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THE EFFECT OF PRETREATMENT AND SUBSEQUENT DRYING ON THE ACTIVITY OF GRAPE OXIDASE[1]

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THOMPSON SEEDLESS GRAPES usually darken during drying in the preparation of raisins. The intensity of this darkening depends to a considerable extent on the treatment of the fresh fruit prior to drying. Raisins prepared by drying grapes in the sunlight without other treatment are characteristically of a dark color similar to the clove-brown of Ridgway (1912).[5] Raisins produced by the cold-dip, mixed-dip, soda-dip (hot lye), sulfur-bleach, or golden-bleach procedures, on the other hand, usually have a light color ranging in hue from cinnamon-buff to sepia as judged by the color standards of Ridgway. Hussein and Cruess (1940) investigated the properties of grape oxidase and suggested that oxidizing enzymes are involved to a considerable extent in the darkening of grapes during the preparation of raisins, and to some extent in the darkening of wines. These authors, however, limited their investigations to the enzyme preparation obtained from fresh, untreated grapes. There is no available published information concerning the effects of the various treatments used in the production of light-colored raisins on the oxidizing enzymes occurring in grapes.

A series of experiments was conducted during the 1939 season in order to determine the effects of the mixed-, soda-, and cold-dip pretreatments, and of sulfuring and drying, on the oxidase activity of raisins made from Thompson Seedless grapes.

EXPERIMENTAL PROCEDURE

Materials Used.—Thompson Seedless grapes were used in all experiments unless otherwise indicated.

The sulfur-bleach and cold-, mixed-, and soda-dip procedures employed were similar to those in commercial use as described by Mrak and Long (1941). The golden-bleach procedure was similar to that used in the preparation of sulfur-bleach raisins, except that the fruit was dried in a dehydrater having a relative humidity of 25 per cent at the hot end, and a dry-bulb temperature of 71.1° C (160° F).

The dipping preparations used were: soda dip, 0.5 per cent solution of

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NaOH brought to 95° C (203° F); mixed dip, an emulsion containing 68 grams of K_2CO_3, 34 grams of NaOH, 50 cc of California olive oil, and approximately 12 liters of tap water brought to 82.2° C (180° F); and cold dip, an emulsion containing 950 grams of an alkali mixture consisting of 95 grams of Na_2CO_3, 855 grams of K_2CO_3, 100 cc of California olive oil, and approximately 20 liters water at 20° C (68° F).

Freshly picked Thompson Seedless grapes were treated in one or another of the dipping preparations for various periods of time, then sulfured or dried according to the particular procedure used in commercial practice.

Enzyme Preparation.—Untreated, freshly dipped, sulfured, and dried grapes were tested for oxidase activity after first making an enzyme preparation from the various samples. The enzyme preparations and measurement were made according to the procedures of Hussein and Cruess (1940) as described briefly in the paragraph below. Treated grapes were stored at –17.7° C (0°F), and the raisins at 0° C (32° F) until used.

To make the enzyme preparation, samples were ground in a mortar containing white sand and acetone and were then filtered. This procedure was repeated a second and third time. The dilute acetone prevented browning, removed auto-oxidizable catechol compounds, tannins, some of the water-soluble materials, and precipitated the enzyme. The solid residue was then extracted with a 0.2 molar citrate buffer of pH 4.5, equal in volume in cubic centimeters to three times the weight in grams of the grape sample used. Then three volumes of 95 per cent alcohol were added and the mixture centrifuged. The resulting precipitate was suspended in the aforementioned citrate buffer.

Quantitative Measurement of Grape-Oxidase Action.—The colorimetric method of Bansi and Ucho (1926) as modified by Hussein and Cruess (1940), was used for the quantitative measurement of oxidase activity. This procedure is as follows: 1 cc of enzyme extract and 1 cc of 1 per cent guaiacol solution (by volume) in 50 per cent ethyl alcohol were added to a test tube containing 5 cc of 0.2 molar acetate buffer of pH 4.5. Then sufficient distilled water was added to make a total volume of 20 cc. Finally 1 cc of 0.1 *N* H_2O_2 was added to the same test tube after which the tube was held at 30° C (86° F) for 1 hour. The reaction was then stopped by the addition of 5 cc of glacial acetic acid. The peroxidase enzyme of the grapes catalyzes the oxidation of the guaiacol by the H_2O_2, a reaction similar to that occurring during the browning of the grape flesh by oxidation catalyzed by the enzyme.

Hussein and Cruess (1940) estimated enzyme activity by observing the extent to which the extracts darkened. This was conveniently done

by measuring the light transmission with an Evelyn photoelectric colorimeter, with a filter having maximum transmission at 5,400 angstrom units. From the transmissions (T) were calculated values of log 1/T, used as the measure of enzyme activity. The greater the value of log 1/T the greater is the enzyme activity.

EXPERIMENTAL OBSERVATIONS

Effect of Various Dips.—The effects of the type and time of dip used on enzyme activity of fresh grapes are shown in table 1. The enzyme

TABLE 1

EFFECT OF VARIOUS DIPPING TREATMENTS ON ENZYME ACTIVITY OF THOMPSON SEEDLESS GRAPES

Type of dip	Dipping period	Per cent of light transmission	Log 1/T values for enzyme activity	Per cent increase or decrease in oxidase activity
Cold dip*	2 minutes	46.25	−0.335	− 2.6
	5 minutes†	59.00	−0.229	−34.8
	10 minutes	64.50	−0.190	−45.0
No dip (check)		45.00	−0.347	....
Mixed dip‡	5 seconds†	40.00	−0.398	+15.8
	30 seconds	56.25	−0.248	−28.0
	60 seconds	75.75	−0.120	−64.5
No dip (check)		45.50	−0.342	....
Soda dip (5 per cent solution of NaOH)	5 seconds†	38.50	−0.415	+14.2
	30 seconds	95.25	−0.022	−94.0
	60 seconds	98.75	−0.004	−99.0
No dip (check)		43.50	−0.362	....

* An emulsion containing 950 grams of alkali mixture (95 grams of Na_2CO_3 and 855 grams of K_2CO_3) and 100 cubic centime ers of olive oil and about 20 liters of tap water.
† Dipping periods commonly used in commercial practice.
‡ An emulsion containing 68 grams of K_2CO_3, 34 grams of NaOH, 50 cubic centimeters of olive oil, and 12 liters of tap water.

activity of grapes treated in the cold dip decreased when the length of dipping period was increased. This decrease in enzyme activity, however, was not directly proportional to the length of dipping period used. The skin of grapes treated in the cold dip did not crack, even after 10 minutes' exposure, but much of the waxy bloom was removed. The amount removed varied according to the length of the dipping period. Each skin was covered with a thin layer of oil which caused the fruit to remain soft and pliable, even when dried. This oil coating may have acted as a layer retarding the entrance of oxygen into the flesh of the fruit, thereby preventing in some degree, darkening of the raisins by oxidation.

Immersion of grapes for an appreciable period of time in the hot dipping solutions reduced the enzyme activity, whereas dipping for a very short time increased enzyme activity. When the dipping period was increased to 30 seconds or more, enzyme activity decreased rapidly as shown in table 1. The most rapid decrease in enzyme activity occurred when the grapes were treated in the soda dip, while the slowest decrease in activity occurred when they were treated in the cold dip. The temperature of the dipping solution was undoubtedly an important factor in accounting for these differences. In order to determine the effect of heat alone on the enzyme activity, dipping tests were conducted in distilled

TABLE 2

EFFECT OF HOT-WATER DIPS ON ENZYME ACTIVITY IN THOMPSON SEEDLESS GRAPES

Temperature of dip, degrees Fahrenheit	Dipping period, seconds	Per cent of light transmission	Log 1/T values for enzyme activity	Per cent increase or decrease in oxidase activity
180	5	45.1	−0.345	+18.0
	30	69.0	−0.162	−43.0
	60	88.2	−0.092	−70.5
No dip (check)	.....	52.0	−0.284	....
205	5	48.2	−0.315	+17.5
	30	95.2	−0.020	−92.5
	60	98.2	−0.006	−98.5
No dip (check)	.....	54.0	−0.268	....

water at 82.2° C (180° F) and 96.1° C (203° F). The results were very similar to those obtained in the experiments with the soda and mixed dips. Grapes dipped for 5 seconds in hot water at 82.2° C (180° F) and 96.1° C (203° F) displayed an increase in enzyme activity similar to that observed in the mixed-dip and soda-dip experiments. When grapes were subjected to the hot-water treatments for 30 or 60 seconds, on the other hand, enzyme activity decreased greatly as indicated in table 2. The skins of most of the grapes subjected to the soda dip, the mixed dip or the 30- and 60-second hot-water dips cracked extensively. The most severe skin cracking occurred when the longest dipping periods were used; the skins of grapes subjected to the 5-second hot-water dip, did not crack. When a grape oxidase preparation was heated for various periods of time at 82.2° C (180° F) and 95° C (203° F) no activation effect was observed (table 3). The enzyme system was almost completely inactivated in 30 seconds. This indicates that the apparently increased activity, observed when grapes were treated in the soda and the mixed dips for short periods of time, was due to some other cause than oxidase

response alone. Futher tests were conducted with Muscat grapes which are larger and have thicker skins than the Thompson Seedless variety. Although the Muscat grapes were dipped in water at 95° C (203° F) for various periods of time up to 60 seconds, in no case did the skins of these grapes show cracking. Furthermore, the data obtained (table 4) show that heating in water for very short periods of time did not increase

TABLE 3

EFFECT OF HEAT ON THE ENZYME PREPARATION OBTAINED FROM THOMPSON SEEDLESS GRAPES

Heating period in seconds at 180° F	Per cent of light transmission	Log 1/T values for enzyme activity	Per cent decrease in oxidase activity
0 (check)	88.00	−0.056	...
2	88.50	−0.054	3.5
5	89.25	−0.050	8.9
10	88.50	−0.054	3.5
15	89.50	−0.046	17.6
20	95.00	−0.022	60.7
30	98.75	−0.005	91.1

TABLE 4

THE EFFECT OF HOT-WATER DIPS ON ENZYME ACTIVITY IN MUSCAT GRAPES

Dipping period in seconds at 203° F	Per cent of light transmission	Log 1/T values for enzyme activity	Per cent decrease in oxidase activity
0 (check)	39.0	−0.409	..
5	47.2	−0.323	22.0
10	86.0	−0.065	84.0
20	91.2	−0.039	90.7
60	95.2	−0.022	94.2

enzyme activity. The enzyme system was almost completely inactivated after 60 seconds of heating. This indicates that skin differences between the two varieties may be partially responsible for the variations in oxidase responses to the dipping treatments of short duration. Variations in oxidase distribution within the individual berry, as well as varietal differences in size of the berries, may also be factors accounting for the observed differences in effects of the heated dips on the oxidase activity of the two varieties.

In order to compare the distribution of enzyme activity in Thompson Seedless and Muscat grapes, individual berries were peeled and the Muscats were seeded. Table 5 shows that enzyme activity was greater

in the skins than in the flesh of the two varieties of grapes used. A much greater difference between enzyme activity in skin and flesh was observed in the Thompson Seedless than in the Muscat grapes. Enzyme activity in Muscat grapes was slightly greater in the skin than in the flesh. In Thompson Seedless, on the other hand, enzyme activity in the skins was over 31 times as great as that in the flesh. The skins of Thompson Seedless grapes showed more activity than either the skin or the flesh of Muscat grapes. The flesh of the former, however, showed less activity than either the skin or flesh of the latter. The relative richness in oxidase of skin as observed here agrees with the data of Hussein and Cruess

TABLE 5

COMPARISON OF ENZYME ACTIVITY OF SKIN AND FLESH OF THOMPSON SEEDLESS AND MUSCAT GRAPES

Variety of grape	Portion of grape tested	Per cent of light transmission	Log 1/T values for enzyme activity	Weight ratio of skin to flesh	Oxidase activity ratio of skin to flesh*
Thompson Seedless	Skin	61.00	−0.215	0.113:1	31.1:1
	Flesh	82.50	−0.083		
Muscat	Skin	76.25	−0.118	0.101:1	7.75:1
	Flesh	75.00	−0.122		

* Solutions used in measurement of enzyme activity of Thompson Seedless skins were diluted twelve times and that for Muscat skins eight times. Corrected values for activity ratio of skin to flesh are given in the last column. The activity ratio of Muscat flesh to Thompson Seedless flesh (corrected value) = 1.47:1; and of Muscat skin to Thompson Seedless skin (corrected value) = 0.37:1.

(1940) concerning the location of the enzyme in the grape. This observed unequal enzyme distribution is probably concerned, to some extent at least, with the variations in enzyme responses when Thompson Seedless and Muscat grapes were treated in the hot-water dips. It may also account for the fact that Muscat grapes that retain a light color have not been produced successfully on a commercial scale by use of the cold, mixed, or soda dips without a subsequent sulfuring treatment.

Effect of Sulfuring.—Sulfur-bleached raisins were dried to a moisture content of about 15 per cent. The oxidase activity in finished raisins varied greatly with the period of exposure and concentration of SO_2 used in the sulfuring treatment. The data in table 6 show that the enzyme activity in the sulfur-bleached raisins decreased with increase in length of the sulfuring period. In these experiments, as in commercial practice, the lightest-colored raisins were obtained when grapes were sulfured for 2 hours and the darkest when they were sulfured for 1 hour or less. Table 7 indicates that the concentration of SO_2 in the sulfuring house at the time of sulfuring also affects the enzyme activity of raisins treated by this method.

Raisins prepared by drying grapes sulfured at the higher concentrations of SO_2, for a given period of time had less enzyme activity than those sulfured at lower concentrations for the same period of time. Apparently the time of exposure and concentration of SO_2 during sulfuring are both important in diminishing oxidase activity in sulfured raisins. Hussein and Cruess (1940) found that very high concentrations of SO_2

TABLE 6

EFFECT OF TIME OF SULFURING ON THE OXIDASE ACTIVITY OF SULFUR-BLEACHED RAISINS

Sulfuring period, minutes*	SO_2 in raisins, p.p.m.	Log 1/T values for enzyme activity†	Per cent decrease in oxidase activity	Color grade of the finished raisins‡
0 (check)	...	−0.025	...	Poor
30	150	−0.023	7.0	Poor
60	300	−0.016	35.7	Poor
90	400	−0.010	59.5	Fair
120	570	−0.005	79.4	Good

* The concentration of SO_2 in the sulfuring compartment was 1.5 per cent by volume, and the temperature, 105°–120° F.
† Represents activity calculated to 1 gram of dry weight.
‡ Color grade judged from standpoint of salability as sulfur-bleached raisins.

TABLE 7

EFFECT OF CONCENTRATION OF SO_2 DURING SULFURING ON THE OXIDASE ACTIVITY OF SULFUR-BLEACHED RAISINS

Concentration of SO_2 in per cent by volume in sulfuring compartment*	SO_2 in raisins, p.p.m.	Log 1/T values for enzyme activity†	Per cent decrease in oxidase activity	Color grade of the finished raisins‡
Check	...	−0.025	...	Poor
0.75	270	−0.020	18.7	Poor
1.50	570	−0.006	77.9	Good

* The length of the sulfuring period was 120 minutes and the temperature 105°–120° F.
† Represents activity calculated to 1 gram of dry weight.
‡ Color grade judged from standpoint of salability as sulfur-bleached raisins.

were required to inactivate a grape-enzyme preparation. Oxidase activity decreased gradually as the concentration of SO_2 added to the solution was increased from 0 to 5,580 p.p.m., but was not entirely inhibited at any concentration used. It is difficult to understand why the enzyme preparation should be so resistant to SO_2. Possibly other factors such as physical condition of the grapes, maturity, and temperature complicate the results.

Effect of Drying.—The treatment of grapes preliminary to drying is the same for golden-bleached and sulfur-bleached raisins. Both are lye-dipped and sulfured; but the former are then dehydrated and the

latter are exposed to the sun for a short time and later dried in the shade. The two products, however, are different in appearance and storage qualities. Sulfur-bleached raisins usually darken more rapidly in storage than do the golden-bleached; this may be attributed to some extent to the higher moisture content in the former. Nevertheless it was thought that oxidase may play a part in this darkening. Consequently, grapes from each of two lots were dehydrated and shade-dried and then compared for oxidase activity. Table 8 shows that the enzyme activity in the

TABLE 8

EFFECT OF SUN-DRYING AND DEHYDRATION ON OXIDASE ACTIVITY IN THE DRIED PRODUCTS

Drying procedure	Per cent of light transmission	Log 1/T values for enzyme activity	Ratio of activity of dehydrated to sun-dried
Dehydrated at 160° F.	90.75	−0.042	0.188:1
In sun 1 day and then dried in the shade.	59.75	−0.224	
Dehydrated at 160° F.	88.75	−0.052	0.213:1
In sun 1 day and then dried in the shade.	57.00	−0.244	

dehydrated product was approximately one fifth of that of shade-dried. This variation in oxidase activity may account for some of the differences in the storage qualities of the two types of raisins.

SUMMARY

Oxidase enzymes cause discoloration of grapes and raisins under certain conditions. Experiments have been conducted to determine the effect of various dipping, sulfuring, and drying procedures on this activity. The commercial cold-, mixed-, and soda-dip treatments decreased the oxidase activity when immersion periods of sufficient length were used. Oxidase activity was stimulated by very short, soda, mixed, and hot-water dips. Sulfuring decreased the oxidase activity, approximately in proportion to the period of exposure and concentration of SO_2 used during sulfuring. Raisins prepared by dehydration had about one fifth the oxidase activity of sulfured grapes dried in the shade, probably because of the relatively high temperature used for dehydration.

The diminishing effect on oxidase activity of some of the commercial dipping treatments may account for the production of light-colored raisins without the use of SO_2.

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