Effect of Papain on Amylograph Viscosity of Flour[1]

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ABSTRACT

Additions of small amounts of the proteolytic enzyme papain lowers the peak viscosity of a flour-water slurry as measured on the amylograph. This effect was found to be due to release or activation of beta-amylase which is inactive under normal conditions used for the amylograph test. The magnitude of this effect is different for different flours.

Alpha-amylase activity is usually low in sound wheat but increases rapidly during sprouting; hence, a low peak viscosity in the amylograph is considered to be an indication of higher levels of alpha-amylase resulting from sprout damage. On the other hand, beta-amylase activity is usually quite high in flour from sound wheat, but its action on starch is presumed to be such that it has little effect on the amylogram. In addition to amylolytic enzymes, other flour components such as starch, gluten, and pentosans, and physical factors such as particle size and condition of starch granules, also affect the amylogram. Their effects are relatively more important in flours from sound wheat in which the level of alpha-amylase is very low. In our studies of the factors related to amylograph viscosity of flour slurries, it was observed that a significant lowering of peak viscosity occurred if the flour slurry was treated with papain before the amylograph test was run. This paper discusses the results of some experiments made to determine the mechanism of this effect.

MATERIALS AND METHODS

The flour used in all but one set of experiments was milled on a Buhler experimental mill, from Canadian hard red spring wheat. It was straight-grade, untreated, and unbleached, with protein and ash contents of 13.5 and 0.44% respectively. Five other flours of similar protein and ash contents but differing in diastatic activities as measured on the amylograph were used in the experiments discussed in the last section of Results and Discussion. The starch and gluten used in some experiments were respectively commercial granular wheat starch and vital gluten, marketed by Industrial Grain Products. The papain was purified powder concentrate obtained from Mann Research Laboratories. It did not show any amylolytic activity on starch. Standardized 2,000°L. beta-amylase was also obtained from Mann Research Laboratories.

Amylograms were obtained on the Brabender VISCO/amylo/GRAPH. In most experiments the final flour:water ratio was 50 g.:450 ml. The 350-cm.g. cartridge was used for all amylograms. Other pertinent details of specific experiments will be given in the section dealing with the results of these experiments.

The method of Schwimmer (1) was used to determine saccharifying ac-

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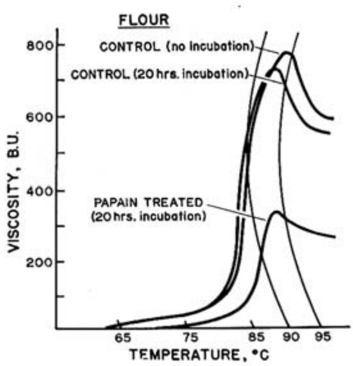
tivity, and the ICC method (2) for dextrinizing activity. All chemicals were of reagent grade.

RESULTS AND DISCUSSION

Preliminary Experiments

A typical example of the effect of papain on the flour amylogram is shown in Fig. 1. An initial flour-water slurry containing 50 g. of flour, 300 ml. of distilled water, 0.03% papain, and 50 μmoles of cysteine was first incubated for 20 hr. at 20°–22°C. Cysteine was included as the reducing agent necessary to activate the papain. A control was incubated similarly but without papain or cysteine. For the amylograph test, an additional 150 ml. of distilled water was added. Figure 1 also shows a normal amylogram for the same flour-to-water ratio (i.e., no preincubation of the slurry).



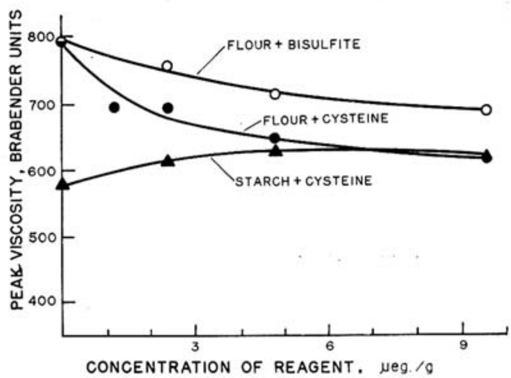


Fig. 1 (left). Effect of papain treatment on flour amylogram.

Fig. 2 (right). Effect of reducing agents on peak amylogram viscosity of flour and starch.

The amylogram for the papain-treated slurry shows a significantly lower peak viscosity than that for the untreated slurry; incubation of the flour-water slurry without papain had little effect on the amylogram. All three curves showed the same gelatinizing and peak viscosity temperature.

Treatment of flour with papain inactivated by boiling, or by oxidation with iodine (3), did not produce any lowering of the peak viscosity. Increase in papain concentration and additions of small amounts of reducing agents, sodium sulfite or cysteine, intensified the lowering of viscosity (Table I).

Reducing agents had an effect similar to that of papain; however, the magnitude of the maximum decrease in viscosity was considerably smaller. The maximum effect is obtained with relatively small amounts of reducing agent (e.g., 200 μM of cysteine). Reducing agents have no effect on the peak viscosity of starch-water slurries (see Fig. 2); that is, these agents have no direct effect on the starch gelatinization characteristics.

A number of hypotheses can be postulated to explain the observed effect of papain on amylograph peak viscosity. Three were examined experimentally. These are, that the lowering of viscosity can result from 1) degradation of flour proteins, 2) increase in starch susceptibility resulting from the deg-

TABLE I
EFFECT OF PAPAIN CONCENTRATION ON PEAK VISCOSITY OF FLOUR AMYLOGRAMS, WITH SULFITE AND CYSTEINE USED AS PAPAIN ACTIVATORS

	PEAK VISCOSITY
	B.U.
Control flour	775
Treated with 0.015% papain	505
Treated with 240 μeq. Na_2SO_3 + 0.008% papain	560
Treated with 240 μeq. Na_2SO_3 + 0.015% papain	500
Treated with 240 μeq. Na_2SO_3 + 0.030% papain	420
Treated with 60 μeq. cysteine + 0.015% papain	350
Treated with 60 μeq. cysteine + 0.030% papain	330
Treated with 60 μeq. cysteine + 0.060% papain	290

radation of the flour proteins, and 3) liberation of amylolytic enzymes that are inactive in the absence of papain.

Direct Effect of Protein Degradation

Flour proteins, especially those that form gluten, have a small positive effect on the amylograph viscosity of starch. Accordingly, when they are hydrolyzed, some lowering of viscosity would be expected.

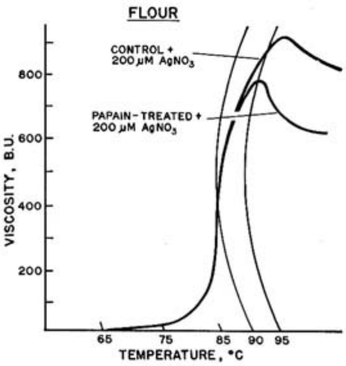
To eliminate enzymes which can affect the viscosity of a flour slurry, silver nitrate was added at the beginning of the viscosity measurement but after incubation with papain. The amount used was sufficient to completely inactivate both the amylolytic and proteolytic enzymes. Although silver nitrate could also have an effect on flour proteins other than inactivation of enzymes, it is presumed that this would not markedly affect the viscosity of the flour-water slurry since the amounts necessary to inactivate the enzymes are very small. Accordingly, the difference in peak viscosities in the curves in Fig. 3 is considered to reflect the decrease due to protein degradation by papain. Similar peak difference was obtained for control and papain-digested samples prepared by freeze-drying of slurries after incubation and then heating at 105°C. for 1 hr. to inactivate the enzymes.

Comparison of Fig. 3 with Fig. 1 shows that the decrease of the amylograph peak viscosity due to degradation of protein by papain is considerably less than that due to the factor that produces the decrease shown in Fig. 1.

Effect of Increase in Starch Susceptibility

Although it is generally accepted that a decrease in amylogram peak for a flour slurry reflects enzymatic degradation of the starch, it seemed advisable to confirm this for the conditions used in the present study. This was done by a combination of experiments involving additions of silver nitrate at various temperatures, and additions of starch to untreated and papain-treated flour slurries.

Figure 4 shows the effects of 200 μM of silver nitrate added to flour slurries after incubation at various temperatures. The slurry was first heated to the temperature shown, held at that temperature for 20 min., and then silver nitrate was added to stop further enzymatic reactions. The remainder of the amylogram was determined in the usual manner.



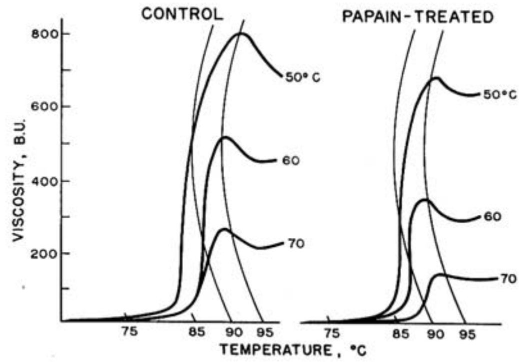


Fig. 3 (left). Effect of silver nitrate on amylograms of normal and papain-treated flour.

Fig. 4 (right). Effect of preincubation at various temperatures on flour amylograms.

Silver nitrate inhibition after incubation at 50°C. produced no additional effect to that obtained by incubation at 20°–22°C. (compare with Fig. 3). However, when the silver nitrate was added after incubation at 60°C. or higher temperature, there was a marked decrease in peak viscosity; the decrease is somewhat greater in the papain-treated slurries than in the controls. It appears that the observed decrease in peak viscosity results from enzymatic degradation of starch that is solubilized above 50°C. but well below the peak gelatinization temperature. The magnitude of this effect seems to depend on the amount of substrate available which appears to increase with temperature of incubation. Papain treatment produces an additional decrease in peak viscosity. This suggests that papain increases the activity or the amount of starch-degrading enzymes.

Table II shows the effect of additions of 10 g. of wheat starch on the peak viscosities of papain-treated, untreated, and untreated plus 150 μM silver nitrate flour slurries. The amount of flour used in each case was adjusted to give a peak viscosity of 350 B.U. for the slurry without the starch. The largest increase (640 B.U.) occurred in the slurry in which the enzymes were inactivated with silver nitrate, whereas the papain-treated slurry showed the

TABLE II

VISCOSITY INCREASE BY ADDITIONS OF 10 G. STARCH TO THE VARIOUS SAMPLES

	Peak Viscosity	
	B.U.	
Flour, 50 g., papain-treated	345	
+ wheat starch, 10 g.		800
Flour, 43 g., control	350	
+ wheat starch, 10 g.		875
Flour, 38.5 g. + 150 μM $AgNO_3$	350	
+ wheat starch, 10 g.		990

TABLE III
EFFECT OF MALT ON PEAK VISCOSITY OF UNTREATED AND PAPAIN-TREATED SAMPLES AFTER HEAT-TREATMENT

	Peak Viscosity	
	Untreated	Papain-Treated
	B.U.	*B.U.*
Dried slurry	410	290
Dried slurry, heat-treated[a]	820	645
Dried slurry, heat-treated[a], + malt[b]	340	240

[a] 1 hr. at 105°C.
[b] 0.03 ml. of 1% solution of 250°L. malt syrup.

smallest increase (455 B.U.). This provides additional evidence that decreases in peak viscosity result from enzymatic degradation of the starch.

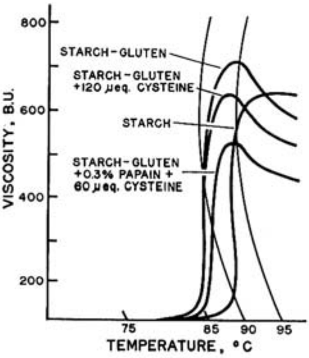
Results in Fig. 4 and Table II indicate that the major part of the lowering of viscosity caused by papain treatment is associated with degradation of the starch during the amylograph test. However, this could result indirectly, as postulated in the second hypothesis given above. Table III gives the results of an experiment designed to examine this possibility. In this experiment, untreated and papain-treated flour slurries were first freeze-dried and then equilibrated to 14–15% moisture content at room temperature. Inactivation of the enzymes in the dried products was accomplished by heating the samples in closed containers for 1 hr. at 105°C. The effect of malt addition on peak viscosity of heat-treated samples is shown in Table III.

The decrease in peak viscosity produced by the added malt is 480 and 405 B.U. for the untreated and the papain-treated samples respectively. The effect that can be attributed to the increase in starch susceptibility resulting from degradation of the flour proteins by papain is 75 B.U. This is considerably less than the over-all effect of papain treatment shown in Fig. 1. It is therefore concluded that the observed effect of papain is primarily due to the release of amylolytic enzymes and the effect due to increased susceptibility of the starch is small.

Effect of Liberation of Amylolytic Enzymes

Results presented above suggest that the action of papain in lowering peak amylograph viscosity of flour slurries is through the liberation or activation of an amylolytic enzyme. Further evidence supporting this possibility is shown in Fig. 5. Here the effects of papain and cysteine treatments on the viscosity of slurries of starch-gluten (4 to 1 by weight) are examined. The peak viscosity for the papain-treated starch-gluten slurry (425 B.U.) is not only lower than that for the control starch-gluten slurry (610 B.U.) but also lower than that for a slurry of starch alone (535 B.U.). The decrease in viscosity of the starch-gluten slurry by cysteine without papain is less than half of that produced by cysteine with papain. Papain and cysteine had no effect on the starch slurry amylogram (not shown). The treatment apparently releases enzymes bound in the gluten which are inactive when the amylogram for a starch-gluten slurry is determined.

So far, nothing has been said about the specific nature of the starch-degrading enzyme released by papain. From the fundamental viewpoint, it



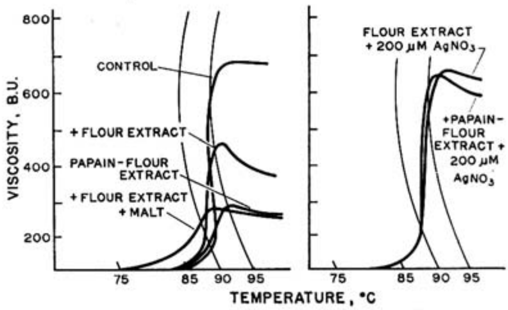


Fig. 5 (left). Effect of papain treatment on amylograms of mixtures of starch and gluten.

Fig. 6 (right). Effect of additions of various flour extracts on the starch amylogram.

would be of significance to determine if this enzyme is alpha- or beta-amylase type.

Liberation of beta-amylase in flour by papain digestion has been reported by many previous workers (1,4,5,6,7), although little attention has been paid to its role in determining the viscosity of flour slurries. It is generally accepted that alpha-amylase is the predominant starch liquefier in such systems. The release of alpha-amylase from wheat proteins by treatment with papain has been suggested (8), but the experimental evidence presented in support of this was quite meager.

The effect of papain was examined on two types of amylolytic activity. An experiment was made to determine which of the two enzymes, alpha- or beta-amylase, might be involved in the effect observed in this study. The amylolytic activities examined were saccharifying and dextrinizing power (see Table IV). Saccharifying activity in flour slurries is almost completely due to beta-amylase. Dextrinizing activity reflects mainly alpha-amylase activity, but is affected by beta-amylase to some extent.

TABLE IV

COMPARISON OF ACTIVITIES OF AMYLOLYTIC ENZYMES AFTER VARIOUS TREATMENTS

	Saccharifying Power[a]		Dextrinizing Power	
	Water Extract	0.5% NaCl Extract	Soluble Starch[b]	Beta-Limit Dextrin
Flour	45	75	0.59	0.37
Flour treated with: 120 μeq. cysteine	75	115	0.75	.37
0.03% papain + 60 μeq. cysteine	190	....	0.95	.35
Gluten	61	95	0.77	.62
Gluten treated with: 120 μeq. cysteine	105	190	1.10	.56
0.03% papain + 60 μeq. cysteine	185	....	1.35	0.58

[a]Schwimmer units (1).

[b]The dextrinizing power on soluble starch is arbitrarily expressed by ICC units (2), and calculations are made with two extinction readings at 5- and 60-min. reaction times.

Extracts from samples treated with cysteine and with cysteine and papain were much higher in the two types of amylolytic activity than the controls, when the determinations were made with soluble starch as substrate and without adding beta-amylase. When the effect of beta-amylase was eliminated by using beta-limit dextrin as substrate for dextrinizing power (column 4), the effect of the papain diminished to a negligible level. This could occur only if the increase in saccharifying and dextrinizing activities produced by papain and cysteine treatments was due to liberation of beta-amylase and not alpha-amylase.

A similar conclusion can also be made on the basis of amylograph results. Figure 6 shows amylograms for starch slurries in the presence of various flour extracts. The curves on the left show that addition of water extract lowers the peak viscosity quite markedly. A further decrease in peak viscosity results when extracts of papain-treated flour slurry are added. Both extracts produced marked decreases in peak viscosity, but had essentially no effect on gelatinization temperature. On the other hand, when a small amount of malt extract was added, both peak viscosity and gelatinization temperature decreased. This suggests that the enzymes involved in producing the effects shown by the two lower curves are different. It is well known that the major starch-liquefying factor in malt is alpha-amylase. Furthermore, when the water and papain extracts of the flour were treated with silver nitrate to inactivate the extracted enzymes, their effect on the starch amylogram was eliminated (see curves on right of Fig. 6).

Further evidence supporting the view that the lowering of the peak viscosity by papain is due to liberation of beta-amylase is shown in Fig. 7. Here the effects of the papain extract of flour and commercial beta-amylase on starch and flour amylograms are compared. The amylograms for flour also show the effect of added malt for comparison. The amount of commercial beta-amylase used was equivalent in activity to the activity of the extract from 50 g. of flour treated with papain.

The effect of commercial beta-amylase on both the starch and flour amylograms was similar to that of the papain-treated flour extract. On the other hand, the effect of malt (on the flour only) was quite different, although its peak-lowering activity was adjusted to approximate that produced by the flour extract and the commercial beta-amylase. Differential inactiva-

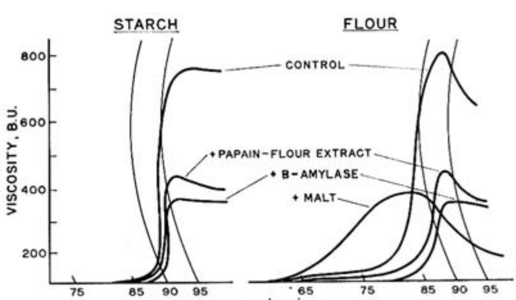


Fig. 7. Effects of beta-amylase and malt on the amylograms of starch and flour.

tion of the beta-amylase in the papain-treated flour extract by the procedure of Kneen *et al.* (9) eliminated most of its peak-lowering activity.

Results presented so far suggest that for some flours, beta-amylase could play an important role in amylograph viscosity. Flours from sound grain which generally have a low alpha-amylase activity would be of this type. In such flours, the decrease in viscosity produced by beta-amylase in the amylograph would be strongly dependent on the gelatinization process, especially since beta-amylase is rapidly inactivated by temperatures in the region where starch gelatinization occurs. Accordingly, it would be expected that the contribution of beta-amylase would be extremely sensitive to agents that affect the gelatinization temperature. In this regard it was found that the presence of 0.6% sodium chloride in the starch slurry eliminates the effect of papain almost completely without affecting the beta-amylase activity. With a modified amylograph technique developed by Crossland and Favor (10), it was shown that such a concentration of salt raises the gelatinization temperature of flour-water slurries 3° to 4°C., and this apparently eliminates the papain effect by inactivating the liberated beta-amylase before sufficient substrate is available for its action. Although there have been a number of studies on the relative stability of alpha- and beta-amylase at various temperatures under different conditions (11,12,13), additional studies are necessary to relate this information to the combined action of these enzymes in the amylograph.

Effect of Papain Treatment of Different Flours

In the final part of this study, the effect of papain treatment on peak viscosity of five straight-grade flours from Canadian hard red spring wheat was examined. The results are summarized in Fig. 8 and Table V.

Figure 8 shows the effects of adding inhibitors to control slurries and to slurries treated with papain. Although the flours differed in their peak viscosities, the effects observed were analogous for all flours. Addition of *p*-chloromercuribenzoate to the controls raised the peak viscosity. Silver nitrate (used with three of the flours) produced a further increase in peak viscosity. Additions of these inhibitors to the papain-treated slurries restored the peak viscosity to the levels obtained for the controls. Silver nitrate seems to be a more efficient inhibitor than *p*-chloromercuribenzoate.

Table V shows the saccharifying activities and peak viscosities of the five flours with normal flour extracts and with those obtained by papain treatment. With all the flours, papain treatment essentially doubled the saccharifying

TABLE V

CHANGE IN SACCHARIFYING POWER AND AMYLOGRAM BY PAPAIN TREATMENT

Flour	Saccharifying Power[a]		Peak Viscosity	
	Normal[b]	Papain-Treated	Normal	Papain-Treated
			B.U.	*B.U.*
A	75	180	645	380
B	72	179	620	350
C	85	223	560	250
D	98	195	345	245
E	129	205	200	120

[a]Expressed in Schwimmer units (1).

[b]Determined on 0.5% NaCl extract.

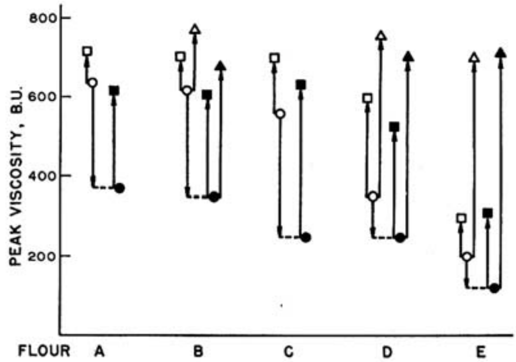


Fig. 8. Effect of papain treatment on peak amylogram viscosity in the absence and presence of *p*-chloromercuribenozate (PCMB) and silver nitrate of various flours. Symbols: open (outlined) and solid (black) indicate, respectively, control and papain-treated. ○, ●, control; □, ■, 150 μM PCMB; △, ▲, 200 μM $AgNO_3$.

activity. The correlation between saccharifying activity and peak viscosity change was low, and there were definite differences in the response of various flours to papain treatment as measured in the amylograph. Accordingly, it appears that different flours could contain different amounts of beta-amylase that is inactive in the absence of papain.

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