DOI : 10.5958/0974-0112.2016.00085.2

Indian J. Hort. 73(3), September 2016: 400-404

Dynamics of biochemicals of Punjab MACS Purple and H-144 from veraison to maturity under Punjab conditions

Pooja, G.S. Kocher* and M.I.S. Gill**

Department of Microbiology, Punjab Agricultural University, Ludhiana 141004

ABSTRACT

Grape [cv. Punjab MACS Purple (PP) and H-144] berries were analysed at different maturity stages for °Brix, anthocyanins, total phenols, ascorbic acid, pH, titratable acidity, tartaric acid, malic acid, total sugars, reducing sugars, glucose and fructose contents to select optimum harvesting stage for quality wine production. An increase in the berry size during the maturation period increased °Brix to 18.0 & 16.4°B and sugars to 17.6 and 15.6% in PP and H-144, respectively. The final glucose and fructose contents were 82.1, 90.2 and 74.2, 77.8 g/l that lead to glucose/fructose ratio of 0.91 and 0.96 in PP and H-144, respectively, while, malic and tartaric acids decreased to 4.0, 4.4 and 3.7, 4.5 g/l in fully ripened berries of PP and H-144, respectively. Total phenolics, anthocyanins and ascorbic acid contents were found to increase during the maturation period. Thus, the study defined 19-20 and 12-15 days of veraison in PP and H-144, respectively for optimum maturity under Punjab agro-climatic conditions.

Key words: Grape, maturity, organic acid, phenolics, sugars.

INTRODUCTION

Enology encompasses a complete process that starts with selection of the grapes and ends with bottling of finished wine. This is because wine quality is largely dependent on the quality of raw material as grape juice contributes important phenolics, flavanoids and other aromatic compounds. Besides, some compounds undergo biochemical transformations to form compounds during fermentation process that are distinctive to wine quality. 'Uvology' is the branch of Science, which deals with the study of composition and mechanical characteristics of grapes, distribution of chemicals in constituent parts of the grapes and compositional changes during ripening (Canja and Padureano, 6). At maturity, grape juice is generally composed of water (74%), sugars (25%, primarily fructose and glucose), organic acids (0.8%, primarily tartaric and malic acids), minerals (0.5%, mainly potassium), phenolics, flavonoids, aromatics and nitrogenous compounds (0.2%) (Conde *et al*., 8). However, due to the abundance and ease of measurement, the primary fruit maturity indicators for wine production are considered to be sugar, acid content and pH.

Veraison period (onset of ripening coupled with colour change in berries) witnesses drastic changes in the overall composition of grape berries leading to an optimal stage, which is referred as technological maturity for producing a particular wine (Antoce, 3). Broadly, technological maturity refers to accumulation of large amounts of sugar and reduced excessive acidity in order to achieve a balanced ratio between the two parameters. Glucoacidimetric index values (ratio between total sugars content and acidity) increase during maturation and at ripening of the grapes, and for wine grape varieties the desirable index has values between 27-34 (Antoce, 3).

Under North Indian conditions, especially in Punjab where pre-monsoon rainfall damage to grape crop is very common, a thorough study to select the optimum maturity stage for harvest of berries for wine production holds important consideration. Punjab MACS Purple is a black grape variety that ripens in the 2nd week of June, while H-144 is a pigmented grape variety (showing purple coloration) with white juice that ripens in the 1st week of July (Gill and Arora, 12). Punjab MACS Purple (H-516) and H-144 hybrid grape cultivars (introduced to north India from ARI, Pune) have shown promising characteristics in terms of fruit yield, juice content, TSS, clusters per vine and their processing potential (Gill *et al*., 11). Starting from the fruit set, onset of veraison to full mature stage, the physico-chemical analysis of berries can save the grape crop to process them into quality wines, besides increasing the economic status of Punjab farmers. Therefore, these two cultivars having wine making potential were selected for the present study to determine fruit parameters at different maturity stages during veraison period.

*Corresponding author's E-mail: gskocher@pau.edu

**Department of Fruit Science, Punjab Agricultural University, Ludhiana 141004

MATERIALS AND METHODS

The sampling site of the grape berries was the Vineyard of Department of Fruit Science, PAU, Ludhiana. Grape berries cv. Punjab MACS Purple and H-144 were hand harvested and randomly collected (10 clusters from each vine and 10 berries from each cluster) from start of veraison to the fully matured stage at regular intervals of three days, in sterile plastic bags. They were then taken to the Industrial Microbiology Laboratory, PAU, Ludhiana for further analysis, whereby berries were crushed in a mechanical juicer to extract juice that was collected in sterile amber bottles for further use.

The physico-chemical analysis of grape berries included estimation of total soluble solids (digital refractometer, Atago), total sugars (Dubois *et al*., 10), pH (Hanna HI96107), titratable acidity, reducing sugars (Miller, 16), ascorbic acid (AOVC, 4, anthocyanins (Zoecklein, 21), malic acid (Goodban and Stark, 13), total phenols (Malik and Singh, 15), glucose and fructose contents. The later were analysed by HPLC (Dionex Corp., CA, USA) using a Shodex SP-0810 column (330 mm × 7.8 mm) fitted with a SP-G guard column (Waters Inc., USA). Degassed deionized water was used as a mobile phase at a flow rate of 1.0 ml / min. The column oven and refractive index detector were maintained at 80° and 55°C, respectively. Fruit juice samples were centrifuged and filtered through Phenomenex 0.45 µm using RC membranes. Peaks were detected by the refractive index detector and quantified on the basis of area and retention time of the sugar standards.

RESULTS AND DISCUSSION

The grape berries (cv. Punjab MACS Purple and H-144) were analysed for different physico-chemical parameters for the whole veraison period, which continued for 19-20 days in Punjab MACS Purple and 12-15 days in H-144. During the veraison period, TSS content of both Punjab MACS Purple (2.8 to 18.0°B) and H-144 (2.1 to 16.4°B) cultivars was found to be increased that improves the sweetness of the berries. Detailed analysis of sugar components revealed that total sugars and reducing sugars were increased with increase in the TSS content from onset of veraison to fully matured stage (Fig. 1 & 2). Glucose and fructose content in the berries of both the varieties increases during veraison to fully mature stage ranging from 16.4 to 82.1, 11.3 to 90.2 g/l in PP and 6.1 to 74.2, 5.2 to 77.8 g/l in H-144, respectively. Results (Fig. 1 & 2) showed that glucose predominates over fructose in early stages upto veraison, while fructose content was higher than glucose content in later maturity stages in both the cultivars. At fully ripened stage G/F ratios were found to be 0.91 and 0.96 in PP and H-144 cultivars, respectively that met the desirable range for wine production. Elsewhere, literature revealed that glucose / fructose ratio varies according to the

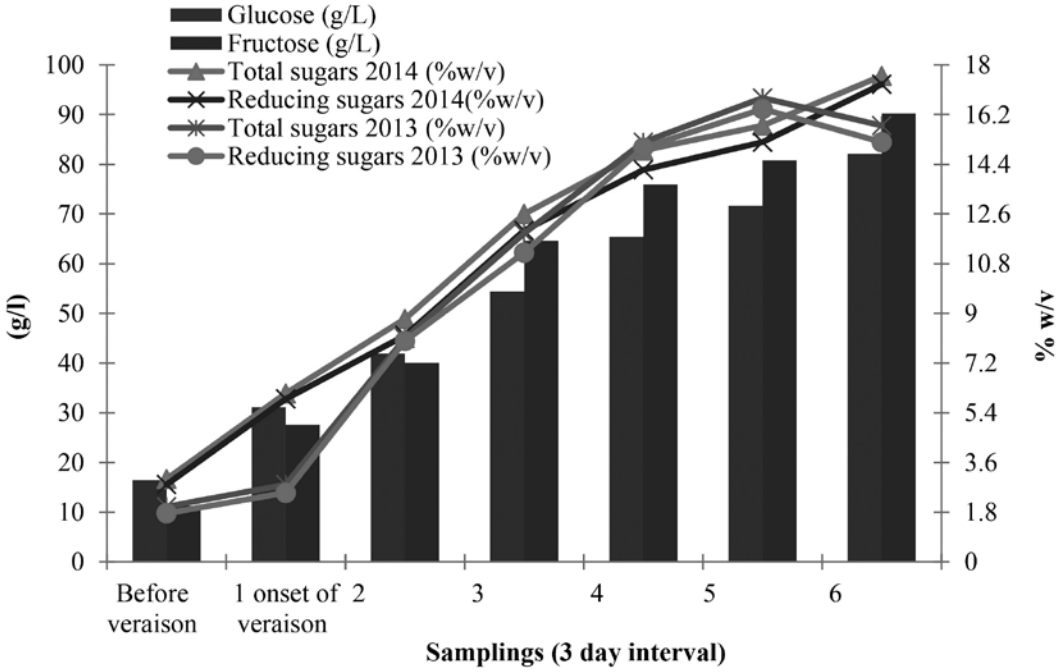

Fig. 1. Different sugars content in Punjab MACS Purple grape berries at various maturity stages.

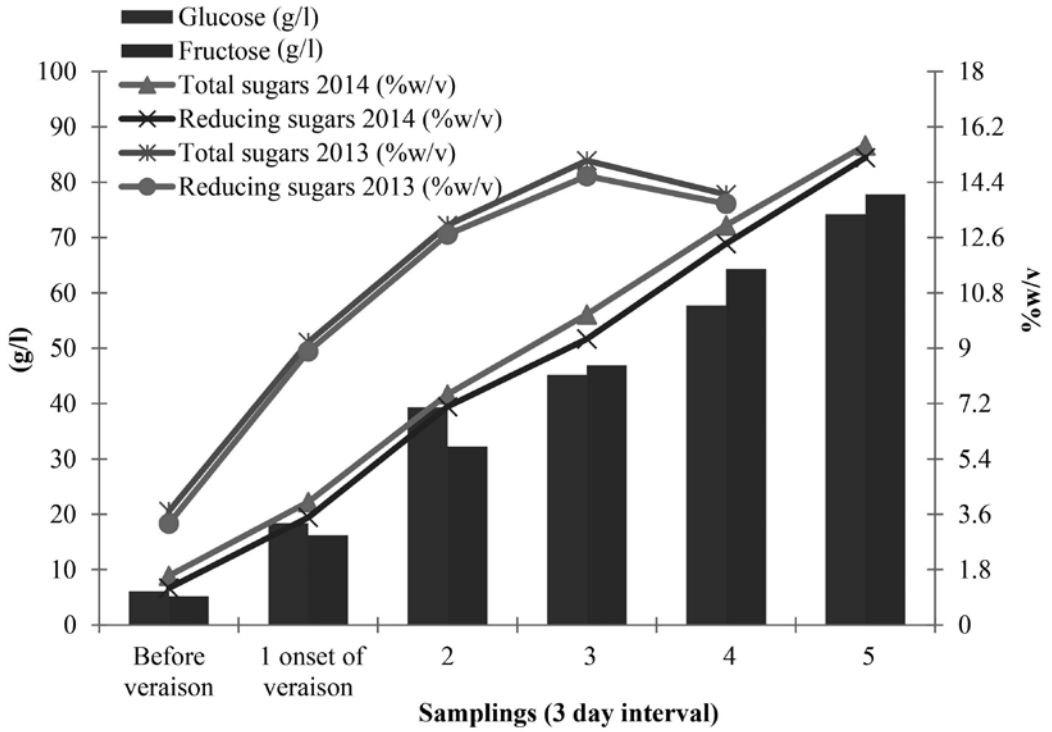

Fig. 2. Different sugars content in H-144 grape berries at various maturity stages.

cultivar, growing condition, and activity of enzymes for sucrose degradation. G/F ratio was reported in the range of 0.74 to 1.55 (Sabir *et al.*, 18) and 1.95 to 1.55 from unripe to ripe stage of berry growth (Soulis and Averginos, 19). The difference may be attributed to different climatic conditions as well as cultivars studied. However, a similar range of 0.74-1.05 and 0.98 to 1.42 was reported by Kliewer (14). Punjab MACS Purple cultivar showed a higher content of sugar compared to H-144. Similarly, Rusjan *et al.* (17) reported an average content of 154-180 g/l sugars in red grapes which was found to be higher as compared to green grape cultivars. Sabir *et al.* (18) reported the total sugars content ranging from 16.3 to 19.8% in five different cultivars of grapes, which increased during the course of maturation initially ranging from 2.2 to 5.4% at unripe stage. Grape varieties like Cabernet Sauvignon, Shiraz, Merlot, Pinot Noir, Chardonnay, Zinfandel *etc*. are popularly used in Australia and America for wine production and are normally harvested after attaining 19-25°B level in the berries (Anon, 2).

However, due to hot weather and abrupt rainfall under North Indian climatic conditions, grape cultivars showed a low °Brix content, which maximally reached to 18°B in PP and 16.4°B in H-144. However, processing of grape into wine requires an initial Brix of 20-22°B so as to achieve a final ethanol content of 12-13% (v/v). Hence, °Brix level needs to be adjusted to 20-22°B (by chaptalizing sugar to the juice) so that the desired ethanol levels may be obtained in the resulting wine. This is also important when there are natural climates/ climate change such as during 2013, whereby both cultivars suffered from pre-monsoon rainfall damage that lead to a sharp decrease in the TSS (°Brix) levels as well as physical damage to berries, making them unsuitable for wine production. Further amelioration of sugar into grape juice is allowed in many countries such as Chile, China, Canada, Germany, Japan, New Zealand *etc.* and prohibited in Australia, Austria, some parts of Germany, California of USA, Italy and South Africa (US Federal Regulations, 20).

The decrease in acidity is another event during veraison period. The berry organic acids, *i.e.* tartaric acid as well as malic acid were found to decrease gradually (Tables 1 & 2) during the veraison period resulting in the optimum acidity range for wine production. This decrease is attributed to an increase in membrane permeability allowing acids to store in cell vacuoles (Kliewer, 14). Recording of °Brix, pH and acidity is an easy criterion for selecting optimum grape berries for wine production includes the calculation of °Brix: acid ratio and °B-pH index that should be in the range of 27-34 and 200-270, respectively (Bisson, 5). During the veraison period, both parameters were

Table 1. Physico-chemical analysis of Punjab MACS Purple berries at different intervals during veraison (2013 and 2014).

Parameter	Samplings of vintage								
	Before veraison	Onset of veraison	3*	6*	9*	12*	15*	18*	$CD_{0.05}$
TSS	2.8 ± 0.2	4.7 ± 2.1	8.8 ± 0.4	12.9 ± 0.4	15.6 ± 0.8	17.1 ± 1.0	17.5 ± 0.3	18.0 ± 0.2	0.385
Titrable acidity (% w/v)	1.7 ± 0.4	1.1 ± 0.3	0.85 ± 0.1	0.7 ± 0.12	0.6 ± 0.02	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.027
pH	2.7	2.9	3.1	3.4	3.6	3.7	3.8	3.8	0.031
Brix-acid ratio (27-34)[D]	1.6	4.27	10.35	18.4	26.0	28.5	29.1	30.0	0.564
Brix-pH index (200-270)[D]	20.4	39.5	84.5	149.1	202.1	234.0	252.7	259.4	0.729
Tartaric acid (g/l)	10.7 ± 0.4	9.9 ± 0.3	7.6 ± 0.5	6.5 ± 0.3	5.5 ± 0.3	4.3 ± 0.6	4.4 ± 0.3	4.4 ± 0.2	0.201
Malic acid (g/l)	9.65 ± 0.5	8.6 ± 0.3	7.1 ± 0.3	6.3 ± 0.5	5.8 ± 0.2	4.2 ± 0.2	4.1 ± 0.1	4.0 ± 0.1	0.083
Total phenols (mg/100 ml)	49.3 ± 3.4	86.4 ± 1.4	102 ± 2.5	133.6 ± 3.4	155.8 ± 1.4	177.2 ± 2.2	175.6 ± 1.9	178 ± 0.4	0.672
Anthocyanins (mg/100 ml)	0.43 ± 0.04	1.91 ± 0.09	5.33 ± 0.22	12.4 ± 0.4	15.3 ± 0.11	20.7 ± 0.15	20.8 ± 0.23	23.2 ± 1.1	0.412
Ascorbic acid (mg/100 ml)	602 ± 4.6	792 ± 2.6	934 ± 4.2	1229 ± 2.4	1289 ± 1.3	1384 ± 2.2	1397 ± 2.4	1428 ± 4.7	0.913

*Days after veraison; [D]desirability range; Date of onset of veraisons- 26/5/13 and 29/5/14.

Table 2. Physico-chemical analysis of H-144 berries at different intervals during veraison (2013 and 2014).

Parameter	Samplings of vintage							
	Before veraison	Onset of veraison	3*	6*	9*	12*	15*	$CD_{0.05}$
TSS	2.1 ± 0.5	4.2 ± 0.1	8.7 ± 0.2	12.6 ± 0.1	14.2 ± 0.3	16.0 ± 0.3	16.4 ± 0.2	0.287
Titrable acidity (% w/v)	1.8 ± 0.2	1.4 ± 0.3	1.0 ± 0.1	0.9 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.061
pH	2.3	2.5	3.0	3.4	3.7	4.0	4.0	0.047
Brix-acid ratio (27-34)[D]	1.1	3.0	8.7	14.0	23.6	32	32.8	0.387
Brix-pH index (200-270)[D]	11.1	26.2	78.3	145.6	194.3	256	262.4	0.679
Tartaric acid (g/l)	11.0 ± 0.7	9.6 ± 0.4	7.3 ± 0.3	6.4 ± 0.1	5.3 ± 0.2	4.4 ± 0.3	4.5 ± 0.2	0.162
Malic acid (g/l)	10.8 ± 0.3	8.9 ± 0.3	6.4 ± 0.8	6.3 ± 0.1	4.9 ± 0.6	3.8 ± 0.3	3.7 ± 0.6	0.128
Total phenols (mg/100 ml)	47.8 ± 1.67	64.8 ± 1.26	76.9 ± 2.5	105.2 ± 1.3	124.2 ± 1.3	134.3 ± 2.11	141.4 ± 2.87	0.339
Anthocyanins (mg/100 ml)	0.0 ± 0.0	0.14 ± 0.05	0.23 ± 0.21	0.57 ± 0.14	0.61 ± 0.23	0.75 ± 0.24	0.78 ± 0.14	0.015
Ascorbic acid (mg/100 ml)	340.9 ± 5.9	539.2 ± 2.7	848.4 ± 3.4	949.7 ± 6.5	1247.7 ± 3.8	1287.9 ± 2.7	1281 ± 1.2	0.754

*Days after veraison; [D]desirability range; Date of onset of veraisons- 26/5/13 and 29/5/14.

found in the desirable range for both the cultivars PP and H-144 (Tables 1 & 2).

The present study further focused on total phenolics, anthocyanins and ascorbic acid in the berries that are known to improve sensory, visuality, aromatic as well as nutritive value of the wine produced from them. All the three components increased during the course of maturation period of berry growth and their final concentrations were found to be 178.0, 23.2 and 1428.0 mg/100 ml in PP and 141.4, 0.78 and 1281 mg/100 ml in H-144 cultivars, respectively. The trends in physico-chemical grape characteristics were also reflected in their appearance (Fig. 3a & b) with increase in size and colour change to dark purple/black in PP and to light purple in H-144. In another study on the growth and ripening of berries by Ana and Maja (1), the phenolic content of mature berries was reported 3272 mg/kg of fresh weight of grape. Similarly, Cantos *et al.* (7) found the total phenolics in the range of 115 (Dominga) to 361 (Flame Seedless) mg/kg of fresh weight of grapes. Anthocyanins content in three grape juices (homemade, commercial and organic grape juice) studied by Burin *et al.* (9) was found to be 241.22, 207.99, 460.45 mg/l. Research studies carried out with Italian grape varieties have also shown that total anthocyanin levels ranged from 102 to 405 mg/l (Cimino *et al*., 9).

Hence, the present study concludes that two grape cultivars under evaluation are good in terms of physical and biochemical composition that is required for quality wine production. Further, it clearly defines the physico-chemical characteristics as well as appearance of grapes for two consecutive years that

veraison period is 19-20 days in PP and 12-15 days in H-144. This information may be helpful in deciding the proper harvesting stage (after 15-17 days in PP and 13 days in H-144 from onset of veraison) for grapes in respect of Punjab purple (H-516) and H-144 cultivars for processing them into quality wines.

ACKNOWLEDGEMENTS

The authors acknowledge DST, New Delhi for providing the financial assistance to Pooja in the form of INSPIRE Fellowship programme and Department of Fruit Science, PAU, Ludhiana for providing grape cultivars for the study.

REFERENCES

1. Ana, T. and Maja, M. 2010. Changes in sugars, organic acids and phenolics of grape berries of cultivar Cardinal during ripening. *J. Food, Agri. Env.* **8**: 223-27.

2. Anonymous, 2013. http://nrcgrapes.nic.in/Recommended_Varieties.htm

3. Antoce, O.A. 2007. *Oenology: Chemistry and Sensorial Analysis* (1st Edn), Universitaria Publishing House, Craiova, pp. 212-15.

4. AOVC. 1996. *Methods of Vitamin Assay*, Association of Vitamin Chemists Inc. (Ed.), Interscience Publishers, New York, pp, 79-81.

5. Bisson, L. 2001. Optimal grape maturity. http://www.practicalwinery.com/julaug01p32.htm

6. Canja, C.M. and Pădureanu, V. 2011. Bulletin of the *Transilvania.* University of Braşov. **4**: 53.

7. Cantos, E., Espin, J.C. and Tomas-Barberan, F.A. 2002. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MSMS. *J. Agric. Food Chem.* **50**: 5691-96.

8. Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. and Geros H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *Food*, **1**: 1-22.

9. Cimino, F. 2007. Radical-scavenging capacity of several Italian red wines. *Food Chem*. **103**: 75-81.

10. Dubois, M., Giles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350-56.

11. Gill, M.I.S., Sharma, J.K., Sachdev, P. and Mehan, V.K. 2008. H-516: A promising grape hybrid under N-Indian conditions. *Acta Hort.* **785**: 23-26.

12. Gill, M.I.S. and Arora, N.K. 2009. Performance of different grape varieties under North Indian conditions. *Indian J. Ecol.* **36**: 15-17.

13. Goodban, A.E. and Stark, J.B. 1957. Rapid method for determination of malic acid. *Anal. Chem.* **29**: 283-87.

14. Kliewer, W.M. 1967. The glucose-fructose ratio of *Vitis vinifera* grapes. *American J. Enol. Vitic.* **18**: 33-41.

15. Malik, C.B. and Singh, M.B. 1991. *Plant Enzymology and Histoenzymology* (1st Edn.), Kalyani Publishers, New Delhi, pp. 231-33.

16. Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**: 426-28.

17. Rusjan, D., Korosec-koruza, Z. and Veberic, R. 2008. Primary and secondary metabolites related to the quality potential of table grape varieties (*Vitis vinifera* L.). *European J. Hort. Sci*. **73**: 124-30.

18. Sabir, A., Kafkas, E. and Tangolar, S. 2010. Distribution of major sugars, acids and total phenols in juice of five grapevine (*Vitis* spp.) cultivars at different stages of berry development. *Spanish J. Agri. Res.* **8**: 425-33.

19. Soulis, T.P. and Avgerinos, A.S. 2006. Changes in the glucose: fructose ratio during the growth period of the Razaki grape. *J. Sci. Food Agr.* **35**: 527-30.

20. United States Federal Regulations, 2004. Title 27, Section 24. 177" (PDF). Department of the Treasury.

21. Zoecklein, B. 2005. *Wine Analysis and Production,* Chapman & Hall, New York.

Received : September, 2015; Revised : July, 2016; Accepted : August, 2016