DETERMINATION OF SUGARS IN DIFFERENT GRAPES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY – ELSD.

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ABSTRACT-The purpose of this current research is quantitative determination of glucose, fructose and sucrose in various grapes. Grape is one of the most important commercial crops and widely consumed. Green, black and red grapes are used for present study which is easily available in the market. Simultaneous determination of sugars was performed using High performance liquid chromatography equipped with ELSD detector. The concentration of fructose and glucose ranged from 8.70-10.44% and 6.17-9.18% respectively. It was found that fructose to glucose ratio varied among variety of grapes. Red grapes contain increased concentration of glucose than fructose. Sucrose, maltose, xylose and lactose were found to be below limit of detection. Fructose and glucose are the major sugars found in all samples.

KEY WORDS: **Glucose, fructose, grape, HPLC-ELSD**

INTRODUCTION

The major components in grapes are water followed by sugars mainly fructose, glucose and sucrose. Grape is an excellent source of nutrients able to contribute to a healthy diet. The monosaccharide is one of the most important parameter for the assessment of the commercial quality of the fruits for human and industrial use [1]. Nowadays, it is consumed due to presence of antioxidants in large quantity. It also contains organic acids, elements and phenolic compounds. Recently, the interest in the composition of berry fruits has grown because of increased awareness of their possible health benefits, as they are rich sources of sugars, micronutrients, and other important substances such as phytochemicals. Infact, recent studies have revealed that berry species have essential positive effects on the human health which could be mainly ascribed to the presence of several taste and health related compounds such as sugars, phenolics, and organic acids [2]. Sugars have major effects on taste and represent an index of consumer acceptability. Modern consumers are more interested in their personal health and expect the foods to be not only tasty and attractive but also safe and healthy.

The sucrose metabolism-related sugars in fruits mainly include glucose, fructose, and sucrose [3]. There are some scientific evidences that sucrose is the primary form of transported sugar in tomatoes [4, 5], but it only accounts for a small percentage, while glucose and fructose, in approximately equal contents, reach over 50% of the total water-soluble sugars in fruits [6]. During grape berry ripening, other metabolic changes occur, such as the accumulation of sugars in the form of glucose and fructose in the berry (flesh and skin) vacuoles, after translocation of sucrose from the leaves [7]. The accumulation of sugar in the form of glucose and fructose within the vacuole is one of the main features of the ripening process in grape berries and is a major commercial consideration for the grape grower, winemaker and dried fruit producer [8, 9]. In this study, grape samples of green, black and red varieties from local market were selected and processed for sugar profiling. A HPLC equipped with evaporative light scattering detector (ELSD) was used for sugar analysis. The aim of this research is to compare glucose and fructose content of different green, black and red varieties of grapes.

MATERIALS AND METHODS

In this study, Sonaka variety for Green, Flame seedless variety for Black and Red globe variety for Red Table grapes was selected. Samples were collected from local market and were immediately processed for sugar analysis. Sugars were analyzed according to the method of Clement et al. [9]. Accordingly, 1g crushed berries sample was taken in centrifuge tubes and to it 10ml 80% methanol was added, vortexed for 1min, then was centrifuged at 5000rpm for 5 min. The supernatant was filtered through 0.22um filter, diluted and analyzed by HPLC-ELSD. For quantification, calibration curves were prepared using standards for each compound fructose, glucose and sucrose [fig 1]. Sugars were analyzed in a chromatograph equipped with Evaporative Light Scattering Detector (ELSD) detector (Agilent technologies). The separation of sugars was performed using a amino column Luna 5µ NH_2 (150 x 4.6) with a mobile phase of 93% acetonitrile and 7% HPLC grade water, degassed and ultrasonicated. The analysis conditions were held constant at a flow rate of 0.8ml/min at 35°C, injection volume was 5µl. The ELSD temperature was 40°C and injection volume 15µl.

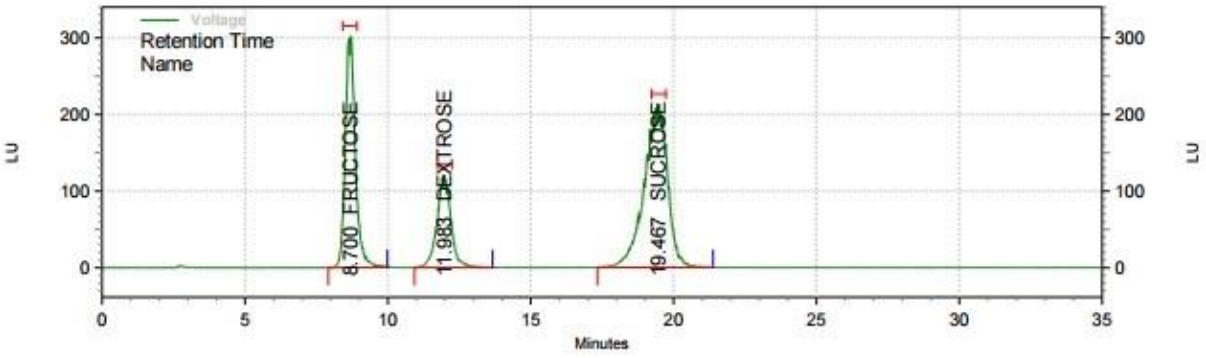

Fig. 1 Chromatogram of standard mixture of fructose, glucose, sucrose.

RESULTS AND DISCUSSION

It was observed that the concentration of fructose ranged from 8.25-10.56%, 8.48-9.02% and 8.47-8.88% in Sonaka, Flame seedless and Red globe variety respectively. The concentration of glucose ranged from 7.0-9.18%, 9.4-9.78% and 6.25-6.57% in Sonaka, Flame seedless and Red globe variety respectively. Comparative graph for fructose and glucose in all three varieties of grapes are shown in Fig. 2, 3 and 4. As expected, fructose was higher than glucose in Sonaka and Red globe grapes but to the contrary glucose was more than fructose in Flame seedless grapes. Sucrose, maltose, xylose and lactose were found to be below limit of detection. Based on the results of this study, fructose and glucose are the major sugars found in all samples. Also, there is a need to understand other biochemical parameters that change the overall flavor of grapes due to color of grapes.

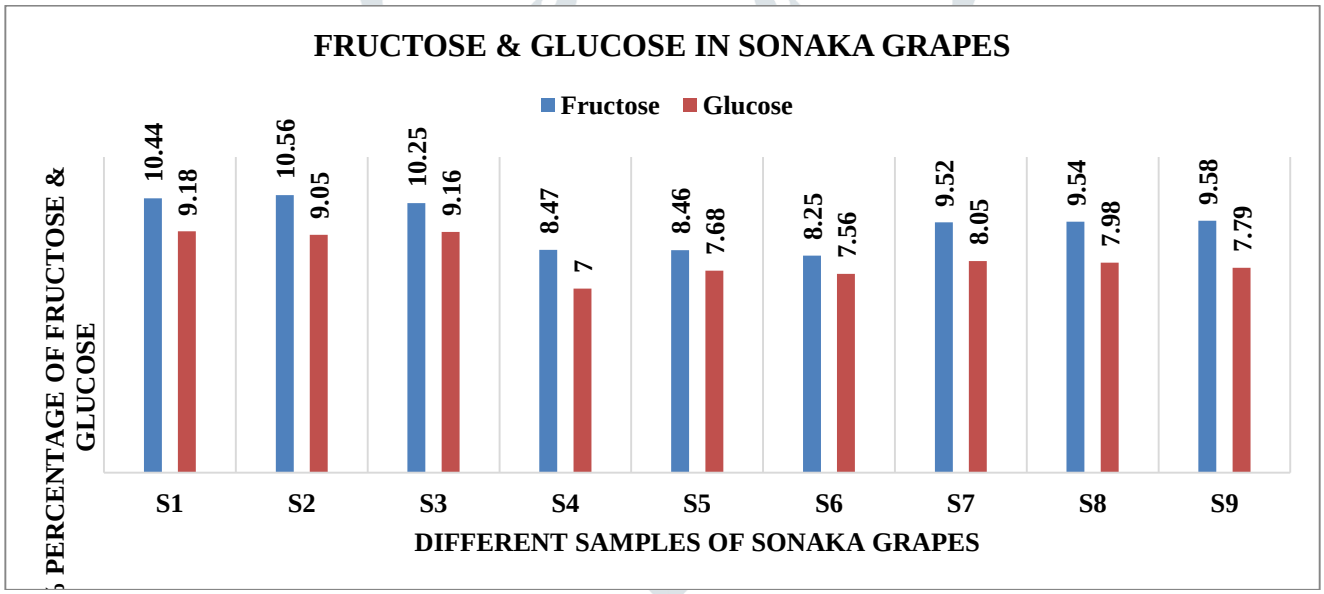

Fig. 2

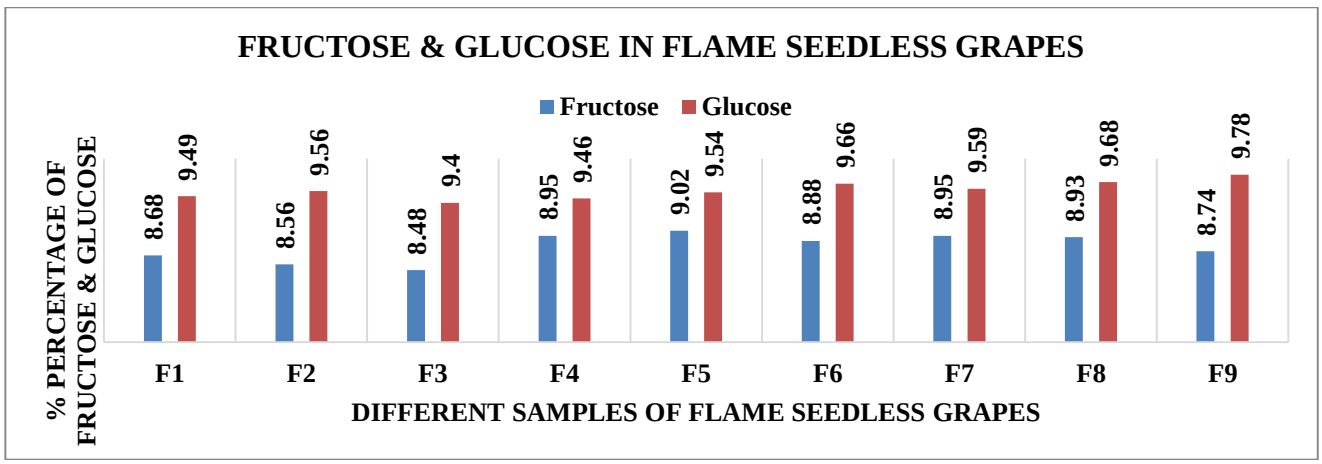

Fig. 3

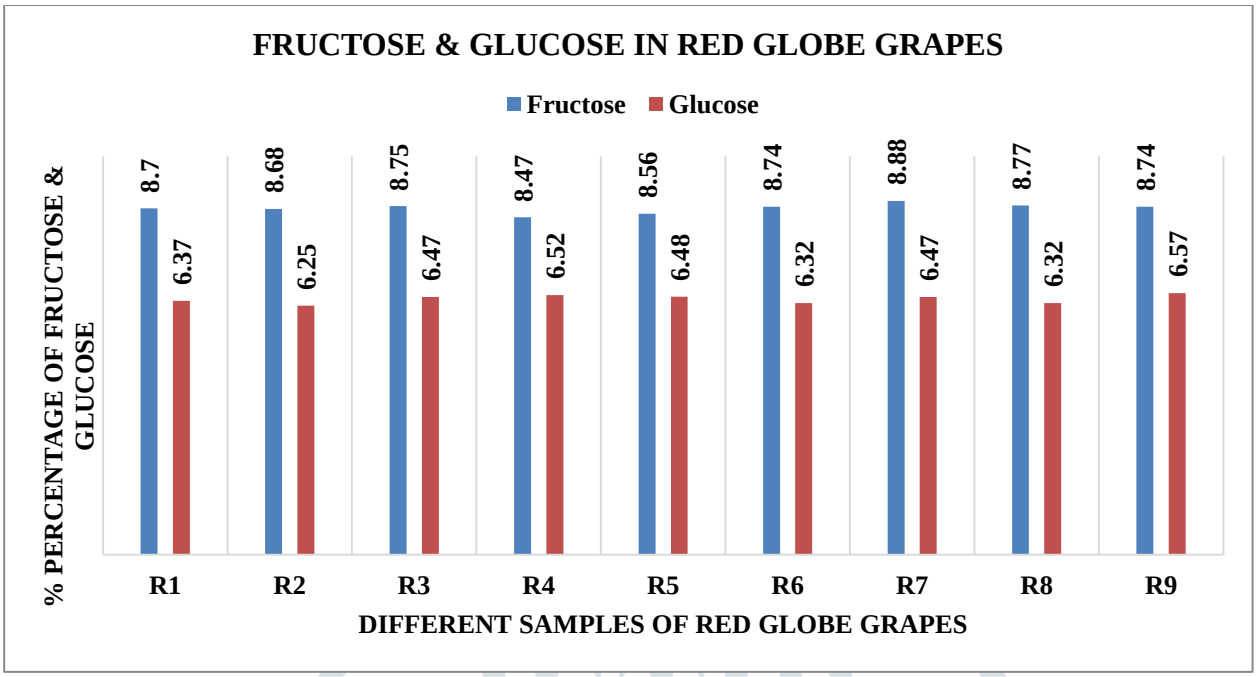

Fig. 4

REFERENCES

[1] Mahmood, S., Mohammed, T., Salih, A., El-Sayed, S. A. 2011. Determination of Fructose, Glucose and Sucrose in Taif Grape using High Performance Liquid Chromatography and analysis of mineral salts.

[2] Badjakov, M. Nikolova, R. Gevrenova, V. Kondakova, E. Todorovska, and A. Atanassov, "Bioactive compounds in small fruits and their influence on human health," *Biotechnology & Biotechnological Equipment*, vol. 22, no. 1, pp. 581–587, 2008.

[3] Lu, Y. M. and Zhang, D. P. "Accumulation of sugars in developing fruits," *Plant Physiology Communications*, vol. 36, no. 3, pp. 258–265, 2000.

[4] Walker, A. J. and. Ho, L. C. "Carbon translocation in the tomato: carbon import and fruit growth," *Annals of Botany*, vol. 41, no. 4, pp. 813–823, 1977.

[5] Bucheli, P. and D´evaud, S. "Sugar accumulation in tomato and partial purification of buffer-insoluble invertase," Phytochemistry, vol. 36, no. 4, pp. 837–841, 1994.

[6] Robinson, N. L., Hewitt, J. D. and Bennett, A. B. "Sink metabolism in tomato fruit. I. Developmental changes in carbohydrate metabolizing enzymes," Plant Physiology, vol. 87, no. 3, pp. 727–730, 1988.

[7] Orak, H. H., "Determination of Glucose and Fructose Contents of Some Important Red Grape Varieties by HPLC" Asian Journal of Chemistry, Vol. 21, No. 4 , 3068-3072, 2009

[8] Shaw, D.V., J. Am. Soc. Horticult. Sci., 113, 770, 1988.

[9] Clement, A., Yong, D. and Brechet, C."Simultaneous Identification of Sugars by HPLC Using Evaporative Light Scattering Detection (ELSD) and Refractive Index Detection (RI). Application to Plant Tissues", Journal of liquid Chromatography, vol 15, issue 5, 1992.

[10] Coombe, B.G., Am. J. Vitic., 43, 101, 1992.

[11] Davies, C. and Robinson, S.P. , Plant Physiol., 111, 275, 1996.

[12] Pablo, M., Paula, R., Daniel, M, Rosa, M. and Bruno, G., "Characterization of sugars and organic acids in Commercial varieties of table grapes", CHILEAN JOURNAL OF AGRICULTURAL RESEARCH 71(3), 2011.

[13] Montesano, D., Cossignani, L., Giua, L., Urbani, E., Simonetti, M. S. and Blasi, F. ,2016, A Simple HPLC-ELSD Method for Sugar Analysis in Goji Berry.

[14] Baldwin, E.A. 2002. Fruit flavor, volatile metabolism and consumer perceptions. p. 89-106. *In* Knee, M. (ed.) Fruit quality and its biological basis. Sheffield Academic Press, Sheffield, UK.

[15] Chervin, C., A. El-Kereamy, J.P. Roustan, A. Latché, J. Lamon, and M. Bouzayen. 2004. Ethylene seems required for the ripening of grape, a non-climacteric fruit. Plant Science 167:1301-1305.

[16] Conde, C., P. Silva, N. Fontes, A.C.P. Dias, R.M. Tavares, M.J. Sousa, *et al.* 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1:1-22.