Anthocyanin Pigments as a Maturity Index for Processing Dark Sweet Cherries and Purple Plums

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Measurement of the anthocyanin pigments in dark sweet cherries and purple plums was developed as a maturity index. Alcoholic-HCl extracts in pH 1.0 buffer were measured in a colorimeter with a 515-mμ filter. Absorbance at 515 mμ was compared to soluble solids, acidity, and soluble solids–acid ratio. Absorbance was found the best index for predicting depth of color in the final processed product.

FOR MANY YEARS there has been a need for a maturity index that could be used in the harvesting of highly pigmented fruits for processing. Considerable work has been done in evaluation of soluble solids, acid, soluble solid–acid ratios, skin color, flesh color, and firmness as maturity indices (*6–9*, *14*, *15*). None has been found reliable for use without adjustment for yearly climatic changes, cultural practices, and other variables.

Most maturity indices for dark sweet cherries (DSC) and purple plums (PP) have been developed to use in harvesting fruit for the fresh market and not for the more mature fruit used for processing. These indices are related to the quality of the canned product only indirectly. Visual evaluation of skin and flesh color is subjective and, at best, roughly quantitative with trained observers.

A large measure of consumer acceptance for processed DSC and PP depends upon attractive, deep colored sirup and fruit. This color comes from the anthocyanin pigments present in the skin of PP and in the skin and flesh of DSC. A quantitative measure of these pigments as they develop during maturation could be useful as an objective maturity index. Any method to be useful in quality control must be relatively quick and simple and should use equipment already available in quality control laboratories or inexpensive.

One of the first quantitative measurements of anthocyanin pigments was made by Sondheimer and Kertesz on strawberries (*12*). The pigment content was determined by the difference in absorbance between strawberry extract in buffer at pH 3.4 and 2.0. Other methods measure the difference in absorbance of acidified extracts before and after bleaching the anthocyanin pigments with sodium sulfite (*11*) or hydrogen peroxide (*4*, *13*). Such methods correct for the presence of chlorophyll and other nonanthocyanin pigments. DSC and PP skins at canning maturity contain relatively minor amounts of nonanthocyanin pigments that may be extracted with acidified alcohol. Acidified alcohol will extract the anthocyanins from Concord grapes (*3*). Absorbance was measured on an aliquot diluted to a suitable concentration with pH 1.0 buffer.

The Hortispect (*5*) and Hunter color and color difference meter (*15*, *16*) have been used to measure flesh color changes of green to amber during maturation of Italian-type prunes used to produce canned PP. These measurements are not a direct measurement of sirup color or appearance of the canned products. Instruments of this type are rather costly and not commonly available in quality control laboratories.

Experimental

Raw Material. Fruit used in these studies was collected during the 1960–62 growing seasons. DSC of the Bing and Lambert varieties were harvested at 2- to 7-day intervals from trees in a variety test block at the Irrigation Experiment Station, Prosser, Wash. The PP were Early and Regular Italian strains grown in two nearby commercial orchards and harvested at weekly intervals. Each season, fruit was harvested from one or more trees of each variety normal in crop load and appearance. On the day of harvest, samples were taken to the laboratory and sorted into two or three lots on the basis of visual skin color. Analytical samples were drawn from each lot and the remainder of the lot was processed unless it was similar in color to some collected previously.

Processing. Number 2 fruit enameled cans were filled with 13.5 ounces of DSC or 12 ounces of PP. The cans were then filled with hot 30% sucrose sirup, steam-exhausted, cooked in a nonagitating boiling water bath, cooled in running water to room temperature, then stored at 60° F. constant temperature until evaluation.

Analytical. Soluble solids and titratable acidity were determined on 500-gram samples of fresh fruit that had been pitted and ground in a blender for 3 minutes. Ten-gram samples of PP were titrated to pH 8.1 with 0.100*N* NaOH. The acidity of DSC was determined by the AOAC method (*2*). Soluble solids were determined upon filtrate from the same pulp with an Abbe-type refractometer.

Absorbance Measurements of Sirup Color. Absorbance of sirups from canned cherries and purple plums was determined by diluting 1 or 2 ml. of sirup to 100 ml. with pH 1.0 buffer and measuring the absorbance in a colorimeter with a 515-mμ filter. The results were multiplied by 50 or 100 to obtain the absorbance of the sirup. All samples were read immediately after dilution with buffer.

Hunter Meter Measurements. Skin color of fresh and processed fruit was measured with a Hunter color and color difference meter having an *Rd* scale. A white tile with $Rd = 80.8$, $a = -0.8$, and $b = +3.2$ was used to standardize the instrument. The individual fruits were presented to the instrument in an optically flat glass cell. The size of the cherries made it desirable to use the standard unit with small area illumination. Ten cherries were presented to the instrument with the suture side down. Five plums were measured on each cheek which had been polished to remove the bloom. Data presented are the average of 10 readings.

Raw Fruit Extract Absorbance Measurement

Dark Sweet Cherries. One hundred grams of pitted DSC were blended with 400 ml. of 70% alcohol containing 0.5% HCl for 3 minutes. A portion of the extract was filtered through paper or a glass wool plug. A 2-ml. aliquot was diluted to 100 ml. with pH 1.0 KCl–HCl buffer. Absorbance was read on a colorimeter equipped with a 515-mμ filter. Results were multiplied by 250 to obtain the absorbance of the raw fruit extract on a fresh weight basis. All data presented here are from an Evelyn colorimeter equipped with macrocells.

Purple Plums. The skins pared from

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400 grams of PP were blended for 3 minutes with 250 ml. of 95% ethanol containing 1% HCl. The slurry was quantitatively transferred to a 500-ml. volumetric flask and made to volume with pH 1.0 buffer. The flask stood for 30 minutes with occasional shaking to allow the color to leach from the skins. The extract was filtered through paper or a glass wool plug; the first 20 ml. were discarded. Five milliliters of the filtrate were diluted to 100 ml. with pH 1.0 buffer and 25 ml. of this solution diluted again to 100 ml. with pH 1.0 buffer. Absorbance was measured at 515 mμ and multiplied by 100 to obtain absorbance of the raw fruit extract on a fresh weight basis. Occasionally the final solution was slightly turbid. For greater precision, a correction for turbidity was sometimes made by subtracting the absorbance at 660 mμ from that at 515 mμ. The turbidity correction should vary from 0.0 to 0.012 on the diluted sample.

Results and Discussion

Color Extraction. One hundred grams of DSC and 400 grams of PP are samples of adequate size to provide an accurate measure of the pigments present and still allow convenient and rapid analysis. Smaller samples do not contain enough individual fruits to make the sample representative of the material from which it was selected. Twenty-one samples of DSC were analyzed in duplicate; the average difference between the duplicates was 5.2%. Likewise, 10 duplicate analyses of PP differed by 4.6% on the average.

An alcoholic solvent containing HCl is required for the highest recovery of anthocyanin pigments from fruit. Methanol, ethanol, and 2-propanol with about 30% water are satisfactory solvents. Only data from ethanolic extracts are presented in this paper. Water in the acidified alcohol prevents solution of compounds that are turbid in pH 1.0 buffer.

Absorbance of DSC extracts made with pH 1.0 buffer containing no alcohol averaged 4% lower than those made with alcoholic solvent. Foaming of PP–pH 1.0 buffer homogenates made it difficult to determine accurately if there was a similar color loss. Extraction with 70% ethanol without HCl gave 11% lower values for absorbance of the extract from raw fruit. The addition of 0.1% NaF to 70% ethanol will increase the recovery of anthocyanins, but the absorbance values are still 3% lower than when HCl is added to the ethanol. The added HCl lowers the pH, changes the anthocyanin equilibrium to the flavylium cation (colored form), and protects against molecular destruction. Blending for an additional 7 minutes in the ethanol–HCl solvent increased the absorbance by 4%. Conversion of some leuco-anthocyanins to anthocyanidins by the heat generated by long

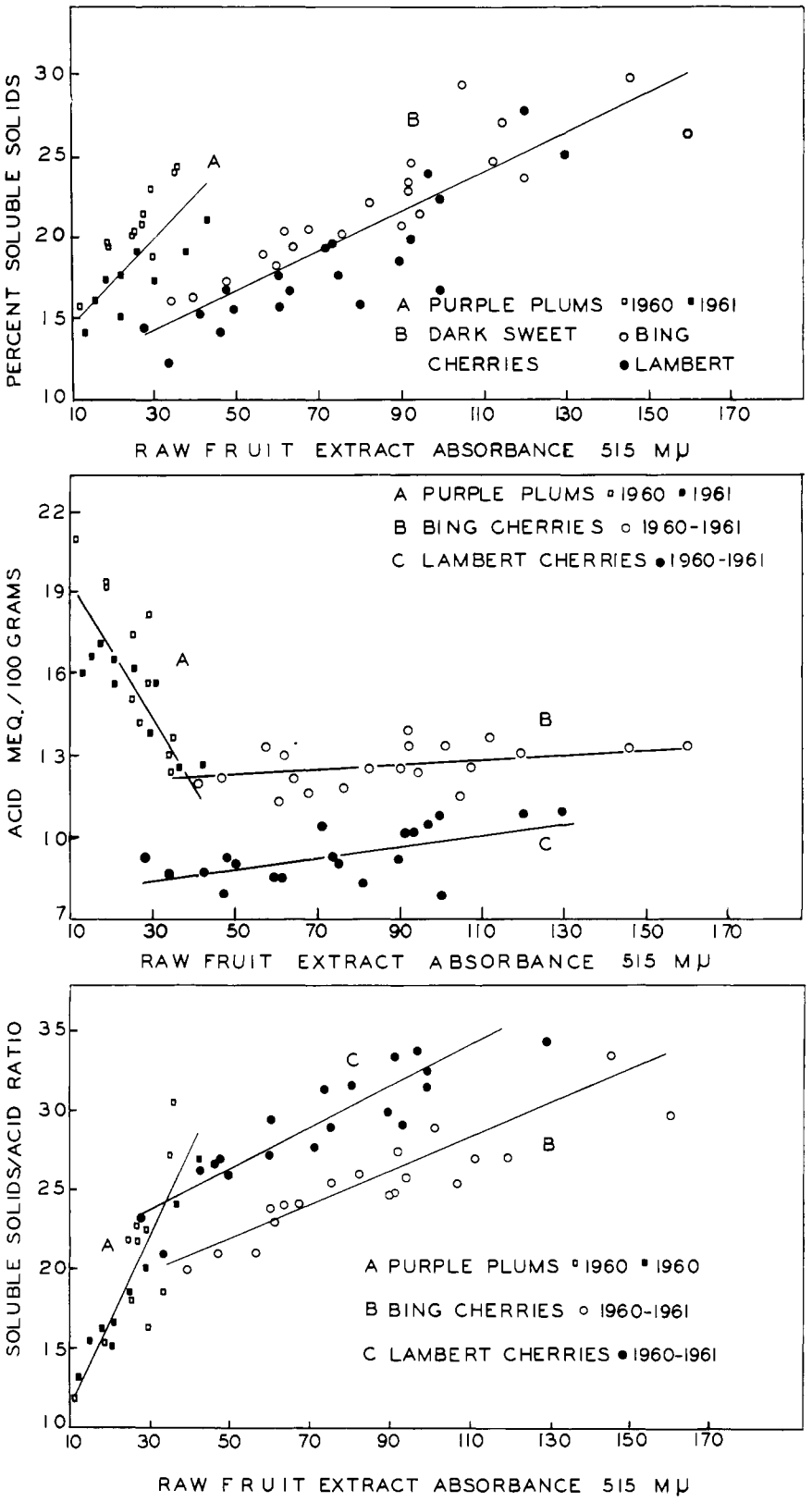

Figure 1. Comparison of raw fruit absorbance with other commonly used maturity indices

Top. Comparison of raw fruit absorbance with soluble solids
A = regression line for PP, $r = +0.717$
B = regression line for DSC, $r = +0.861$

Middle. Comparison of raw fruit absorbance with titratable acid content
A = regression line for PP, $r = +0.777$
B = regression line for Bing cherries N.S.
C = regression line for Lambert cherries, $r = +0.496$

Bottom. Comparison of raw fruit absorbance with soluble solids–acid ratio
A = regression line for PP, $r = +0.888$
B = regression line for Bing cherries, $r = +0.779$
C = regression line for Lambert cherries, $r = +0.912$

blending in the presence of HCl probably caused the increase.

Extraction time is not critical for DSC. One, 3, or 5 minutes give the same absorbance because most of the pigment is in the flesh and the skins disintegrate easily. PP have all the anthocyanin pigments in skins which are difficult to blend and penetrate with solvent. Three-minute blending does not extract all the color. However, making up to volume with pH 1.0 buffer and mixing occasionally for 30 minutes before filtering completed pigment extraction. In later trials, it was found that blending for 5 minutes, making to volume, and filtering immediately gave the same absorbance.

Comparison among Methods of Measuring Maturity. In 1960 and 1961, anthocyanin pigment extractions from 43 harvest samples of DSC and 20 harvest samples of PP were compared with soluble solids, titratable acids, and sirup color of the canned product. Early Italian prunes were added to the study in 1961. Limited samples of both cherry varieties and Early Italian prunes were sampled in 1962 for comparison of the extract absorbance and Hunter color and color difference meter values.

Four to six harvests were made at 2- to 7-day intervals for each of the DSC varieties. Five and six harvests were made at weekly intervals for the PP. These harvests covered a maturity range from commercial fresh market ripeness to a point past canning ripe when cullage became excessive.

Comparisons of the absorbance of extract from raw fruit and three older maturity indices are presented in Figure 1. The data for both DSC and PP show a significant correlation between raw fruit extract absorbance and the other maturity indices, with the exception of titratable acidity of Bing cherries. The different acid contents of Bing and Lambert cherries require that these varieties be separated in evaluation of the data.

The absorbance of extract from raw fruit and soluble solids contents shows a positive relationship when varieties and years are grouped together. Individual regression lines for the varieties by separate years give somewhat closer agreement. These data indicate that the Bing variety has higher soluble solids for the same amount of color than does Lambert. Samples of both cherry varieties and PP taken in 1960 were higher in soluble solids for the same amount of color than in the succeeding year. The cherries came from the same trees in both seasons but the plums were harvested from different orchards. Seasonal variations caused the changes between the two years in cherries, but probably do not account for all the difference in the PP. These data indicate that the relationship of total color and soluble solids is subject to environmental conditions.

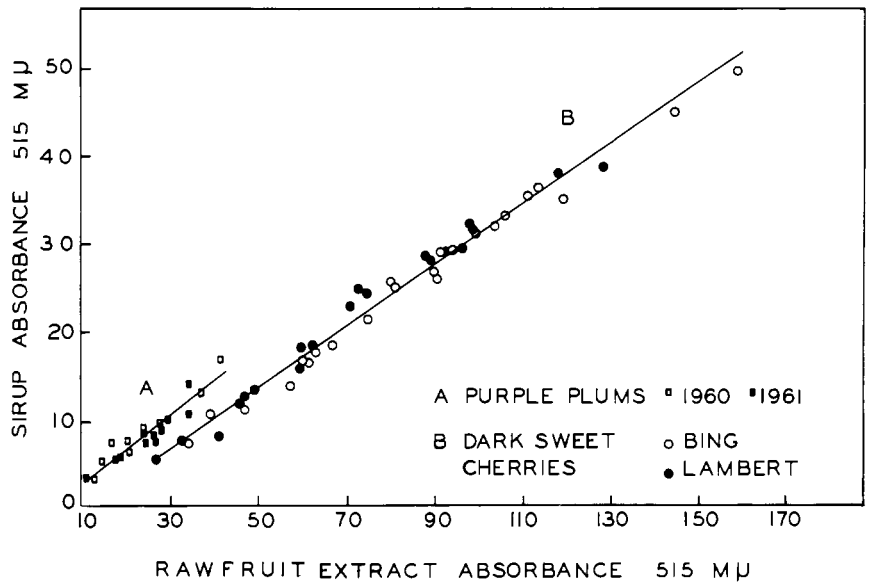

Figure 2. Comparison of the absorbance of the raw fruit extract and sirup from canned fruit

A = regression line for PP, $r = +0.950$
B = regression line for DSC, $r = +0.991$

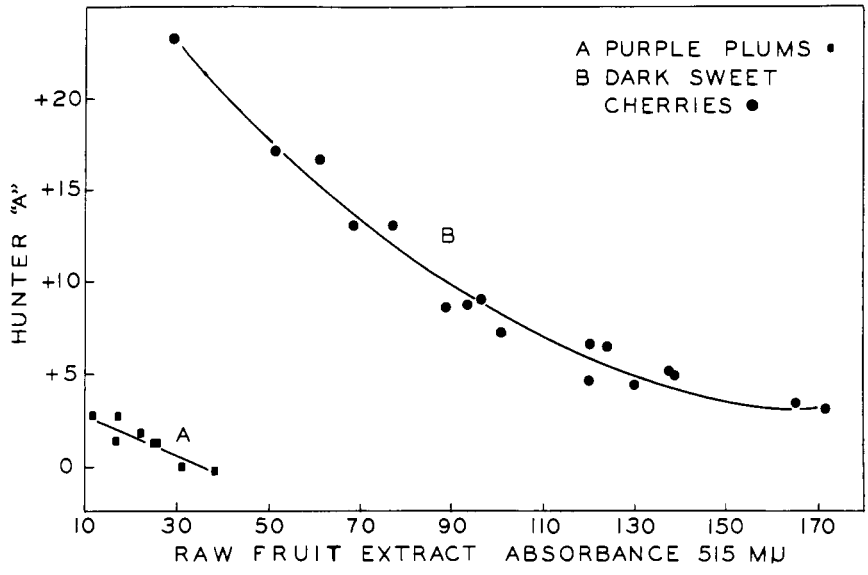

Figure 3. Comparison of HC and CDM a values and raw fruit extract-absorbance

A = regression line for PP, $r = -0.967$
B = curve for both varieties DSC

Figure 1 (middle) shows the relationship between raw fruit extract absorbance and titratable acidity. These data indicate that titratable acid might be more useful than soluble solids as a maturity index for purple plums. However, others have found titratable acidity unreliable (*14*). It would require annual adjustment because of yearly variations in total acid and in the rate of change during maturation. Titratable acid could not be used for DSC. There is little change in acid contents during maturation. Bing and Lambert have different acidity levels.

Figure 1 (bottom) shows that soluble solids–acid ratio correlates more closely with absorbance of raw fruit extract than do either soluble solids or acid alone. Part of this improvement is due to the separation of the Bing and Lambert varieties of DSC. A separate set of standards for the two major dark sweet cherry varieties used by processors and possibly for other varieties acceptable canning would be inconvenient.

Fresh Fruit Absorbance and Sirup Absorbance. Figure 2 shows the relationship of the canned fruit color measured by absorbance of the sirup with the color of the raw fruit extract as measured by absorbance. Sirup absorbance was

measured 2 and 5 months after processing for PP and DSC, respectively. Sirup absorbance measured in a pH 1.0 buffer and at a standardized time after processing is a quantitative measure of color in the sirup and canned fruit. Sirup absorbance and raw fruit extract absorbance have the highest correlation coefficient of any factors examined in this study. The 43 samples of both varieties of DSC taken during the two years have a correlation coefficient of $r = +0.991$. The correlation coefficient of 20 PP samples taken during the two years is $r = +0.950$. The regression lines for the PP and DSC have the same slope and would come very close to being superimposed upon each other in the overlapping areas if both fruits had the same fill weight and had been stored for the same time before measuring sirup absorbance.

During 1962, the Hunter color and color difference meter was used to evaluate the skin color of a series of fruit at successive stages of maturity for each variety of DSC and the Early Italian strain of PP. Data are presented in Figures 3 and 4 for Hunter color and color difference meter *a* and *b* only because change in the *Rd* value was small. The data show a significant negative correlation between the three Hunter measurements and raw fruit absorbance for DSC. Only the correlation of absorbance with Hunter *a* was significant for purple plums. The plotted data show that a curved line fits the DSC points best because the change in Hunter color and color difference meter *a* and *b* during maturation is not uniform. The greatest rate of change occurs before canning maturity is reached. These data closely parallel visual observations of the fresh fruit.

Raw Fruit Absorbance and Sensory Evaluation. At the time sirup absorbance was measured, all of the processed samples were opened and evaluated visually for the depth of color in sirup and fruit. Visual rankings were approximately in the same order as that of the raw product extract absorbance and sirup absorbance. An example of this ranking is given in Table I. Samples visually ranked out of absorbance order often contained nonuniform individual fruits. Others came from cans with flaws in the fruit enamel causing a darkening of fruit and sirup.

Flavor was evaluated by the authors at the time sirup absorbance and color were measured. The wide range of cut out soluble solids made the ranking of DSC and PP flavor beyond the scope of this paper. However, the flavor increased in intensity as color increased and the fruit became more mature. These observations confirm Wiley's observations (*15*) that per cent transmittance of canned sirup, canned skin color, and taste panel color scores, among other

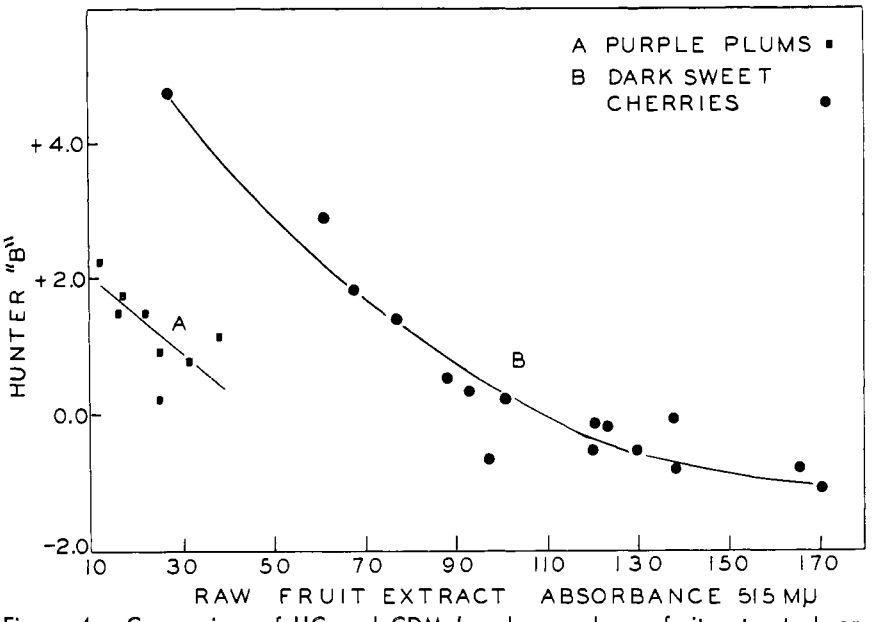

Figure 4. Comparison of HC and CDM *b* values and raw fruit extract-absorbance

A = regression line for PP N.S.
B = curve for both varieties DSC

Table I. Comparison of Visual Ranking of Canned Color with Canned Sirup Absorbance and Fresh Fruit Measurements for Lambert DSC

		Fresh Fruit			
Visual Depth of Color	*Sirup Absorbance*	*Absorbance*	*Soluble solids, %*	*Titratable acid, meq./100 g.*	*Soluble solids–acid ratio*
1	5.3	27	14.4	9.2	23
2	8.0	41	15.1	8.6	26
3	13	47	16.7	9.2	27
4	13	49	15.5	8.8	26
5	18	63	16.7	—	—
6	18	60	17.6	8.9	30
7	24	75	17.5	9.0	29
8	25	73	19.6	9.3	31
9	29	89	18.6	9.2	30
10	29	91	22.8	10.2	34
11	29	96	23.7	10.5	34
12	38	120	27.7	10.8	38
Rank correlation coefficient[a]	1.00	0.986	0.979	0.820	0.973

[a] $P < 0.01$.

parameters, are significantly correlated to taste panel flavor scores for PP. Wiley and Worthington (*16*) correlated fresh fruit soluble solids–acid ratio, per cent soluble solids, pressure test, and Hunter *a* values of the flesh to panel flavor scores of the canned product. None of Wiley's measurements on fresh fruit is a direct measure of color or flavor in the canned product. Measuring the raw fruit extract absorbance is more useful because it is both a direct measure of amount of color present and an indication of maturity, therefore flavor.

There seems to be very little available information related to maturity indices for processing DSC. Hartman and Bullis (*10*) found that Bings and Lamberts with a Balling reading of 20% were sufficiently mature to eat fresh. Allen compared subjective color observation with soluble solids for several dark sweet varieties grown in northern California and concluded that color was a more reliable index of maturity than soluble solids (*1*). Both studies were aimed at fresh market maturity. They concluded that soluble solids and color are closely related, with soluble solids more dependent upon such factors as size of fruit, season, and variety.

This study has reconfirmed the relationship of color to soluble solids for DSC and extended it to the maturities used for processing. Again, it would seem best to use a measurement that is directly related to at least one of the most important quality factors, color or flavor. Raw fruit extract absorbance is most closely related to color of the processed product and parallels development of flavor during maturation.

Application of Method

No minimum absorbance that will produce a fancy canned product has been proposed because of differences between colorimeters, cell sizes, and individual processor preferences. Using an Evelyn colorimeter with a 515-mμ filter, a raw fruit absorbance of 90 or higher for DSC gives a processed product with deep color that an industry panel graded fancy. Bings will attain somewhat more color than Lamberts. Raw fruit absorbancy of 25 or higher for PP likewise gives a processed product with good color that an industry panel graded fancy.

Raw fruit absorbance has proved useful in this laboratory for evaluation of unfamiliar varieties, strains, or selections for processing. Certain DSC varieties have a larger percentage of pigment near the skin as ripening develops. Processing releases this pigment into the sirup and remainder of the flesh, resulting in lighter color than would be expected from the external appearance. The amount of color in PP is difficult to estimate visually once the surface of the fruit has become colored. Increases in anthocyanin pigment content are not reflected by corresponding changes in visual color or Hunter color and color difference meter values.

Raw fruit absorbance coupled with field observations could be used to determine when commercial harvests should start and which orchards should be picked first. Normally, a few days' delay will increase the color of fruit from borderline maturity to a satisfactory level. Raw fruit absorbance can be used to produce a pack standardized with respect to color for each grade.

Each processor will decide what amount of color he wishes his products to have. A trial pack will establish these levels in terms of absorbance of fresh fruit extracts and serve as a standard for subsequent harvests.

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GRAPE FLAVOR AND ODOR

Detection of an Undesirable Anomaly in Concord Grape by Gas Chromatography

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Concord grape essence was analyzed by gas-liquid chromatography. Compositional criteria established were used for the determination of an organoleptically detectable anomaly. Atypical group grapes were defined by a tenfold increase in *n*-valeraldehyde content, which can be detected organoleptically. The sensation recorded is one of an increased sweet and fruity flavor.

A STANDARD of high quality in fresh fruit is important in the preparation of all fruit products. Any anomaly in fresh fruit which could lower this standard is viewed with concern. In 1958, in the Niagara Peninsula, Ontario, Canada, grapes which were small, light in color, and different in flavor from typical Concord grapes were found on a small percentage of vines (*7*), and the juice from such grapes was inferior to that from normal ones. This fruit grew on seemingly healthy vines and in most cases adjacent vines bore typical normal grapes. The atypical grapes remained in this state until they dropped because of overmaturity. Total acidity, Brix, and pH of the atypical grapes (*4*) indicated that no consistent variation in any of these factors could be associated with the abnormality; therefore, a comparison by gas chromatography of some components of normal and atypical grapes was initiated to discover possible sites of flavor differences.

Holley, Stoyla, and Holley (*1*) used classical chemical methods to identify eight volatile constituents of Concord grape, but no gas chromatographic study of whole grape juice has been reported. Thus, methods were needed for gas chromatographic analysis of volatile components and identification of some of these compounds before a comparative study could be made.

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Experimental

Identification of Some Volatile Components. Frozen grape juice from normal typical grapes harvested in 1960 was used. The juice had been pasteurized and stored at −22° C. It was thawed and passed through a high speed separator at 1300 × g to remove chloroplasts and tartrates. The essence from 2000 ml. of cleared juice was extracted into ethyl chloride. Complete removal of taste and odor, as judged by a panel of five expert tasters, was facilitated by fourfold extraction using 200 ml. of ethyl chloride (*3*) each time. The four extracts were combined and dried 12 hours