Food Chemistry 43 (1992) 193–197

Relationship between anthocyanins and sugars during the ripening of grape berries

M. L. González-SanJosé

Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, E–28006 Madrid, Spain

&

C. Diez

Institut d'Estudis Avançats de les Illes Balears (CSIC), Campus Universitario, E–07071 Palma de Mallorca, Spain

(Received 23 October 1990; revised version received and accepted 18 February 1991)

The relationship between the sugars in the skin and in the must and the anthocyanins during the ripening process in two varieties of red grapes used in wine making was examined. The sugars in the must were directly related to the total anthocyanins in the must and closely related to the derivatives of peonidin, while the sugars in the skin were closely related to each of the anthocyanins and to the other phenolic compounds present in the skin.

INTRODUCTION

Sugars are the initial precursors in the biosynthesis of anthocyanins (Hrazdina *et al.*, 1984; Teusch *et al.*, 1987; Ruhnan & Forkmann, 1988). A relationship between the two must, therefore, exist. However, researchers who have undertaken work on this topic have not always come to identical conclusions.

A large number of papers have concluded that accumulation of anthocyanins takes place, on the whole, about a week after the massive increase in the level of sugars in the berry; these sugars are mainly concentrated in the pulp (Pirie & Mullins, 1976; Kluba & Mattick, 1978; Flora & Lane, 1979; Pirie & Mullins, 1980; Hrazdina *et al.*, 1984). In addition, several in-vitro studies carried out on isolated tissues have demonstrated that saccharose, as well as other sugars, stimulates anthocyanin synthesis (Gianfagna & Berkowitz, 1986).

The controversy between the published results is polarized between two facts. The first one is the role of sugars in the anthocyanin synthesis.

Some workers such as Pirie & Mullins (1977), have suggested that the sugars in the skin play a role as regulators in the synthesis of anthocyanins and, in general, of phenolic compounds. Champagnol (1984) concluded that the sugar content regulated the intensity of berry coloration and that the factors that affected the sugar content affected berry colour as well. Others, such as El-Manawaty (1981), state that sugars play an extremely important role in anthocyanin synthesis, chiefly in the synthesis of diglycosyl derivatives, but do not refer to any regulatory role; yet others, such as Wicks & Kliewer (1983) deny the regulatory role of sugar in anthocyanin synthesis. If sugars do have such a role, changes in the anthocyanin content should also be reflected in the carbohydrate content. However, many external factors, such as light and ethephon (2-chlorethanephosphonic acid), bring about changes in the anthocyanin concentration without altering the concentration of soluble solids.

The experiments of the above authors indicated that the total sugar content in the skin of the berries increased during grape ripening, even though the sugars were employed in the synthesis of the anthocyanins. The sugar concentration in the skin did not fall because, during ripening, sugars were translocated to the skin from the vacuoles, cytoplasm and walls of the cells in the pulp. This suggests that the sugar level in the skin is a factor which only limits anthocyanin synthesis.

The second fact is the type of sugar related to anthocyanin synthesis.

Some workers report that there is a direct relationship between anthocyanin content and the level of

Food Chemistry 0308-8146/92/$05.00

soluble carbohydrates in the skin of grape berries (Pirie & Mullins, 1977; Popescu *et al.*, 1986), but it is not very clear if this relationship exists between the level of sugars in the pulp and the skin anthocyanin content.

Jackson (1986) carried out a study of a variety of factors affecting the content of soluble solids and colour in grapes and reported that changes in colour and in the Brix levels took place in parallel, decreasing with crop load (the number of berries) and increasing with the crop load: leaf area ratio, which was indicative of the influence of the content of soluble solids on colour.

In contrast, Piergiovanni & Volonteiro (1983) reported that the progressive increase in anthocyanins did not appear to be related to the sugar content in the pulp. They drew this conclusion after noting that there was no increase in anthocyanin synthesis in years in which the ripening index of the grapes was high, in other words, in which there was a high proportion of sugar in the must. These results agree with Pirie & Mullins (1977) and Hrazdina (1986), who stated that there was no evidence of a direct relationship between changes in the content of soluble solids in the berry and anthocyanin synthesis, although the onset of the rapid accumulation of sugars coincided with the beginning of anthocyanin synthesis and with activation of the phenylpropanoid and flavonoid enzyme systems.

Regarding all these previous results, we have studied the relationship between sugar levels in the skin and in the pulp of grape berries and phenolic content in the skin during ripening, trying to resolve, mainly, the second point of this controversy, namely whether or not sugar level in the pulp is related to the synthesis of phenolic compounds in the skin.

Factor analysis is applied to phenolic variables and sugar levels for two varieties of *Vitis vinifera*, Cencibel and Garnacha, which are two of the most important varieties for Spanish red wine elaboration.

Based on the composition of grapes, during ripening, reported by González-SanJosé (1989), the variables analysed were: total phenols, low polymeric phenols, procyanidins, *o*-diphenols, total anthocyanin, individual anthocyanins, skin sugars and °Brix of must.

MATERIALS AND METHODS

Plant material

Samples were collected from two experimental vineyards of *V. vinifera*, varieties Cencibel and Garnacha, run by the Comunidad Autonoma of Madrid. Random samples were taken in 'Z' pattern design to prevent border and centre effects, and the bunch was taken from each vine selected by lot from among the four cardinal points of the compass. This was carried out three times a week, from 'veraison' to maturity, in the years 1986 and 1987.

For quantitative analysis, four groups of 100 berries each were randomly selected from all the samples collected in each of 16 days of sampling; therefore, the sample size was 56 cases (16 × 4) in each grape variety (González-SanJosé *et al.*, 1990). Cases were each group of 100 berries.

Analytical procedures

The grapes were peeled manually and the pulps were separated from the skins. These were lyophilized (Virtis Lyophilizer Model 6201–6230) and pulverized. The pulps were crushed to obtain the must where °Brix were measured.

Sugar extract was prepared by maceration of 1·0 g of lyophilized skins in hot water ($T < 60$°C).

Phenol and anthocyanin extracts were obtained by maceration of 2·5 g of lyophilized skins in methanol–formic acid (95:5).

The total anthocyanins (ACY), total phenols, low polymeric phenols, procyanidins and *o*-diphenols were analysed, respectively, by means of colours at different pH values, using Folin-Ciocalteus' reagent, according to the method of Masquellier *et al.* (1965), via transformation into red pigments by heating in an acid medium, and by the method of Swain & Hillis (1959). All these methods were described previously (Paronetto, 1977).

Individual anthocyanins were analysed by HPLC (González-SanJosé *et al.*, 1988).

Skin sugars were analysed in accordance with the Métodos Oficiales de Análisis (1973).

Statistical analysis

Factorial analysis was applied to all the samples without regard to grape variety or the sampling year.

The factor matrix was estimated from the correlation matrix by means of principal component analysis, using program 4M from the statistical package BMDP83 (Jenrich & Mundie, 1983). Factors were rotated using the Varimax method for ease of interpretation. Program 4M was run on a CYBER 155/855 computer (Control Data Corp., Arden Hills, California, USA).

RESULTS AND DISCUSSION

Analytical results obtained for each variable in each day of sampling give a coefficient of variation less than 5%. The range, mean, standard deviation, standard error of mean and coefficient of variation of evaluated variable were reported by González-SanJosé (1989).

Analytical data were evaluated by factor analysis, a useful statistical method for analysing, describing and interpreting multidimensional data matrices, i.e. statistical data consisting of observations of more than one

Table 1. Correlation coefficients between variables and °Brix levels in the must and total sugars in the skin

Variable	°Brix	Skin sugars
°Brix	1·000	—
Skin sugars	0·122	1·000
Total phenols	0·198	0·708
Low polym. phenols	0·310	0·720
o-Diphenols	0·030	0·516
Procyanidins	0·029	0·753
Skin ACY	0·456	0·615
Must ACY	0·764	0·460
Dp-3-glu	0·341	0·658
Cy-3-glu	0·340	0·628
Pt-3-glu	0·269	0·729
Pn-3-glu	0·569	0·687
Mv-3-glu	0·400	0·734
Dp-3(6-ac)-glu	0·256	0·765
Cy-3(6-ac)-glu	0·158	0·653
Pt-3(6-ac)-glu	0·401	0·766
Pn-3(6-ac)-glu	0·552	0·594
Mv-3(6-ac)-glu	0·393	0·588
Dp-3(6-pcoum)-glu	0·250	0·650
Mv-3(6-caf)-glu	0·293	0·693
Cy-3(6-pcoumn)-glu	0·344	0·594
Pt-3(6-pcoum)-glu	0·307	0·593
Pn-3(6-pcoum)-glu	0·601	0·667
Mv-3(6-pcoum)-glu	0·461	0·639

Cy = cyanidin; Dp = Delphinidin; Mv = malvidin; Pn = peonidin; Pt = petunidin; Glu = glucoside; Ac = acetyl; pcoum = *p*-coumaryl; caf = caffeyl; ACY = total anthocyanins.

Table 2. Factor scores for rotated factors from factor analysis of all the samples collected over the ripening period. (Rotated factor scores of less than an absolute value of 0·250 have been set to 0.)

Variable	Factor 1	Factor 2	Factor 3
Dp-3(6-ac)-glu	0·968	0·000	0·000
Dp-3(6-pcoum)-glu	0·962	0·000	0·000
Dp-3-glu	0·952	0·000	0·000
Pt-3-glu	0·949	0·000	0·000
Cy-3(6-ac)-glu	0·930	0·000	0·000
Cy-3-glu	0·925	0·000	0·000
Pt-3(6-pcoum)-glu	0·924	0·000	0·000
Mv-3(6-ac)-glu	0·921	0·307	0·000
Mv-3(6-caf)-glu	0·918	0·254	0·000
Mv-3-glu	0·893	0·317	0·000
Skin ACY	0·882	0·390	0·000
Pt-3(6-ac)-glu	0·879	0·303	0·000
Total phenols	0·863	0·000	0·000
Procyanidins	0·828	0·000	0·000
Mv-3(6-pcoum)-glu	0·822	0·412	0·000
Pn-3-glu	0·772	0·518	0·000
Pn-3(6-pcoum)-glu	0·738	0·549	0·000
Cy-3(6-pcoum)-glu	0·718	0·000	0·000
Skin sugars	0·716	0·000	0·585
Must ACY	0·629	0·606	0·000
Pn-3(6-ac)-glu	0·587	0·556	0·000
°Brix	0·000	0·836	0·000
o-Diphenols	0·000	0·000	0·834
Low polym. phenols	0·433	0·000	0·743

Key: see footnote to Table 1.

variable, which usually correspond to random variables (Morrison, 1976).

The correlation matrix derived from statistical analysis (Table 1) allowed the interpretation of the relationships between the variables.

It is interesting to note that the correlations between the sugars in the skin and the families of phenolic compounds in the skin, and all the individual anthocyanins considered, had values greater than 0·5. Moreover, the values of the correlations between the Brix levels in the must and the total anthocyanins in the must, and the peonidin derivatives in the skin, were also greater than 0·5.

These results support the conclusion that the sugars in the skin are directly related to the families of phenolic compounds in the skin and to the total and individual anthocyanins and that the sugars in the pulp are directly related to the anthocyanins in the must and to the peonidin derivatives in the skin.

It is important to note that both grape cultivars used are not teinturier grapes. The anthocyanin levels in must were never greater than 10 mg/100 berries. These pigments are present in the must because some cells of the most external cellular layer of the mesocarp are coloured (which can be observed after the berries are peeled manually).

Factor analysis attempts to explain, by means of a linear model, a large set of observed variables on the basis of a small number of hypothetical variables termed factors. Such factors should be regarded as the dimensionality effect that relates and explains the relationships and associations that exist between the variables.

The factor analysis, applied to analytical values, defined three factors with eigenvalues greater than unity, into which the variables evaluated have been grouped. These factors, together, explained 94·69% of the total variance.

Table 2 presents the rotated loadings for factors, with rotated factor loading less than, or equal to an absolute value of 0·250 set to zero.

The rotated factor loading shows that factor 1 (F1) was closely correlated with the individual anthocyanins, the total anthocyanins in the skin, the total polyphenols, the procyanidins and the sugars in the skin and somewhat less closely correlated with the anthocyanins in the must. It was also correlated to a certain extent with the low-polymer polyphenols. Factor 2 (F2) was mainly correlated with the Brix levels, as well as with the total anthocyanins in the must and the peonidin derivatives. Factor 3 (F3) was closely correlated only with the *o*-diphenols and with the low-polymer polyphenols, and it was somewhat less closely correlated with the sugars in the skin.

These results demonstrate that, although the sugars are metabolites that are the precursors of the phenolic compounds only the sugars present in the skin are

closely related to the phenolic compounds in the skin, which is reflected by F1, with which the Brix levels can be said to be unrelated (actual values of the rotated factor score = 0·103).

In addition, F2 suggests that the sugars in the must, and hence the sugars in the pulp, inasmuch as the grapes were peeled before crushing, were mainly related to the anthocyanins in the must and to the peonidin derivatives in the skin. Although these last variables were mainly correlated with F1, the factor scores for F2 were also significant, which was not the case for the rest of the anthocyanins considered. In other words, the variability that can be attributed to ripening had a similar effect on all the variables in this factor, and this was indicative of the interrelationships among them.

Since synthesis of the anthocyanins is localized in the same tissues in which the anthocyanins are present (Ran & Forkmann, 1986), it is likely that synthesis of the peonidin derivatives took place mainly in the innermost layers of the epidermis or even in the subepidermis. That is, the peonidin derivatives, like the rest of the anthocyanins, were synthesized from the sugars present in the skin; however, they may also have been synthesized from sugar translocated from the pulp, in line with the redistribution of sugars described by Wicks & Kliewer (1983). This could account for the association with F2.

This phenomenon might be related to the fact that synthesis of peonidin derivatives is quite pronounced in in-vitro cultures (Lofty *et al.*, 1988). In such cultures, the tissues are maintained in a fluid that is rich in sugars, thus resembling the pulp, and there may be a certain relationship between the availability, or the greater mobility, of the sugars and activation or stimulation of the enzyme systems that lead to the production of peonidin and its derivatives. To date no hypothesis has been put forward in this regard, although the prevalence of derivatives of peonidin in the pulp of teinturier varieties has been described by Bakker & Timberlake (1985). Furthermore, the pulp also contains low concentrations of saccharose, which activates anthocyanin synthesis (Gianfagna & Berkowitz, 1986).

CONCLUSIONS

Only the sugars present in the berry skin are closely and directly related to the synthesis of phenolic compounds in the skin.

Although soluble carbohydrates present in the pulp are unrelated to phenolic compounds present in the skin, the peonidin derivatives are an exception, as they would be synthesized, mainly, in the most internal cellular layer of the epidermis, where they would be able to be synthesized from sugars present in the skin and from sugars translocated from the pulp.

REFERENCES

Bakker, J. & Timberlake, C. F. (1985). The distribution of anthocyanins in grape skin extracts or Port wine cultivars as determined by HPLC. *J. Sci. Food Agric.*, **36,** 1315–24.

Champagnol, F. (1984). Elements de physiologie de la vigne et de viticulture generale. *Rev. Agron.*, **327,** 124–45.

El-Manawaty, H. K. E. (1981). Relation of anthocyanin pigments concentration and sugar formation in some pigmented grape varieties. *Agric. Res. Rev.*, **56,** 83–7.

Flora, L. F. & Lane, R. P. (1979). Effects of ripening and harvest date on several physical and compositional factors of cowart Muscadine grapes. *Am. J. Enol. Vitic.*, **30,** 241–6.

Gianfagna, T. J. & Berkowitz, G. A. (1986). Glucose catabolism and anthocyanin production in apple fruit. *Phytochemistry*, **25,** 607–9.

González-SanJosé, M. L. (1989). Comportamiento de compuestos del metabolismo secundario en la maduracion de la uva de *Vitis vinifera.* PhD Thesis, Universidad Autonoma de Madrid, Spain.

González-SanJosé, M. L., Diez, C., Santa-Maria, G. & Garrido, J. L. (1988). Analisis por cromatografia liquida de alta eficacia de los pigmentos autocianicos de uvas de *V. vinifera* L. *An. Quim.*, **84**(C), 290–4.

González-SanJosé, M. L., Barron, L. J. R. & Diez, C. (1990). Evolution of anthocyanins during maturation of Tempranillo grape varieties using polynomial regression models. *J. Sci. Food Agric.*, **51,** 337–43.

Hrazdina, G. (1986). Glucosylation of flavonoid compounds in *Hippeastrum. Bull. Liaison Groupe Polyphenols*, **13,** 27–32.

Hrazdina, G., Parsons, G. F. & Mattick, L. R. (1984). Physiological and biochemical events during development and maturation of grape berries. *Am. J. Enol. Vitic.*, **35,** 220–7.

Jackson, D. I. (1986). Factors affecting soluble solids, acid, pH and color in grapes. *Am. J. Enol. Vitic.*, **37,** 179–83.

Jenrich, R. & Mundie, J. (1983). *Statistical Software.* University of California Press, Los Angeles.

Kluba, R. M. & Mattick, L. R. (1978). Changes in non volatil acids and other chemical constituents of New York State grapes and wines during maturation and fermentation. *J. Food Sci.*, **43,** 717–20.

Lofty, S., Fleuriet, A., Ramos, T. & Macheix, J. J. (1988). Metabolisme des anthocyanols dans les suspensions cellulaires du raisin cultivées *in vitro. Bull. Liaison Groupe Polyphenols*, **14,** 258–61.

Masquellier, J., Michaud, J. & Triaud, J. (1965). Fractionnement des leucoanthocyanes du vin. *Bull. Soc. Pharm. Bourdeaux*, **104,** 145–8.

Métodos Oficiales de Análisis (1973). Ministerio de Agricultura, Madrid, Spain.

Morrison, D. (1976). *Multivariate Statistical Methods*, International Student Editions. McGraw-Hill Kogakusha, Tokyo, pp. 1–90.

Paronetto, L. (1977). *Polifenoli e Tecnica Enologica.* Selepress. Milano, pp. 73–131.

Piergiovanni, L. & Volonteiro, G. (1983). Studio della frazione antocianica delle uve. Variazioni di composizione durante la maturazione. *Technol. Alimentari-Imbottigliamento*, **6,** 22–8.

Pirie, A. J. G. & Mullins, M. G. (1976). Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate and abscisic acid. *Plant Physiol.*, **58,** 468-72.

Pirie, A. J. G. & Mullins, M. G. (1977). Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. *Am. J. Enol. Vitic.*, **28,** 204–9.

Pirie, A. J. G. & Mullins, M. G. (1980). Concentration of phenolics in the skin of grape berries during fruit development and ripening. *Am. J. Enol. Vitic.*, **31,** 110.

Popescu, I. V., Ionita, A., Tudorache, E., Marimescu, E. & Biciurescu, A. (1986). Quantitative and qualitative evolution of anthocyanins during grapes maturation in case of some Vitis cultivars. *Bull. Liaison Groupe Polyphenols*, **13,** 491–3.

Ran, D. & Forkmann, G. (1986). Anthocyanin synthesis in tissue cultures of *Callistephus chinesis* (china aster). *Plant Cell Reprod.*, **5,** 435–8.

Ruhnan, B. & Forkmann, G. (1988). Flavin 3,4-diols in anthocyanin biosynthesis, enzymic formation with flower extracts from *Callistephus chinesis*. *Phytochemistry*, **27,** 1085–9.

Swain, T. & Hillis, W. E. (1959). The phenolics constituent of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.*, **10,** 63–8.

Teusch, M., Forkmann, W. & Seyffert, W. (1987). Genetic control of hydroxycinnamoyl-coenzyme A: anthocyanidin-3-glucoside hydroxycinnamoyl-transferase. *Phytochemistry*, **26,** 2181–7.

Wicks, A. S. & Kliewer, W. M. (1983). Further investigations into the relationship between anthocyanins, phenolics and soluble carbohydrates in grape berry skins. *Am. J. Enol. Vitic.*, **34,** 114–16.