Starch Functionality as Affected by Amylases from Different Sources

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ABSTRACT

For an evaluation of changes in starch functionality, different amounts of α-amylases (1, 2, or 8 SKB units per gram) from bacterial, fungal, and cereal sources were incubated for various times (4, 8, or 16 hr) with starch isolated from wheat flour. Water-binding capacity, swelling power, solubility, gelatinization temperature ranges, and amylograph pasting characteristics were measured, scanning electron microscopic studies of the starches were conducted, and baking characteristics of the treated starches were evaluated. Enzyme treatment affected water-binding capacity. Swelling power at 90° C was reduced and starch solubility increased due to the enzyme treatment. Gelatinization temperatures of the starch decreased after treatment with bacterial and cereal enzyme treatments. Scanning electron microscopy showed slight starch damage as surface modifications due to enzyme treatment. Amylograph measurements indicated that enzymatic treatment resulted in decreased consistencies from treated starches. Bread-baking quality of starch improved with low-level treatment of enzyme from each source. Grain and texture of the breads were better than those of bread baked with a control starch. High levels (8 SKB units/g) of enzyme treatment showed detrimental effects on bread-baking performance. Because the interfering secondary activities were eliminated by the experimental design, the observed changes are attributable to amylase activities only.

Use of amylases in the baking industry is of long standing. Its original purpose was to generate fermentable sugars by amylolysis of starch to improve volume and general bread characteristics.

Johnson and Miller (1949) studied the role of commercial malted wheat and fungal amylase in bread making. Loaf volume increased when α-amylase concentrations were increased up to 20 times (20×) the normal concentrations found in flour. In general, grain improved with increasing amylase levels up to five times (5×) the normal concentration. Texture generally improved up to the 5× concentration, but a moist gummy crumb was produced at the 20× concentration. This crumb characteristic was less pronounced with the fungal amylase because of inactivation at lower temperatures.

Johnson et al (1949) evaluated malted wheat flour and fungal and bacterial amylase supplements for breadmaking purposes. Wheat flour supplement was found to be the most satisfactory when flours were supplemented to similar gassing powers. The fungal preparation produced bread with open grain and harsh texture caused by excessive proteinase activity. Sticky, gummy bread was characteristic of the bacterial supplement because of excessive starch degradation during baking. In addition, flours were supplemented to give equivalent maximum viscosities, determined with an amylograph. Again, the fungal preparation produced excessive protein degradation, and the bacterial supplement yielded a sticky, gummy crumb. Johnson and co-workers (1949) concluded that consideration should be given to the relationship between thermal inactivation of the α-amylase and the temperature of starch gelatinization and to the relative potency of the α-amylase and proteolytic enzymes present in the supplement.

Miller and co-workers (1953) compared cereal, fungal, and bacterial α-amylases as breadmaking supplements in relation to bread staling. All three types of amylases decreased the rate of firming with age as a result of the production of fractions that absorbed moisture during starch retrogradation. Bacterial amylase supplementation produced a sticky, gummy crumb and the largest quantity of extractable dextrins.

Dough rheological properties and baking qualities as affected by cereal or fungal amylase supplementation of wheat flours were also investigated by Maninder and Bains (1976). Supplementation was beneficial because of the low indigenous α-amylase activities in the flours studied. Decreased viscosities, as measured by the amylograph, were most apparent in the flour supplemented with cereal amylase as compared to the fungal amylase-supplemented flour. Maninder and Jorgensen (1983) found improvements in loaf volume and bread quality with fungal α-amylase supplementation. Gelatinization temperatures and amylograph peak viscosities decreased with increasing amylase levels.

This amylase action has become less important with modern bakery formulas, which contain relatively high levels of fermentable sugars to support vigorous fermentation. Consequently, the improving effects of amylases used today are attributed to other reactions contributing to better quality, e.g., reduction of damaged starch or action of other enzymes present in commercial preparations.

Because commercial enzymes contain secondary activities, it is difficult to resolve whether the primary activity or the associated enzymes contribute to the improving role. An alternative approach is to use starch as a substrate, attempt to modify it by the action of amylases, and investigate the changes effected by the amylolysis; this approach was used in the present study. Figure 1 shows the overall plan of investigation.

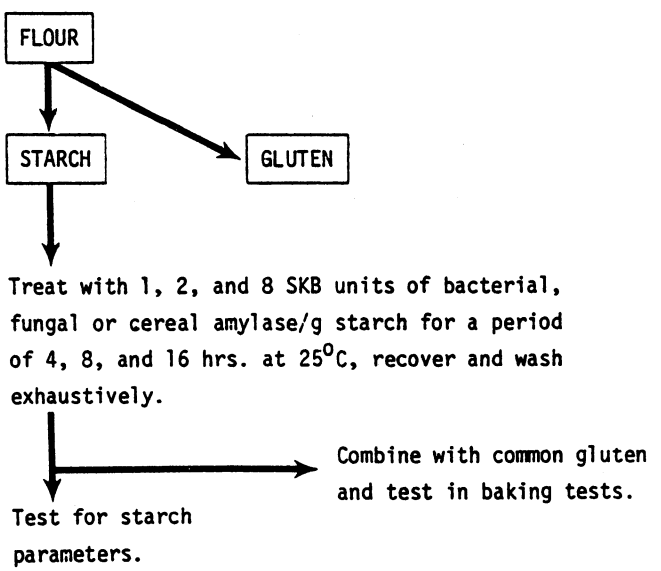

Fig. 1. Plan of investigation.

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MATERIALS AND METHODS

Fractionation of Flour and Enzyme Treatments

Untreated hard red winter wheat patent flour was separated into starch and gluten using the dough procedure (Kulp and Bechtel 1963, Lorenz et al 1983). The gluten was hand-washed from the dough, frozen immediately, and kept in frozen storage until needed for baking. The method of starch isolation was described previously (Kulp et al 1983). Starch was freeze-dried and stored under ambient laboratory conditions.

Three different α-amylase preparations were obtained from Sigma Chemical Corporation, St. Louis, MO: *Bacillus subtilis* α-amylase, *Aspergillus oryzae* α-amylase, and α-amylase from barley malt (*Hordeum vulgare*). These enzymes differ in heat stability characteristics, that of barley malt being active even above the gelatinization temperature range of starch, fungal amylase losing activity before starch reaches gelatinization temperature, and bacterial amylase surviving baking.

Starch was suspended in phosphate buffer (pH 5.2) and treated with 1, 2, or 8 SKB units of each α-amylase for 4, 8, or 16 hr at 25° C, then centrifuged and washed several times with distilled water to remove enzymes.

Proximate Composition of Starches

Nitrogen and crude fat of the control and selected treatment starches were determined by AACC methods 46-12 and 30-20 (AACC 1976). Amylose was determined as described by Juliano (1971).

Scanning Electron Microscopy (SEM)

Samples of the control starch and intermediate (2 SKB/8 hr) and high (8 SKB/16 hr) treatment levels for each enzyme source were examined with a Philips 505 scanning electron microscope. Conductive copper adhesive tape was glued onto aluminum specimen stubs and oven-dried for 10 min. Silver paint was applied along the edges of the tape before removal of the copper tape's protective covering. Starch samples were applied to the sticky prepared surface. A 200 Å gold-palladium coating was applied by a Technic Hummer V. The samples were viewed and photographed at magnifications ranging from 600 to 2,500×.

Physicochemical Properties

The water-binding capacity, swelling power, and solubility were determined for all samples in order to assess the effect of treatment on these properties.

Water-binding capacity was determined using the method of Medcalf and Gilles (1965). This method was modified by use of 20 ml of 2.5 μM silver nitrate solution per gram of starch to inactivate any residual α-amylase activity (Kulp et al 1972).

Swelling power and solubility were determined at temperatures of 70 and 90° C according to the procedure of Leach et al (1959). One gram of starch was suspended in 10 ml of distilled water in a tared 15-ml centrifuge tube. After 30 min in a thermostatically controlled water bath, the tubes were centrifuged at 5,000 rpm for 10 min. The supernatants were drained into pretared moisture dishes, evaporated to dryness in a 100° C moisture oven, and cooled to room temperature in a desiccator prior to reweighing, and swelling was calculated as the weight of the sedimented paste per gram of dry starch. Five replicates were done for each sample.

Pasting properties of the starches were evaluated with the Brabender Visco/amylo/Graph type VA-VE. Starch (37.8 g, 9% mb) and 420 ml of liquid were used. Ten milliliters of 2.5 μM $AgNO_3$ solution were added to inactivate residual enzyme activity (Kulp et al 1972). The bowl speed was 75 rpm with a 700-cmg torsion spring. The suspension was heated from 25 to 92° C at a controlled rate of 1.5° C per minute, held at 92° C for 15 min, and then cooled to 35° C. Consistency, as measured in Brabender units (BU), was recorded at 92° C, after 15 min at 92° C, and at 35° C.

The gelatinization temperatures of selected enzyme-treated starches were determined by the procedure of Schoch and Maywald (1956) using a polarized light microscope equipped with a Kofler hot stage. Temperatures were recorded for 2, 50, and 90% loss of birefringence.

Bread Baking

Starch-gluten breads. Each starch sample was recombined with gluten isolated from the original flour. The remix procedure of Irvine and McMullan (1960) was slightly modified. The formulation used for each pup loaf consisted of 50 g of wet gluten, 85 g of starch, 10 g of sucrose, 1.5 g of salt, 0.5 g of mineral yeast food, 3 g of shortening, 3 g of yeast, 4 g of nonfat dry milk, 20 ppm of $KBrO_3$ and water as needed. Doughs were fermented 75 min at 88° F and 80% rh, rounded and bench rested for approximately 10 min before they were molded, proofed to height at 100° F and 90% rh, and baked at 425° F for 18 min. Specific loaf volume was measured by rapeseed displacement. External and internal loaf characteristics were also evaluated. Breads were scored as follows, using a 100 point scale: crust color, 7; symmetry, 7; break, 6; crumb color, 10; flavor, 15; grain, 20; texture, 20; and volume, 15. The maximum number of possible points is indicated after each bread characteristic.

Breads from enzyme-supplemented flour. Samples of the original flour were used to bake breads in which amylase supplementation was the same as the amounts used in starch treatment. Each enzyme source was used to supplement the flour dough to achieve 1, 2, or 8 SKB units per gram of starch. The amounts calculated were based on flour being 70% starch and the individual activities of the enzymes. Flour dough formulation was as follows: 100 g of flour, 6 g of sucrose, 3 g of shortening, 1.5 g of salt, 2.5 g of active dry yeast, 0.5 g of mineral yeast food, 50 ppm $KBrO_3$, and water as required. Fermentation, molding, proofing, and baking conditions were described in the previous section. Specific volumes and bread scores were evaluated as previously described.

Statistical Evaluation

The statistics used to analyze water-binding capacity, swelling, and solubility data included a factorial analysis of variance to determine differences between the treatments and one-way analysis to ascertain differences between the untreated control starch and individual treatment levels. Treatment levels are defined as the responses from the individual enzyme sources for each combination of amount and length of time (1/2, 1/8, 1/16, 2/4, 2/8, 2/16, 8/4, 8/8, and 8/16).

RESULTS AND DISCUSSION

Proximate Compositions of Starches

The composition of starches recovered after enzyme treatment is given in Table I. The treated starches did not differ in tested protein, crude fat, or amylose contents from the untreated control starch, being essentially of the same purity regardless of conditions and type of enzyme used in the treatment.

SEM

The scanning electron micrographs of control and selected enzyme-treated starch at intermediate (2 SKB/8 hr) and high (8

TABLE I
Proximate Analyses of Starch and Flour

Sample[a]	Nitrogen (%)	Protein (%)	Crude Fat (%)	Amylose (%)
Control	0.05	0.28	0.10	20.9
B 1/4	0.05	0.29	0.13	21.0
B 8/16	0.05	0.30	0.20	21.1
F 1/4	0.05	0.28	0.13	21.3
F 8/16	0.05	0.27	0.16	21.7
C 1/4	0.04	0.26	0.06	21.7
C 8/16	0.05	0.30	0.16	21.0
Flour	2.29	13.03	...	...

[a] B = Bacterial, F = fungal, C = cereal. First number shows SKB units per gram of starch; second number shows incubation period (hr) at 25° C.

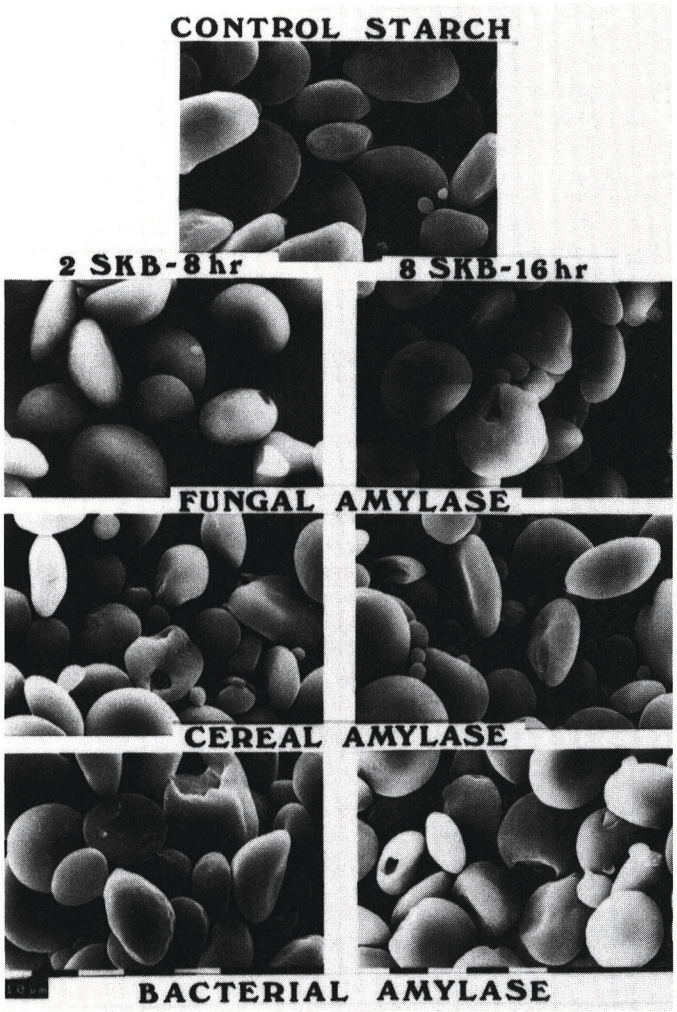

Fig. 2. Scanning electron micrographs of control and selected enzyme treated starches.

SKB/16 hr) treatment conditions with all three enzymes are shown in Figure 2. The untreated control starch granules appear intact, with no erosion or cracks. The α-amylase-treated starches, regardless of enzyme source, show some surface modification. Reports in the literature indicate that α-amylolysis produces radial and tangential channels emanating from the equatorial groove of the granules (Sandstedt 1954, Gallant et al 1972). These effects could not be detected—the changes did not progress far enough. The bacterial amylase-treated starches showed greater attack by the enzyme than the other treated starches.

Physicochemical Properties

Water-binding capacities, swelling powers, and solubilities of experimental starches are given in Table II. All tests were conducted with added $AgNO_3$. Generally, water binding was increased; swelling power was increased slightly at 70° C and decreased slightly at 90° C. Solubility was most affected. It increased at 70° C over the control for all enzymes except the fungal amylase; at 70° C all treated starches were more soluble than the control.

Statistical evaluation of the data showed that for water-binding capacity source of enzyme, amount, and time of incubation were significantly different with amount being least significant. All swelling and solubility values are significant, except that length of incubation as the main effect did not significantly affect swelling and solubility at 90° C. When compared in one-way analysis of variance versus control, it was evident that swelling and solubility patterns are significantly altered when compared with control. The least changes appear to occur in water-binding capacity.

Amylograph data (Table III) were obtained by pasting experimental starches in water containing $AgNO_3$ to inactive residual enzyme activity. This method permits us to estimate the changes in starch without the interference of amylase. It is evident that all of the treated starches lost some thickening power. Most affected was the bacterial amylase-treated starch, followed by that treated with cereal or fungal amylases. However, a discernible degree of change from control was observed in all treated starches.

Gelatinization temperature ranges determined on a Kofler hot stage, using loss of birefringence as a reference index are shown in Table III. Treatments with bacterial and cereal α-amylases, in

TABLE II
Physicochemical Properties

Sample[a]	Water-Binding Capacity (%)	Swelling Power		Solubility (%)	
		70° C	90° C	70° C	90° C
Control	101.6 ± 5.8	6.44 ± 0.03	10.42 ± 0.09	2.60 ± 0.23	1.37 ± 0.11
B 1/4	109.0 ± 5.7	6.79 ± 0.10	9.49 ± 0.24	3.55 ± 0.11	3.01 ± 0.32
B 1/8	103.6 ± 2.2	6.75 ± 0.03	9.62 ± 0.28	3.68 ± 0.08	2.80 ± 0.44
B 1/16	104.2 ± 6.0	6.56 ± 0.05	9.99 ± 0.28	3.46 ± 0.14	2.09 ± 0.37
B 2/4	105.3 ± 5.2	6.91 ± 0.05	9.59 ± 0.26	4.44 ± 0.09	2.69 ± 0.02
B 2/8	105.3 ± 2.4	6.76 ± 0.02	9.48 ± 0.23	4.17 ± 0.16	3.29 ± 0.16
B 2/16	100.7 ± 7.2	6.76 ± 0.04	9.59 ± 0.15	4.08 ± 0.24	3.35 ± 0.18
B 8/4	108.7 ± 2.3	7.24 ± 0.03	9.32 ± 0.13	5.79 ± 0.11	4.34 ± 0.23
B 8/8	106.3 ± 2.7	7.09 ± 0.06	9.36 ± 0.18	5.12 ± 0.29	3.86 ± 0.36
B 8/16	111.7 ± 4.4	7.08 ± 0.03	8.97 ± 0.50	4.15 ± 0.17	3.46 ± 0.25
F 1/4	103.6 ± 5.2	6.63 ± 0.05	9.94 ± 0.17	2.60 ± 0.04	3.27 ± 0.12
F 1/8	102.1 ± 4.2	6.50 ± 0.04	9.57 ± 0.34	2.83 ± 0.09	3.61 ± 0.39
F 1/16	101.5 ± 3.7	6.59 ± 0.02	9.52 ± 0.12	2.78 ± 0.11	3.30 ± 0.28
F 2/4	104.1 ± 3.5	6.64 ± 0.02	9.38 ± 0.11	2.58 ± 0.09	3.27 ± 0.32
F 2/16	101.3 ± 5.7	6.58 ± 0.03	9.30 ± 0.11	2.48 ± 0.04	3.84 ± 0.12
F 8/4	101.8 ± 5.0	6.56 ± 0.04	9.21 ± 0.24	2.53 ± 0.04	3.97 ± 0.37
F 8/8	106.3 ± 4.6	6.61 ± 0.04	9.25 ± 0.17	2.50 ± 0.07	3.43 ± 0.26
F 8/16	103.8 ± 4.8	6.60 ± 0.04	9.23 ± 0.25	2.43 ± 0.12	2.82 ± 0.14
C 1/4	99.5 ± 2.3	6.71 ± 0.07	9.51 ± 0.19	3.17 ± 0.15	2.96 ± 0.30
C 1/16	118.7 ± 8.2	6.71 ± 0.05	8.81 ± 0.10	2.95 ± 0.19	3.73 ± 0.22
C 2/4	96.4 ± 3.5	6.63 ± 0.04	8.81 ± 0.29	2.70 ± 0.11	3.44 ± 0.42
C 2/16	117.1 ± 5.5	6.66 ± 0.06	9.46 ± 0.24	3.19 ± 0.14	3.29 ± 0.14
C 8/8	99.7 ± 3.1	6.58 ± 0.10	9.28 ± 0.12	3.10 ± 0.08	3.36 ± 0.16
C 8/16	113. ± 7.4	6.99 ± 0.19	8.94 ± 0.17	4.59 ± 0.19	3.90 ± 0.12

[a] B = Bacterial, F = fungal, C = cereal. First numbers shows SKB units per gram of starch; second number shows incubation period (hr) at 25° C.

general, resulted in lower gelatinization temperatures when compared to the control and fungal α-amylase-treated starches. The latter was virtually identical in gelatinization temperature to the control.

Bread Baking

Starch-gluten breads. Starch-gluten flour breads are shown in Figure 3 and scores are given in Table IV. Specific volumes of bread baked with starches treated with bacterial α-amylase increased with increasing enzyme amounts. No trends were observed for specific volumes of breads made from fungal and cereal enzyme treated starches.

High treatment (8 SKB units) levels of bacterial enzyme produced deleterious effects on break and shred and grain characteristics. Starch treatment with fungal α-amylase resulted in a slight decrease in score for break and shred, and high levels (8 SKB units) yielded lower scores for grain characteristic. In general, crust color, crumb color, and flavor were not affected by enzyme treatment, regardless of enzyme source.

A slight improvement in texture was apparent in the bacterial and fungal enzyme-treated starch breads with increased length of incubation at the low treatment level ([1 SKB unit/g]/16 hr). No further improvement was observed; the scores remained similar with increasing enzyme amounts and lengths of incubation. No trend in texture scores was observed for the cereal enzyme-treated starch breads.

Grain scores for the bacterial and fungal enzyme-treated starch breads generally increased and then decreased at high α-amylase treatment levels. The texture scores did not follow this trend; no deleterious effect was noted at the higher treatment levels.

Total scores increased and then decreased with increasing enzyme amounts for the bacterial and fungal enzyme-treated starches. No trend was apparent for the cereal enzyme-treated starch breads. Total scores reflected reported trends. Excessive α-amylase activity has been reported to produce detrimental effects in bread (Johnson and Miller 1949).

Assuming that residual α-amylase activity was present, the thermostability of the *Bacillus subtilis* (bacterial) enzyme is illustrated by the decreased break and shred, grain, and total scores at the high (8 SKB units) treatment levels. Remaining active throughout baking, continued starch hydrolosis by the bacterial α-amylase resulted in a very open grain and lower scores.

Overall, total scores of breads baked with enzyme-treated starches were higher than those for control breads (except for high treatment with bacterial amylase). Both cereal and fungal amylase-treated starches performed better than the control starch. These data substantiate the claim that amylases are improvers of grain, texture, and total bread quality.

Enzyme-supplemented flour breads. Enzyme-supplemented flour breads are shown in Figure 4 and bread scores are given in Table V.

Addition of α-amylase to flour resulted in higher specific volumes than those of the enzyme-treated starch breads. Increasing enzyme amounts generally resulted in increased specific volume scores. Removed during starch isolation, the water-soluble components were not present in the starch-gluten breads, which contributed to the difference in volume of the breads.

Low levels (1 and 2 SKB units/g) of enzyme supplementation of flour yielded increased total scores when compared to the unsupplemented flour control bread, for all enzyme sources. The high supplement level (8 SKB units/g) of the bacterial and fungal enzymes resulted in lower total bread scores than the total bread score of the control. The cereal enzyme supplement at the high level did result in a lower total bread score when compared to the low enzyme supplement levels, but a slightly better score than the unsupplemented control flour bread. Again, the detrimental effect of excessive α-amylase activity in bread was demonstrated, with

TABLE III
Amylograph Consistencies and Gelatinization Temperature Ranges of Selected Starches

	Consistency (BU)			Loss of Birefringence (°C)		
		After 15 min				
Sample[a]	At 92°C	at 92°C	At 35°C	2%	50%	90%
Control	325	445	490	54.6	60.0	65.3
B 1/4	260	375	440	52.0	57.6	62.3
B 2/8	240	380	415	...	...	...
B 8/16	240	345	395	52.6	57.3	62.3
F 1/4	...	...	...	55.6	60.3	65.3
F 1/8	320	440	500	...	...	...
F 2/8	310	445	485	...	...	...
F 8/16	250	385	465	54.0	61.0	65.6
C 1/4	255	415	455	51.3	56.3	61.3
C 2/8	240	375	410	...	...	...
C 8/16	255	375	430	52.3	57.6	63.3

[a] B = Bacterial, F = fungal, C = cereal. First number shows SKB units per gram of starch; second numbers shows incubation period (hr) at 25°C.

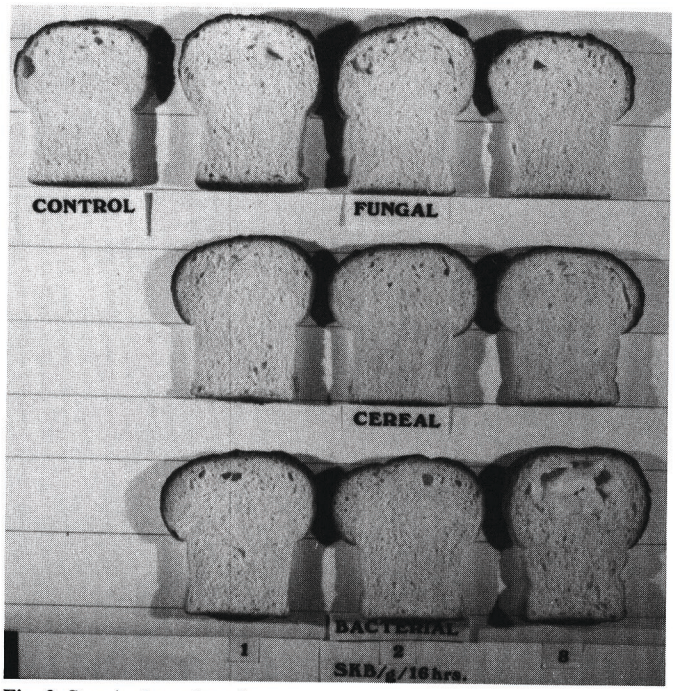

Fig. 3. Starch-gluten breads.

TABLE IV
Starch Gluten Bread Scores

Sample[a]	Specific Volume (cm^3/g)	Flavor	Grain	Texture	Total
Control	5.05	14	15	16	85
B 1/4	5.10	14	18	16	88
B 1/16	5.12	14	19	18	91
B 2/4	5.26	14	18	17	90
B 2/16	5.46	14	19	18	92
B 8/4	6.18	14	17	18	88
B 8/16	6.07	14	9	18	80
F 1/4	4.89	14	17	16	86
F 1/16	5.34	14	18	18	90
F 2/4	5.30	14	18	18	90
F 2/16	5.18	14	17	18	88
F 8/4	5.30	14	16	18	88
F 8/16	5.15	14	16	18	88
C 1/4	5.58	14	18	18	92
C 1/16	5.56	14	18	16	89
C 2/4	5.05	14	18	16	88
C 2/16	5.55	14	19	19	94
C 8/4	5.28	14	17	17	88
C 8/16	5.19	14	18	18	90
Maximum score		15	20	20	100

[a] B = Bacterial, F = fungal, C = cereal. First number shows SKB units per gram of starch; second number shows incubation period (hr) at 25°C.

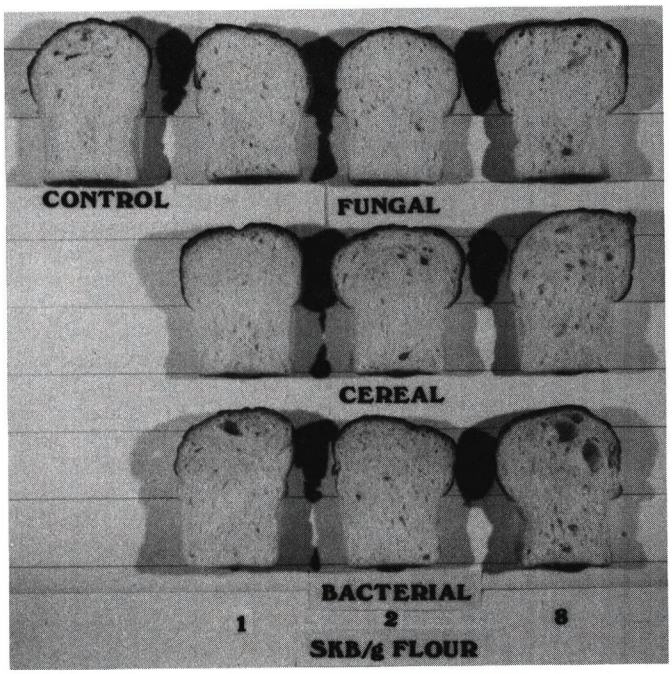

Fig. 4. Enzyme-supplemented flour breads.

TABLE V
Enzyme-Supplemented Flour Breads

Sample[a]	Specific Volume (cm^3/g)	Break	Grain	Texture	Total
No enzyme, control	5.84	4	17	15	82
B1	6.13	5	17	17	87
B2	6.07	6	18	17	90
B8	6.17	5	9	16	77
F1	5.78	6	16	18	88
F2	5.94	6	16	18	88
F8	6.89	4	12	17	80
C1	6.07	5	17	18	89
C2	6.10	4	17	18	86
C8	6.22	5	13	17	83
Maximum Score		6	20	20	100

[a] Sample designation: letters indicate enzyme source: B = bacterial amylase, F = fungal amylase, and C = cereal amylase. Numbers indicate SKB units per gram of starch.

the grain characteristic being most affected. Holes and irregular cell structure were obvious in the 8 SKB units/g supplementation for all enzyme sources and resulted in low grain scores.

In general, decreases in the break and shred, and the grain and texture scores were apparent at the high levels of all enzyme sources, with a greater decrease because of the bacterial α-amylase. Enzyme supplementation of flour did not result in improvements in crust color or flavor. A slight decrease in crumb color scores was observed with the high levels of bacterial and fungal enzyme supplements because of a more open grain.

CONCLUSIONS

The study evaluated effects of three types of amylases on starch properties and concomitant effects on bread quality. Because the interfering secondary activities were eliminated by the experimental design, the observed changes are attributable to the amylase activities only. These are: 1) changes in starch properties, mainly solubility at 70° C and pasting properties, indicating a slight loss in thickening power; 2) changes in starch appeared to be limited to amorphous region of the granules; 3) changes in starch were reflected in certain improvements of bread, e.g., volume, texture, and grain; and 4) effects of overtreatment with bacterial amylase were caused by changes in the starch.

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