[Reprinted from FOOD TECHNOLOGY, 1952, Vol. VI, No. 3]

Behavior of the Anthocyanin Pigments in Concord Grapes During Heat Processing and Storage[a]

L. V. L. SASTRY AND R. G. TISCHER[b]
Agricultural Experiment Station, Iowa State College, Ames, Iowa

(Manuscript received August 18, 1951)

The anthocyanin pigment concentrations of grape juice samples obtained on processing Concord grapes in glass jars at temperatures of 170° F., 210° F. and 250° F. for process times varying from 5 to 100 minutes were determined after storage of the samples for 0, 2, 4, and 6 months at 70 ± 0.5° F. The treatments during storage included the presence of air or nitrogen in the headspace of the container, or storage of the juice samples in the dark or exposed to light when the samples were preserved by pasteurization or addition of toluene. The period of storage exerted the maximum influence on the deterioration of the pigment. Both the mono- and the diglucosides of the anthocyanidin decreased on storage. Decrease in the pigment concentration on processing and storage was more striking in the case of anthocyanin chloride solutions than in grape juice samples.

Grape juice is one of the most popular fruit beverages and its production in the U. S. during 1949-50 was about 2.8 million cases. The color of the clear fruit juice is ranked as an important factor for judging quality. The intense color of Concord grape juice has been attributed principally to the presence of the water-soluble anthocyanin pigments, modified to some extent by the other components of the juice.

Considerable work has been reported on the variation in the color content of grape juice with different processing methods and storage. Hartmann and Tolman *(4)* pointed out that for *Vitis labrusca* varieties a temperature of 150° F. (65.5° C.) should not be exceeded during the heating process as an excessive amount of tannin is extracted from the seeds at high temperatures. Using the grapes of Petite Sirah (a *V. vinifera* variety of red grapes grown in California), Joslyn, Farley and Reed *(5)* observed that the color obtained by heating for 5 minutes at 158° F. matched with that obtained for 18 minutes at 140° F. (60° C.), about 40 minutes at 122° F. (50° C.), 60 minutes at 104° F. (40° C.), and about 2 minutes at 170° F. (76.5° C.). Amerine and DeMattei *(1)* studied the effect of respiration, various gas treatments, and heat on the rate of extraction of the color from uncrushed carignane grapes. No conclusive results on the color extraction were noted due to storage under gas using oxygen, nitrogen, ethylene, and carbon dioxide prior to processing. The best results for color extraction were obtained by dipping the whole grapes in hot water at 203° to 208° F. (95° to 97.5° C.) from 1 to 3 minutes before crushing and processing. Tischer *(10)* described a high temperature process for

[a] Work on this project was made possible through a grant from the Council Bluffs Grape Growers Association, Council Bluffs, Iowa.

[b] Journal Paper No. J-2001 of the Iowa Agricultural Experiment Station, Ames, Iowa Project No. 1051.

the extraction of Concord grape juice in the absence of air using a steam retort. It was reported that no serious losses in juice quality were indicated in the different juices as evidenced by chemical analyses and triangular difference palatability tests.

In studying the visual color changes occurring in Concord grape juice during storage, Tressler and Pederson *(11)* observed that these changes could be reduced by eliminating air from the bottle and further noted that light had a detrimental effect. Pederson and Tressler *(7)* noted that on storage the color of the grape juice changed from reddish purple to a brick red and then to a dull brown simultaneously with clouding and sedimentation. Powers and Esselen *(8)* stated that the effect of light was only of minor importance in the deterioration of glass packed foods in comparison with the deteriorative changes caused by heat and oxygen. Nebesky, Esselen, McConnell and Fellers *(6)*, studying the effects of various factors on the stability of the color in a variety of fruit juices during storage, observed that cherry, grape, and tomato juices were much more stable and exhibited less color deterioration than strawberry, raspberry, and currant juices. Working with purified pigment solutions from strawberries and currants, they observed that high storage temperatures and the presence of oxygen accelerated color deterioration with both pigments, while a low storage temperature preserved the color.

The present study was made to determine the effects of various factors on the stability of the colored components in grape juice prepared from grapes processed for different lengths of times and at different temperatures. The behavior of the principal color components of grape juice on storage under controlled experimental conditions was also investigated.

EXPERIMENTAL PROCEDURE

A cylindrical retort, the temperature of which was accurately regulated, was used for processing. A multi-point, strip-chart, potentiometer type temperature recorder recorded the temperatures inside and outside the containers during the process. Molded plastic, copper-constantan thermocouples, of 2-inch length, were employed as the sensing elements.

Fresh ripe grapes were harvested in the morning from the horticultural farm at Iowa State College, and were processed within a period of 24 hours. Preliminary processing studies indicated that in general, increased color concentrations were obtained with high process temperatures and long process times. The main experimental design was based on these preliminary studies. The process times were chosen such that their logarithmic values were approximately in an arithmetic progression, and the process temperatures were likewise fixed on the assumption that the effect of process times was logarithmically related to process temperatures. Process temperatures of 170° F. (76.5° C.), 210° F. (99° C.), and 250° F. (121° C.) were employed for process times ranging between 5 and 100 minutes.

The order in which the processes were conducted was randomly chosen to minimize experimental errors arising from the temporary storage of the raw product at room temperature before processing. Further, 3 separate replications of each of the processes were done using crushed grapes filled into glass jars of 1 pint capacity. Processing was done by immersing the glass jars completely in water in the retort. The juice samples were collected by centrifuging the cooked mass in a basket centrifuge for 10 minutes at 1500 r.p.m. with cheese cloth serving as a filter. The juice samples were filled into 8 test tubes leaving a headspace of about 1 inch. Toluene was added to 4 test tubes in each sample to prevent fermentation. The remaining 4 test tubes in each sample were pasteurized at 180° F. (82° C.) for 1 minute. In each of the 2 groups of 4 tubes, nitrogen was filled into the headspace of 2 of the pasteurized samples and 2 of the toluene treated samples. In the resulting 4 groups of 2 tubes each treated alike, 1 tube in each group was exposed to light and the other was left in the dark at a constant temperature of 70° ± 0.5° F. (21° C.).

Use of this split-plot factorial arrangement made it possible to have three paired treatment comparisons in each set of tubes as shown in Table 1.

TABLE 1
Split-plot arrangement of three treatments for eight-tube samples of processed Concord grape juice

Tube no.	Paired treatment comparisons		
	Pasteurization (P) vs. toluene (T)	Nitrogen (N) vs. air (A)	Light (L) vs. dark (D)
1	P	N	L
2	P	N	D
3	P	A	L
4	P	A	D
5	T	N	L
6	T	N	D
7	T	A	L
8	T	A	D

Processing at 210° F. (99° C.) and 250° F. (121° C.), and storage studies on the pigment concentrations in the juice samples were also conducted with grapes stored in the refrigerator at 40° F. (4.5° C.) for 1 month. Processing and storage studies on the pigment concentrations were also conducted with clear pigment solutions dissolved in McIlvaine's buffer solution of pH 3.4 (contained in glass jars).

The investigation also included a study of the behavior of the purified anthocyanin pigment on processing and storage. For this purpose the pigment solution was prepared by dissolving 4.25 g. of the anthocyanin pigment, isolated as the chloride from Concord grapes by the method suggested by Anderson *(2)*, in 2 liters of McIlvaine's buffer solution of pH 3.4. Equal volumes of 25 ml. of the pigment solution were processed in glass jars for times and temperatures identical with those used for processing the grapes. The concentrations of the clear pigment solutions before and after the storage periods of 2 and 4 months were determined after diluting 1 ml. of the clear solution to 50 ml. with 2% HCl.

For work involving paper chromatography, Whatman No. 4 filter papers (strips 22.5" length, 0.75" width) were used. Initially the solvent used for separation was allowed to flow to the top of the paper strips by capillary action. This procedure facilitated the removal of some yellow colored impurities present in the paper. As these impurities moved along with the solvent front, the ends of the strips containing the colored impurities were cut off. As suggested by Bate-Smith *(3)* the upper layer of the butanol, acetic acid, and water mixture (4 : 1 : 5 by volume) was used as the partitioning solvent. Resolution was affected by ascending chromatography using a few drops of the acidified grape juice sample, the number of drops depending on the pigment concentration of the juice sample. Three consecutive runs were made on each sample, each of 4 hours' duration at a temperature of 70° F. ± 0.5° F., the paper being air dried after each run. This procedure seemed to give a better resolution of the individual bands than when separation was made using a single pass of 24 hours' duration.

The anthocyanin concentrations of the pigment solutions were determined by utilizing the property of the clear pigment solutions of pH 1.0 or less that the optical density observed at a wavelength of 515 mμ, bears a linear relationship to the pigment concentration over a limited range. Interference due to presence of soluble brown pigments formed on storage; the thermal breakdown products; and the grape juice components, such as sugar, pectin, tartaric, malic, and tannic acid, were determined. The optical densities of the clear solutions observed at a wavelength of 515 mμ and at pH 1.0 provided a fair indication of the amount of anthocyanin pigment in grape juice and in solutions of pure pigment isolated from grapes when the samples were suitably diluted.

RESULTS AND DISCUSSION

The yields of juice obtained from fresh grapes and from grapes stored at 40° F. (4.5° C.) for 1 month are included in Table 2. An examination of the data shows that while there is a progressive increase in the yields with fresh grapes with increasing process times at

TABLE 2
Yields of grape juice resulting from processes at 170°, 210°, and 250° F.

Temperature	Time	Average yield
° *F.*	*minutes*	%
170	31.5	53.6
170	39.7	54.9
170	50.0	60.0
170	63.0	60.5
170	79.0	61.8
210	12.5	62.6
210	15.8	65.4
210	20.0	62.3
210	25.0	66.2
210	31.5	61.9
210 [c]	31.5	63.8
210 [c]	63.0	63.9
210 [c]	100.0	67.8
250	5.0	62.5
250	6.3	65.8
250	7.9	62.3
250	10.0	67.7
250	12.5	73.8
250 [c]	12.5	68.5
250 [c]	31.5	72.7
250 [c]	63.0	79.6
250 [c]	100.0	79.1

[c] Processes on grapes stored at 40° F. for one month.

170° and 250° F., no such trends are noticeable with processes at 210° F. The data also indicate that with grapes stored at 40° F. for 1 month the yields were higher when the processing was at 250° F., compared to the yields obtained after processing at 210° F. No differences in yields were realized when the time of processing was increased beyond 63 minutes at a process temperature of 250° F.

The concentrations of pigment in grape juice and in purified pigment solutions resulting from processing at 170°, 210°, and 250° F. are presented in Figure 1 in terms of optical densities. From these data it is evident that the purified pigment solution was partially destroyed during processing. In addition, the rate of destruction was proportional to the time and temperature of the process. The values presented for the optical density of grape juice indicate that when grapes were processed at 170° F. under the conditions imposed in this investigation, no progressive increase in concentration of the color was obtained by increasing the process time from 31.5 minutes to 79 minutes. When the processing was done at 210° F. the color content increased progressively when the process time was increased from 12.5 to 31.5 minutes, while a further increase in process time up to 100 minutes did not bring about an increase in the pigment concentrations of the juice samples. The concentration of pigment in juice samples processed at 250° F. for the relatively short times usually considered necessary at this temperature was similar to that found for the two lower temperatures but exhibited no clear influence of time of processing. For times longer than 63 minutes at 250° F. the pigments were brownish red and the juice was slightly turbid while those processed for times less than 63 minutes were red in color. The juice samples prepared by processing at 170° F. were purple. This difference in color may be due to the presence of the other components present in the juice samples which modify the color due to the anthocyanin pigment. This assumption was made as the pH of the different samples did not vary enough to suggest pH as the cause for differences in color.

The shapes of the absorption curves of the juice samples determined over a range of 400-600 mμ using a Coleman spectrophotometer exhibited maxima at 515 mμ for samples adjusted to pH 1.0 with the exception of the juice samples prepared with process times of more than 63 minutes at 250° F., the optical densities of these juices at 400 mμ were almost equal to those at 515 mμ. Apparently as the anthocyanin pigment was destroyed with increasing process time at 250° F.

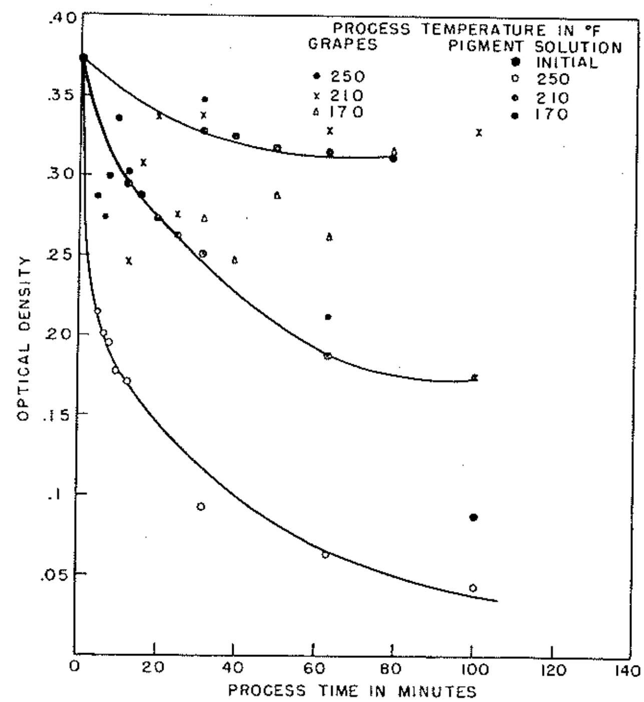

Figure 1. Variation of pigment concentrations of grape juice samples and pigment solutions immediately after processing, with process times. Pigment concentrations are expressed as average values of optical densities at 515 mμ at pH 1.0 of solutions (1:100, 1:50) of juice samples and pigment respectively.

beyond 63 minutes, the optical densities at 515 mμ were lowered and the degradation products of the anthocyanin possessed higher absorption values around 400 mμ. Other changes such as a Maillard reaction, deterioration of pectic substances or of ascorbic acid may also have caused the maxima to shift.

The concentration of pigments in the grape juice samples resulting from storage for 0, 2, 4, and 6 months are presented in Figures 2 and 3. The results indicate that the pigment concentrations were higher in every case when nitrogen formed the headspace and the sam-

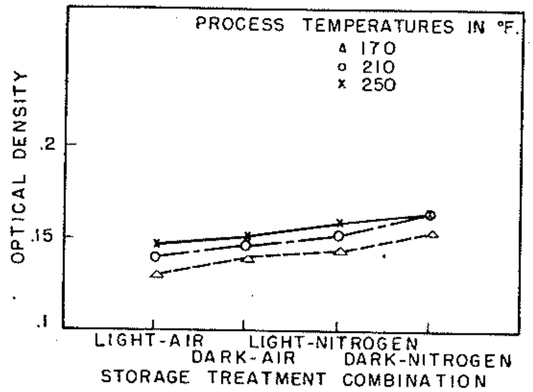

Figure 2. Variation of pigment concentrations of grape juice samples with storage treatments averaging over other variables except process temperature. Pigment concentrations are expressed as average values of optical densities at 515 mμ at pH 1.0 of solutions (1:100). Storage temperature was 70 ± 0.5° F.

ples were stored in the dark. However, the data shown in Figure 4 definitely lead to the conclusion that the period of storage exerted the maximum influence on the deterioration of the pigment and suggest that the rate of deterioration of pigment is proportional to the temperature of processing. The deterioration in the anthocyanin content of pure pigment solutions on heat and storage treatment appeared to be greater than the deterioration occurring in the juice samples wherein the pigments exist along with the other juice components. However, the formation of brown pigments during storage may have obscured the true relationship between grape juice and pure pigment.

The pigment concentrations in the juice samples after storage for 6 months could not be determined exactly as larger dilutions of the juice samples with dilute acid were employed to reduce the interference of the turbidities developed. The loss of pigment occurred primarily in the first 2 months while further storage periods did not involve comparable deterioration. During the storage period the juices changed from purple to light brownish red. A brown sediment settled to the bottom of the test tubes and the sediment increased with the period of storage. No effort was made to secure a quantitative estimate of the brown sediment. The data indicate that the amount of air dissolved in the juice samples is apparently sufficient to cause deterioration of the pigments, possibly by oxidation, as the presence of nitrogen in the headspace yielded results similar to those obtained with oxygen in the headspace. Pasteurization of grape juice samples or the addition of toluene brought about identical changes in the pigment concentrations as a result of storage period and storage treatments. Similar changes were observed in samples (processed from grapes stored at 40° F. for 1 month) processed for the longer times at temperatures of 210° F. and 250° F.

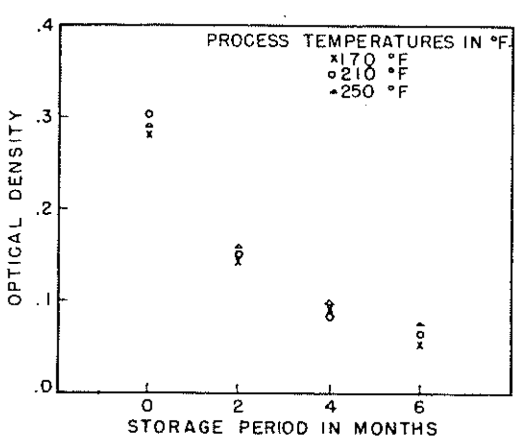

Figure 3. Variation of pigment concentrations of grape juice samples with storage period averaging over other variables except process temperature. Pigment concentrations are expressed as average values of optical densities. Storage temperature was 70 ± 0.5° F.

A study of the changes occurring in the pigment components during storage was made by use of a chromatographic technique using paper strips. With freshly prepared juices it was noted that two major bands were prominent on the paper column. The lower band with an R_f value of 0.12 was purple in color and the upper band with an R_f value of 0.21 was red in color. It has been suggested that the lower one was the diglycoside and the upper one the monoglycoside of the anthocyanidin *(9)*. With each juice sample the R_f values were determined under identical conditions and the average values were found to be 0.12 for the purple band and 0.21 for the red band. Spraying ammoniacal silver nitrate on the papers indicated faint brown bands, moving along with the solvent front and reducing silver nitrate in the cold. These brown bands were observed only with juices processed at temperatures of 250° F. for process times greater than 10 minutes. However, at the sites where the spots were initially placed, brown discolorations reducing ammoniacal silver nitrate in the cold were noted showing that some types of brown discoloration could not be moved by the solvent front.

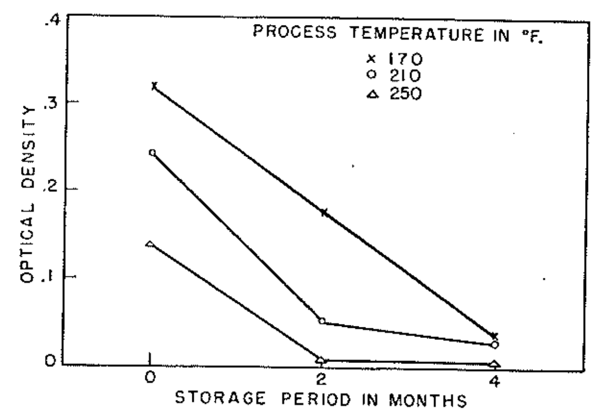

Figure 4. Variation of pigment concentrations of pigment solutions of pH 3.4 with storage period averaging over values for process times at each process temperature. The pigment concentrations are expressed as average values of optical densities at 515 mμ at pH 1.0 of solutions (1:50). Storage temperature was 70 ± 0.5° F.

At the end of each storage period each juice sample was separated by use of paper chromatograms under identical conditions. The intensities of the two bands were reduced progressively with storage and the lower band became much less intense than the upper one. A brown band also appeared at the top of the column moving along with the solvent front and this band was especially noticeable with the juice samples obtained after processing at higher temperatures.

On exposure of the papers to ammonia, two yellow bands at the top, one below the other, became prominent. Another band with an R_f value of 0.52, was revealed by a yellow color. All these bands reduced ammoniacal silver nitrate in the cold in the course of a few minutes. The brown discolorations at the site where the samples were placed on the paper columns, increased with storage. No special fluorescent properties were observed when these columns were examined under ultraviolet light, though the colors of the bands looked brighter than under daylight. Spraying dilute ferric chloride solution on the developed and air dried chromatogram produced a change in the color of the top band moving along with the solvent, to an intense yellow which faded rapidly. A light greenish band of R_f value 0.52, changing to grey in the course of 1 hour, was also noticed on spraying ferric chloride solution on the chromatogram.

SUMMARY

No regular variations in the anthocyanin pigment concentrations were noted in the grape juice samples on processing Concord grapes in glass jars at 170° F. for process times ranging from 31.5 to 79 minutes. When the processing was done at 210° F., the pigment concentration increased progressively as the process time was increased from 12.5 to 31.5 minutes, while a further increase in process time up to 100 minutes did not further increase the pigment concentrations of the juice samples. The pigment in juice samples processed at 250° F. for times longer than 63 minutes was found to undergo destruction.

These results indicate that the period of storage exerted the maximum influence on the deterioration of the pigment as compared to the presence of air or nitrogen in the headspace of the container, or storage of the samples in the dark or exposed to light. The amount of air dissolved in the juice samples is apparently sufficient to deteriorate the pigments, possibly by oxidation.

Both the mono- and the diglucosides of the anthocyanidin were found to decrease as a result of storage.

Studies on the effect of different processes on solutions of the major anthocyanin pigment (monoglucoside) indicated a gradual destruction of pigment with increased process time even at the lower tempratures of processing. On storage, the pigment concentrations diminished with simultaneous formation of gelatinous sediments in the solutions. The deterioration in the anthocyanin content on processing and storage was found to be more striking in the case of anthocyanin chloride solutions than in grape juice samples.

LITERATURE CITED

1. Amerine, M. A., and DeMattei, W. Color in California wines. III. Methods of removing color from the skins. *Food Research*, 5, 509 (1940).
2. Anderson, R. J. Concerning the anthocyans in Norton and Concord grapes. A contribution to the chemistry of grape pigments. *J. Biol. Chem.*, 57, 795 (1923).
3. Bate-Smith, E. C. Paper chromatography of anthocyanins and related substances in petal extracts. *Nature*, 161, 835 (1948).
4. Hartmann, B. G., and Tolman, L. M. Concord grape juice, manufacture and chemical composition. U. S. Dept. Agr., *Bull.*, 656 (1918).
5. Joslyn, M. A., Farley, H. B., and Reed, H. M. Color retention in fruit products. *Ind. Eng. Chem.*, 21, 135 (1929).
6. Nebesky, E. A., Esselen, W. B., Jr., McConnell, John E. W., and Fellers, C. R. Stability of color in fruit juices. *Food Research*, 14, 261 (1949).
7. Pederson, C. S., and Tressler, D. K. Improvements in the manufacture and the preservation of grape juice. N. Y. State Agr. Expt. Sta., *Bull.*, 676 (1936).
8. Powers, J. J., and Esselen, W. B., Jr. Should the glass packer be concerned about light? *Glass Packer*, 21, 612, 643 (1942).
9. Sastry, L. V. L. Nature of pigments in Concord grapes and their behavior during heat processing and storage. Unpublished Ph.D. Thesis. Ames, Iowa, Iowa State College Library, 1951.
10. Tischer, R. G. A high temperature process for the extraction of Concord grape juice. *Food Technol.*, 5, 160 (1951).
11. Tressler, D. K., and Pederson, C. S. Preservation of grape juice. II. Factors controlling the rate of deterioration of bottled Concord juice. *Food Research*, 1, 87 (1936).