Pak. J. Bot., 36(2): 389-394, 2004.

CHANGES IN SUGAR COMPOSITION IN CHERRY LAUREL (CV OXYGEMMIS) FRUIT DURING DEVELOPMENT AND RIPENING

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Abstract

Changes in the soluble sugar composition in cherry laurel (*Laurocerasus officinalis* 'Oxygemmis') fruit during development and ripening were studied by gas chromatography-mass spectrometry (GC-MS). The sugars identified and quantified in the fruit were fructose, glucose and sucrose, and sorbitol as sugar alcohol. Fructose and glucose were the major sugars while sucrose were in much lesser amounts. From 23 to 58 days after flowering (DAF), the levels of fructose and glucose started to decrease rapidly and reached to a minimum level of 1.3 and 0.8% of fresh weight, respectively. The decrease in these two sugar levels followed an increase in fructose and glucose levels beginning from 65 to 86 DAFs as 23.6 and 20.8% of fresh weight, respectively. The level of sorbitol decreased to a minimum value at 51 DAF, and then increased rapidly after 58 DAF to its highest level as 13.4% of fresh weight. Besides these sugars sucrose was not detected between 23 and 44 DAFs, but increased rapidly until 58 DAF and then decreased after this stage. The rapid increase in the levels of fructose, glucose and sorbitol were determined from 79 to 86 DAFs which is the harvest season of this cultivar.

Introduction

Free sugars are one of the most important constituents for determining the quality of fruits and vegetables. There have been numerous studies investigating the free sugar composition, metabolism and physiology of many horticultural crops and fruits (Shaw, 1988). Most fruits accumulate and store either sucrose or hexoses. Fruits such as peach (Moriguchi *et al*., 1991), mango (Hubbard *et al*., 1991), certain melons (Hubbard *et al*., 1989) and some citrus (Lowell *et al*., 1989) store relatively high levels of sucrose. However, small fruits, such as blueberries and strawberries, often store primarily glucose and fructose (Shaw, 1988).

The genus *Laurocerasus* L. (cherry laurel) is represented by only one species in the Rosaceae family. *L. officinalis* Roem. (syn: *Prunus laurocerasus* L.) is an evergreen plant up to 6 m in height. The fruits of wild cherry laurels are ovoid, 8 mm in diameter (12 mm in some cultivars), dark purple or black in mature form (Browicz, 1972). The fruits of both the wild and cultivated plants are very poisonous in its early stages but can be used, when ripe, to prepare various alcoholic drinks which have a pleasant almonds taste (Milan, 1984). Many cultivars of the plant have been described in different countries (Dirr, 1990).

Three cultivars viz., Oxygemmis, Globigemmis and Angustifolia and the wild forms of *L. officinalis* are commonly present in Turkey, especially in the Black Sea region. The first two cultivars, 'Oxygemmis' and 'Globigemmis', were first recorded in Turkey (Var, 1992) and are abundantly grown for their large sweet fruits. The cultivars differ in quality of sweetness among the other described cultivars in public consumption. However, the

fruits of cultivated plants are sold in both fresh and dry forms in the big stores and local markets. In recent years, the fresh and dried fruits have been used for making jam, dried fruit pulp, marmalade and adorning alcoholic drinks. The fruits of this plant are well known as folk medicinal plants and used in the treatment of some illnesses (Baytop, 1984). Overall these benefits, the annual production of cherry laurel fruits of Turkey is not well documented.

Several studies have been reported on the soluble sugar ompositon of cherry laurel fruits (Ayaz *et al*., 1996, 1997; Ayaz, 1997). Our knowledge of the sugars that accumulate during fruit development and ripening of cherry laurel is limited. The aim of the present work was to study the changes of soluble sugar contents that occur during fruit development and ripening of 'Oxygemmis'. We chose to work with the cultivar since this is one of the principal cultivars grown in the Black Sea region in Turkey.

Materıals and Methods

Fruit material: Cherry laurel fruits (*Laurocerasus officinalis* Roem.'Oxygemmis') were harvested from various parts of the young trees during early morning in the vicinity of Trabzon Turkey (approx. 500 m above sea level, latitute 10°E). During each cherry laurel collection stage, one kg fruit was collected randomly depending on their color and maturity indices. Ten stages of fruit development have been determined ranging from fully green to fully reddish-black. These samples were obtained on a continuing basis at seven-day intervals after flowering, between April and June 1999 (Table 1). Fruits harvested in early morning were maintained below 12°C until arrival at the laboratory. In the laboratory, the fruits were kept in a frezeer (-20°C) for further analysis.

Table 1. Harvest dates, days after flowering (DAF) , state of maturity and color of cherry laurel cv. *Oxygemmis*) fruit.

Harvest no	Harvest date	Days after flowering (DAF)	State of maturity, color of fruit
1.	April 23, 1999	23	Unripe, dark green
2.	April 30, 1999	30	Unripe, dark green
3.	May 7, 1999	37	Unripe, dark green
4.	May 14, 1999	44	Unripe, greenish
5.	May 21, 1999	51	Unripe, light green
6.	May 28, 1999	58	Unripe, light green
7.	June 4, 1999	65	Halfripe, green-reddish
8.	June 11, 1999	72	Half raw, reddish
9.	June 18, 1999	79	Ripe, red
10	June 25, 1999	86	Fully ripe, reddish-black or dark black

Extraction of soluble sugars: The extraction of soluble sugar was made according to method of Kader *et al*., (1993). A sample 1 g of frozen cherry laurel fruits were ground in lquid N_2 and extracted in boiling 80% ethanol (1:10, w/v) to which 100 mg mannitol was added as an internal standard. Extracts were centrifuged, the supernatant decanted and the pellet re-extracted twice. The combined supernatant was partitioned against chloroform, and aqueous fraction was dried under vacuum, resuspended in water, and passed through Dowex-1 and Dowex-50 ions resins (Sigma). The resulting fraction was dried under vacuum and kept in a freezer (-20°C) for further analysis.

Preparation of oxime-trimethylsilyl derivatives of sugar extracts: Trimethylsilylated (TMS) oximes of sugar extracts were prepared according to Biermann & McGinnis (1989). A ca. 50 mg portion of sample was weighed and dissolved in 2.0 ml of pyridine. A 50 µl portion of the pyridine solution was transfered to a vial for pyridine stock solution containing 3% w/w hydroxylamine hydrochloride, and a known amount of methyl α-D-glucopyranoside added as an internal standard (ca. 250 µg/200 µl). The sample solution was kept at 70°C for 30 min. After cooling at room temperature 300 µl of HMDS (hexamethyldisilizane) and 200 µl of TMCS (trimethylchlorosilane) was added for silylation. Silylation was carried out at room temperature for 30 min before analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis of sugars: The GC-MS conditions were selected according to procedure of Chapman & Horvat (1989). The GC analysis was performed with a Varian 3300 instrument equipped with a flame ionization detector (FID). The GC-column was an HP-1 capillary column (25 m x 0.32 mm i.d., 0.17 µm film thickness). Injector and detector temperatures were 225 and 280°C, respectively, and the column oven temperature was initially held at 150°C for 4 min, then programmed to 192°C at 4°C/min and held for 0.5 min., and then programmed to rise 240°C at 10°C and hold for 7 min. Hydrogen was used as the carrier gas at a flow rate of 42 cm/s. A Merck-Hitachi D-2000 integrator was used for the peak area measurements. Mass spectrometry was used for sugar identification. The GC-MS analysis was performed with an HP 5890-5970 instrument using similar GC column operated at the same temperatures as in the GC-FID. Mass spectrometer conditions: ion source temperature, 150°C; scan rate, 200 amu/s; ionization energy 70 eV. The interface temperature between the GC and mass spectrometer was 200°C.

A mixture containing known amounts of the internal standard and pure reference sugars (glucose, fructose, sucrose, sorbitol, inositol and raffinose) was analysed to obtain correction factors. Invidual sugars were identified by comparison of their retention times and mass spectra with those of TMS derivatives of authentic compounds prepared in the same manner. Sugars were quantitated by using methyl α-D-glucopyranoside as an internal standard. Sugars were expressed at weigth percent of fresh weight.

Statistical analysis: The extraction and determination of sugars were made in triplicate. Analysis of variance of data was evaluated by the Statistical Analysis System (STATGRAPHICS Ver. 5.0 program). Duncan's Multiple Range Test was employed to determine the statistical significance of differences among the means. Statistical analysis in Table 2 indicates significant changes ($P = 0.05$) in sugars during fruit development and ripening.

Results and Dıscussıon

The sugars identified and quantitated during development and ripening of 'Oxygemmis' fruit were fructose, glucose and sucrose. Sorbitol was also identified as a sugar alcohol. Fructose and glucose were the major sugars detected while sucrose was found in much lesser amounts.

All measurements began 23 DAF (23 April) which was the earliest stage at which fruits could conveniently be obtained. From 23 to 58 DAFs, fructose and glucose level decreased rapidly to a minimum value of 1.3 and 0.8% of fresh wt, respectively. These

Table 2. Changes in the soluble sugar contents (% fresh weight) of cherry laurel (*Laurocerasus officinalis* Roem. 'Oxygemmis') fruits during development and ripening.

Days after flowering (DAF)	Sugars			
	Fructose	Glucose	Sucrose	Sorbitol
23	15.6±1.1 c	20.9±0.5 a	n.d.	4.7±0.8 d
30	12.7±2.0 d	16.4±1.2 b	n.d.	4.4±0.4 d
37	13.9±1.1 cd	16.6±1.4 b	0.1±0.0 d	5.8±0.2 c
44	7.0±1.3 f	5.9±0.6 d	0.2±0.0 cd	3.1±0.3 e
51	3.5±0.9 g	2.5±0.1 e	0.3±0.1 b	0.5±0.0 e
58	1.3±0.4 h	0.8±0.2 f	0.6±0.1 a	2.9±0.4 f
65	6.3±1.1 f	3.5±1.2 e	0.1±0.0 d	6.0±0.7 c
72	9.7±0.6 e	11.9±1.3 c	0.1±0.0 cd	8.6±0.5 b
79	17.9±1.3 b	16.0±0.4 b	0.3±0.1 b	12.9±0.5 a
86	23.6±0.2 a	20.8±0.7 a	0.2±0.0 bc	13.4±0.2 a

Results are the means of three replicates of extractions and determination. For comparison among means the analysis of variance (ANOVA) was used. Means in rows with different letter significantly different at $P = 0.05$. n.d.; detected (< 0.1 %).

decreases were followed by an increase in the level of both fructose and glucose from 65 to 86 DAFs to a maximum level as 23.6 and 20.8% of fresh wt, respectively. As a major sugar alcohol sorbitol was detected in all developmental stages beginning 4.7% of fresh wt at 23 DAF and decreased to a minimum level 0.5% of fresh wt at 51 DAF and then gradually increased to a maximum level 13.4% of fresh wt at 86 DAF. Sucrose was not detected from 23 to 30 DAFs and the level increased rapidly until 58 DAF to a maximum level 0.6% of fresh wt, and then decreased to a minimum level of 0.1 and 0.1% of fresh wt, respectively at 65 and 72 DAFs. At 79 DAF, the level of sucrose increased suddenly to 0.3% fresh wt and then remained low at 86 DAF in the over ripened fruits.

These results are in accord with the published data that glucose and fructose were generally considered the main sugars in mature cherry laurel fruits, and however, a lesser amount of sucrose was also noted in some other cultivars and the wild forms of cherry laurel (Gogolishvili, 1971; Romero-Rodriguez *et al.,* 1992; Ayaz *et al*., 1996; Ayaz, 1997; Ayaz *et al*., 1997). The absence of sucrose can be explained by the decomposition effect of invertase during the ripening of some fruits. During further ripening, it can be accounted for by the fact that sucrose may be synthesized in the fruits at early weeks of development, but later stages it was enzymatically hydrolyzed to glucose and fructose when translocated to the flesh of the fruit (Zimmermann, 1954).

In 'Oxygemmis' fruit used in the presence study, sucrose was not synthesized at early weeks of development but at later stages significantly ($P = 0.05$) different quantities were reported, even if it was low. Nielsen *et al.*, (1991) proposed that acid invertase is the main sucrose cleaving enzyme during early development, whereas SS (sucrose synthetase) is responsible for the cleavage of sucrose during the late phase of growth until the fruits start to ripen. In plums (DeVilliers *et al*., 1974), apricot (Bieleski & Redgwell 1985) and peach (Ishida *et al*., 1985) fruits, the same explanation about lesser amounts or absence of sucrose have been reported.

Dini *et al*., (1989) reported that soluble sugar content varies considerably within and among species depending on the age, maturity and environmental conditions. Variation in carbohydrate content due to non-genetic factors is well known. Anticipation of gross

quantitative and qualitative changes, associated with varying environmental and developmental conditions, has discouraged chemotaxonomic evaluations of carbohydrate evidence. Both quantitative and qualitative differences in plant tissues arise owing to their different functions, but may not be consistent (Smith, 1976).

Sometimes sorbitol produced during growth of the fruit on the tree is not accumulated but continuously converted into fructose, sucrose and glucose and its actual concentration in the fruit remains constant (Ackermann *et al*., 1992). The sorbitol amount in 'Oxygemmis' fruit as well as 'Globigemmis' (Ayaz *et al*., 1996) showed the same decreases and increases in parallel to the levels of individual sugars at all development stages. However, the accumulation of the sugars is linked to the conversion of sorbitol translocated from the leaves to the fruit of apple during cell expansion, fructose and sucrose being the favored products (Berüter, 1985).

From this study, we enlarged the scant information about sugar accumulation during fruit development of cherry laurel fruits that were commonly used in consumption in Turkey (Black Sea Region). However, as a continuation of one part of chemical analyses of cherry laurel fruits have been undertaken in our lab, our results might be of use to consumers and food technologists.

References

Ackerman, J., M. Fischer and R. Amado. 1992. Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). *Journal of Agricultural and Food Chemistry*, 40: 1131-1134.

Ayaz, F.A., E. Bertoft and M. Reunanen. 1996. Changes in the low molecular weight carbohydrate content of *Laurocerasus officinalis* Roem. cv. Globigemmis during fruit development. *Bulgarian Journal of Plant Physiology,* 22: 25-29.

Ayaz, F. 1997. Studies on water soluble sugar and sugar alcohol in cultivars and wild forms of *Laurocerasus officinalis* Roem. *Pakistan Journal of Botany,* 29: 331-336.

Ayaz, F.A., A. Kadioglu, M. Reunanen and M. Var. 1997. Sugar composition in fruits of *Laurocerasus officinalis* Roem. and its three cultivars. *Journal of Food Composition and Analysis,* 10: 82-86

Baytop, T. 1984. *Türkiye'de Bitkiler ile Tedavi (Geçmişte ve Bugün)*. İstanbul Universitesi Yayınları, No. 3255, Ecz. Fak., No. 40, İstanbul. p 260.

Berüter, J. 1985. Sugar accumulation and changes in the activities of related enzymes during development of the apple fruit. *Journal of Plant Physiology*, 121: 331-341.

Bieleski, R.L. and R.J. Redgwell. 1985. Sorbitol versus sucrose as photosynthesis and translocation products in developing apricot leaves. *Australian Journal of Plant Physiology,* 12: 625-668.

Biermann, C.J. and D.G. McGinnis. 1989. *Analysis of Carbohydrates by GLC and MS.* CRC Press, Inc., USA.

Browicz, K. 1972. *Laurocerasus* Duhamel. *In*: *Flora of Turkey and the East Aegean Islands*. (Ed.): P.H. Davis, Edinburgh University Press, Edinburgh, Vol. 4: pp 6-8.

Chapman, G.W.Jr. and R. J. Horvat. 1989. Determination of nonvolatile acids and sugars from fruits and sweet potatoes by capillary GLC and GLC-MS. *Journal of Agriculture and Food Chemistry,* 37: 947

DeVilliers, O. T., J. T. Meynhardt and J. A. DeBruyn.. 1974. The metabolism of sorbitol and sugars in santa rosa plums. *Agroplantae,* 6: 51-54.

Dini, A., D.F. Simone, E. Ramundo and F. Senatore. 1989. Oligosaccharides in five different *Vicia faba* L., cultivars. *Biochemical Systematics and Ecology*, 17: 559-561.

Dirr, M. 1990. *Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses*, pp 301-306. StipesPublishing Company, Champaign, Illionis.

Gogolishvili, Z. M. 1971. Study of sylvan cherry laurel fruit. *Trudy Gruzinskii Nauchno Ssledovatel'skii Institut Pischevoi Promyshlennosti*, 5: 133-136.

Hubbard, N.L., S.C. Huber and D.M. Pharr. 1989. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.). *Plant Physiology,* 91: 1527-1534.

Hubbard, N.L., D.M. Pharr and S.C. Huber. 1991. Sucrose phosphate synthase and other sucrose metabolizing enzymes in fruits of various species. *Physiologia Plantarum,* 82: 191-196.

Ishida, M., A. Inaba and Y. Sobajima. 1985. Translocation of labelled compounds from into shoot and fruit with their conversion in peach trees. *Science Reports of Kyoto Perfectural University,* 37: 163-171.

Kader, F., B. Rovel and M. Metche. 1993. Role of inveratse in sugar content in highbush blueberries (*Vaccinium corymbosum* L.). *Lebensmittel Wissenschaft und Technologie,* 26: 593-595.

Lowell, C.A., P.T. Tomlinson and K.E. Koch. 1989. Sucrose metabolizing enzymes in transport tissues and adjacent sink structures in developing citrus fruit. *Plant Physiology,* 90: 1394-1402.

Milan, S.P.A. 1984. *The Mcdonald Encyclopedia of Medicinal Plants*. Mcdonalds Co. Ltd., London, p 252.

Moriguchi, T., Y. Ishizawa, T. Sanada, S. Teramoto and S. Yamazaki. 1991. Role of sucrose synthase and other related enzymes in sucrose accumulation in peach fruit. *Journal Japanese Society of Horticultural Science,* 60: 531-538.

Nielsen, T.H., H.C. Skjaerbaek and P. Karlsen. 1991. Carbohydrate metabolism during fruit development in sweet pepper (*Capsicum annuum* L.) plants. *Physiologia Plantarum,* 82: 311-319.

Romero-Rodriquez, M.A., M.L. Vazquez-Oderiz, J. Lopez-Hernandez and J. Simal-Lazano. 1992. Studies on chemical composition, physical characteristics and maturity indices of cherry laurel (*Prunus laurocerasus* L.) and elder berries (*Sambucus nigra* L.). *Industrie-Alimentari*, 31: 911-912.

Shaw, D.V. 1988. Genotypic variation and genotypic correlation for sugars and organic acids of strawberries. *Journal of American Society of Horticultural Science*, 113: 770-774.

Smith, P.M. 1976. *The Chemotaxonomy of Plants. Evidence from Carbohydrates*, Chapter 7, pp 77-89, Edward Arnold Pub., London .

Var, M.1992. Kuzey Batı Karadeniz Bölgesi Doğal Odunsu Taksonlarının Peyzaj Mimarlığı Yönünden Değerlendirilmesi Üzerine Araştırmalar. Doktora Tezi. Karadeniz Teknik Üniversitesi Fen Bilimleri Enstitüsü, Trabzon, Turkey.

Zimmermann, M. 1954. Über die Sekretion Saccharosespaltender Transglucosidasen in Pflanzlichen Nektar. *Experienta,* 10: 145.

(Received for publication 21 October 2001)