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STUDIES ON WATER SOLUBLE SUGAR AND SUGAR ALCOHOL IN CULTIVARS AND WILD FORMS OF *LAUROCERASUS OFFICINALIS* ROEM.,

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Abstract

Water soluble sugar and sugar alcohol composition in fruits of *Laurocerasus officinalis* cvs. Oxygemmis, Globigemmis and wild form of *L. officinalis* Roem., have been examined. Gas chromatography-mass spectrometry studies exhibited that the main sugars in *L. officinalis* and related cultivars are fructose, glucose and sorbitol and sorbitol and mannitol as sugar alcohols. Fructose, glucose and sorbitol were in significantly higher levels in cultivars and the wild form with highest value 74.4% of dry weight of total water soluble sugar in Oxygemmis and the highest level of sugar alcohol (42.6%) in the wild form with least in Angustifolia (19.9% of dry weight).

Introduction

The genus *Laurocerasus* (cherry laurel) of the family Rosaceae, represented by only one species *L. officinalis* Roem., (syn: *Prunus laurocerasus* L.) is an evergreen plant of up to 6 m in height. The fruits of cherry laurel are ovoid, 8 mm in diameter (12 mm in some cultivars), dark purple or black in mature form (Browicz, 1972). The fruits of the wild form are not eaten as food due to their bitter taste (Flint, 1983). The fruits of both the wild and cultivated plants are very poisonous in their early stages but can be used, when ripe to prepare various alcoholic drinks with pleasant taste of almonds (Milan, 1984). The cultivated plants have large, sweet fruits which are sold in both fresh and dried forms. Of the many cultivars reported from different countries (Dirr, 1990; Pamay, 1992), 3 cultivars viz., Oxygemmis, Globigemmis and Angustifolia and the wild form of *L. officinalis* are commonly present in Turkey, especially in the Black Sea Region where Oxygemmis and Globigemmis were first recorded in Turkey (Var, 1992). There are reports on the chemical compositions of the fruits of some cherry laurels such as primverosides (Weinges *et al.*, 1991), volatile compounds (Mchedlidze & Kharebava, 1988), seed fatty acid (Ayaz *et al.*, 1995), and ethanol soluble sugar (Ayaz *et al.*, 1997). We identified and quantified ehtanol soluble sugars in the fruits of the three cultivars. The present paper describes the water soluble sugars and sugar alcohols in *L. officinalis*.

Materials and Methods

Fruit Material: Cherry laurel fruits of cv. Oxygemmis, Globigemmis, Angustifolia and

the wild form of *L. officinalis* L., were harvested in early morning in mid August, 1995 from young trees from the vicinity of Trabzon (Turkey). The seeds were removed from the mesocarps and the mesocarps were dried at 60°C under vacuo for 24 h. After drying, 250 g of the fruit sample (mesocarps) was ground in a Wareing blender. For the extraction, 10 g ground fruit sample was used in triplicate.

Extraction of Low Molecular Weight Carbohydrates: Sugars were extracted according to the method of Ganter *et al.*, (1992). Dry ground fruits (10g) was suspended in water (40 ml) and shaken at 4°C overnight. The aqueous solution was centrifuged at 15.000 x g and the supernatant was concentrated under reduced pressure below 40°C and lyophilized. The lyophilized sugar sample was stored for further analysis.

Preparation of Oxime Trimethylsilyl Derivates of Sugar Extracts: Trimethylsilylated oximes of sugar extracts were prepared according to method of Biermann & McGinnis (1989). A 50 mg portion of the sample was weighed and dissolved in 2.0 ml of pyridine. A 50 μl portion of the pyridine solution was transferred in a vial for pyridine stock solution containing 3% w/w hydroxylamine hydrochloride and a known amount of methyl α-D-glucopyranoside as an internal standard (ca. 250 μg/200 μl) was added. 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) were added for silylation. The silylation was allowed to be completed at room temperature for 30 min., before analysis.

Standard Materials: Sugar (glucose, fructose, sucrose, sorbitol, mannitol, inositol and raffinose) standard was obtained from Sigma Chemical Co. Hydroxylamine hydrochloride, HMDS (hexamethyldisilizane), TMCS (trimethylchlorosilane) and α-D-glucopyranoside were obtained from Fluka (Buchs, Switzerland). A mixture containing certain amounts of the internal standard and pure reference sugars (glucose, fructose, sucrose, mannose, sorbitol, inositol and raffinose) was analysed to obtain correction factors (GC detection responses vs. the internal standard for each analysed sugar).

GC-MS Analysis of Sugars: The GC analysis were 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), and the column oven temperature was initially held at 100°C and then gradually increased to 290°C at 8°C/min heating rate. Hydrogen was used as the carrier gas at a flow rate of 55 cm/s. A Merck-Hitachi D-2000 integrator was used for the peak area measurements. Sugar identifications were based on retention time from analysis of reference sugars. Mass spectrometry was used in identification. The GC-MS analyses were performed with an HP 5890-5970 instrument using similar GC-column operated at the same temperatures as in the GC-FID.

GC-MS Analysis of Sugar Alcohols: The extracted samples were analysed by alditole acetate method (Wolfrom & Thompson, 1963). The analysis was started with total hydrolysis in 1 M trifluoroacetic acid (TFA) for 5 h at 100°C. The monomeric sugars were converted to alditoles in aqueous sodium borohydrate (100 mg/ml), allowing to processed at 4°C overnight. Thereafter excess sodium borohydrate was destroyed by multiple evaporation with acetic acid: methanol (1/200, v/v). Finally, the alditols were acetylated in acetic acid anhydride at 100°C overnight. Insitol was used as an internal standard.

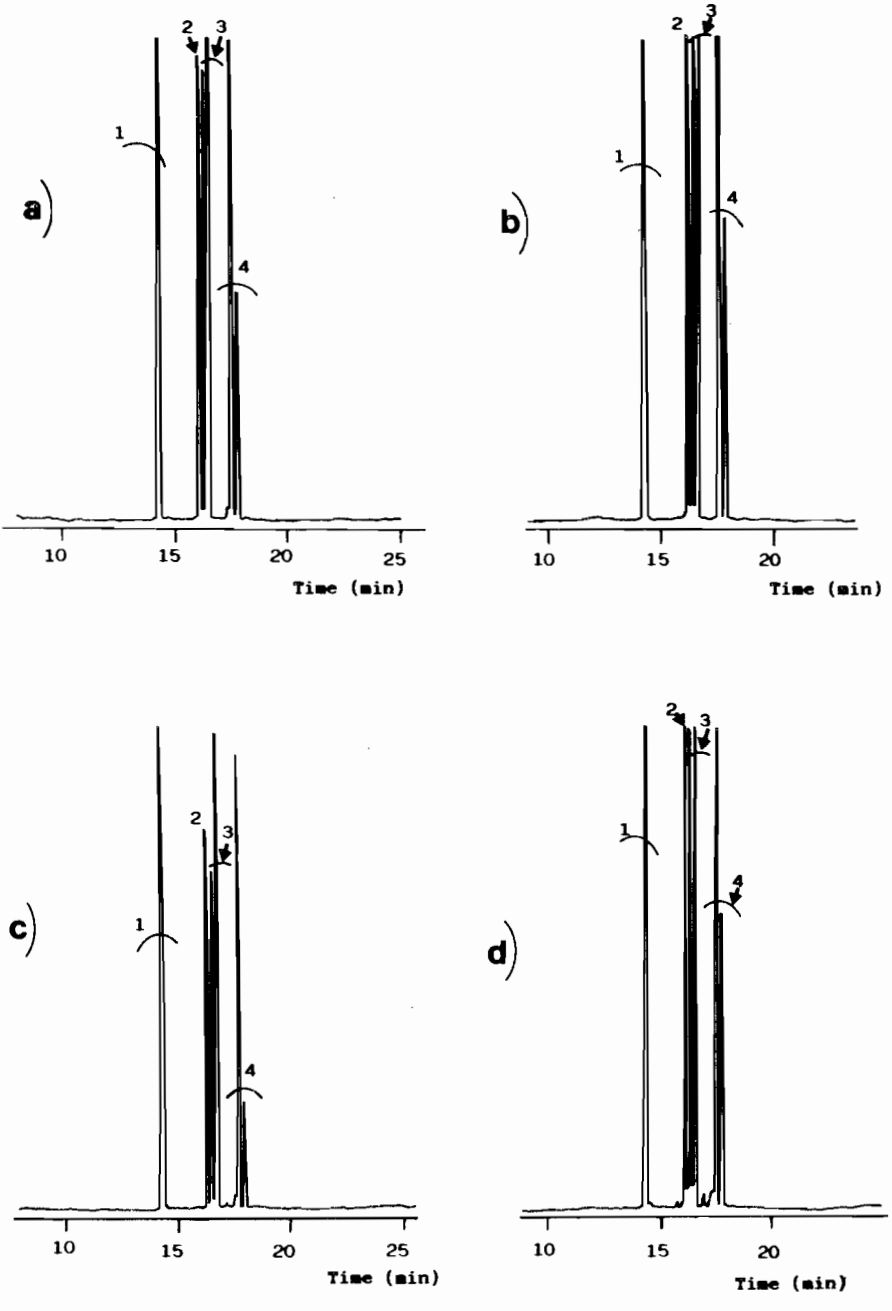

Fig.1. Gas chromatograms of water soluble sugar extracts of cultivars and the wild form of *L. officinalis* Roem. a) cv. Oxygemmis, b) cv. Globigemis, c) cv. Angustifola, d) the wild form of *L. officinalis*.
1. internal standard (as α-D-glucopyranoside). 2. sorbitol, 3. fructose and 4. glucose.

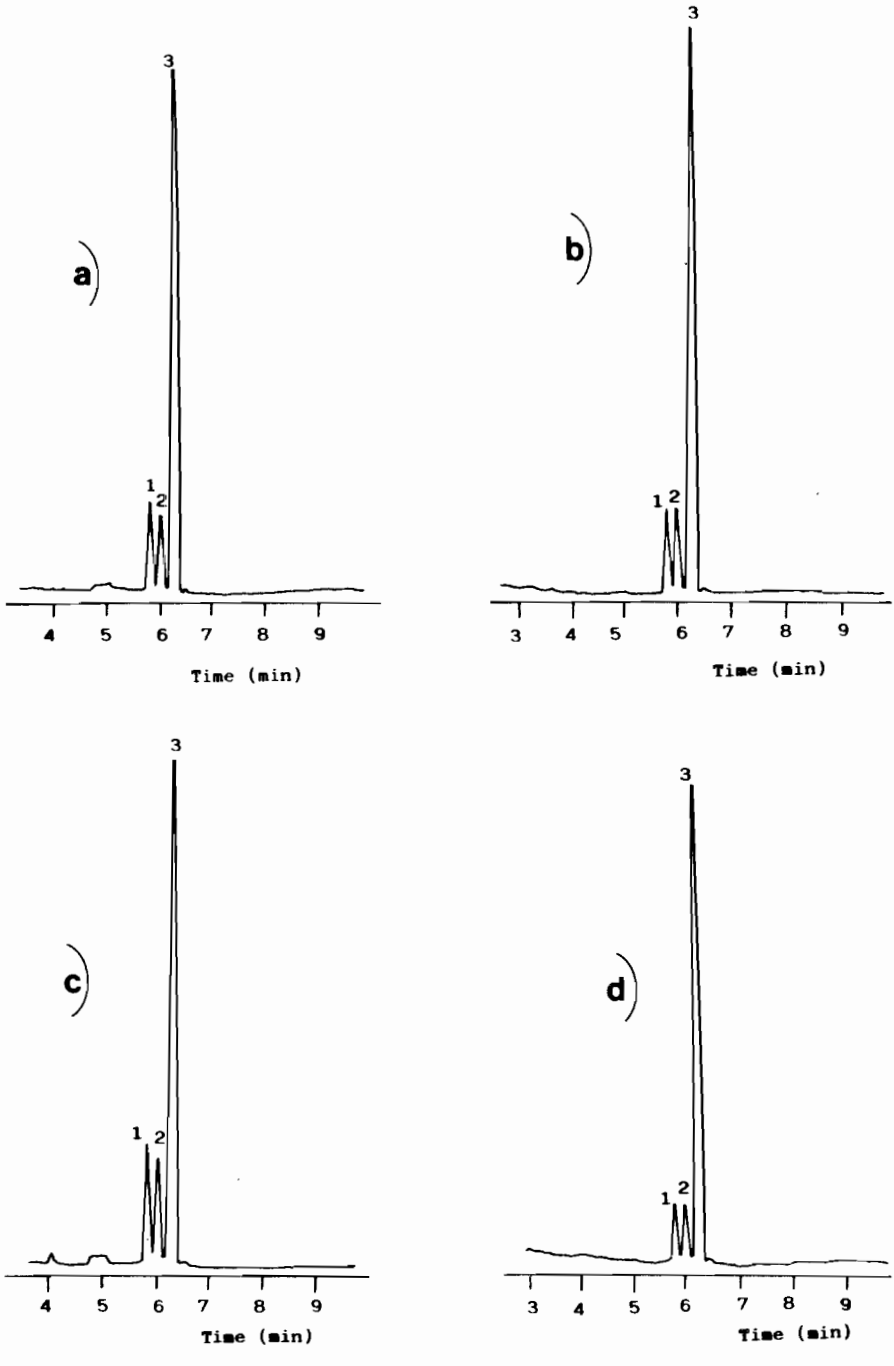

Fig.2. Gas chromatograms of sugar alcohols of cultivars and the wild form of *L. officinalis* Roem. a) cv. Oxygemmis, b) cv. Globigemmis, c) cv. Angustifola, d) the wild form of *L. officinalis*.
1. internal standard (as inositol), 2. mannitol, 3. sorbitol.

Gas chromatography-mass spectrometry (GC-MS) analyses were performed with an HP 5890-5970 GC-MS instrument equipped with an HP-1 capillary column (25 m, 0.32 mm i.d., film thickness 0.17 μm). Helium was used as carrier gas at a flow rate of 35 cm/s, and the MS was operated at 70 eV impact energy. The GC oven temperature was initially held at 100°C and then gradually increased to 280°C at 6°C/min heating rate.

Results and Discussion

Fructose, glucose and sorbitol were found higher in Oxygemmis than in Globigemmis, Angustifolia and the wild form. Globigemmis and the wild form showed more fructose and glucose than in Angustifolia (Table 1). The proportions of fructose, glucose and sorbitol were approximately 1.8:2.1:1.0 for Oxygemmis, 2.2:2.5:1 for Globigemmis, 2.5: 2.8:1 for Angustifolia and 3.4:3.4:1 for the wild form.

The major sugar alcohols found in chery laurels were sorbitol and mannitol with highest level of sorbitol was obtained in the fruits of wild form while Angustifolia showed the least amount (Table 1). However, no significant difference were observed in mannitol content among cultivars and the wild form. Although the wild form of cherry laurel has a rich sugar alcohol content, the bitter taste can probably be caused by the high levels of some hydroxy acids (Milan, 1984; Baytop, 1984).

According to our results glucose and fructose found to be present within range 23.1-14% are generally considered as the main sugars in mature cherry laurel fruits. These results were consistent with the observations (Ayaz *et al.*, 1997), whereas the level of ethanol soluble sugars were generally found to be lower than water soluble sugars in cherry laurel fruits.

The results showed that the relative distribution of water soluble sugars were very different from ehtanol extraction. However, the fruits of Oxygemmis were character-

Table 1. Sugar contents in the fruits of cultivars and the wild form of *Laurocerasus officinalis* Roem. Total sugar is sum of fructose, glucose and sorbitol.

Species	Sugars (% dry weight)				Sugar alcohols (% dry weight)	
	Fructose	glucose	sorbitol	total	sorbitol	mannitol
L. officinalis cv. Oxygemmis	28.1±0.14	32.0±0.41	15.3±0.13	74.4	29.6±0.04	4.0±0.1
L. officinalis cv. Globigemmis	26.1±0.02	30.2±0.01	12.0±002	68.3	39.8±0.5	5.4±0.01
L. officinalis cv. Angustifolia	21.0±0.02	23.0±0.03	8.3±0.01	52.3	19.9±0.2	3.8±0.06
L. officinalis (wild form)	25.0±0.03	25.3±0.03	7.4±0.02	57.3	42.6±0.6	4.9±0.3

ized by a high percentage of total soluble sugar (74.4%), followed by Globigemmis (68.3%), the wild form (57.3%) and Angustifolia (52.3%). Although the total water soluble sugar content of the wild form was higher than in Angustifolia, but its fruit is generally not eaten as food (Flint, 1983; Baytop, 1984, 1989; Milan, 1984). The best cultivars for sugar quality seem to be Oxygemmis and Globigemmis.

Alditoles are widely distributed in higher plants. The most common are mannitol, sorbitol (glucitol) and glucitol (dulcitol). The distribution of some of the alditoles are so unique that they have been suggested as valuable chemotaxonomic markers (Keller, 1989). The identifications and quantifications of water soluble sugars and sugar alcohols in cherry laurel fruits imply that those can be used as chemotaxonomic markers as in the case of ethanol soluble sugars (Ayaz *et al.*, 1997).

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