CHARACTERISTICS OF THE TYROSINASE SYSTEM IN POTATOES WHICH BLACKEN AFTER BOILING

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(WITH THREE FIGURES)

The discoloration of white or Irish potatoes after cooking appears to be associated with both instability of the protein and unusual activity of the tyrosinase system (11, 13). Merkenschlager (7) attributed the abnormality jointly to accumulation of tyrosine and unusual activity of tyrosinase. This difference from normal tubers is correlated with more rapid reddening and subsequent greater darkening of frozen, ground tissue that is exposed to the air while thawing. The present paper covers an investigation of some of the enzymic factors that may contribute to this abnormality.

Experimentation

Tuber groups were selected that were uniform in appearance but different in respect to discoloration after cooking, as shown by testing longitudinal sections. These were washed without soaking, rinsed with distilled water, and dried. They were then frozen with solid carbon dioxide, ground in a meat cutter, and allowed to thaw under nitrogen. The latter precaution prevented melanin formation and was taken because of Raper and Wormall's (10) finding that formation of melanin at pH 6 sometimes results in the precipitation of a melanin-enzyme complex. When completely thawed, the tissue was pressed and the sap was stored under nitrogen at 0° until used. During this period starch settled out. Unless otherwise stated, borate, phosphate, and phthalate buffers were prepared as directed by Clark (2), with the exception that sodium salts were substituted for those of potassium in all cases.

Determination of Tyrosinase Activity

A modification of the method of Raper and Wormall (10) was used. Ten-ml. portions of press sap were placed in 500-ml. round bottom flasks containing 100 ml. of a buffered 0.05 per cent. tyrosine solution, 10 ml. of toluene and a few drops of capryl alcohol. The flasks were placed in series for aeration in a water bath maintained at 20° C. A strong current of air, saturated with water, toluene, and capryl alcohol at 20° C. was drawn through the system. At the beginning and at intervals, 5-ml. portions of the test fluid were removed, acidified with 1 ml. of 5 per cent. acetic acid, placed in a boiling water bath for 15 minutes, plugged with cotton, and

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allowed to stand twenty-four hours. They were then filtered, and the precipitate of melanin and protein washed with hot, dilute acetic acid. Thirteen ml. of a saturated sodium carbonate solution were added and the samples allowed to stand one hour. Five ml. of phenol reagent were then added, the sample was diluted to 50 ml., and tyrosine determined as directed by Folin and Marenzi (3) for tryptophane. The rate of loss of tyrosine was taken as a measure of tyrosinase activity.

In determining the influence of boiled juice a portion of the press sap was placed on a boiling water bath, stirred until flocculation of protein was complete, filtered, and cooled. When testing the ash, a portion of the sap was evaporated to dryness on a steam bath under a current of air, then ashed carefully. The ash was dissolved in dilute hydrochloric acid, adjusted to the desired pH with sodium hydroxide, and made up to the original volume. An amount of either boiled juice or dissolved ash equivalent to the amount of crude enzyme preparation (10 ml. of press sap) was then added to the reaction flask and an equivalent amount of distilled water to the controls.

![Graph](image)

**Fig. 1.** Comparison of tyrosinase activity in different buffers and at different pH values.

A—unbuffered.
B—0.05 M phosphate at 6.0 pH.
C—0.05 M borate at 6.0 pH.
D—0.05 M phosphate at 8.0 pH.
E—0.05 M borate at 8.0 pH.
Effect of different buffers

Early in this investigation it was found that the buffer used had a marked influence on the activity of the enzyme. Figure 1 illustrates the relative activity of tyrosinase preparation in the presence of 0.05 M phosphate and 0.05 M borate at pH values of 6.0 and 8.0. The pH of the unbuffered control (5.97) did not change during the course of the reaction. At pH 6.0 phosphate accelerated the reaction continuously while borate apparently produced an initial accelerating action, which declined sharply after a lapse of several hours. In view of the results at pH 6.0 it seems reasonable to suppose that the difference in activity at pH 8.0 may be attributed both to acceleration by phosphate and to retardation by borate.

An inhibiting action was exerted by phthalate at pH 6.0 throughout the course of the reaction. Acetate and citrate buffers (0.05 M) at pH 6.0 had no effect during the first several hours, but in later stages the reaction ceased. It is believed that this was caused by removal of the enzyme by combination with the melanin, which precipitates at that pH. A mannitol-boric acid buffer prepared according to Britton (1) had no effect at pH 7.0.

The concentration of the buffer influenced the rate of enzyme action. Tyrosinase preparations were slightly less active in 0.1 M than in 0.05 M phosphate but a similar increase in borate concentration had a marked inhibitory effect. In some cases enzymatic action was arrested completely by 0.5 M borate. The color changes accompanying the oxidation of tyrosine are much more rapid in phosphate than in borate buffers. In the latter the red stage is prolonged. Unbuffered solutions adjusted to the same pH exhibit intermediate changes.

Tyrosinase activity in normal and discoloring tubers

Some doubt was cast upon the validity of our earlier determinations of tyrosinase activity (13) by the discovery that the borate buffers used were ineffective in preventing changes in pH. Addition of potato sap to borate buffer of pH 8.0, in the proportions used earlier, resulted in a final pH of 7.0 or lower. The addition of sap of abnormal tubers caused a smaller change than did that of normal tubers, but this difference was seldom over 0.2 pH.

The relative rates of oxidation of tyrosine in phosphate solutions of pH 6.95 and 7.8 were determined. This increase in pH of almost one unit produced an activity in sap from normal tubers equivalent to that in juice of potatoes which discolor. The effect of pH in borate solutions was not tested but comparison of the rates obtained in different experiments gave no indication of greater sensitivity to pH changes.

For more conclusive evidence on the relative tyrosinase activity in nor-

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mal and discoloring tubers, determinations in which the pH was carefully controlled were made on several lots of potatoes. The pH was adjusted by addition of sodium hydroxide and was determined in each case at the beginning and at the end of an experiment by means of a glass electrode. The results of experiments using both phosphate and borate solutions at pH 7 showed markedly greater oxidation of tyrosine by the sap of abnormal tubers. Similar results were obtained with phthalate, acetate (pH 6.0), mannitol-boric acid (pH 7.0), phosphate (pH 8.0), and unbuffered solutions. In every case the tyrosinase in press sap of discoloring tubers was more active than that of normal ones, regardless of the buffer used or of the pH at which the experiment was conducted.

These results show rather conclusively that potatoes which discolor when cooked possess an unusually high tyrosinase activity. The fact that a difference in rate of oxidation of tyrosine is always obtained, regardless of the buffer or of the pH used in testing, is further evidence that we are measuring an actual inequality either of the states of activation or of the quantities of enzyme in the two kinds of tubers. It is possible that any one buffer might react with some component of one type of potato sap, causing the formation of a complex that affects the enzyme but is non-existent in the tuber itself. If such were the case, it seems unlikely that the same effect would be produced by all the buffers used at the various pH values.

**Comparative effect of buffers on the tyrosinase of normal and discoloring tubers**

It was observed that the greatest differences in tyrosinase activity between normal and discoloring tubers were evident in the unbuffered solutions near pH 6.0 (the pH of the sap) and in certain concentrated buffers. The addition of phosphates, borates, or phthalates of 0.05 M concentration tended to decrease the divergence. When the phosphate concentration was increased to 0.1 M, the difference was further lessened. A similar increase in borate concentration gave the opposite effect. The borate exerted a greater inhibiting action on the tyrosinase in the sap of normal tubers than on that in discoloring ones, resulting in an accentuation of the difference between the two. A still further increase in the concentration of the borate buffer resulted in a complete inactivation of the enzyme in the juice of normal tubers, while that in the sap from discoloring tubers remained moderately active. In one experiment the latter oxidized 18.4 mg. of tyrosine in 24 hours in 0.5 M borate as compared to 33.4 mg. when in 0.1 M borate. This effect may be either partly or entirely due to a difference in enzyme quantity or state of activation, to factors in the sap that modify the action of the buffer on the enzyme, or to other factors whose effect on the enzyme is modified by the buffer. Thus experiments with purified enzyme preparations might give entirely different results.
Effect of Tuber Factors on Tyrosinase Activity

Haehn (5) noticed that boiled potato juice had an activating effect on tyrosinase. He attributed this response to the presence of certain salts and seems to have neglected the effect of additions of the ash upon pH. Raper and Wormall (10) also presented evidence for the presence of an activator in boiled sap, but found that it was not present in the ash. In view of these results, we believed it possible that the presence of some such activator in the one, or perhaps of an inhibitor in the other, might account for the difference between discoloring and normal potatoes.

An activator of tyrosinase was found to be present in boiled juice of abnormal tubers. The addition of such juice to a tyrosine-tyrosinase system caused a marked acceleration in the rate of enzyme action, while the boiled juice of normal potatoes had little or no activating effect. This effect was greatest in unbuffered solutions, slightly less marked in borate, less apparent in phthalate and mannitol-boric acid buffers, and non-existent in phosphate.

The activating effect of boiled juice is illustrated in figures 2 and 3 which summarize the results of several experiments. Maximum acceleration due

![Graph showing effects of boiled sap on tyrosinase activity in 0.05 M borate buffer at pH 8.0.](Figure 2)

**FIG. 2.** Effects of boiled sap on tyrosinase activity in 0.05 M borate buffer at pH 8.0.
A—Normal sap.
B—Normal sap + boiled normal sap.
C—Normal sap + boiled discoloring sap.
D—Discoloring sap.
E—Discoloring sap + boiled normal sap.
F—Discoloring sap + boiled discoloring sap.
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Fig. 3. Effect of boiled sap on tyrosinase in unbuffered solutions at pH 6.0.

A—Normal sap.
B—Discoloring sap.
C—Normal sap + boiled discoloring sap.
D—Discoloring sap + boiled discoloring sap.

To boiled juice was obtained when the press sap of normal potatoes was used as the source of the enzyme. It was noted that no activator could be detected in potatoes that cooked white, while those that cooked slightly grey often contained the activator in limited amounts. Some of these samples exerted a slight inhibitory action, but the effect was never of such magnitude as to be of significance.

In considering only the results of figure 2, one might conclude that this activator is the only factor responsible for the difference in the tyrosinase activity of the two types of tubers. That this probably is not the case is indicated by the fact that the activator has no effect in phosphate-buffered systems, yet the sap of abnormal tubers is always higher in tyrosinase activity than that of normal ones when tested in such buffers. There was evidence that borates also affect the activator. A greater acceleration due to the addition of boiled juice was apparent in 0.1 M than in 0.05 M borate. In 0.5 M borate the activating effect is yet more pronounced. An enzyme preparation from normal tubers that showed no tyrosinase activity in 0.5 M borate was moderately active if boiled juice from discoloring tubers was added.
The effect of the activator is less apparent at higher pH values than at lower ones. Its nature is as yet unknown. It is rather unstable, as its ability to affect the enzyme lessens upon standing at temperatures as low as 5°. It is partially destroyed by prolonged boiling. When boiled juice containing the activator was dialyzed against distilled water at 5° C. or lower, its ability to activate was lost more rapidly than when stored at the same temperature. The activator is seemingly organic in nature, for it could not be detected in the ash. It is possible, however, that an active inorganic constituent would be altered during the ashing process and thus become inactive. The fact that the compound is unstable also is an indication that it is not an inorganic salt.

The possibility that the accelerating effect of boiled juice might be caused by the slightly increased substrate concentration was considered, for the boiled juice added contained some tyrosine. In some experiments, tyrosine was added to the controls so that their final substrate concentration was equal to, or in excess of, that in the test solution. The results were essentially the same as those previously obtained. It could also be possible that the apparent acceleration was due to oxidation of compounds in the juice that are more rapidly oxidized than tyrosine itself. In practically all cases, however, the flasks to which boiled juice was added contained less tyrosine at the end of the run than did the controls. This was true even though the controls contained less tyrosine at the beginning than did the test solutions. The effect noticed must then be a true acceleration of enzyme action.

**Effect of specific substances on tyrosinase activity**

Our preliminary evidence (13) that potassium may act as an inhibitor of tyrosinase has not been supported by further tests. Addition of amounts of potassium chloride sufficient to equalize the potassium content of the added saps had no effect on the tyrosinase activity.

We have included tests of iron because of the results of TINCKLER (12) and of MADER and MADER (6) indicating that this metal plays a rôle in discoloration after cooking. The addition to digests of either ferrous or ferric chloride in 0.01 per cent. concentration resulted in slight acceleration of tyrosinase activity in 0.1 M phosphate buffer at pH 7.0. As the activator in boiled juice has no effect in phosphate solutions, and since both forms of iron are active but the activator does not appear in the ash, iron is probably not the activator described above.

The addition of a small amount of catechol to a digest in borate buffer resulted in initial rapid melanin formation, as indicated by darkening, but the ultimate disappearance of tyrosine was not altered. It thus appears probable that the acceleration reported by PUGH (9) for both catechol and dihydroxyphenylalanine was temporary and followed by the action of an
inhibitor, as found by GRAUBARD and NELSON (4). As abnormal tubers contain high proportions of free amino acids it seemed possible that the observation of NOBUTANI (8) that these accelerate the oxidation of tyrosine might be significant in blackening after cooking. The addition of glycine in the proportion of one part to five of tyrosine in a borate-buffered digest practically inhibited the oxidation during the first several hours. At the end of 24 hours however the loss of tyrosine was the same as in the controls.

Summary

1. The tyrosinase activity of potato sap is affected by the particular buffer used in its determination. Phosphate accelerates the oxidation of tyrosine, while borates and phthalates inhibit the reaction. The inhibition is proportional to the concentration of the buffer.

2. The fact that the tyrosinase activity of tubers which discolor is higher than that of normal tubers was further substantiated. The difference in activity was apparent in several different buffers, in unbuffered solutions, and also at different pH values. Greater differentiation was apparent in either borate or unbuffered solutions than in phosphate buffers.

3. An activator of tyrosinase was found in the boiled sap of abnormal potatoes. It is not present in the ash and is lost in prolonged boiling or upon standing, and in dialysis. The activating effect was apparent in unbuffered solutions and in those buffered with borate, but non-existent in phosphate buffers. Since differentiation of the two types of potatoes is apparent even in phosphate buffers, the activator in boiled juice is not the only factor contributing to high tyrosinase activity in abnormal potatoes.

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