STUDIES ON OXIDASE ACTIVITY IN POTATO TUBERS

III. ON THE OXIDATION OF p-CRESOL AND CATECHOL BY MACERATED TISSUE *

by

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The primary aim of this work was to develop a quantitative method for the determination of the oxidase activity of white potatoes. The discoloration of potato dice is presumably the result of the oxidation of free tyrosine by the enzyme tyrosinase, forming first a pink and then a grayish-black pigmentation attributed to melanin formation.

The first procedure suggested by our laboratory was the „Direct Colorimetric Method" [1] in which potato dice were macerated in a Waring Blendor, agitated for a given period, suspension filtered to complete clarity, and the resulting color determined photometrically. The depth of the color formed under standard conditions was employed as a measure of the enzymatic discoloration tendency, being a function of the substrate as well as of the enzyme available for the reaction.

In a second study [2], standard potato dice were macerated in the presence of o-phenylenediamine. An orange pigment was produced which was presumed to be of a phenazine nature and formed by the condensation of the o-phenylenediamine with one of the products of tyrosine oxidation. This compound was provisionally termed „*Tyrophenazine*". The orange color could be stabilized in 80% acetone, or by extraction with butyl alcohol, and the resulting depth of color, as measured photometrically, was employed as an index of the enzymatic coloration tendency.

In a third procedure [3], the „tyrophenazine" formed by the interaction of o-phenylenediamine and potato macerated juice was determined fluorometrically in a Pfaltz & Bauer Fluorophotometer. This method yielded results in all respects parallel to the colorimetric o-phenylenediamine method.

In the present paper we describe two further procedures for the assay of oxidase activity in white potatoes, based on macerating the potato dice in the presence of p-cresol and catechol, and determining the resulting depth of color formation photometrically after stabilization in 80% acetone.

Further we have studied the correlation of the respective methods of assay.

I. EXPERIMENTAL

In the cresol experiments, 50 grams of representative potato dice are blended in a Waring Blendor in the presence of 80 ml of water and 20 ml of 1% p-cresol solution. After 3 minutes blending, the mixture is filtered through cotton or glass wool into a 250 ml

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Erlenmeyer flask which is maintained at 25—30° C. The color becomes first orange-red, then bright red. After 15 minutes from the beginning of blending, 10 ml of the maceration juice are pipetted into 40 ml of acetone which stops the reaction. By this procedure, various colloidal materials are precipitated, and a readily filterable solution is obtained.

The acetone solution is filtered through Whatman # 5 filter paper and the depth of color in the clear filtrate is determined in a Klett-Summerson photoelectric colorimeter, using a blue # 42 filter. The same procedure was employed when catechol served as the substrate, except that 5 ml of the maceration extract were added to 45 ml acetone.

The Direct Colorimetric Method was carried out as previously described [1]. The colorimetric and the fluorometric o-phenylenediamine methods were carried out in accordance with the procedures published earlier in this series [2, 3].

Blanching was carried out in a colander which was inserted into a boiling water bath and withdrawn after a certain number of seconds, after which the dice were cooled in an ice bath.

The dihydroxyphenylalanine and the tyrosine determinations were carried out according to ARNOW [4]. Green Mountain potatoes were the variety employed in all determinations.

2. OBSERVATIONS AND RESULTS

a. *Assays with p-Cresol*

To determine the reproducibility of the method, ten different samples from a given batch of dice were tested by the above method. The results are shown in Table I.

TABLE I

REPRODUCIBILITY OF P-CRESOL EXPERIMENTS

Test No.	Color Reading	Deviation	% Deviation
1	335	—1	0.3
2	325	—11	3.3
3	345	9	2.7
4	350	14	4.2
5	350	14	4.2
6	310	—26	7.6
7	350	14	4.2
8	315	—21	6.2
9	335	—1	0.3
10	345	9	2.7
Average	336	12	3.6%

It will be seen that the maximum deviation from the mean is 7.6% and the average deviation less than half this figure.

The influence of time on the degree of coloration of two batches of dice under the previously described conditions was next studied. The results are shown in Fig. 1.

It will be seen that most of the color formation occurs in the first five minutes, and that the rate of increase after 15 minutes is slow.

The influence of blanching on the degree of coloration was studied in four samples of Green Mountain potatoes. Fig. 2 shows the progressive decline of color formation in proportion to the blanching time and its complete inhibition.

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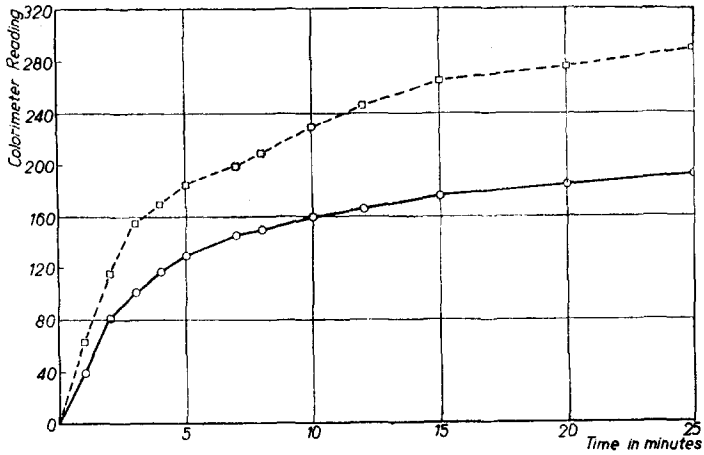

Fig. 1. Effect of Time on p-Cresol Coloration in Potato Maceration Juice.

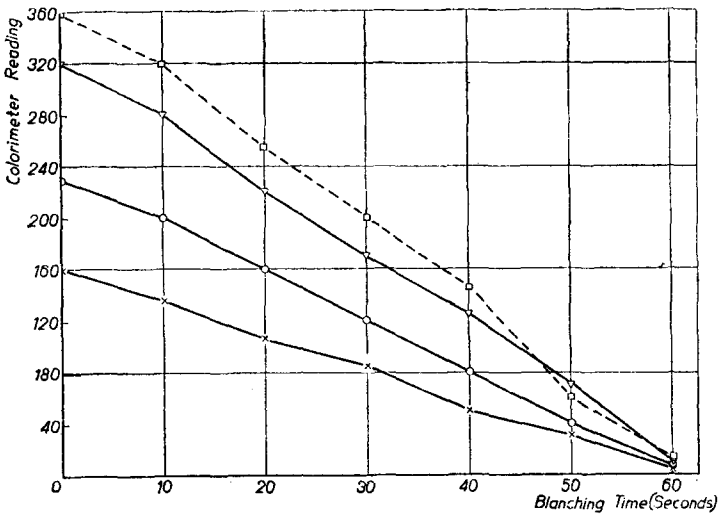

Fig. 2. Influence of Blanching on p-Cresol Coloration in Potato Maceration Juice.

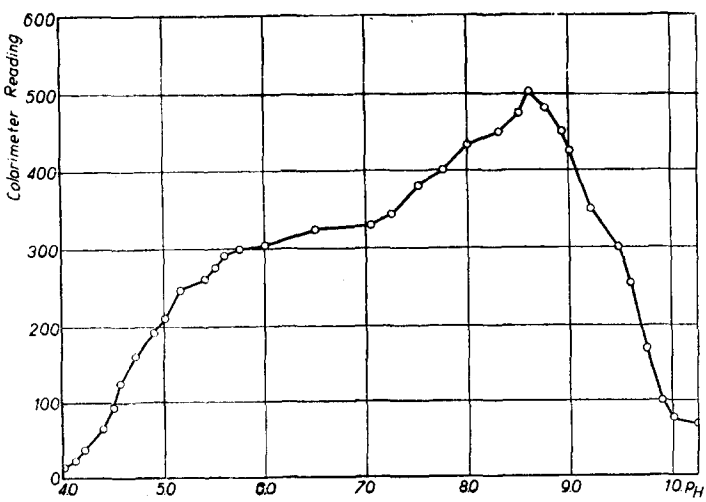

Fig. 3. Effect of pH on p-Cresol Coloration of Potato Maceration Juice.

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The influence of p_H on the cresol coloration was studied in the range of p_H 3.9 to p_H 10.5 as shown in Fig. 3. It will be seen that the maximum occurs under these conditions at p_H 8.6.

To determine the influence of temperature on the reaction, a series of determinations were carried out at temperatures ranging from 5° to 65° C. The results are shown in Fig. 4. It will be seen that the maximum coloration occurs in the temperature range of 35° to 40° C. and is progressively diminished as the temperature is raised or lowered beyond this range.

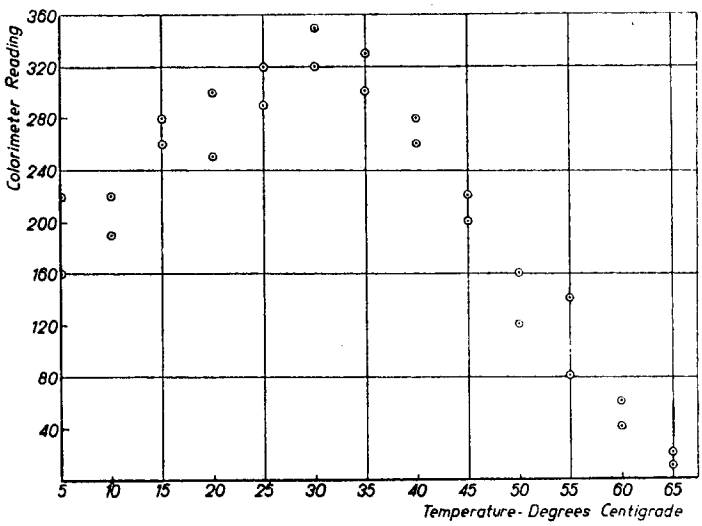

Fig. 4. Effect of Temperature on p-Cresol Coloration.

b. *Experiments with Catechol*

The reproducibility of the catechol method was studied in 12 samples. It will be seen from Table II that the reproducibility is somewhat less than in the case of p-cresol.

TABLE II

REPRODUCIBILITY OF CATECHOL METHOD

Catechol	Color Reading	Deviation	% Deviation
Trial # 1	280	—13	4.5
2	260	—33	11.2
3	270	—23	7.8
4	270	—23	7.8
5	300	7	2.4
6	300	7	2.4
7	315	22	7.5
8	311	16	5.5
9	300	7	2.4
10	305	12	4.1
11	275	—18	6.1
12	311	16	5.5
Average	293	16.4	5.1

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The rate of color formation in the reaction of catechol and potato maceration juice was studied with two samples of Green Mountain potatoes.

It will be seen from Fig. 5 that in contrast to the other substrates previously employed, maximum color formation is attained within the first 2 minutes.

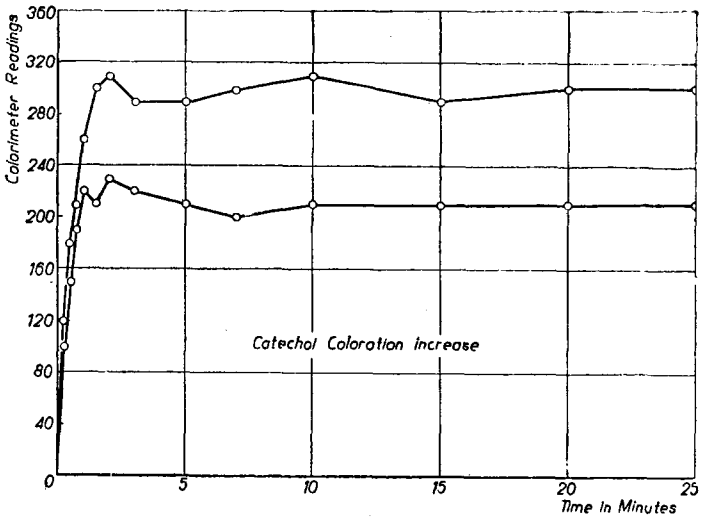

Fig. 5. Effect of Time on Catechol Coloration in Potato Maceration Juice.

The influence of blanching on the catechol oxidation is shown in Fig. 6. It will be noted that this curve is essentially similar to the curve obtained with p-cresol, and to the corresponding blanching curves previously reported for the „Direct Colorimetric Method" and the o-phenylenediamine method [1, 2, 3].

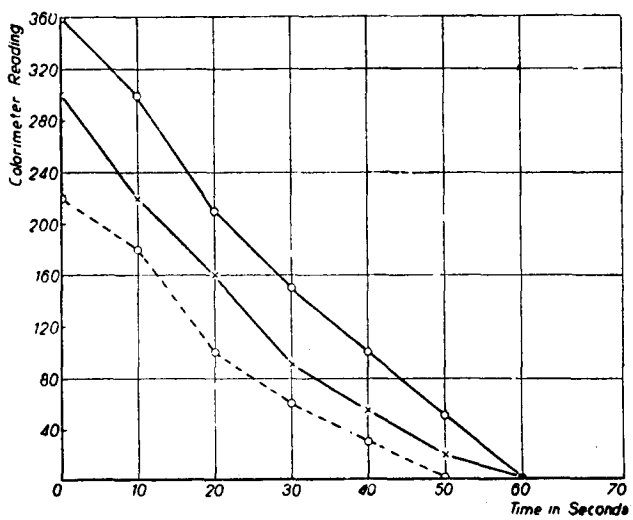

Fig. 6. Influence of Blanching on Catechol Coloration in Potato Maceration Juice.

The catechol p_H activity curve (shown in Fig. 7) differs from the corresponding p-cresol curve principally in the location of the maximum at p_H 6.0 (close to the hydrogen

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ion concentration of the maceration juice) rather than on the alkaline side as with p-cresol.

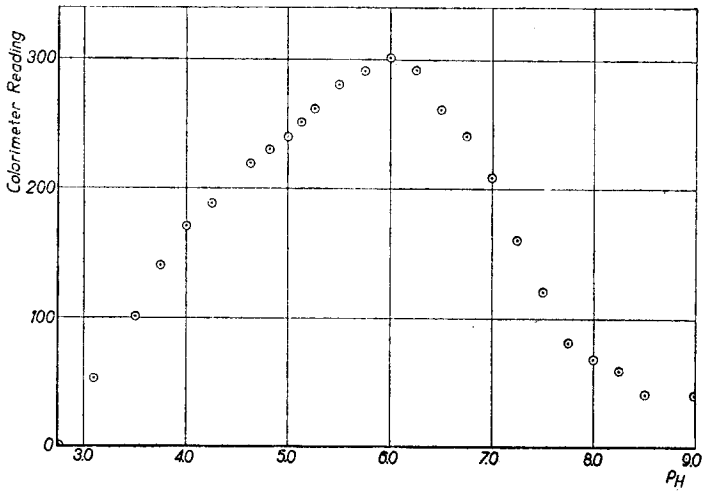

Fig. 7. Influence of pH on Catechol Coloration.

Fig. 8 shows the influence of temperature on the oxidation of catechol by potato maceration extract. This curve differs from the corresponding p-cresol curve chiefly in the lower temperature range. Here, although the lower temperature retards the rate of color formation, the inhibiting effect of the catechol on the enzyme is also retarded so that the reaction proceeds for a longer period, although at a slower rate.

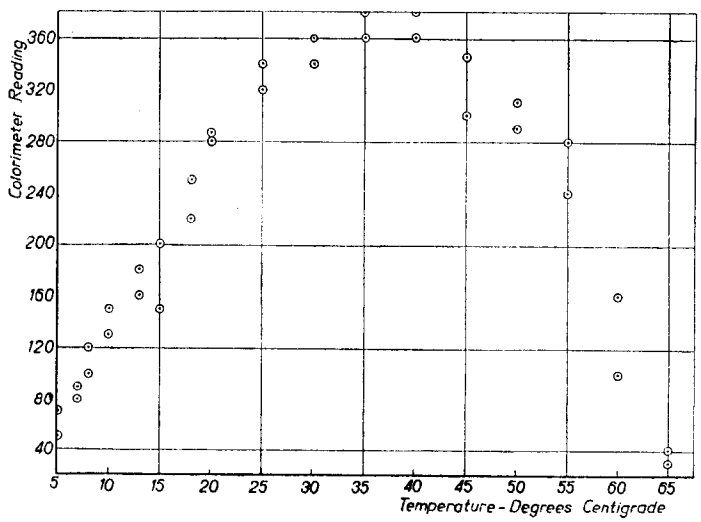

Fig. 8. Effect of Temperature on Catechol Coloration.

c. *Correlation of the Catechol and Cresol Assays with the o-Phenylenediamine and the Direct Colorimetric Method*:

Representative samples of fifty batches of potatoes were assayed by each of the following methods: α The direct colorimetric method [1]; β the o-phenylenediamine colori-

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metric method [2]; γ the o-phenylenediamine fluorometric method [3]; δ the catechol method, and ε the cresol method described above.

Duplicate determinations were carried out in each instance.

The coefficients of correlation were then calculated according to MILLS [5] with the results shown.

TABLE III

CORRELATION OF THE VARIOUS METHODS

	Direct Colorimetric	o-Phenylene-diamine Colorimetric	o-Phenylene-diamine Fluorometric	Catechol
Cresol	+ .54	+ .65	+ .63	+ .98
Catechol	+ .48	+ .58	+ .59	—
o-Phenylenediamine Colorimetric . . .	+ .84	—	+ .99	—
o-Phenylenediamine Fluorometric . . .	+ .82	—	—	—

It will be seen that a substantial positive correlation exists between all the methods. It is higher between the o-phenylenediamine and the direct colorimetric method, shown graphically in Fig. 9, than between either of these methods and the cresol or catechol assays (see Fig. 10 and 11). Very high correlations were found between the cresol and the catechol methods as shown in Fig. 12. Virtually complete correspondence was found between the o-phenylenediamine colorimetric and fluorometric methods (see Fig. 13), as previously reported [3].

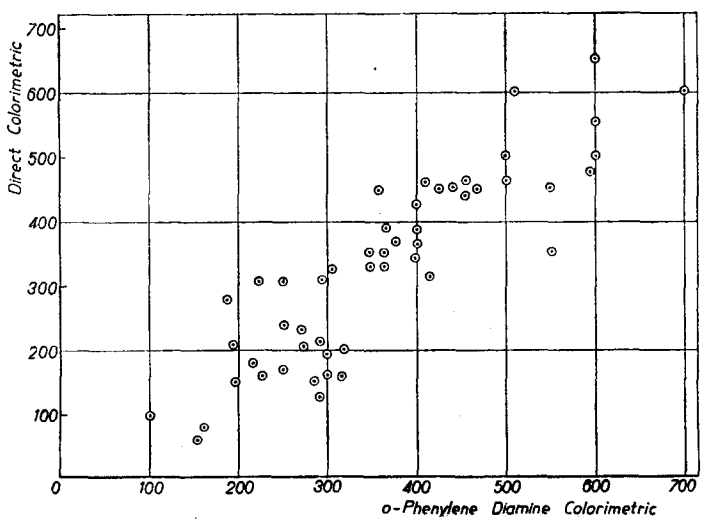

Fig. 9. Relation of o-Phenylenediamine and Direct Colorimetric Methods.

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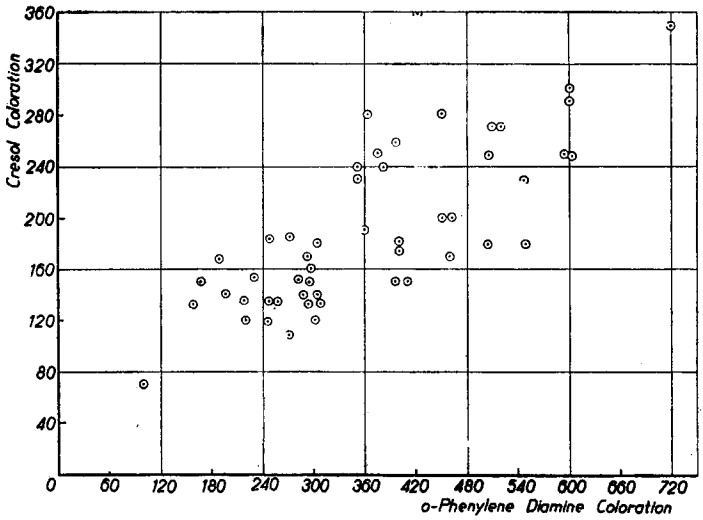

Fig. 10. Relation of o-Phenylenediamine and Cresol Coloration in Potato Maceration Juice.

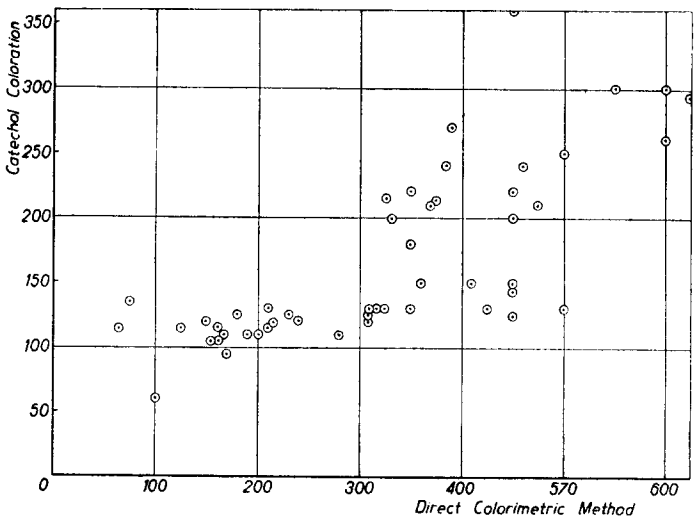

Fig. 11. Relation of Direct Colorimetric Method and Catechol Coloration in Potato Maceration Juice.

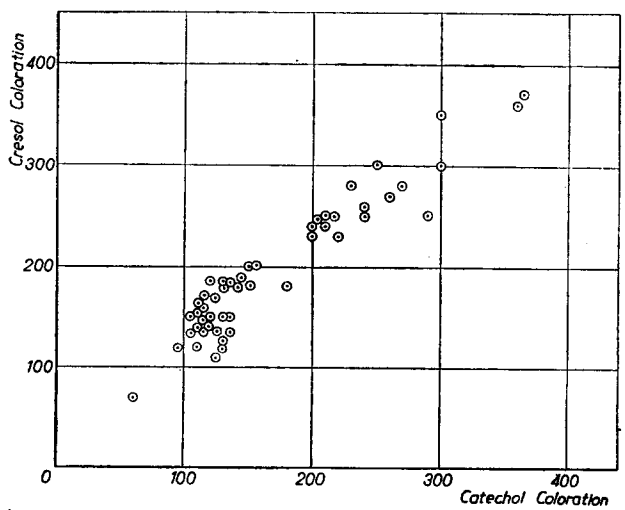

Fig. 12. Relation of Cresol and Catechol Coloration in Potato Maceration Juice.

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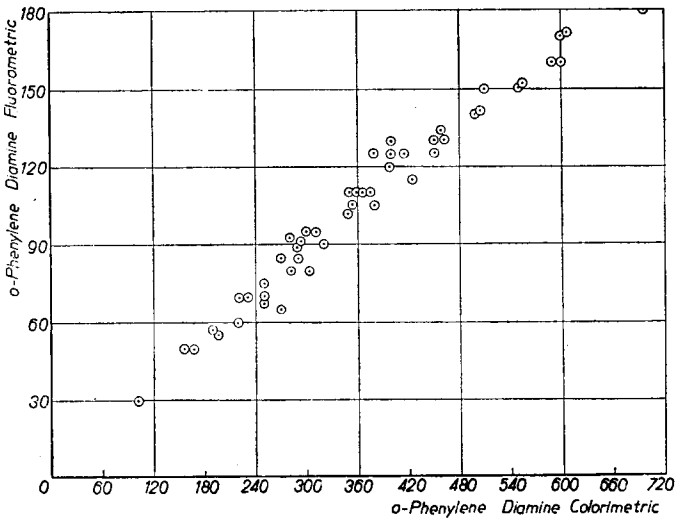

Fig. 13. Relation of o-Phenylenediamine Colorimetric and Fluorometric Method.

3. DISCUSSION

The direct colorimetric method represents perhaps the most reliable means of determining the enzymatic discoloration tendency of white potatoes since it duplicates the phenomena which actually occur in discoloration without the addition of any external indicator or substrate. The method is a measure of both enzyme and substrate taking part in the reaction.

In the direct colorimetric method, however, especially with blanched materials, it is somewhat difficult to secure a completely clear filtrate for photometric assay.

The o-phenylenediamine methods are believed to depend upon the formation of a condensation product („Tyrophenazine") between the o-phenylenediamine and an oxidation product formed during tyrosine oxidation. It is thus also dependent on potato substrate as well as on enzyme concentration. The degree of fluorescence produced by the „Tyrophenazine" reaction was found to have a positive correlation of .80 with the dihydroxyphenylalanine content and of .19 with the tyrosine content of the fresh potato dice.

Since the direct colorimetric and o-phenylenediamine methods measure both enzyme and potato substrate concentrations involved in the discoloration, and the catechol and cresol methods merely the extent of enzyme action on an added substrate, it might be supposed that the two former methods would show a higher correlation with each other than with the latter methods. This was indeed found to be the case (see Table III).

The very high correlation between the cresol and catechol techniques is in agreement with the views of NELSON AND DAWSON [6] and others that the cresolase and catecholase activity of potato tubers are due to a single enzyme.

SUMMARY

1. Studies were made of potato oxidase action by determining the depth of color formed by the reaction of catechol and p-cresol with potato maceration juice under standard conditions. Reproducibility was somewhat greater with p-cresol than with catechol.

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2. The p-cresol oxidation proceeds steadily at a diminishing rate. Catechol color formation reaches its maximum at about 2 minutes, presumably due to enzyme inhibition by the catechol and thereafter shows little variation.

3. Maximum color formation with catechol occurred at the natural pH of the juice. Maximum color formation with p-cresol was achieved in slightly alkaline solution which probably represents not the optimum pH of the reaction but the point at which enzymatic inactivation overcomes a greater chemical color formation in alkaline solution.

4. With both substrates, the reaction is diminished by blanching in boiling water and completely extinguished in a sixty-second blanching period.

5. The temperature curves of the catechol and cresol reactions are essentially similar except in the low temperature range.

6. The coefficients of correlation of the catechol and cresol assays with the previously described direct colorimetric method and the o-phenylenediamine colorimetric and fluorometric methods were determined in 50 separate batches of potato dice. Almost complete correlation was found between cresol and catechol assays and between the o-phenylenediamine colorimetric and fluorometric methods. High positive correlations were found between the direct colorimetric method and the two o-phenylenediamine methods. Substantial positive correlations were found between the cresol and catechol assays and the other three methods.

7. The o-phenylenediamine fluorescence method showed a high correlation with the dihydroxyphenylalanine content, and a slight positive correlation with the tyrosine content of the fresh potato.

RÉSUMÉ

1. L'action de l'oxydase de la pomme de terre a été étudiée en déterminant l'intensité de la coloration formée dans des conditions standard par la réaction de la catéchine et du p-crésol avec le jus de pomme de terre macéré. La reproductibilité était un peu plus grande avec le p-crésol qu'avec la catéchine.

2. L'oxydation du p-crésol diminue progressivement dans le cours du processus. La coloration de la catéchine atteint son maximum au bout de 2 minutes environ, probablement à cause de l'inhibition de l'enzyme de la catéchine; après celà elle ne subit plus que de légères modifications.

3. La coloration maximum avec la catéchine s'est produite au p_H naturel du jus. La coloration maximum avec le p-crésol a été obtenue dans une solution légèrement alcaline, laquelle représente probablement non pas le p_H optimum de la réaction, mais le point auquel l'inactivation enzymatique empêche une plus grande coloration chimique dans la solution alcaline.

4. Avec les deux substrats la réaction est diminuée en les blanchissant dans l'eau bouillante; elle est complètement éteinte par un blanchiment de 60 secondes.

5. Les courbes de température des réactions de la catéchine et du crésol sont essentiellement similaires, excepté aux basses températures.

6. Le coefficient de corrélation des essais avec la catéchine et le crésol effectués par la méthode colorimétrique directe décrite antérieurement et par les méthodes colorimétriques et fluorométriques à l'o-phénylènediamine a été déterminé dans 50 échantillons de pommes de terre en cubes. La corrélation s'est averée presque totale entre les essais du crésol et de la catéchine et entre les méthodes colorimétriques et fluorométriques à l'o-phenylènediamine. On a trouvé des corrélations positives entre la méthode colorimétrique directe et les deux méthodes basées sur l'oxydation de l'o-phénylènediamine. Des corrélations évidemment positives ont été trouvées entre les déterminations au crésol et au catéchine et les trois autres méthodes.

7. La méthode à la fluorescence, mettant en jeu l'o-phénylènediamine s'est montrée en bon accord avec la teneur en dihydroxyphénylalanine, et en accord probable avec la teneur en tyrosine de la pomme de terre fraîche.

ZUSAMMENFASSUNG

1. Die Wirkung der Kartoffeloxydase wurde untersucht durch Bestimmung der Stärke der Färbung welche durch die Reaktion von Katechin und p-Kresol mit

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Kartoffelmazerationssaft inter Standard-Bedingungen entsteht. Die Reproduzierbarkeit war etwas grösser mit p-Kresol als mit Katechin.

2. Die p-Kresoloxydation verläuft regelmässig mit abnehmender Geschwindigkeit. Die Katechinfärbung erreicht ihr Maximum nach ca. 2 Minuten; dies wird wahrscheinlich durch die Hemmung des Enzyms durch das Katechin verursacht. Danach zeigt sie wenig Veränderung.

3. Die maximale Färbungsbildung wurde mit Katechin bei dem natürlichem pH des Saftes erreicht. Die maximale Färbungsbildung mit p-Kresol wurde in einer leichtalkalischen Lösung erreicht, welche wahrscheinlich nicht den optimalen pH der Reaktion darstellt, sondern den Punkt bei dem die enzymatische Inaktivierung eine stärkere chemische Färbungsbildung in alkalischer Lösung übersteigt.

4. Mit beiden Substraten vermindert die Reaktion durch Bleichung in kochendem Wasser und sie verschwindet vollständig nach einer Bleichungsdauer von 60 Sekunden.

5. Die Temperaturkurven der Katechin- und Kresolreaktionen sind im wesentlichen gleich, ausgenommen bei niedrigen Temperaturen.

6. Der Korrelations-Koeffizient der Katechin- und Kresolversuche mit der bereits beschriebenen direkten kolorimetrischen Methode und den kolorimetrischen und fluorometrischen o-Phenylendiaminmethoden wurde in 50 verschiedenen Partien von Kartoffelwürfeln bestimmt.

Die Korrelation zwischen den Versuchen mit Kresol bezw. Katechin und o-Phenylendiamin war beinahe vollständig. Zwischen der direkten kolorimetrischen Methode und den beiden o-Phenylendiaminmethoden wurden hohe positive Korrelationen festgestellt. Bedeutende positive Korrelationen wurden zwischen den Kresol- und Katechinversuchen und den anderen drei Methoden festgestellt.

7. Die o-Phenylendiaminfluoreszenzmethode wies eine hohe Korrelation mit dem Gehalt an Dihydroxyphenylalanin, und eine schwach positive Korrelation mit dem Tyrosin gehalt der frischen Kartoffel auf.

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