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OBJECTIVE METHOD OF ESTIMATING ANTHOCYANIN CONTENT FOR DETERMINING COLOR GRADE OF GRAPES

INTRODUCTION

THE USDA COLOR GRADE of hand-harvested grapes is determined visually (USDA, 1965). Such a subjective technique is not satisfactory for grading the color of mechanically-harvested grapes because the skins are often separated from the pulp and the harvest consists of a mixture of skin, pulp, whole fruit and juice in a 4 ft X 4 ft X 4 ft plastic-lined bin. Fresh grapes must have adequate color if they are to produce concentrated frozen juice of satisfactory color. The USDA color grade of the frozen concentrated sweetened juice is based on absorbance of the juice (pH 3.2) at 430 and 520 nm (USDA, 1957). Absorbance at 430 nm indicates degree of degradation, and at 520 nm indicates the anthocyanin (Acy) content.

Since the color of concentrated frozen juice is based on Acy content, juice color should be satisfactory if the fresh grapes contain adequate Acy. This study was to establish a rapid objective optical method for estimating Acy content, which could be used to grade the color of mechanically-harvested grapes. The study was designed so that macerates prepared for USDA grade determination of soluble solids could also be used for Acy analyses. For soluble solids determination, samples are collected randomly from bins and macerated together.

MATERIALS & METHODS

HAND-HARVESTED CONCORD GRAPES of different maturities were air-shipped from Westfield, N.Y., to Beltsville, Md. 58 stem-free samples, each about 250g, were macerated in a Waring Blendor under a stream of nitrogen for 60 sec. Part of each macerate was analyzed for soluble solids content and another was measured optically to determine the feasibility of estimating the Acy content. The remainder of the sample was placed in a 4 oz jar and frozen at −20° until analyzed for Acy content within a 2-month period.

Soluble solids

Total soluble solids of juice from the macerates was determined with a Bausch and Lomb temperature-controlled refractometer.

Optical measurements

Optical measurements for estimating Acy included reflectance and absorbance of light. Light reflectance was measured with a Hunter-Lab Model 25 Color Difference Meter, standardized with a white standard tile. A 6.5 cm diam sample cup, containing macerate to a depth of 2.5 cm was placed on the measuring port (4.9 cm diam) of the instrument and the Hunter L, a_L and b_L values were recorded.

Light absorbance (absorbance = 1/T, which includes absorbance, reflectance and scatter of light) of 1-cm deep macerate was determined with a USDA Spectrophotometric Difference Meter (SDM) as described by Birth and Norris (1965). From the absorption spectra of a few grape macerates differing in maturity, two wavelengths 630 and 690 nm, were selected for measuring the absorbance. Most of the absorbance at 630 nm was assumed to be due to anthocyanins. Other factors which absorbed at 630 nm, were assumed to be absorbing at all other wavelengths, thus the difference in absorbance at 690 nm from that at 630 nm was used as the SDM reading of the anthocyanin content.

Total anthocyanin content

The total Acy content was determined by a method described for cranberries (Fuleki and Francis, 1968) with modification. A 100-g sample of frozen macerate was thawed and remacerated with 200 ml of extracting solution (95% ethanol, conc HCl and water, 85:2:13). The macerate was vacuum-filtered through a Buchner funnel containing Celite Analytical Filter Aid on Whatman No. 1 filter paper. The residue on the filter pad was washed repeatedly with extracting solution until the washings were clear. The filtrate was brought to 500 or 1000 ml with extracting solution. A 2-ml aliquot of filtrate was transferred to a 100 ml volumetric flask, brought to volume with extracting solution, and mixed. The pH of the solution was measured and adjusted to 1 if necessary, and the solution was stored in the dark at room temperature for 2 hr. Absorbance was measured with a Bausch and Lomb 505 recording spectrophotometer. The total Acy content was calculated from absorbance at 540 nm by using the extinction value established for cyanidin 3-galactoside ($E^{1\%}_{1cm}$ = 958) as suggested by Fuleki (1972).

RESULTS & DISCUSSIONS

THE VISUAL COLOR of the fresh grapes ranged from acceptable to unacceptable based on USDA grading standards. The Acy content of these Concord grapes of different maturities ranged from 30–120 mg/100g fresh weight (gfw). Grapes of minimum acceptable color contained about 55 mg Acy/100 gfw. Most of the optical measurements of the macerated samples were correlated with a geometric function of the Acy content.

The difference between the absorbance at 630 and 690 nm [ΔA (630–690 nm)] of the macerated samples correlated with the log of the Acy content (r = 0.982) (Fig. 1). Grapes with minimum acceptable color (55 mg/100 gfw) had a ΔA (630–690 nm) of about 0.94. This value could be used as the

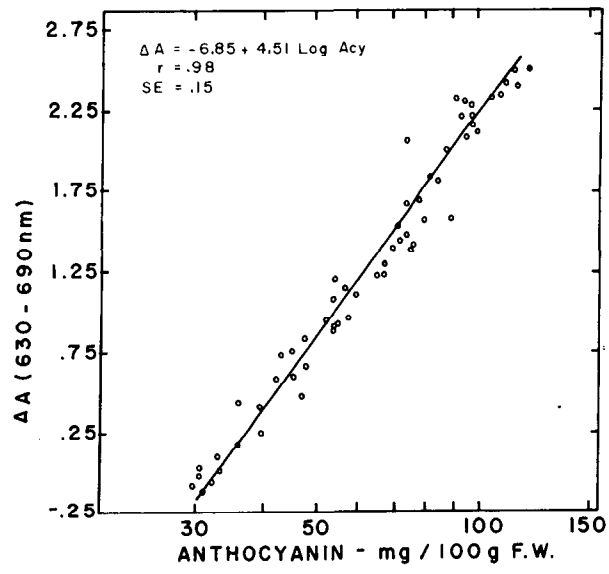

Fig. 1.—Relationship between anthocyanin concentration and ΔA (630–690nm) of Concord grape macerates.

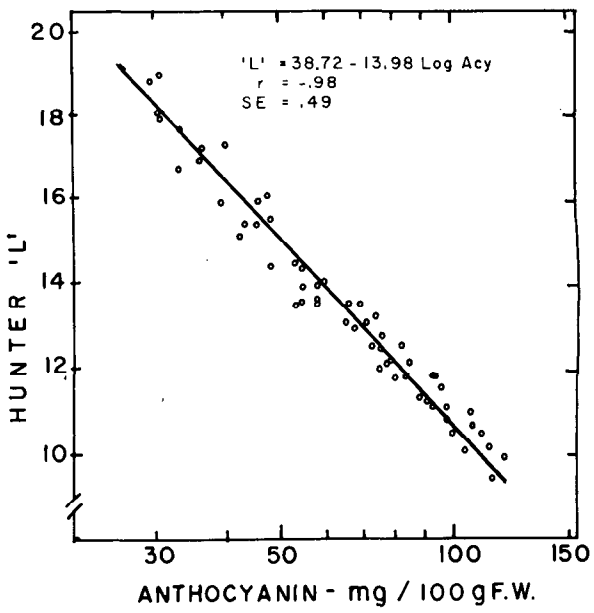

Fig. 2—Relationship between anthocyanin (Acy) concentration and Hunter 'L' values of Concord grape macerates.

separating point between acceptable and unacceptable grapes. If the color requirement of the frozen concentrates was lowered and an additional class was formed, the grapes could be separated into unacceptable, minimal and satisfactory color by using ΔA values of 0.7 and 1.5 as the separating points. Grapes with ΔA greater than 1.5 cannot be separated clearly into additional classes due to the increased deviation of the values from the regression line.

Of the Hunter meter readings, only "L," which measures lightness and darkness, correlated with the log Acy content (r = −0.980) (Fig. 2). The "L" value of 14 could be used as the separating point between acceptable and unacceptable grapes. In contrast to ΔA values, "L" values could be used to separate the grapes containing 50–100 mg Acy/100 gfw into two or three classes. Grapes containing less than 50 mg Acy/100 gfw cannot be separated clearly into additional classes due to the increased deviation of values from the regression line.

The soluble solids content correlated with Acy content (r = 0.966). This close association between soluble solids content and Acy content has been reported (Clore et al., 1965) and was anticipated due to the increased concentration of both with ripening. However, the regression equation for estimating Acy content from soluble solids content would not necessarily be the same for grapes from different geographical locations. The soluble solids content of grapes from the Southern and Northern United States would be about the same at harvest, but the Acy content of grapes from Southern United States generally would be less than that from Northern United States due to differences in temperature. Thus, the soluble solids content would not be a good index for estimating the Acy content for grade standards.

CONCLUSION

THE Acy CONTENT of freshly macerated grapes could be estimated objectively and rapidly by optical methods. The light-absorbance technique was effective for estimating Acy concentration between 40–70 mg/100 gfw. The light-reflectance technique was effective when the concentration range was between 50 and 100 mg/100 gfw. Since the Acy content of grapes with minimum color was about 55 mg/100 gfw either technique could be used for determining color acceptability of grapes to be processed.

REFERENCES

Birth, C.S. and Norris, K.H. 1965. The difference meter for measuring interior quality of foods and pigments in biological tissues. USDA Tech. Bull. 1341.

Clore, W.J., Neubert, A.M., Carter, G.H., Ingalsbe, D.W. and Brummund, V.P. 1965. Composition of Washington-produced Concord grapes and juices. Wash. Agr. Expt. Sta. Tech. Bull. 48.

Fuleki, T. 1972. Changes in the chemical composition of Concord grapes grown in Ontario during ripening in the 1970 season. Canadian J. of Pl. Sci. 52: 863.

Fuleki, T. and Francis, F.J. 1968. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanins in cranberries. J. Food Sci. 33: 72.

USDA, AMS. 1957. U.S. Standards for Frozen Concentrated Sweetened Grape Juice. Federal Register 22, F.R. 6077, Wash., D.C.

USDA, AMS. 1965. U.S. Standards for grades of American (Eastern type) bunch grapes. Federal Register 30, F.R. 3371, Wash., D.C.

Ms received 3/29/75; revised 6/14/75; accepted 6/18/75.

Presented at the 34th Annual Meeting of the Institute of Food Technologists, New Orleans, La., May 12—15, 1974.

We acknowledge Karl Norris, Instrumentation Research Laboratory, AMRI, for the spectral analysis of the grapes.

Mention of a specific brand name does not imply endorsement by the authors or institutions at which they are employed to the exclusion of others not mentioned.