmann and ADMI preferments were slight over a 24-hour period. The protease activity in the sugar preferment declined rapidly to 50 and 75 percent of the original for Rhozyme A-4 and malted wheat flour, respectively, during the first six hours, and then remained nearly constant for the remaining 18 hours of the fermentation period. A very similar trend was observed for protease activity values determined by the gelatin viscosity technic. The work of Miller and Johnson (10) suggests that the decrease in protease activity was due to the high acidity developed in the sugar preferments (Fig. 2) in which no buffer was present.

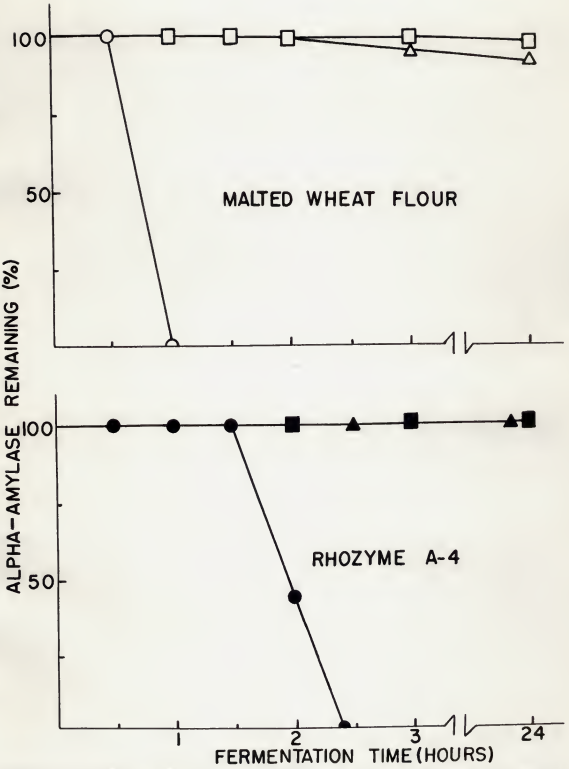

Fig. 1. Effect of fermentation time on the amount of alpha-amylase activity retained in different pre-ferments. Open and closed symbols represent the use of malted wheat flour and Rhozyme A-4, respectively. The triangles, circles and squares represent ADMI, sugar and Fleischmann pre-ferments, respectively.

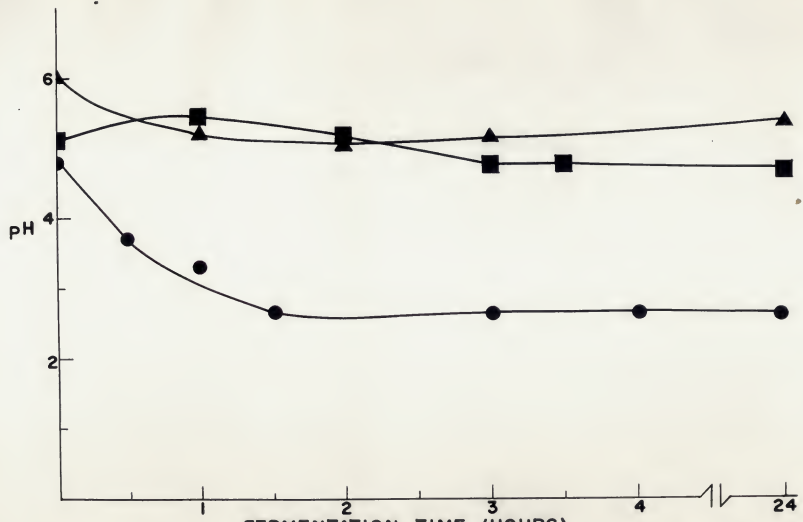

Fig. 2. Effect of fermentation time on pH of preferments. The triangles, circles and squares represent ADMI, sugar, and Fleischmann preferments, respectively.

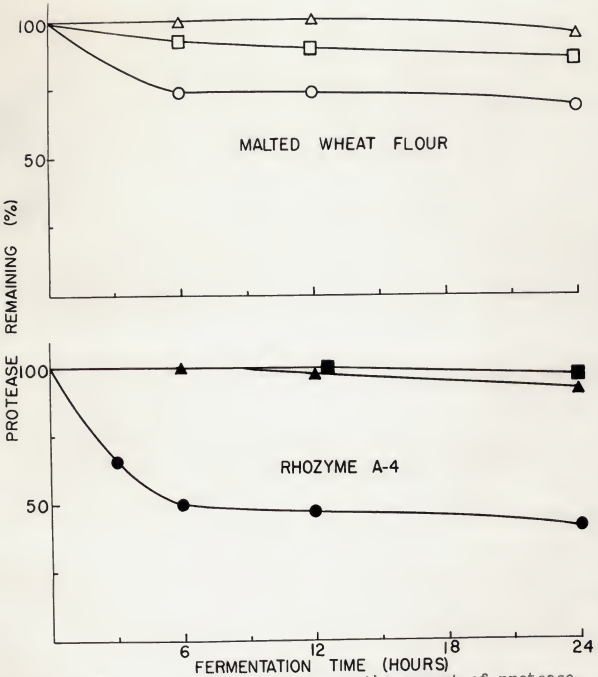

Fig. 3. Effect of fermentation time on the amount of protease activity retained in different preferments. Open and closed symbols represent the use of malted wheat flour and Rhozyme A-4, respectively. The triangles, circles and squares represent ADMI, sugar and Fleischmann preferments, respectively.

The stabilities of alpha-amylase and protease were studied in preferments maintained at 30° C. (86° F.); although the ADMI process specifies prefermentation at 38° C. (100° F.). It is believed that similar stability results would have been obtained at the higher temperature. Miller, et al. (13) found that the amylases from fungal and malted wheat flour were stable at least to 65° C. (149° F.). Miller and Johnson (10) found that the proteases from fungal and malted wheat flour at pH 5.0 were stable during 30 minutes of heating at 50° C. (122° F.). Furthermore, the analysis of protease activity are usually conducted at 40° C. (104° F.).

The enzyme activity decreased markedly in the sugar preferment. Preliminary experiments showed that inferior bread resulted when this preferment was employed. Accordingly, further experiments using the sugar preferment were not performed.

The data in Table 4 summarize the results for the sponge and ADMI processes using various concentrations of Rhozyme A-4 with strong and weak flours. While the Fleischmann preferment was not used, it is believed that results similar to those using the ADMI process would have been obtained. The sponge method, as might be expected because of the longer time for enzyme action, was much more sensitive to the action of protease. Using the sponge method, the flours tested required 6 to 9 mg. of Rhozyme A-4 per 700 grams of flour to produce the best quality bread. In the absence of added enzyme supplement, the bread lacked volume, satisfactory break and shred, and grain and texture. Excess

enzyme supplementation was even more damaging to these bread attributes.

With the preferments, whether the enzyme was added to the preferment or at the dough stage, the Rhozyme A-4 requirements for the flours were not critical. Satisfactory bread was produced using as high as 27 mg. of Rhozyme A-4 per 700 grams of flour. Generally, three times as much enzyme was required for the preferment processes as for the sponge process. The use of Rhozyme A-4 was beneficial in all cases.

A Study of Factors That May Be Produced in Preferments That Enhance Bread Quality

The baking data presented in Table 5 indicate that nearly comparable bread was produced with all methods when a good baking quality flour was used. In the case of the no-time preferments slightly inferior bread was produced when either the ADMI or Fleischmann process was used. When poor baking quality flours were used, the sponge process produced superior bread while all preferment processes produced nearly comparable bread, but inferior to that produced with the sponge process. The differences in bread quality produced using the sponge process and preferment processes seemed to be largely a matter of flour quality rather than the effect produced by the fermentation of the preferments. The baking results with preferment fractions indicate that bread quality enhancing agents were not predominately present in either the yeast cells or preferment liquor. Bread produced using new

Table 4. Summary of bread scores for bread baked using ADMI preferments and sponge methods with three weak and three strong hard red winter wheat flours.

Mg. Rhozyme A-4 per 700 g. flour	Stage enzyme added	Bread scores[1]	
		Strong flours	Weak flours
		Sponge Method	
0	Sponge	90.0	86.7
6	Sponge	95.0	----
9	Sponge	94.0	87.7
12	Sponge	93.0	----
27	Sponge	----	78.0
		ADMI Preferment Process	
0	Preferment	92.5	74.0
9	Preferment	93.8	74.7
18	Preferment	94.5	----
27	Preferment	94.3	78.7
9	Dough	92.0	76.7
18	Dough	93.3	----
27	Dough	93.3	78.0

[1]Volume, 20; Break and shred, 5; Crumb color, 5; Grain and texture, 35; Absorption, 10; Dough handling properties, 15; Mixing tolerance, 10; Total, 100 points.

yeast and spent preferment liquor was comparable in quality to that produced using either a control preferment or one consisting of old yeast and new preferment liquor. The slightly inferior bread generally produced when using the ADMI or Fleischmann no-time process may be due to the short time allowed for the ingredients of the preferments to react with the flour proteins. It is possible that time of fermentation in the presence of the flour was responsible for part of the differences observed between sponge and preferment bread quality. Since, the preferment processes are essentially a straight dough procedure, it is not

surprising to find that the sponge process employing weaker flours offered greater tolerance to the baking procedure.

In the fermentation of sugar in preferments, many products are produced. Some of these products produced in fermentation may have a beneficial effect on bread quality; however, from this work it appears that if any enhancing agents are produced they are of insufficient quantity to be of any significant importance in improving bread quality.

Table 5. Summary of bread scores for bread made with three good baking quality and three poor baking quality flours and using the sponge method, ADMI and Fleischmann preferments.

Flour No.	Type of Baking Process								
		ADMI				Fleischmann			
	A	B	C	D	E	F	G	H	I
				Three good quality flours					
1	95	97	94	90	91	91	86	92	85
2	95	92	93	91	84	93	88	92	88
4	93	94	94	95	88	93	92	93	89
Average	94.3	94.3	93.6	92	87.6	92.3	88.6	92.3	87.3
				Three poor quality flours					
8	91	88	89	88	83	84	84	88	86
9	83	77	75	76	75	77	75	79	81
10	88	82	80	82	80	84	80	82	82
Average	87.3	82.3	81.3	82	79.3	81.6	79.6	83	83

A represents sponge; B and F represent control preferment; C and G represent new yeast and spent preferment liquor; D and H represent old yeast and fresh preferment liquor; E and I represent fresh yeast and fresh preferment liquor.

Effect of Using ATP in Preferments

Since the availability of labile phosphate may be limited in preferments, ATP might be expected to enhance yeast fermentation of sugar in the preferments. If this were the case, then the addition of ATP should increase the rate of fermentation or possibly eliminate the need for fermentation completely in the preferment. To investigate this phenomena, ATP was added in various amounts to no-time ADMI preferments for bread baking. The results are shown in Table 6. No improvement in bread quality was produced by the addition of ATP using a good baking quality flour. This suggested that ATP is already produced in excess by the yeast cells, and therefore, is not a limiting factor in preferment fermentation.

Table 6. Bread scores obtained using ADMI no-time preferments and various quantities of ATP with a good baking quality flour.

Quality characteristics	Maximum score	Mg. of ATP per 700 g. flour						
		0	25	50	75	100	125	150
Volume	20	18	18	18	17	19	18	18
Br & shred	5	5	5	5	5	5	5	5
Cr color	5	5	5	5	5	5	5	5
Gr & tex.	35	28	26	26	28	28	28	28
Absorption	10	9	9	9	9	9	9	9
Do. hand.	15	13	13	13	13	13	13	13
Mix. tol.	10	10	10	10	10	10	10	10
Total	100	88	86	86	87	89	88	88

SUMMARY

The stability of alpha-amylase and protease from both malted wheat flour and fungal Aspergillus oryzae in three different preferments was determined. The optimum relative protease supplementation to use in ADMI and Fleischmann preferments as compared to the sponge method was determined. The sugar preferment was not studied extensively due to the lack of a buffering agent in the preferment and consequent decrease in pH accompanied by a loss in both alpha-amylase and protease activity. A study was made to determine if any constituents were produced in the ADMI or Fleischmann preferment that might enhance the quality of the bread. Experiments were designed to demonstrate whether these constituents were present in the yeast cells or preferment liquor. The use of adenosine triphosphate (ATP) in ADMI no-time preferments was investigated. It was concluded that:

1. The activities of alpha-amylase and protease derived from either malted wheat flour or fungal Aspergillus oryzae remained essentially constant in the ADMI and Fleischmann preferments during 24 hours of fermentation.

2. In the sugar preferment, the alpha-amylase activity from the malted wheat flour and the fungal preparation declined rapidly and no activity remained after 2.5 hours.

3. In the sugar preferment, after six hours the protease activities of the malted wheat flour and the fungal preparation were 75 and 50 percent, respectively, of the original and they

remained essentially constant for the following 18 hours of fermentation.

4. The loss of both amylase and protease in the sugar preferment apparently was due to a decrease in pH in the unbuffered system.

5. No difference in bread quality was observed whether the enzyme supplements were added to the ADMI or Fleischmann preferments or later at the dough stage.

6. The optimum protease supplementation for the ADMI and Fleischmann preferments was two to three times that required using the sponge method.

7. The preferment methods tolerated larger quantities of protease than the sponge method.

8. Although bread quality enhancing agents may be produced in preferments, their effect on improving bread quality does not appear to be significant.

9. When using good baking quality flours, either the ADMI or Fleischmann process, produced bread which was nearly equal in quality to that produced using the sponge process.

10. When using poor baking quality flours, the sponge process produced bread that was superior to that produced using either the ADMI or Fleischmann preferment processes.

11. No beneficial affects were noted when adenosine triphosphate (ATP) was added to ADMI no-time preferments.

ACKNOWLEDGMENTS

The author would like to express his thanks and acknowledge his indebtedness to Doctor John A. Johnson and Doctor Byron S. Miller for their assistance, encouragement, and many helpful suggestions in supervising this research problem and in the preparation of the manuscript. Thanks are also due Mr. Don Miller and Mrs. Bette Hays for their technical help and assistance in performing the baking experiments and typing; and to the other members of the Department of Flour and Feed Milling Industries who were so generous with their assistance.

Acknowledgment and thanks are given to Rohm and Haas Company, Philadelphia, Pennsylvania, for the financial assistance which made this research possible.

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THE APPLICATION OF ENZYMES IN THE STABLE FERMENT PROCESS FOR COMMERCIAL BREAD PRODUCTION

by

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of Agriculture and Applied Science, 1952

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1956

The activity of both malted wheat flour and fungal (Aspergillus oryzae) alpha-amylase and protease in the American Dry Milk Institute (ADMI) and Fleischmann preferments remained essentially constant for 24 hours of fermentation. In the sugar preferment the alpha-amylase activity of both malted wheat flour and the fungal preparation declined rapidly after 30 minutes and two hours, respectively. No alpha-amylase activity from the malted wheat flour or the fungal preparation remained after 2.5 hours. In the sugar preferment, after six hours the protease activities of the malted wheat flour and the fungal preparation were 75 and 50 percent, respectively, of the original. During the following 18 hours of fermentation there was only a slight loss of protease activity. The loss of both protease and alpha-amylase activity in the sugar preferment apparently was due to a decrease in pH.

No difference in bread quality could be observed whether the enzyme supplements were added to the ADMI and Fleischmann preferments or later at the dough stage. The optimum protease supplementation for the two preferments appeared to be three times that required with the sponge method. The preferment methods tolerated large quantities of protease.

The effect of fermentation on the bread making capacity of preferments was studied. Two preferments were employed. No significant bread enhancing agent was found in either the ADMI or Fleischmann preferments using a four hour fermentation period.

When using good baking quality flours, either the ADMI or Fleischmann preferment process, produced bread which was nearly equal in quality to that produced using the sponge process. When using poor baking quality flours, the sponge process produced bread that was definitely superior to that produced using either the ADMI or Fleischmann preferment process.

No beneficial effects were noted when adenosine triphosphate was added to ADMI no-time preferments.

Date Due