Enzymatic Browning, Reflectance Measurements, and Effect of Adenosine Triphosphate on Color Changes Induced in Plant Slices by Polyphenol Oxidase

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Studies on prevention of enzymatic color formation by adenosine triphosphate were made directly on plant slices by color difference (reflectance) measurements. Adenosine triphosphate prevented or diminished the color development in potatoes, apples, avocados, mushrooms, and peaches, but had no effect on the intrinsic activity of polyphenol oxidase enzyme(s). Its effectiveness increased with concentration except when a considerable amount of substrate, such as dihydroxyphenylalanine, was added. The color-preventing action of adenosine triphosphate differed from that of ascorbic acid in that the latter reduces the quinones formed in the reaction catalyzed by polyphenol oxidase, while adenosine triphosphate, as such, is not a reducing agent. A number of organic and inorganic phosphates and other substances were ineffective in preventing enzymatic color formation in potato.

CUT OR BRUISED PLANTS often turn visibly dark. Potato as well as other vegetables and fruits darken as a consequence of polyphenol oxidase activity in the injured tissue (*7*). (Polyphenol oxidase enzymes are also known as phenolase, polyphenolase, catecholase, tyrosinase, and cresolase.) Mono-, di-, or polyphenols serve as substrates for these enzymatic oxidations, followed by nonenzymatic steps resulting in colored compounds of high molecular weight (*3, 8*). The over-all reactions for tyrosine are:

$$\text{Tyrosine} + \frac{1}{2}\,O_2 \xrightarrow[\text{I}]{E} \text{Dihydroxyphenylalanine (DOPA)} + \frac{1}{2}\,O_2 \underset{\text{II}}{\overset{E}{\rightleftharpoons}} \text{Dihydroxyphenylalanine-Quinone} \rightarrow \text{Leuco Compound} + \frac{1}{2}\,O_2 \rightarrow \text{Dopachrome} \rightarrow \text{Etc.} \rightarrow \text{Dark polymers (Melanin).}$$

Only steps I and II are mediated by the polyphenol oxidase enzymes(s) designated as *E* (*3*).

The initial quinone (product of II) is spontaneously reduced by ascorbic acid and other reducing agents, which prevent subsequent oxidation and polymerization of quinones. For this reason, enzymatic darkening of fruits and vegetables begins only after all ascorbic acid has been oxidized. Freshly browned slices may be partly decolorized by added ascorbic acid.

A preliminary communication (*5*) showed that adenosine triphosphate (ATP) interferes with enzymatic color formation in potato. This action resembles that of ascorbic acid in some respects but not in others. The data presented here and in an earlier paper (*5*) indicate that the effect of adenosine triphosphate is not a direct one either on the polyphenol oxidase nor on the initial products of the reaction catalyzed by this enzyme.

In the course of study of color prevention in potato slices by adenosine triphosphate, a quantitative method, based on reflectance, was adapted for measuring color formation. The reflectance measurements were made directly on the slices. What this method lacks in precision is compensated by speed and convenience.

The direct measurement of enzymatic darkening in vegetable and fruit slices and data on the effect of adenosine triphosphate and other compounds on the darkening of such slices are presented here.

Materials and Methods

Experiments were performed on slices of potato (Russet Burbank, White Rose, and Kennebec, stored at 4.5° C. prior to use), apples (Winesap, Newtown Pippin, and Greening, stored at 10° C. prior to use), and other vegetables and fruits. Departures from the described procedure and additional experimental details accompany the tables and graphs.

Experimental Procedure

Slices 0.25 inch in thickness were cut mechanically, when possible, scored with a stiff nylon brush, then moistened with water or solution(s) used for treatment. All readings were taken with the Hunter Color and Color Difference meter. The slices were marked to ensure the same orientation at each reading.

Materials and reagents were chilled before use. A few drops of reagent, pH 6.1 to 6.5, were added at intervals to keep slices moist. Distilled water or dilute substrate, dihydroxyphenylalanine, was added to the controls. The slices were kept in covered chambers on ice or in a refrigerator.

The treated surface of each slice was placed directly over the 1-inch instrument opening and properly oriented. An initial reading was recorded and others followed at time intervals. Three separate readings were obtained on each area:

Rd = per cent reflectance; *a* = redness *vs.* greenness; *b* = yellowness *vs.* blueness.

The freshly cut surface of a potato has a total reflectance of about 35 to 60% as compared with a pure white magnesium oxide standard. When exposed to air, the surface turns reddish brown, then gray with a decrease in per cent reflectance, *Rd*. Darkening was magnified on adding a substrate dihydroxyphenylalanine. Color development decreased, however, when ascorbic acid or adenosine triphosphate at pH 6.1 to 6.5 was added. The values were calculated as differences from initial values; that is, the first measurement was subtracted from all subsequent measurements. As the slices darkened, the *Rd* values diminished, while *a* generally increased first and later decreased. The changes in *b* were small and therefore are not reported. The results are expressed as: decrease in per cent reflectance, $-\Delta Rd$, with time, or change in redness, Δa, with time.

Variations between individual tubers (or fruits) were sizable; therefore, all color measurements on slices were replicated two to five times per experiment.

Factors Which Influenced Measurements. The degree of bruising increased the rate of darkening and pos-

sibly the degree of final darkening. This was evident from differences in reflectance on the two opposite faces of the same cut surface, when differently scored. It was found best to score slices rapidly and to treat and measure the initial values as quickly as possible. Extremely rapid darkening occurred in eggplant and in mushroom slices.

Results

Prevention of Darkening. Figure 1 shows the interference of adenosine triphosphate with darkening and with reddening on the surface of potato slices. Control slices turned dark; slices treated with 0.1*M* adenosine triphosphate or 0.1*M* ascorbic acid did not darken or redden appreciably within 160 minutes. Redness in the control slices increased rapidly to a maximum and then decreased. The curves in Figure 1 are fairly typical for potato slices treated with ascorbate or adenosine triphosphate as compared with controls.

Decolorization. Figure 2 shows the decreased reflectance and a corresponding increase in redness in untreated samples. When 0.1*M* adenosine triphosphate was added to the slices at the beginning of the experiment, almost no darkening occurred (base line, Figure 2). Where it was added after the slices became reddish brown, the reflectance increased and redness decreased, because

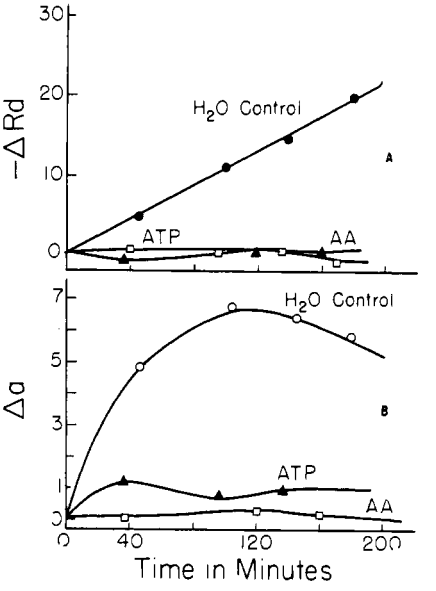

Figure 1. Effect of adenosine triphosphate (0.1M ATP, pH 6.2) and of ascorbic acid (0.1M AA, pH 6.2) on color development in slices of White Rose potatoes

A = decrease in per cent reflectance, $-\Delta Rd$, with time
B = change in redness, Δa, with time

added adenosine triphosphate decolorized much of the redness. Decolorization with adenosine triphosphate did not occur in slices kept under nitrogen. Oxygen was not required for the decolorization of red intermediates with ascorbic acid, but oxygen was required for decolorization with adenosine triphosphate. Yet prevention by adenosine triphosphate of total color, *Rd*, and of redness, *a*, with time, in most experiments, paralleled those by ascorbate (Figure 2).

Effect of Adenosine Triphosphate Concentration. In general, the higher the concentration, the more effectively was the color development prevented. The results obtained on slices of White Rose potatoes with natural substrates are shown in Figure 3, while the results with added dihydroxyphenylalanine are shown in Figure 4.

In addition to the concentrations of adenosine triphosphate presented in Figure 3, the results with $4 \times 10^{-3}M$ and $8 \times 10^{-4}M$ adenosine triphosphate were omitted from the graphs for the sake of clarity. The omitted curves nearly coincided with each other and fell in the expected order, close to and slightly above the curve obtained by treatment with $2 \times 10^{-2}M$, and somewhat below that with $1.6 \times 10^{-4}M$ adenosine triphosphate. Both total reflectance and redness were affected by the adenosine triphosphate concentration.

The effect of adenosine triphosphate concentration on color development in

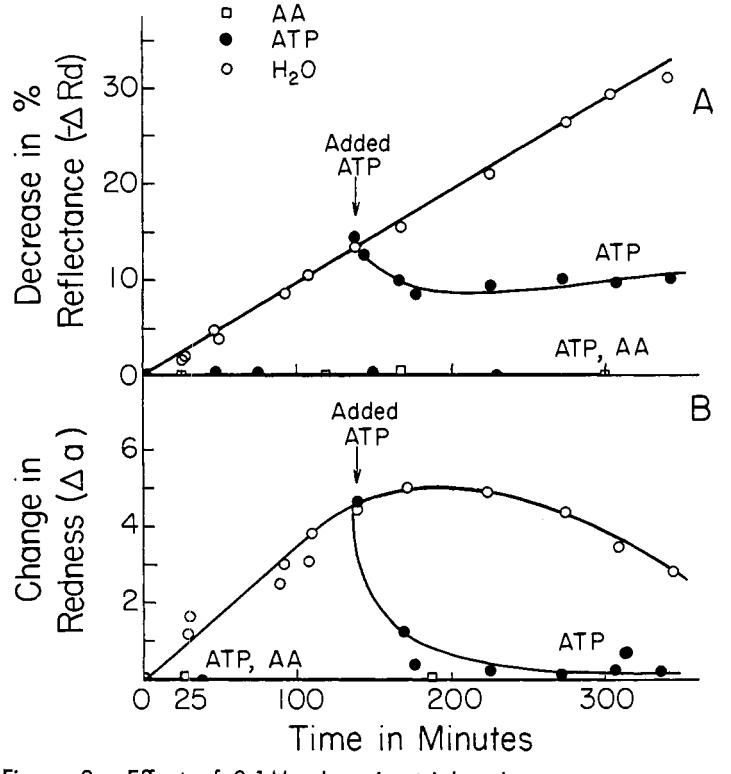

Figure 2. Effect of 0.1M adenosine triphosphate (ATP) and 0.1M ascorbic acid (AA), both at pH 6.1, on color formation in White Rose potato slices. ATP was added to some of the control slices after 138 minutes of color development

A = decrease in per cent reflectance, $-\Delta Rd$, with time
B = change in redness, Δa, with time

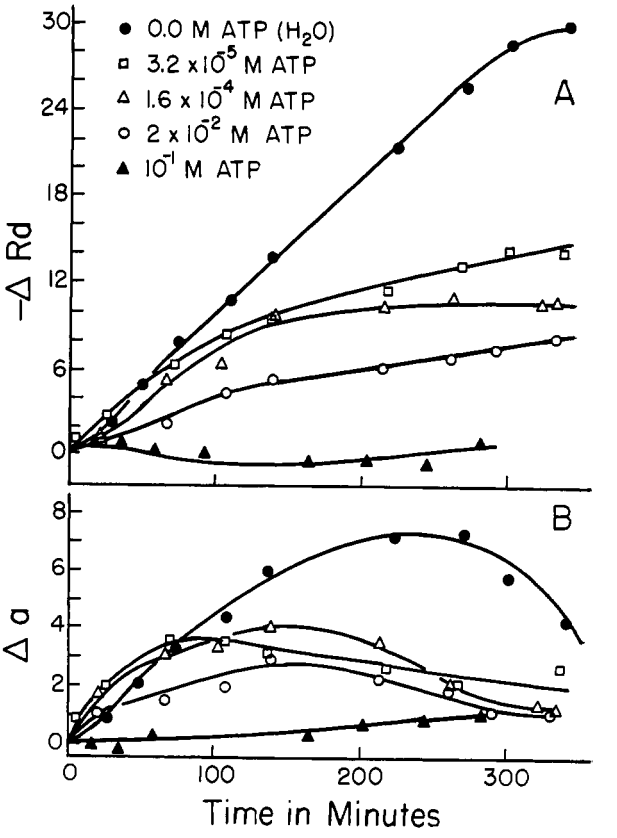

Figure 3. Effect of adenosine triphosphate (ATP) concentration, pH 6.2, on color development in White Rose potato slices

A = decrease in per cent reflectance, $-\Delta Rd$, with time
B = change in redness, Δa, with time

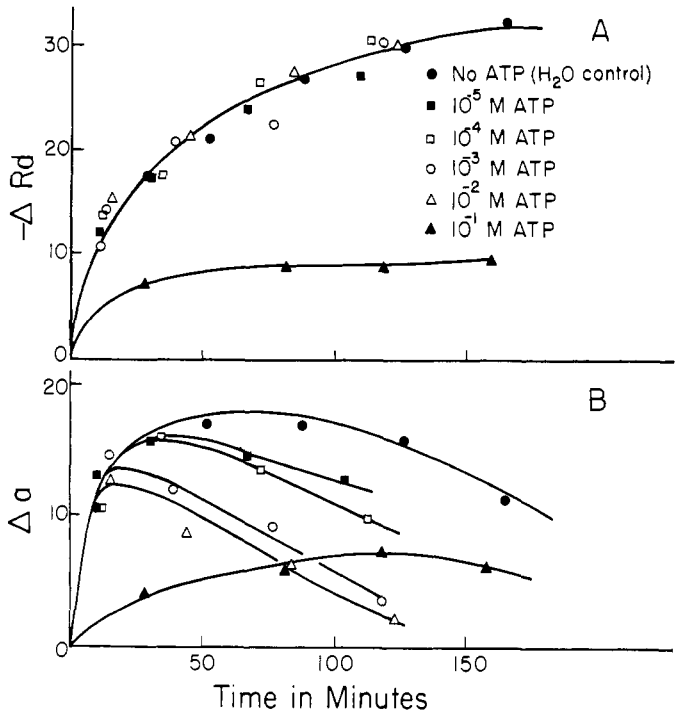

Figure 4. Effect of adenosine triphosphate (ATP) concentration, pH 6.2, on color development in White Rose potato slices at a constant level of dihydroxyphenylalanine (DOPA) 2.5 × $10^{-3}M$

A = decrease in per cent reflectance, $-\Delta Rd$, with time
B = change in redness, Δa, with time

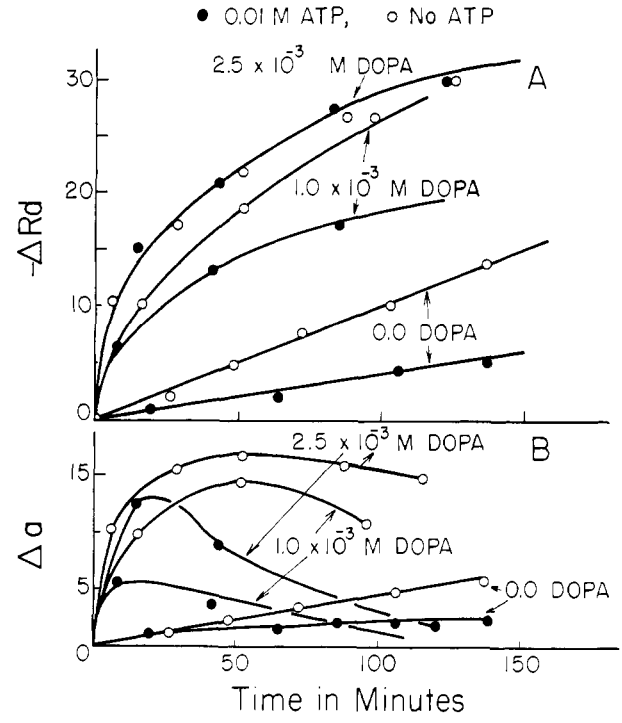

Figure 5. Effect of several substrate dihydroxyphenylalanine (DOPA) concentrations on color development with and without adenosine triphosphate (ATP) (0.01M) using White Rose potato slices

A = decrease in per cent reflectance, $-\Delta Rd$, with time
B = change in redness, Δa, with time

potato slices treated with dihydroxyphenylalanine is shown in Figure 4. With 2.5 × $10^{-3}M$ dihydroxyphenylalanine, the effect on total reflectance was negligible at concentrations of adenosine triphosphate less than $0.1M$, in contrast to results with no added substrate (Figure 3). The effect of lower concentrations of adenosine triphosphate in the presence of dihydroxyphenylalanine on development of redness is shown in Figure 4. The initial rates for redness were the same for controls as for slices treated with 10^{-5} to $10^{-2}M$ adenosine triphosphate. Maximum redness was attained sooner with higher concentrations of ATP than with lower, but the lower concentration of adenosine triphosphate also resulted in less change in redness. The gradual decrease in redness from a maximum proceeded along parallel lines in water controls and in slices treated with 10^{-5} to $10^{-2}M$ adenosine triphosphate. In slices treated with $0.1M$ adenosine triphosphate (Figure 4), redness increased slowly and then decreased slightly in the 2.5 hours during which measurements were made.

When the results in Figure 4 are compared with those in Figure 3, the opposite effects of adenosine triphosphate and of added dihydroxyphenylalanine become apparent. These data suggest that substrate concentration influenced the effective minimum concentration of adenosine triphosphate necessary to prevent or reduce formation of enzymatic color. Results reported in Figure 5 confirmed this point.

Effect of Dihydroxyphenylalanine Concentration. The effect of differences in concentration of added substrate, dihydroxyphenylalanine, on changes in reflectance and redness in the presence of a constant level of adenosine triphosphate is illustrated in Figure 5. The prevention of color formation in potato slices by $0.01M$ adenosine triphosphate was greatest with little or no added dihydroxyphenylalanine. The effectiveness of adenosine triphosphate decreased as the amount of added dihydroxyphenylalanine increased. At a concentration of 2.5 × $10^{-3}M$ of dihydroxyphenylalanine, $0.01M$ adenosine triphosphate had no effect on reflectance (the curves for treatments with and without adenosine triphosphate coincided). At the lower substrate concentrations, $10^{-3}M$ dihydroxyphenylalanine and no dihydroxyphenylalanine (water), the $-\Delta Rd$ and Δa were lower in slices treated with adenosine triphosphate than in the controls without it. Thus its effect on enzymatic color formation was diminished by increasing amounts of substrate.

The net effect of adenosine triphosphate and of dihydroxyphenylalanine on darkening of slices from several varieties of potato is summarized in Table I. The prevention of color by adenosine triphosphate was most pronounced in the White Rose potatoes of 1953 with or without added dihydroxyphenylalanine. Analogous results were obtained in a single experiment with Kennebec potatoes. The net effect of adenosine triphosphate on formation of enzymatic color in Russet potatoes was less pronounced.

Effect of pH. The optimum activity of polyphenol oxidase from potato and from other plants lies in the pH range from 6 to 8 (*7*, *9*). The plant slices had a high buffering effect and tended to bring the pH of added reagents to the level of the plant tissue used. Adenosine triphosphate, itself a buffer, added to total buffering capacity.

Effect of Other Substances. The effect of several substances on enzymatic darkening of potato slices was tested. Some were chemically related to adenosine triphosphate—e.g., adenosine 5′-mono- and diphosphates. Other phosphates—inorganic pyro-, ortho-, tetra-, and hexametaphosphates—were tried, as were phytate, α- and β-glycerophosphate, phosphoglycerate, and glucose-6-phosphate. Malate, fumarate, succinate, mannitol, sucrose, glucose, and ethylenediamine tetraacetate were also tried. None of these substances (which were tested either because of their sequestering action or biological importance) at concentrations up to $0.1M$, affected the rate of enzymatic color formation under the conditions used.

Prevention of Enzymatic Darkening in Apples. Slices of 3 varieties of apples (Newtown Pippin, Winesap, and Green-

ing) darkened less than controls, when treated with adenosine triphosphate (or ascorbate). All procedures were the same as for potato slices. Typical results with Winesap apple slices are shown in Figure 6. The reactions of all three varieties of apples to treatment with adenosine triphosphate or with ascorbate were in general similar to those of potato slices. In all three apple varieties, adenosine triphosphate decreased the formation of dark color and of redness. Greater darkening occurred in control slices when dihydroxyphenylalanine was added than without added substrate. The negative values for Δa, frequently obtained with apple slices treated with adenosine triphosphate or ascorbate, indicate a fast initial rate of reddening—i.e., a high initial value—combined with slow subsequent decolorization.

Redness increased in apple slices at first; then it either decreased slightly or remained essentially constant. The redness values were drastically reduced in all apple slices treated with adenosine triphosphate in the presence of dihydroxyphenylalanine as compared with slices treated with dihydroxyphenylalanine alone.

The net effects of adenosine triphosphate and of dihydroxyphenylalanine on darkening of apple slices are given in Table II. The net effect of dihydroxyphenylalanine was more pronounced on change in redness than on decrease in total reflectance. The net effect varied also with the varieties.

Avocado. Avocado slices darkened less in the presence of adenosine triphosphate than in its absence (Figure 7 and Table III). Reflectance values for slices treated with adenosine triphosphate or ascorbic acid were similar and parallel. In experiments on slices with added dihydroxyphenylalanine, darkening was at first slightly greater in the controls but became nearly the same in treated and control slices after 20 hours. Treatment of avocado slices with detergent D-40 (sodium alkyl arylsulfonate) improved the penetration of ascorbic acid and of adenosine triphosphate solutions. The high fat content and/or easy separation of cells of the ripe avocado may have been responsible for the poor penetration into the tissues of both ascorbate and of adenosine triphosphate when no detergent was added. The detergent itself had no measurable effect on the darkening of the avocado slices.

In contrast to potato and apple slices, there was little change in redness in avocado slices with or without dihydroxyphenylalanine when treated with adenosine triphosphate or with ascorbic acid. Redness developed slowly in avocado, while graying proceeded more rapidly.

Table I. Net Effect of Adenosine Triphosphate[a] and of Dihydroxyphenylalanine[a] on Enzymatic Darkening of Potatoes

Variety, Season, and Storage	No. of Expts.	Treatments	N.S.[a] Only				N.S.[a] + 2 × 10^{-3}M DOPA				Difference—i.e., DOPA–N.S.			
			2 hours		5 hours		2 hours		5 hours		2 hours		5 hours	
			—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa
Russets, 1955, 1 mo., 4.5° C.	5	H_2O	5.4	3.9	8.3	5.4	13.8	8.5	18.7	6.3	8.4	4.6	10.4	0.9
		ATP	3.3	1.6	5.6	2.5	9.4	2.4	13.3	1.4	6.1	0.8	7.7	−1.1
		H_2O–ATP	2.1	2.3	2.7	2.9	4.4	6.1	5.4	4.9	2.3	3.8	2.7	2.0
Russets, 1953 and 1954, 1 to 8 mo., 4.5° C.	4	H_2O	4.5	3.9	7.6	5.5	13.7	8.4	18.7	6.2	9.2	4.5	11.1	0.7
		ATP	2.7	1.4	4.7	2.0	9.5	2.4	13.2	1.4	6.8	1.0	8.5	−0.6
		H_2O–ATP	1.8	2.5	2.9	3.5	4.2	6.0	5.5	4.8	2.4	3.5	2.6	1.3
White Rose, 1953, 1 to 8 mo.[b] at 4.5° C.	3 to 5	H_2O	11.8	5.1	28.9	5.2	28.6	11.6	...	...	16.8	6.5	...	...
		ATP	1.0	0.8	0.6	0.9	13.8	4.4	...	...	12.8	3.6	...	...
		H_2O–ATP	10.8	4.3	28.3	4.3	14.8	7.2	...	...	4.0	2.9	..	..
Kennebec 1954 at 4.5° C. 9 mo. + period at RT (sprouts)	1	H_2O	...	...	...	...	13.6	8.3	...	...	..	...	...	...
		ATP	...	...	...	...	0.4	0.4	...	...	...	...	...	...
		H_2O–ATP	...	...	...	...	13.2	7.9	...	...	...	...	...	...
Av. of all varieties or net[c] ATP effect		H_2O–ATP	5.1 (14)	3.1 (14)	7.4 (11)	3.4 (11)	6.8 (12)	6.4 (12)	5.4 (9)	4.8 (9)	2.8 (12)	3.5 (12)	2.7 (9)	1.7 (9)

[a] ATP, 0.01- to 0.1M; DOPA, 2 × $10^{-3}M$; N.S. = natural substrate—i.e., no added substrate.
[b] Several experiments after 1 month, some after 4 months, and others after 8 months of storage at 4.5° C.
[c] Average H_2O–ATP was weighted according to number of experiments in each category. Numbers in parentheses equal number of experiment.

Table II. Net Effect of Adenosine Triphosphate[a] and of Dihydroxyphenylalanine[a] on Enzymatic Darkening of Apples

Variety, Season, and Storage	Treatment	N.S.[a] only				N.S.[a] + 2 × 10^{-3}M DOPA				Difference—i.e., DOPA–N.S.[b]			
		2 hours		5 haurs		2 haurs		5 hours		2 hours		5 hours	
		—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa	—ΔRd	Δa
Newtown Pippin (3)	H_2O	4.3	1.8	4.8	2.0	8.2	7.6	13.4	5.8	3.9	5.8	8.6	3.8
	ATP	− 0.5	−1.0	− 1.4	−1.8	2.6	− 1.7	3.9	− 2.3	3.1	− 0.7	5.3	− 0.5
	H_2O–ATP	4.8	2.8	6.2	3.8	5.6	9.3	9.5	8.1	0.8	6.5	3.3	4.3
Greening (1)	H_2O	18.0	8.3	20.0	9.7	14.0	9.0	21.0	12.0	−4.0	0.7	1.0	2.3
	ATP	13.8	5.2	13.2	6.2	5.0	− 6.0	7.5	− 6.5	−8.8	−11.2	− 5.7	−12.7
	H_2O–ATP	4.2	3.1	6.8	3.5	9.0	15.0	13.5	18.5	4.8	11.9	6.7	15.0
Winesap (1)	H_2O	10.0	5.0	11.0	5.6	17.5	6.8	26.5	5.8	7.5	1.8	15.5	0.2
	ATP	7.5	2.0	11.5	1.8	10.5	− 2.4	14.5	− 2.5	3.0	− 4.4	3.0	− 4.3
	H_2O–ATP	2.5	3.0	− 0.5	3.8	7.0	9.2	12.0	8.3	4.5	6.2	12.5	4.5
Av. of all varieties or net[b] ATP effect (5)	H_2O–ATP	4.2	2.9	5.0	3.7	6.6	10.4	10.8	10.2	2.3	7.5	5.8	6.5

[a] ATP, 0.04M; DOPA, 2 × $10^{-3}M$; N.S. = natural substrate—i.e., no added substrate.
[b] Averages of H_2O–ATP, weighted according to number of experiments in each category shown in parentheses.

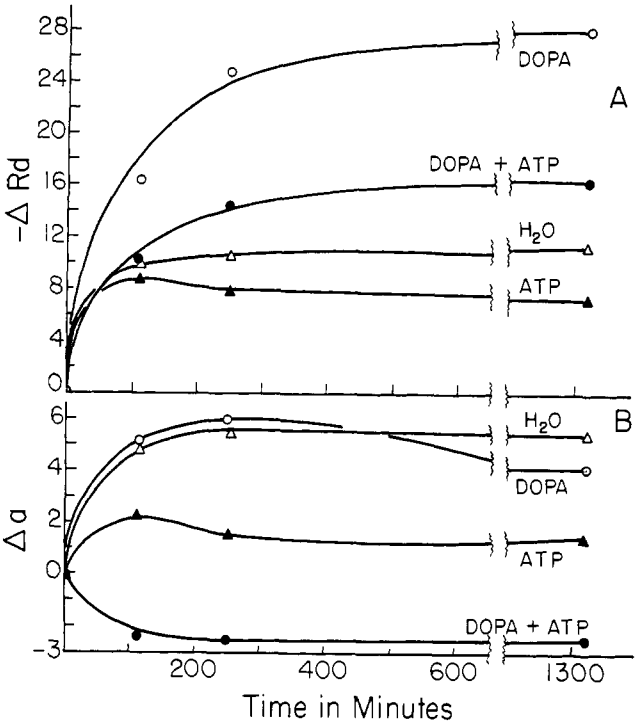

Figure 6. Effect of 0.04*M* adenosine triphosphate (ATP), pH 6.3, on color development in Winesap apple slices, with or without 10^{-3}*M* dihydroxyphenylalanine (DOPA)

A = decrease in per cent reflectance, $-\Delta Rd$, with time
B = change in redness, Δa, with time

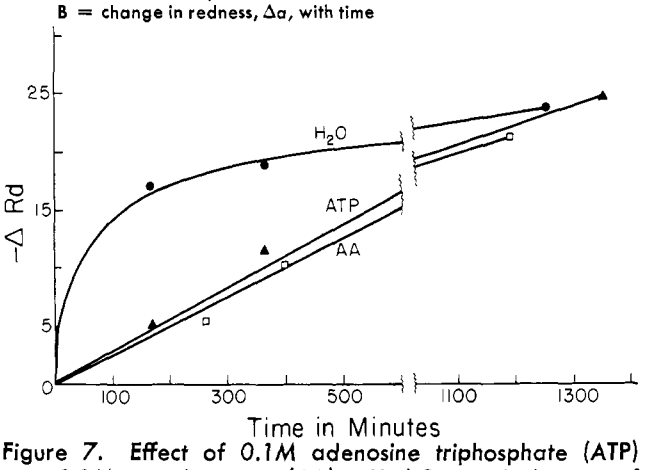

Figure 7. Effect of 0.1*M* adenosine triphosphate (ATP) and 0.1M ascorbic acid (AA), pH 6.2, on darkening of avocado slices, previously treated with detergent D-40 (sodium alkanesulfonate); 2 drops of 0.025*M* dihydroxyphenylalanine (DOPA) were added to all slices. Decrease in per cent reflectance, $-\Delta Rd$, with time

Neither ascorbic acid nor adenosine triphosphate affected the development of red color significantly; therefore, redness data were omitted. The net effect of adenosine triphosphate on reflectance is shown in Table III. Nearly a maximum adenosine triphosphate effect was observed in avocado after 100 minutes, as no further increase had occurred after 300 minutes.

Mushrooms. The results with cut and scored mushroom caps were in general similar to those with potato and apple slices. Under the experimental conditions used, the formation of colored products in the reaction catalyzed by polyphenyl oxidase was very rapid with or without added substrate. Therefore, the initial *Rd*, *a*, and *b* values already included considerable progress in enzymatic browning with the formation of some irreversibly colored compounds. Nevertheless, Table III shows that less color was produced in the mushrooms treated with adenosine triphosphate than in controls. The change in redness in slices treated with adenosine triphosphate was quite small. The decolorizing action of adenosine triphosphate on mushrooms was far less than on slices of potato or apple. These results suggest that the formation of quinones in mushrooms was probably more rapid than the formation of reducing compounds by the action of adenosine triphosphate. The net effect of adenosine triphosphate on reflectance in mushrooms is reported in Table III.

Sweet Potatoes. Although sweet potato roots are known to contain polyphenol oxidase (*1*), measurement of color changes on slices was difficult. The enzyme appears to be localized directly under the skin and in or near vascular bundles. Scoring the surfaces did not improve the distribution of the enzyme, while the resulting powdery layer of starch hindered measurement of color. The Red Velvet variety contained a red-purple color which also hindered measurements of enzymatic darkening. Thus, little or no additional darkening was observed in the control slices, even when dihydroxyphenylalanine or catechol was added as substrate; nor was there any apparent effect of adenosine triphosphate or ascorbate on sweet potato color.

Eggplant. Eggplant slices darkened rapidly and without reddening after cutting and scoring. The few experiments showed that neither ascorbate nor adenosine triphosphate interfered with darkening under test conditions. Either these reagents are not readily absorbed or the nature of the substrate intermediates is not affected by ascorbic acid. Moderate changes in concentration of reagents and addition of dihydroxyphenylalanine and/or detergent did not alter the results. Enzymatic darkening was slightly more rapid and attained a higher level with added dihydroxyphenylalanine, indicating the presence of polyphenol oxidase. It is also possible that the darkening of eggplant is so rapid after cutting that by the time ascorbate or adenosine triphosphate was added, the reactions have progressed beyond the point of reversibility.

Peach. Peach slices treated with adenosine triphosphate or with ascorbate and untreated controls showed the same effects as did potato and apple slices—i.e., the enzymatic darkening was diminished or prevented either by ascorbate or by adenosine triphosphate. Only preliminary experiments were carried out; therefore no quantitative data were presented.

Discussion

The procedure described proved useful in the study of prevention by adenosine triphosphate of the enzymatic browning normally produced by polyphenol oxidase activity.

The efficiency of adenosine triphosphate in preventing color formation in slices of potato depended on such factors as concentration of adenosine triphosphate, presence and concentration of added substrate, and temperature at which the slices were kept.

Up to a certain point, the higher the concentration of adenosine triphosphate, the more complete was the inhibition of color. More adenosine triphosphate was required to inhibit color when dihydroxyphenylalanine was added as substrate. Again, the more dihydroxyphenylalanine

Table III. Net Effect of 0.1*M* Adenosine Triphosphate on Enzymatic Darkening ($-\Delta Rd$) of Avocado and Mushroom Slices

	$-\Delta Rd$ of Avocado[a]		$-\Delta Rd$ of Mushroom	
Treatment	*100 minutes*	*300 minutes*	*100 minutes*	*300 minutes*
H_2O	15.3	18.8	10.4	12.5
0.1*M* ATP	3.7	8.4	6.8	7.7
H_2O-ATP	11.6	10.4	3.6	4.8

[a] D-40 detergent was added to all slices of avocado.

was added, the more adenosine triphosphate was required. These observations suggest a quantitative relationship between the substrate for the polyphenol oxidase reaction and the adenosine triphosphate effect. By measuring redness in potato slices, it was possible to demonstrate inhibition of color with as little as $10^{-5}M$ adenosine triphosphate. Higher concentrations of adenosine triphosphate reduced redness with no apparent change in initial rates.

Addition of dihydroxyphenylalanine increased darkening in all slices. At the same time, increasing the amount of dihydroxyphenylalanine counteracted the color-preventing action of adenosine triphosphate. This could be expected if addition of adenosine triphosphate resulted in an increase in quinone-reducing material. If enough substrate were added to build up a reserve of quinone, then the rate of forming reducing substance by adenosine triphosphate, at its maximum, might be insufficient to reduce all the quinone formed. Therefore, darkening would proceed. This probably occurred in the case of eggplant and, to some extent, of mushroom. A similar situation was created in potato slices to which large amounts of substrate were added, showing that the effectiveness of adenosine triphosphate in preventing formation of color depends also on the amount of substrate present.

Nearly all experimental data indicate that the inhibiting effect of adenosine triphosphate upon color development paralleled that of ascorbate, which reduces the quinones formed in the early stages of oxidation (*7*). Neither ascorbic acid nor adenosine triphosphate is a true inhibitor, as both permit enzymatic oxidation to go on (*2*, *4*).

Color differences between adenosine triphosphate-treated and untreated potato slices were apparent in the first hour and often persisted after holding overnight. The early stage (reddish brown) of enzymatic color formation in potato slices was reversed by addition of adenosine triphosphate in the presence of air, but not under vacuum nor under nitrogen. Ascorbate, however, decolorized initial reddish brown color in the absence of air. This was true when any one or a combination of the following was also added to the slices at pH 6.2: phosphate buffer, succinate, and dihydroxyphenylalanine. Oxygen, therefore, appears necessary for the production of quinone-reducing substances by adenosine triphosphate in living tissue. Moreover, active cell particles also seem necessary for the adenosine triphosphate effect (*4*, *5*).

The prevention of browning by adenosine triphosphate was maintained much longer in slices kept cold than in slices left at room temperatures. A possible explanation is that in the cold, adenosine triphosphate is decomposed more slowly by hydrolyzing enzymes present in potato tissue (*10*). The low temperature also prolongs the metabolic activity of the cytoplasmic particles, mitochondria (*6*). Adenosine triphosphate acts indirectly via small and very labile potato cell particles (*5*), which are laborious to isolate and harder to keep in an active state. In slices, some of the same enzymatic phenomena found in carefully prepared particles could be demonstrated with far less time and effort. Direct measurement of color changes in enzymatic and other reactions upon slices is rapid and convenient.

In all plant materials studied, there was a positive correlation between the anatomical location of vascular tissue and of seeds and the location of the darkest areas. Such localization of polyphenol oxidase activity was most pronounced in sweet potato, avocado, and eggplant. To establish whether or not adenosine triphosphate interferes with color formation in slices of sweet potato and eggplant, considerable modifications in method and extensive studies would have been required.

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BEET SUGAR IMPURITIES

Colorimetric Determination of Saponin as Found in Beet Sugars

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The unsightly flocculent precipitate that sometimes forms when impure beet sugars are placed in acid solution has recently been studied. A saponin in the beet root, if not removed during the refining process, is responsible in part for this apparently harmless, but unsightly floc. An accurate, rapid colorimetric method has been developed for evaluation of finished sugars and for refinery-control measures. Visual examination of numerous sugars for floc gave results that compared well with the quantities of saponin found.

THE COMPOSITION of acid-insoluble substances in beet sugars has been studied recently (*1*, *7*) and earlier references to the subject were included. There remained the problem of sugar refining-control measures, on which work is continuing. However, to have control, it is necessary to have available rapid and accurate analytical methods. Walker (*6*) developed a colorimetric method based upon the reaction of sugar beet saponins with antimony pentachloride. West and Gaddie (*8*) tested this method extensively to correlate results with the observed floc precipitate.

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