earliness, and by larger size, of seed plots. The effects of these factors were often greater than the effects of tuber-unit planting.

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POTATO QUALITY VI. RELATION OF TEMPERATURE AND OTHER FACTORS TO BLACKENING OF BOILED POTATOES[1]

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INTRODUCTION

In certain of the important potato growing regions of New York State the problem of tuber blackening after boiling is serious because of its frequent and widespread occurrence and the discrimination of consumers. The discoloration appears principally in the stem-end and is present only after boiling. Several investigators (8, 18, 25) have reported that the discoloring pigment is melanin which is formed by the oxidation of certain phenolic compounds. However, Robison (17) employing solubility tests stated that the pigment could not be melanin. Nutting's (12) work also indicates that the dark pigment could not be melanin but that the precursor of this pigment is of the flavone-type substances. However, neither the mechanism by which the oxidation of the phenols to melanin or the change of flavone-type substances to the black pigment takes place in the boiled tubers, nor the environmental factors which cause the production of tubers which blacken, have been reported by these authors.

Two of the present authors have previously reported on the relation of various factors to the occurrence of blackening (9, 10, 20, 21, 22.) In the five-year period in which these investigations were in progress, more than 600 samples of potatoes grown under widely varying environmental conditions were studied. Briefly, the results obtained, some of which are as yet unpublished, show that varying levels

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or combinations of various levels of nitrogen, phosphorus, potassium, lime, and manure had no consistent or predictable effect upon the occurrence of blackening, except as they may have altered the stage of maturity with reference to weather conditions, particularly temperature. Likewise, soil reaction, soil moisture, soil type, and deficiencies of boron, copper, zinc, manganese, and magnesium had no noticeable consistent effect.

The fact that light markedly affects the balance between soluble and insoluble nitrogen in plants (11) caused the authors to investigate the effect of shading the potato vines, upon the severity of blackening and the concentration of phenolic compounds in the tubers (9). Shading increased the soluble nitrogen, total phenols, and tyrosine fractions and increased the severity of surface blackening. The amount of stem-end blackening was not appreciably increased, however.

Because of the fact that mineral nutrition, soil moisture, and light were found to exert very little influence on blackening, the study of the relation of temperature to blackening was begun. Later, a high correlation was found between the pH of a tuber or portion of a tuber and the degree and extent of blackening. Therefore, studies were initiated and are reported here on the effects of storage temperature, hydrogen ion concentration of the boiling medium, gas storage treatments, and other factors on hydrogen ion concentration of the raw tubers and blackening of the cooked tubers.

Experimental Methods and Results

With the exception of eight potato samples taken from the experimental plots of the Department of Horticulture, Florida Agricultural Experiment Station at Gainesville, Florida, all the samples used were grown by the Department of Vegetable Crops, Cornell University. After harvest the samples were placed in 40° or 50° F. storage where they remained until the various tests were made.

The boiling tests were conducted on 6 to 8 pared tubers with a standardized procedure. Precautions were taken to use the same size and kind of porcelain-lined containers, similar amounts of water, approximately the same length of time for boiling, and uniform gas flames. After boiling, the tubers were allowed to stand in air for one-half hour after which they were rated according to the amount of discoloration shown. A rating of 10 was given to samples which showed no blackening with decreasing numbers for increased blackening. Samples exhibiting intermediate blackening were given appropriate ratings. For example, a rating of 9 was given for slight discoloration, ratings of 8 and 7 for appreciable blackening, and ratings of 6, 5, and 4 to samples which darkened severely. In this paper no ratings below 4 are reported.

The mean temperatures reported were calculated from temperature records kept on the plots where the samples were grown or from the temperature records of a nearby U. S. Department of Commerce Weather Station.

Effect of Temperature During the Growing Season on Occurrence of Blackening

In 1940 potato variety tests were located in four widely separated regions in New York, in Steuben, Tompkins, Suffolk, and Wayne counties. At the time of harvest samples of Pioneer Rural, Green Mountain, Sebago, Irish Cobbler, Chippewa and Earlaine varieties were taken from each of the four locations. In table 1 are presented for each variety the rating on blackening and the approximate mean temperature to which the vines were exposed during the last month of growth. The vines of all the varieties grown in Wayne and Suffolk counties were dead by the 20th of August; whereas, in Steuben and Tompkins counties, the early-maturing varieties (Earlaine, Chippewa, and Irish Cobbler) remained alive until about the 10th of September, and the late-maturing varieties (Pioneer Rural, Green Mountain, and Sebago) lived until they were killed by freezing temperatures in late September and early October. Thus, from the dates of maturity and the calculated mean temperature for the last month of the growing season, it may be clearly seen that there were wide differences in temperature under which the tubers of the different varieties and from different locations matured. Likewise, from the boiling test results, it may be seen that tubers from none of the varieties blackened when they matured under relatively high temperatures, and that the severity of blackening was increased as the mean temperature became lower. The late maturing varieties from Steuben and Tompkins counties matured under the lowest mean temperatures, 52° and 59° F., respectively, and blackened severely. Particularly severe was the blackening of these varieties from Steuben County.

Relation of Date of Planting to Occurrence of Blackening

It was thought that if the date of planting influenced the date of maturity, the amount of blackening might also be affected. Pioneer

Rural variety of potatoes was planted on the 6th and 20th of June, 1940, at Ithaca, N. Y. The dates of maturity were approximately the 20th of September and the 5th of October, respectively. The results presented in table 2 show that the four samples taken from those planted on the 6th of June matured under a mean temperature 6° F.

TABLE I.—*Rating on blackening and the approximate mean temperature during the last month of growth for each of six varieties grown in four counties of New York.*

Variety	County Where Grown							
	Steuben		Tompkins		Suffolk		Wayne	
	Color*	Mean Temp.	Color	Mean Temp.	Color	Mean Temp.	Color	Mean Temp.
		Degrees F.		Degrees F.		Degrees F.		Degrees F.
Pioneer Rural	4	52	8	59	**	70	10	72
Grn. Mountain	6	52	6	59	10	70	10	72
Sebago	5	52	8	59	10	70	10	72
Cobbler	7	57	10	63	10	70	10	72
Chippewa	9	59	9	63	10	70	10	72
Earlaine	10	59	9	63	10	70	10	72

**Pioneer Rural variety not grown in Suffolk County.
*10 = white one-half hour after boiling, 1 = extreme blackening one-half hour after boiling.

higher than those planted on the 20th of June, and showed much less discoloration. The average color rating for the four samples from the earlier planting was 8.89, indicating much less blackening than is reflected in the average rating of 5.63 for the samples from the later planting.

In the similar experiment conducted in Steuben County, the planting dates were the 15th of May , the 1st and 15th of June, 1940; however, the date of maturity was not appreciably affected. The vines on all plots remained green until they were frozen the latter part of September. The calculated mean temperature of the last month of the growing season was 52° F. The average ratings on discoloration for 12 samples from each date of planting, arranged in order of planting dates, were 8.5, 8.0 and 8.0.

Relation of Date of Harvest to Occurrence of Blackening

In Steuben County samples of tubers from each of the above dates of planting were harvested on three dates,—16th of August, 7th of September, and 5th of October, 1940. At the first two dates of harvest, the tubers were immature. In table 3 are presented ratings

Table 2.—*Relation of date of planting, date of maturity, and mean temperature prior to maturity, to blackening of Pioneer Rural potatoes.*

Sample Number	Planted June 6, Mature Sept. 20		Planted June 20, Mature Oct. 5	
	Color	Mean Temperature Aug. 20 to Sept. 20	Color	Mean Temperature Sept. 5 to Oct. 5
1	9	61° F.	6	55° F.
2	8.5		5	
3	10		6.5	
4	8		5	
Average Color Rating	8.89		5.63	

on blackening and the mean temperatures for the three-week periods prior to harvest. The samples taken at the last harvest were mature by the 1st of October, and therefore, the mean temperatures for the period from the 7th of September to the 1st of October are given. At the first harvest, little or no discoloration was found in any of the samples; at the second harvest, there was considerable blackening; and, at the last harvest, the greatest amount of blackening was noted. The increase in severity of blackening parallels the decrease in temperature to which the tubers were exposed in the field.

The question may be raised whether the immaturity of the tubers from the first and second harvest might account for the presence of less blackening. In an attempt to answer this question, immature samples were harvested from potato plots at Gainesville, Florida on the 10th of April, and mature samples on the 15th of May, 1941. In this way the reverse of the temperature gradient of the

TABLE 3.—*Relation of date of harvest and mean temperature during three-week period prior to maturity to blackening of Pioneer Rural potatoes.*

Sample	Harvested 8/16/40		Harvested 9/7/40		Harvested 10/5/40	
	Color	Mean Temp. July 24 to Aug. 16	Color	Mean Temp. Aug. 16 to Sept. 7	Color	Mean Temp. Sept. 7 to Oct. 1
1	9.5	64° F.	8	55° F.	4	52° F.
2	9		7		8	
3	10		6		6.5	
4	10		7.5		5	
Average Color Rating	9.63		7.13		5.89	

previous experiment was obtained. As the tubers were becoming more mature the mean temperature was increasing. The results presented in table 4 show that in this case the immature tubers became discolored while the mature tubers did not, with the same relationship to temperature exisiting as in the previous experiment.

TABLE 4.—*Relation of date of harvest and mean temperature previous to date of harvest to blackening of Katahdin potatoes at Gainesville, Florida.*

Sample	Harvested April 10, 1941		Harvested May 15, 1941	
	Color	Mean Temperature March 15 to April 10	Color	Mean Temperature April 20 to May 15
1	8	62° F.	10	76° F.
2	8		10	
3	6.5		10	
4	7		10	
Average Color Rating	7.4		10	

Summary of Cooking Tests

In the course of these investigations 232 samples including 10 varieties grown in 1940 were examined for discoloration,—the total including samples grown at six different locations in New York. The conditions of light, moisture, soil type, nutrition, and temperature under which these samples grew and matured varied widely. The color ratings were grouped and averaged according to the temperatures under which the samples matured. These results appear in table 5. In spite of such variations among the samples as caused by varietal and environmental conditions, little or no blackening was found in samples which matured at temperatures of 70° F. or higher, and blackening was by far the most severe when the tubers matured at temperatures below 60° F.

TABLE 5.—*Summary of the effect of temperature on blackening of samples taken during 1940 season.*

Temperature Range	Number of Samples	Average Color Rating
50° - 60° F.	113	6.56
60° - 70° F.	60	8.61
70° - 80° F.	59	9.91

Relation of Temperature in Storage to Blackening

In previous experiments it had been shown that storage temperatures of 32°, 40°, 50°, and 70° F. did not markedly affect the severity of blackening. However, when it appeared that there was a positive correlation between relatively high temperature in the field and the absence of discoloration in the tubers, it was decided to store tubers at temperatures higher than those of the previous experiments. On the 4th of February, 1941, 150 tubers grown in Steuben County, and known to blacken severely, were divided into three 50-tuber samples which were placed at temperatures of 68°, 86°, and 104° F. The 104° F. temperature was maintained in a force-draft oven and the continual removal of CO_2 (carbon dioxide) and the supplying of O_2 (oxygen) was probably responsible for the fact that the tubers did not develop blackheart until after 3 days. Undoubtedly those tubers at 104° F. lost more moisture than those at lower temperatures and with no forced draft. Seven-tuber samples for cooking tests and chemical analyses were removed from each storage at the end of 12 hours, and at 24-hour intervals for the next four days. At the end of the 4½ day period, the remaining tubers in each of the treatments were returned to 50° F. where they remained for seven days, after which time cooking tests were again made. The seven-tuber samples were halved longitudinally and one-half of each tuber was used for cooking tests and the other one-half for analyses. The seven half-tubers saved for analyses were halved in the opposite direction and the stem end halves used for preparing samples for analysis for sugars and phenolic compounds.

The effect of relatively high temperature on the severity of blackening is shown in table 6. At 104° F. the amount of discoloration progressively decreased to such an extent that after 4½ days the cooked sample was given a rating of 9 and the amount of blackening was estimated to be about one-twentieth of that present before treatment. This experiment was repeated several times with similar results. In some cases practically no blackening was found after 1 and 2-day treatments. The boiled tubers which had been stored at 104° F. showed more yellowing; however, the stem-end blackening practically disappeared. Storage at 86° F. reduced the amount of darkening, but not as much nor so rapidly as did they at the higher temperature. The results also show that the differences in amount of discoloration in the treated samples still existed after storage for 7 days at 50° F.

TABLE 6.—*Relation of storage at relatively high temperatures for short periods to severity of blackening.*

Duration of Storage in Hours	Color Ratings		
	Storage Temp. 68° F.	Storage Temp. 86° F.	Storage Temp. 104° F.
12	5	5.5	6.5
36	5	6	7.5
60	4.5	6	8
84	5	6.5	8
108	5	6.5	9
Treatments stopped and samples returned to 50° F. Color rating 7 days later.	4.5	7	8.5

Because of the fact that the development of a yellow color in the cooked tissue paralleled the decrease in the amount of blackening, it was thought possible that an increase in the amount of reducing sugar might have inhibited the oxidation of phenolic compounds. The results presented in table 7 do not support this idea. The temperature treatments caused no appreciable change in the per cent of reducing sugars. However, the tubers stored at 104° F. almost doubled in total sugar, indicating an increase in the per cent of sucrose. It does not seem likely that this increase in sucrose was instrumental in preventing discoloration.

TABLE 7.—*Relation of storage temperatures to percentages of total and reducing sugars in raw tubers on basis of fresh weight.*

Hours in Storage	Storage Temp. 68° F.		Storage Temp. 86° F.		Storage Temp. 104° F.	
	Total Sugars	Reducing Sugars	Total Sugars	Reducing Sugars	Total Sugars	Reducing Sugars
12	.76	.46	.65	.32	1.07	.55
36	.71	.24	1.15	.55	.94	.32
60	.64	.34	.72	.48	.96	.37
84	.73	.51	.60	.24	1.14	.45
108	.74	.43	.73	.42	1.38	.43

According to Raper (16), the oxidation of tyrosine by tyrosinase involves the formation of 3, 4 dihydroxyphenylalanine (dopa), and that the latter compound is auto-oxidizable to melanin. It was thought that possibly dopa might be the precursor of other black pigments and its concentration in the tubers related to blackening. Dopa was estimated, using a color reaction suggested by Arnow (1), and using catechol as the standard. The results are calculated as per cent dopa on the basis of fresh weight. Tyrosine was estimated according to the method of Folin and Marenzi (5) and also is expressed as per cent of fresh weight. The results presented in table 8 show that neither the amount of tyrosine, nor of dopa, was appreciably altered by storage at relatively high temperatures, indicating that in this experiment the concentration of neither compound was directly related to blackening. It may be possible, however, that with hydroxybenzene compounds present, whether or not they result in subsequent blackening, depends on the hydrogen ion concentration of the tissue.

Relation of Storage Temperatures to Hydrogen Ion Concentration of Tubers and Blackening after Boiling

Tubers which are susceptible to blackening after boiling blacken first and more intensively at the stem end than in the remaining portion of the tuber and the hydrogen ion concentration of the stem end is lower than the remainder of the tuber. Tubers which darken after boiling in water can be prevented from turning black by boiling in an acid medium or by storage at 100° F. for 3 days; hence, the hydrogen ion concentration of the tissue of tubers was determined after storage at various temperatures.

Table 8.—*Relation of storage temperature to percentages of tyrosine and dopa in raw tubers on basis of fresh weight.*

Hours in Storage	Storage Temp. 68° F.		Storage Temp. 86° F.		Storage Temp. 104° F.	
	Tyrosine	Dopa	Tyrosine	Dopa	Tyrosine	Dopa
12	.034	.010	.034	.011	.030	.008
36	.030	.009	.028	.012	.028	.009
60	.029	.011	.029	.010	.030	.011
84	.030	.010	.028	.012	.032	.012
108	.033	.012	.029	.008	.038	.010

A glass electrode was used to determine the pH of the tissue. Lengthwise strips of tissue were removed from a number of tubers. One hundred gram samples were ground in a power liquidizer with 100 c.c. of water. Additions of water to the sample during grinding did not change the pH of the sample.

Rate of respiration was obtained of tubers stored at 34°, 52°, 68°, 80°, 90° and 100° F. by the method described by Platenius (15).

The redox potential of the tissue of tubers which had been stored at 80°, 90°, and 100° was obtained at various times during the storage period. These data were very inconsistent within treatments and are not presented here.

The respiration and pH data are presented in table 9.

The pH of the tubers stored at temperatures below 90° did not change during the course of these experiments. At 90° and 100° storage, however, the tuber tissue increased in acidity. Likewise, only those tubers which had been stored at 90° and 100° decreased in intensity and extent of blackening after boiling.

The respiration data show that the amount of oxygen absorbed increased with an increase in storage temperature up to 90° F. Between 90° and 100°, the amount of oxygen absorbed remained about the same.

TABLE 9.—*Rate of respiration and change in pH of tubers stored at various temperatures.*

Storage Temperature	Time in Storage	(Moles per 1000 Gms. Tuber per Hour)		$CO_2:O_2$ Ratio	pH
		CO_2 Respired	O_2 Absorbed		
° F.	Hours	10^{-5}	10^{-5}		
34	41	1.46	1.26	1.16	6.16
	81	1.19	1.10	1.08	
	134	1.04	1.01	1.03	
52	20	2.86	2.20	1.30	6.14
	41	2.56	2.14	1.19	
	81	2.82	2.54	1.11	
	134	2.48	2.40	1.03	
68	20	5.44	4.78	1.14	6.12
	41	5.16	4.92	1.05	
	81	4.4	3.92	1.12	
	133	3.8	3.56	1.07	
80	22	6.35	5.9	1.08	6.19
	40	6.74	6.5	1.04	6.14
	57	5.58	5.1	1.09	6.07
	76	5.21	4.67	1.12	6.19
90	22	8.79	6.19	1.42	6.19
	40	10.9	9.44	1.16	5.90
	58	8.5	7.04	1.21	5.95
	76	...	...	...	5.95
100	22	...	...	...	6.19
	43	12.12	7.87	1.54	5.87
	58	12.18	8.81	1.38	5.58
	76	9.86	5.34	1.84	5.60

The evolution of carbon dioxide increased with increase in temperature from 34° to 100° F. The increase in the $CO_2 : O_2$ ratio was significant only at the 90° and 100° temperatures. The high ratio of $CO_2 : O_2$ at the high temperatures indicates that some anaerobic respiration occurs. When anaerobic respiration occurs there are a number of incomplete oxidation products formed which may include acetic, formic, oxalic, proprionic and other organic acids in addition to carbon dioxide. The decrease in pH of the tissue occurs probably as a result of the accumulation of some of these acids.

Smith (19) and others since that time have shown that tubers treated with ethylene chlorohydrin have a greatly accelerated rate of respiration. Therefore, tubers treated with ethylene chlorohydrin and subsequently stored at high temperatures will have a higher respiratory rate resulting in a deficiency of oxygen within the tubers and a larger amount of anaerobic respiration than in tubers untreated and stored at the same temperature. This possibly would result in a greater accumulation of organic acids, a lowered pH of the tuber. and a decrease in blackening. It was thought that possibly, with ethylene chlorohydrin treatment, subsequent storage temperatures could be lower than 90° to 100°, yet result in decreased blackening of the tubers.

TABLE 10.—*Effect of temperature and ethylene chlorohydrin treatment on pH and color of boiled potatoes.*

Treatment and Storage Temperatures		Reaction of Tuber Tissue (pH)	Color of Boiled Tubers
Untreated	50°	5.99	Slightly blackened
Ethylene Chlorohydrin	50°	6.04	Slightly blackened
Untreated	70°	5.95	Slightly blackened
Ethylene Chlorohydrin	70°	6.20	Moderately blackened
Untreated	100°	5.92	Slightly blackened
Ethylene Chlorohydrin	100°	5.25	White

Tubers of the Smooth Rural variety were treated with ethylene chlorohydrin vapor (1 cc. per liter of air space at 70° F. for 24 hours). Others were untreated but kept at the same temperature. Some lots of each were then placed at 50°, 70° and 100° F. for two days. The pH of the tissue was determined and the color of the boiled tubers observed at the end of the two-day period. The results are shown in table 10.

At 50° and at 70° the pH of the tubers which had been treated with ethylene chlorohydrin was slightly higher than that of untreated tubers, although the difference at 50° is not significant. Apparently the increase in respiration of ethylene chlorohydrin treated tubers at 50° and 70° is largely aerobic, probably resulting in an increased concentration of carbon dioxide within the tubers. This increase in carbon dioxide may be sufficient to increase slightly the pH of the tuber tissue. The difference in pH between the treated and untreated tubers at 50° apparently was not sufficient to result in any differences in blackening of the tubers. At 70°, however, the pH change was greater and the treated tubers blackened to a greater extent than the untreated. The increased rate of respiration of the treated tubers at 100° undoubtedly was accompanied by an increase in anaerobic respiration and the formation of organic acids. This accumulation of organic acids probably resulted in the low pH of the tuber tissue which was accompanied by an absence of blackening in the boiled tubers. Blackheart, however, was present in all tubers which had been treated with ethylene chlorohydrin and subsequently stored at 100° F.

Tubers of the Smooth Rural variety grown in 1940 and which had been stored until the 12th of August, 1941 at 50° F. were then placed in constant temperature compartments at 80°, 90° and 100° F. After 72 hours, one lot of tubers was removed from each compartment, pared and boiled. Tubers which had been stored at 80° F. blackened as much as those at 50° F. Tubers stored at 90° and 100° for 72 hours did not blacken when boiled. After 144 hours at 80° tubers still blackened after boiling, whereas those at 90° and 100° did not blacken. These latter tubers showed a tendency to remain hard longer during the boiling process than those stored at the lower temperatures.

In order to determine more accurately the minimum length of time necessary for potatoes to be stored at 90° to prevent subsequent blackening in old tubers, a large lot of the 1940 crop of Smooth Rural tubers was placed at 90° F. in September, 1941. These tubers had been stored at 50° F. ever since being harvested,—11 months earlier. It took approximately 6 hours for the interior of the tubers to reach the temperature of of the air in the compartment. At 8-hour intervals, lots of tubers were removed from the 90° temperature and boiled for determination of degree and extent of blackening. The following results were obtained:

Length of Storage at 90° F. (Hours)	Color of Tubers One-Half Hour after Boiling
0	Very black
8	Very black
16	Very black
24	Very black
32	Very black
40	Very black
48	Very black
56	Moderately black
64	Moderately black
72	Slightly black

A storage period longer than 72 hours for old tubers at 90° did not result in complete disappearance of the blackening as it did when the tubers were physiologically younger.

To determine whether the change in pH and degree of blackening after boiling induced by high storage temperature was temporary or permanent, lots of Smooth Rural tubers of the 1940 crop were placed at 100° for 3 days in August, 1941 and then removed to 50° storage for 6 weeks.The tubers blackened considerably and had a reaction of pH 6.16 before placing at 100° F. At the end of 3 days at 100° the reaction was pH 5.60 and the tuber did not blacken after boiling. After 6 weeks at 50° those previously stored at 100° F. had a reaction of pH 6.05 and blackened slightly but not to so great an extent as those stored constantly at 50° and with a reaction of pH 6.17.

Gas Storage Treatments

Since anaerobic respiration in the tubers would result in accumulation of certain organic acids and consequently in a decreased pH of the tissue, tubers of the Mesaba variety, which were known to blacken after boiling, were placed in an atmosphere and at a temperature which would be conducive to anaerobic respiration. Other gases and combinations of gases were employed that resulted in an increased pH of the tubers. The tubers were then boiled and observed for degree and extent of blackening. The length of storage under these conditions was 48 hours. The results of these studies are presented in table 11.

When the tubers were stored in nitrogen gas, the carbon dioxide produced was absorbed by NaOH (Sodium hydroxide) in the bottom of the container. There was a definite decrease in pH of these tubers within 48 hours. When stored very much longer than this the tubers decomposed. A high temperature was employed to hasten the effects of the gases since the high temperatures increase the rate of respiration whether aerobic or anaerobic. The decrease in pH was accompanied by an absence of blackening.

TABLE 11.—*Effect of storage temperatures and gases on pH and blackening of tubers after boiling.*

STORAGE GAS	TEMPERATURE DEGREES F.	pH OF TISSUE AFTER 2 DAYS STORAGE	DEGREE OF BLACKENING OF BOILED TUBERS
Air	50	6.25	Slight
Air	100	6.22	Slight
100% N_2 CO_2 absorbed	100	5.47	None
60% CO_2 40% O_2	100	6.38	Very black
60% CO_2 40% N_2	100	5.75	Grayish cast*

*Appears to be a different type of darkening.

Tubers stored in air at 100° F. for 2 days did not decrease in pH or in degree of blackening. Many of our other experiments show that at 100° F. the increase in acidity and the decrease in blackening usually occur between the second and fourth days of storage.

Carbon dioxide was mixed with several other gases as a storage medium (23). Thornton (23) found that when potatoes are stored in carbon dioxide with oxygen present, the tubers increased in pH. The above data corroborate his findings and also show that when the same percentage of carbon dioxide is mixed with nitrogen instead of with oxygen and tubers stored in it, that the pH of the tissue decreases.

When no oxygen or an insufficient amount is present anaerobic respiration occurs resulting in the formation of several organic acids. These acids may accumulate in sufficient quantity to offset the effects of the accumulation of CO_2 so that the resultant tissue is more acid rather than less acid. This probably is what has occurred at 100° F. with a combination of 60 per cent carbon dioxide and 40 per cent nitrogen gases. When stored in 100 per cent nitrogen with the carbon dioxide of respiration absorbed by NaOH the tubers became still lower in pH probably because carbon dioxide is not present to counteract some of the acidifying effects of the organic acids.

Effect of Hydrogen Ion Concentration of Boiling Medium on Blackening of Tubers

Since it was found that storing tubers at high temperatures and in certain gas concentration decreased the blackening after boiling and that the hydrogen ion concentrations of these tubers was increased, it was conceived that possibly the severity and extent of blackening could be altered by the use of various substances in the boiling medium. Tinkler (24) found that potatoes steamed over water containing a little ammonium carbonate were dark in color after cooking and if potatoes which normally blacken are cooked in water containing a little acetic acid, they were usually better than if cooked in tap water. Nutting and Pfund (13) also found that citric acid, lemon juice or vinegar in the cooking water reduced stem blackening.

(*Hydrochloric acid*) *HCl*. Tubers which blackened when boiled in water did not blacken when boiled in a solution of 0.1 N HCl. The starch of the tubers, however, appeared to have gelatinized considerably resulting in decreased mealiness. Solutons of 0.001 N HCl prevented blackening and did not gelatinize the starch as much as the 0.1 N solution. HCl solutions of 0,0001 N and less did not have much effect either on the blackening or texture of the tubers. This was probably because of the buffer action of the potato preventing an appreciable change in the pH of the tissue. The pH of the raw tuber before boiling in 0.0001 N HCl was 6.20 and after boiling it had the same hydrogen ion concentration.

Since it was desirable to test the relation of H^+ concentration of the cooking medium and of the cooked tuber to blackening, it seemed important to use chemicals with the same cations and anions with the exception of H^+ and OH^- ions in order to eliminate the possible effects caused by different ions. NaH_2PO_4 (Sodium dihydrogen phosphate) of pH 4.5 and Na_2HPO_4 (Disodium hydrogen phosphate) of pH 9.6 seemed desirable reagents for this purpose.

(*Sodium dihydrogen phosphate*) NaH_2PO_4. After boiling potatoes in buffer solution of .05M NaH_2PO_4 of pH 4.5 the tubers did not blacken. The color and texture of these tubers were similar to tubers which had been placed at 100° F. for 3 days.

(*Disodium hydrogen phosphate*) Na_2HPO_4. A buffered solution of Na_2HPO_4 of pH 9.6 caused an increase in the intensity of blackening but did not greatly gelatinize the starch of the tuber. Tubers of the Mesaba variety blackened very extensively when boiled in a solution of Na_2HPO_4 of pH 9.6. The tubers had a reaction of pH 6.61 after boiling. When boiled in a solution of KH_2PO_4 (Potassium dihydrogen phosphate) of pH 4.5 no blackening of the tubers resulted. The reaction of the tubers in this case was pH 5.85.

Sodium hydroxide (NaOH). Low concentrations of NaOH had no apparent effect on either darkening or texture of the tubers probably because of the buffer action of the tuber tissue preventing a significent change in the hydrogen ion concentration of the tissue. A 0.0001 N NaOH solution of pH 10.0 caused no change in degree of blackening. A 0.1N NaOH solution considerably gelatinized the starch and greatly increased the degree of blackening. These tubers, however, turned quite yellow at first and did not blacken until after several hours. The blackening appeared to be more of the blue-black shade than that appearing naturally.

In order to determine if OH ions would cause blackening to occur in tubers which did not normally blacken, tubers which when boiled in water showed no blackening, were boiled in NaOH solutions of various concentrations. These data are shown in table 12.

TABLE 12.—*Effect of concentration of NaOH in the boiling solution on blackening of tubers.*

NaOH CONCENTRATION	COLOR OF TUBERS AFTER BOILING
0	White
0.006 N	White
0.013 N	Very slightly blackened
0.026 N	Some yellowing followed by slight blackening
0.032 N	Medium blackened
0.064 N	Black Turned yellow, then black
0.096 N	Very black

Several hours elapsed before the tubers which had been boiled in the 0.013 N solution became blackened.

When the boiling medium was made more alkaline by several other reagents the results were as shown in table 13.

TABLE 13.—*Effect of OH ion concentration in the boiling solution on blackening of tubers.*

BOILING MEDIUM	COLOR OF TUBERS AFTER BOILING
Water	White
Na_2CO_3	Very blue-black
Na_2HPO_4	Slightly blackened
$NaHCO_3$	Very blue-black
NH_4OH	Slightly blackened
NH_4OH*	Moderately black

*Further added as it boiled out.

Tubers boiled in the solutions of Na_2CO_3 (Sodium carbonate) and $NaHCO_3$ (Sodium bicarbonate) did not blacken until several hours after boiling.

EFFECT OF BOILING TUBERS IN BASIC, ACIDIC, REDUCING, AND OXIDIZING SUBSTANCES ON SUBSEQUENT BLACKENING

Since it has been shown that boiled tubers turn black after exposure to air and are prevented from blackening by confining in an oxygen-free atmosphere (24) it seemed likely that the oxygen in the air was necessary to develop the blackening. Tinkler thus showed that this blackening is an oxidation process. Hence, potatoes that blacken when exposed to the air after boiling probably would turn black while being boiled in an oxidizing solution. Conversely, tubers boiled in a reducing solution possibly would be prevented from blackening or retarded in this process even after exposure to air

$KBrO_3$ (Potassium bromate) was employed as an oxidizing agent and SnC_2O_4 (Stannous oxalate) as a reducing agent. Potatoes were boiled in these solutions alone as well as when combined with Na_2HPO_4 or NaH_2PO_4. SnC_2O_4 brought to the same pH as the tuber was used because it supplies a small but constant amount of reducing material because of its relatively low solubility in water. Neither the SnC_2O_4 nor the $KBrO_3$ solutions had any appreciable effect on the texture of the tubers. The results of the effects on blackening are shown in table 14.

TABLE 14.—*Effects of boiling tubers in reducing and oxidizing agents at various hydrogen ion concentrations on subsequent blackening of the tubers.*

BOILING SOLUTION	BLACKENING OF TUBERS AFTER BOILING
SnC_2O_4 (brought to same pH as tuber)	None
$SnC_2O_4 + Na_2HPO_4$	Very slight
NaH_2PO_4	Slight
Water	Moderate
Na_2HPO_4	More than with water
$KBrO_3 + NaH_2PO_4$	Moderate intensity, but extensive
$KBrO_3$	Very extensive, brown-black
$KBrO_3 + Na_2HPO_4$	Very black with brownish tinge

When tubers which had been boiled in water were further boiled in 0.05N HCl the black color disappeared and starch was gelatinized to some extent. Tubers which had been boiled in a solution of NaH_2PO_4 turned blacker when boiled in 0.05N NaOH and exposed to air for several hours. Tubers which blackened when boiled in tap water became white after being boiled in a solution of SnC_2O_4. However, tubers which had been boiled in SnC_2O_4 solution did not turn black when subsequently boiled in $KBrO_3$ solution. The blackening caused by boiling in $KBrO_3$ solution appeared in the stem ends of the tubers first and later over the entire tuber when a strong solution was used. There was a brownish tinge to this type of blackening.

More work should be done on the relationship between reducing and oxidizing agents before their effects on blackening can be stated. It may be possible that all reducing agents prevent tubers from blackening and that oxidizing agents increase the blackening. A strong reducing agent which does not precipitate in basic solution should be used to make comparisons between solutions of various hydrogen ion concentrations as to its effects on blackening.

OXIDATION-REDUCTION POTENTIALS OF TUBER TISSUE

One hundred grams of tubers were ground in a liquidizer with 100 c.c. of water. A platinum electrode in the tuber tissue served as one-half cell and connected with a calomel half cell by an agar-KCl salt bridge.

The oxidation-reduction potential was very inconsistent and time variable. There was no consistent relationship between the oxidation-reduction potential and blackening of the tubers after boiling.

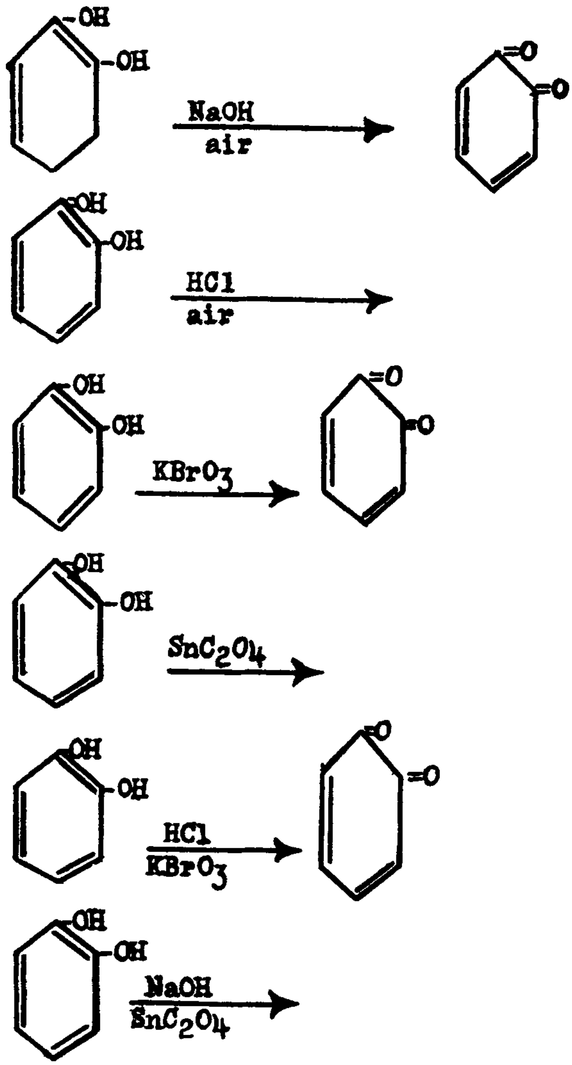
Reducing Properties of Tubers

Lengthwise segments of tissue were removed from a number of tubers and 100-gram samples ground for 2 minutes at high speed in an electric liquidizer with 50 c.c. of a mixture of 2 per cent H_3PO_4 (Phosphoric acid) and 2 per cent H_2SO_4 (Sulphuric acid). The acid inactivated the enzymes in the tissue and prevented formation of pigments by enzymatic action. Ascorbic acid is stabilized by this acid mixture.

Using 50 c.c. portions at a time, 300 c.c. of the mixture was used to quantitatively transfer the tuber tissue to a 500 c.c. graduate. After standing for one hour the mixture was filtered and aliquots used for analyses. The liquidizer beat air bubbles into the mixture which adhered to small potato particles causing them to rise to the surface. With the use of a large pipette the clear solution was removed and filtered by suction. Twenty-five c.c. of this solution was diluted with 50 c.c of water, 25 c.c. of 0.01 KIO_3 (Potassium iodate) and 5 c.c. concentrated HCl added immediately and the solution allowed to stand for 15 minutes. Five c.c. of 5 per cent KI (Potassium iodide) was added and the solution titrated with 0.01 N $Na_2S_2O_3$. (Sodium thiosulphate). The time of the KIO_3 oxidation is very important. There was no difference between tubers stored at 50° and those stored at 100° F. for 3 days in reducing power toward KIO_3.

Possible Compounds Responsible for Blackening

From the results of the experiments mentioned previously in this paper, one may conclude that potatoes which would blacken after boiling in water can be prevented from blackening by boiling in solutions such as hydrochloric acid and stannous oxalate. Increased blackening can be obtained by boiling tubers in solutions such as sodium hydroxide or potassium bromate. Blackening also results when the tubers are boiled in a solution of hydrochloric acid and potassium bromate but no blackening occurs when they are boiled in a solution of sodium hydroxide and stannous oxalate. There are certain organic compounds which are quite common in potatoes which may react with the above chemicals resulting in the color changes noted. These compounds are hydroxybenzenes.



Taking orthodihydroxybenzene as an example of this group, the following indicates their possible reactions:

(orthobenzoquinone)

Not so much tendency to form quinoid structure.

No tendency to form quinone.

No tendency to form quinone.

The quinone compounds are usually colored substances. The data obtained substantiate the possibility of the formation of such quinoid structures, especially since the reactants are present in potatoes.

Discussion

From the results it is apparent that temperature is related to the occurrence of blackening. Tubers maturing under low temperatures (50°-60° F.) were found likely to blacken whereas those maturing at higher temperatures (70°-80° F.) seldom showed any discoloration. High temperatures in storage for 3 to 4 days prevented blackening. The question which arises, therefore, is what changes take place in the tubers, because of high temperature, which decrease or prevent blackening? It would be logical to assume that the effects of temperature on the blackening mechanism would be similar for tubers maturing in the field and in storage. The effect of temperature in storage was found not to be related to the amounts of tyrosine or dopa, and other results (unpublished) indicate that high concentrations of dopa are not necessarily associated with severe blackening. The pH of the tuber, however, may influence this reaction. It was thought possible that high temperatures might reduce dopa to tyrosine but the data obtained do not substantiate this.

In the light of the results of the present paper, together with those obtained by others, the authors offer suggestions concerning the effect of temperature on one possible mechanism involved in the production of the black pigment. Figge (4) reports that the production of melanin from tyrosine by tyrosinase is regulated partially by the oxidation-reduction potential. It is possible that the formation of other black pigments from hydroxy compounds also is regulated by the oxidation-reduction potential. Fredrich (6) found that tubers stored at warmer temperatures showed lower oxidation-reduction potentials than those stored at cooler temperatures. It seemed possible, therefore, with a lower oxidation potential caused by the high temperatures, that either the precursors of the black pigment would not be formed or, if formed, would not be further oxidized. In the present studies, however, no consistent relationship was found between the oxidation-reduction potential and blackening of the tubers after boiling. It is suggested that in future studies the effect of temperature on the presence of the oxidized and reduced forms of glutathione, ascorbic acid, cytochrome, iron, and copper be studied. At high temperatures and with O_2 limiting, these substances would tend to occur in the reduced state and thus not aid in oxidative reactions.

The possible rôle of the phenolic compounds in respiration and formation of black coloration might also be considered. According to the theories on the mechanism of respiration of Palladin (14) and Blackman (2), phenolic compounds act as the principal hydrogen acceptors in the respiratory process and molecular O_2 is used primarily in oxidative regeneration of these hydrogen acceptors. In more recent studies, Boswell and Whiting (3) report that a system, involving phenolic compounds as the hydrogen acceptors, accounts for two-thirds of the total respiration in potato tissue. Since at high temperatures respiration would proceed at a faster rate, the amount of O_2 in the tissues would become proportionately less at high temperature. Magness (7) found almost twice as much O_2 in potato tissue at 11° C. as at 22° C. Thus, at high temperatures and with O_2 limiting, phenolic compounds would tend to remain in a reduced state. With the phenolic compounds in such a reduced condition and with oxygen limiting it seems probable that they would tend not to be oxidized to the black substance in the boiled potatoes, assuming that phenolic compounds are precursors of the black color.

Both of the above hypotheses depend upon high temperatures to place in a reduced condition the various compounds which form the total oxidation-reduction system, as well as to reduce the phenolic compounds which may be the precursors of the black pigment.

The present studies show that the black color does not appear or, if present, will disappear when the tuber tissue has a high hydrogen ion concentration. Conversely, the black color appears or increases in intensity in tissue of lower hydrogen ion concentration. Almost without exception, the stem ends of tubers which blacken have a lower hydrogen ion concentration than the opposite ends of the same tubers. Also, this type of blackening always occurs first and in greater intensity at the stem end than in the apical end of the tubers. These color reactions appear regardless of whether the pH differences occur as a result of growing conditions, storage temperatures, certain gas storage, or boiling media. It is pointed out that possibly the hydroxybenzene compounds of potato tubers are the precursors of the black coloration.

It is not assumed from the evidence obtained in these experiments that among the many environmental factors affecting growth and composition of plants, temperature is the sole factor influencing the occurrence of blackening. It is believed that interactions between light and nutrition may also be important. The results are complicated by the fact that as temperatures were decreasing, days were becoming shorter, light becoming less intense, quality of the light changing, and the per cent of sunshine decreasing. However, as shown in previous work, altering light intensity, widely varying the types of nutrition, varying soil moisture and reaction, had little effect on blackening and it is now believed that any effect they might have would be expressed principally at relatively low temperatures.

The results warrant some suggestions to growers in regions where the problem of blackening is serious. Those who have had to contend with the problem have been puzzled about the cause of blackening and have been seeking means of preventing it. The use of varieties that mature earlier than Pioneer Rural, Green Mountain, and Sebago, wherever feasible, is suggested. Earlier planting dates are suggested where the date of maturity can be influenced by the date of planting. Any means of maturing the potatoes before low temperatures prevail in the fall should tend to reduce blackening. Storing potatoes at 90°-100° F. for 3 or 4 days within several weeks preceding their consumption will greatly reduce or prevent blackening and will prevent its recurrence for several weeks, even though they are subsequently stored at low temperatures.

Summary

The results of the authors' previous work on the problem of blackening of boiled potatoes are reviewed briefly. Nutrition, soil moisture, soil reaction and light had been found to exert little effect on the occurrence of stem-end blackening with the exception of their effect on time of maturity.

In the 1940 experiments, 232 samples of potatoes grown in six different regions of New York State were examined for discoloration after boiling. In spite of variations among the samples caused by variety and environmental conditions, little or no blackening was found in samples which matured under mean temperatures of 70° F. or higher, and samples which matured under mean temperatures of 60° F. or less usually blackened. The regions in which the problem of blackening was most severe had the lowest mean temperatures during the time when the tubers were maturing.

In regions where the problem of blackening is serious the use of earlier maturing varieties than the Pioneer Rural, Green Mountain, and Sebago, wherever feasible, is suggested. Earlier planting dates are suggested where the date of maturity can be influenced by the date of planting. The results indicate that any means of maturing the potatoes before low temperatures prevail in the fall will tend to reduce the amount of blackening.

Exposure of tubers, known to blacken severely, to temperatures of 100° F. for 3 to 4 days prevented practically all blackening in the tubers when they were boiled. Storage temperatures of 90° for several days also reduced the amount of blackening. Analyses for total sugars, reducing sugars, tyrosine, and dihydroxyphenylalanine were made, but the results did not help to explain why high temperatures in storage reduced the amount of blackening.

A discussion of the possible effect of temperature on the blackening mechanism is presented.

A high correlation exists between the hydrogen ion concentration of tuber tissue and the degree of blackening. The pH of tubers which blacken is higher at the stem end than at the apical end and the blackening occurs first and to a greater degree at the stem end than at the apical end. The hydrogen ion concentration of tubers can be increased and blackening decreased by storage at high temperatures, storage in certain gases or boiling in certain acidified solutions. The hydrogen ion concentration can be decreased and blackening increased by storage in certain gases or boiling in certain alkaline solutions.

There was no consistent relationship between the oxidation-reduction potential of the tissues and subsequent blackening of the cooked tubers. No differences in reducing power toward KIO_3 were found among tubers stored at 50° and those stored at 100° F.

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