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Research Article

Determination of Some Low Molecular Weight Carbohydrates in the Fruits of Wild Cherry Laurel *(Laurocerasus officinalis* Roem.) Using Gas Chromatography

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Abstract: Some low-molecular-weight carbohydrates extracted with ethanol and water from the fruits of *Laurocerasus officinalis* Roem. (*Rosaceae*), from two different regions in Trabzon, were analysed using gas chromatography. In one population, the analysis in the ethanol extraction showed the occurence of fructose, glucose, sorbitol and sucrose in concentrations (w/w) of 25.20; 23.00; 14.00 and 0.024 %, respectively. The same sugars, in the water extraction were found in concentrations (w/w) of 24.62; 25.59; 7.43 and 0.114 %, respectively. In the other population, these values were 25.44; 23.23; 14.24 and 0.023 for the ethanol extraction, and 24.74; 24.98; 7.18 and 0.108 % for the water extraction, respectively. Mannitol was not detected in either extraction.

Key Words: *Laurocerasus officinalis,* glucose, fructose, sorbitol, sucrose, gas chromatography

Yabani Karayemiş (*Laurocerasus officinalis* Roem.) Meyvalarındaki Bazı Düşük Molekül Ağırlıklı Karbohidratların Gaz Kromatografisi ile Belirlenmesi

Özet: Bu çalışmada *Laurocerasus officinalis* Roem. (*Rosaceae*) meyvalarında etanol ve su ekstraksiyonuyla bazı düşük molekül ağırlıklı karbohidratlar gaz kromatografisi ile analiz edildi. Analizler sonucu bir populasyonun etanol ekstraksiyonunda fruktozun % 25.20 (a/a), glukozun % 23.00, sorbitolun % 14.00 ve sakkarozun % 0.024 olduğu tespit edildi. Aynı bölgedeki su ekstraksiyonundaki benzer şekerlerden fruktozun % 24.62; glukozun % 25.59; sorbitolun % 7.43 ve sakkarozun % 0.114 olduğu belirlendi. Diğer populasyonda aynı değerler etanol ekstraksiyonunda sırasıyla % 25.44; 23.23; 14.24 ve 0.023 olarak, su ekstraksiyonunda ise % 24.74; 24.98; 7.18 ve 0.108 olarak bulundu. Her iki ekstraksiyonda mannitola rastlanılmadı.

Anahtar Sözcükler: *Laurocerasus officinalis,* glukoz, fruktoz, sorbitol, sakkaroz, gaz kromatografisi

Introduction

Cherry laurel *(Laurocerasus officinalis* Roem., syn: *Prunus laurocerasus* L.) is an evergreen, shrub or small tree of up to 6 m in height. The fruits of the cherry laurel are ovoid, 8 mm in diameter (12 mm in cultivated varieties...) and dark purple or black when mature (1). The fruits of this plant (wild) are not eaten as a fresh fruit due to their bitter taste. Nevertheless, the cultivated plants have large sweet fruits, and these fruits are eaten in both fresh and dry form.

L. officinalis is represented by only one species in the family *Rosaceae* (1). Many cultivars of the plant have been reported in different countries (2, 3, 4). The plants are commonly distributed in the Black Sea Region, 20-1700 m above sea level and sparsely in Balıkesir and Hatay, Turkey (1). The fruits of the cherry laurel and its cultivated forms are widely used as a herbal medicine in Turkey and used in the treatment of stomach ulcers, digestive complaints and bronchitis (seeds), eczema, haemorrhoids and as a diuretic (fruits). It is used externally for its antipuriginous (5,6) and analgesic effect on local pain (7,8). The cherry laurel and some of cultivars have been studied for fatty acids in their seeds (9), volatile-constituent concentrations in the oils in the leaves and fruits (10), and benzyl-ß-primveroside in the green fruits (11). Apart from these papers, no studies on the sugar composition of the fruits of the cherry laurel have been carried out. More detailed knowledge of the sugar content of the wild forms is required for the selection of cherry laurel cultivars with improved nutritional quality. Thus, in this study, certain low-molecular-weight carbohydrates in the fruits of *L. officinalis* were examined using gas chromatography.

Materials and Methods

Material

Ripened fruits of cherry laurel were collected at random (1 kg) in their natural habitats in mid-August from trees in two different regions of Trabzon, Akcaabat, approx. 1000 m above sea level, (Akçaköy) and Çaykara, 700 m above sea level, (Akdoğan). These are referred to as population 1 and population 2, respectively. The harvested fruits were transported to the laboratory in cold storage (below 4ºC). The seeds were removed from the mesocarps, and the mesocarps were dried in vacuo at 60ºC for 24 hrs prior to extraction.

Extraction

The extraction of low-molecular-weight carbohydrates was performed using ethanol and water.

In the ethanol extraction, the fruit material was ground using a mortar and pestle, and the resulting powder was homogenized and extracted according to the method of Booij et al. (12). A known quantity of homogenized fruit material (powder) was defatted by extraction for 8 h with benzene in a soxhlet apparatus (8 h). The residue (defatted) was extracted under reflux with 80 % (v/v) aqueous ethanol for 4 h. After cooling, the suspension was centrifuged and concentrated by evaporation to a dry state in vacuo at 50ºC. The dried sample was then analysed with gas chromatography (GC).

In the water extraction, a ground fruit sample was prepared in the way as in the ethanol extraction. Sugar extraction was performed according to the method of Ganter et al. (13). The ground sample was suspended in water overnight at 4ºC. The suspension was then centrifuged and concentrated under reduced pressure below 40ºC. The extract was dried in vacuo at 50ºC. The dried sample was analysed with GC.

Analysis

A ca 50 mg portion of the sample was weighed and dissolved in 2 ml of pyridine. A 50 µl portion of the pyridine solution was transferred to a vial for the preparation of the trimethylsilylated oximes of the sugars. First, 200 µl of pyridine stock solution containing 3 % w/w hydroxylamine hydrochloride and a known quantity of methyl α-D-glucopyranoside as an internal standard (ca. 250 µg/200 µl) was added. The sample solution was then kept at 70ºC for 30 min. After cooling at room temperature, 300 µl HMDS (hexamethyldisilizane) and 200 µl of TMCS (trimethylchlorosilane) were added for silylation. The silylation was completed at room temperature for 30 min before analysis (14).

A mixture containing a known amount of the internal standard and pure reference sugars (glucose, fructose, sorbitol, mannitol, sucrose) was further analysed in order to determine correction factors (GC detection responses vs...) the internal standard for each set of analysed sugars.

Gas Chromatography

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), and the column oven was programmed to start at 100ºC rising to 280ºC at 6ºC/min. Hydrogen was used as the carrier gas at a flow rate of 1.8 ml/min. A Merck-Hitaichi D-2000 integrator was used for the peak-area measurements. Sugar identifications were based on retention times from analyses of reference sugars. Mass spectrometry (MS) was used in the identifications. The GC-MS analyses were performed with an HP 5890-5970 instrument using similar GC-column operated at the same temperatures as the GC-FID.

Results and Discussion

The low-molecular-weight carbohydrate compositions of the fruits of *L. officinalis* are given in Table 1. Gas chromatograms of the sugars from the reference mixture, ethanol and water extraction in both populations are also given in Figs 1 and 2.

Fructose, glucose and sorbitol were identified and quantified as major sugars in the ethanol extract of *L. officinalis* fruit. In the ethanol extraction the ranges of concentrations (w/w) found for the fruits from population 1 and 2, respectively, were as follows: fructose

Table 1. Contents of some low molecular weight carbohydrates from two populations of *L. officinalis* (% dry weight).

	Ethanol extraction		Water extraction	
Sugars	Population 1	Population 2	Population 1	Population 2
Fructose	25.20±0.26*	25.44±0.49	24.62±0.08	24.74±0.48
Glucose	23.00±0.14	23.23±0.53	25.29±0.32	24.98±0.09
Sorbitol	14.00±0.21	14.24±0.31	7.43±0.40	7.18±0.18
Sucrose	0.024±0.003	0.023±0.11	0.114±0.019	0.108±0.02

* Standard deviation of means (three repetitions)

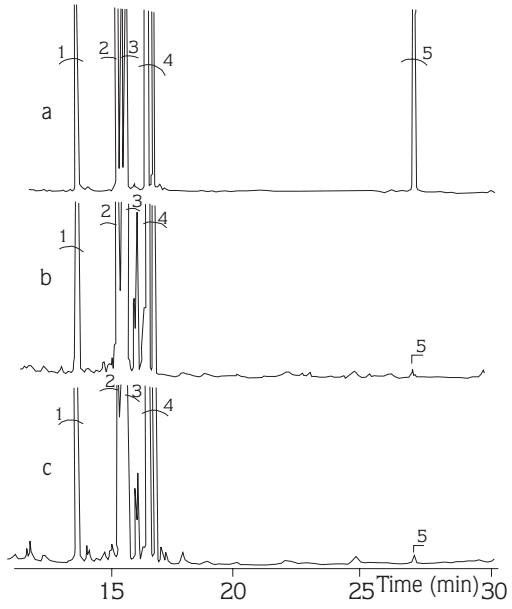

Figure 1. GC chromatograms of some monosaccharides and sucrose from the the fruits of *L. officinalis* in ethanol extraction. a. reference mixture, b. population 1, c. population 2 (1.ISTD (methly α-D-glucopyranoside), 2. sorbitol, 3. fructose, 4. glucose, 5. sucrose.

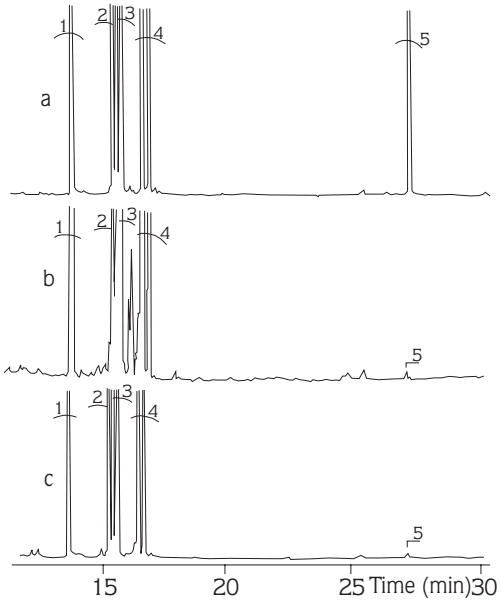

Figure 2. GC chromatograms of some monosaccharides and sucrose from the fruits of *L. officinalis* in water extraction. a. reference mixture, b. population 1, c. population 2 (1.ISTD (methly α-D-glucopyranoside), 2. sorbitol, 3. fructose, 4. glucose, 5. sucrose.

25.20-25.44; glucose 23.00-23.23; sorbitol 14.00-14.24 and sucrose 0.024-0.023 %. Fructose was found in the greatest quantities in this extraction.

In the water extract of *L. officinalis* fruit, fructose, glucose, surbitol and sucrose were identified. In this extract, glucose was the sugar found in the greatest quantities. The sugar values from population 1 and 2, respectively, were as follows: fructose 24.62-24.74; glucose 25.29-24.98; sorbitol 7.43-7.18 and sucrose 0.114-0.108 %.

The lowest level was found to be that of the sucrose in both populations of fruits. However the sucrose level in the water extraction was higher than in the ethanol extraction.

The results show that the method of extraction can alter the relative distribution of low-molecular-weight carbohydrates in fruits of *L. officinalis.* The variations in the level of sucrose may also be due to the activity of enzymes during extraction (15). The differences generally occurred according to the amount of sorbitol. Otherwise, the amount of fructose and glucose were similar. Then are several studies in the literature of ethanol and water extraction of low-molecular - weight carbohydrates using HPLC and GC analysis. Ganter et al. (13) found that the content of fructose, glucose, galactose and sucrose in a water extraction was higher than in ethanol extraction in seeds of *Mimosa scabrella* Benthan. In the same study, sugar alcohols, such as sorbitol and mannitol, were found in lower quantities in the water extraction than in the ethanol extraction. Comparatively low molecular - weight - carbohydrate detection in marine macroalgae indicates that the amount of sorbitol, mannitol and dulcitol in the ethanol extraction were higher than in the water extraction in the species (16). The increase in sorbitol content in the ethanol extraction may be due to the solubilization effect of alcohol.

Figures 1 and 2 show that fructose and glucose display two peaks because of their different forms. Consequently, the areas of both peaks were added for the calculation of these sugar amounts. The sugar composition in population 1 was found to be similar to that of population 2. The results that low-molecular-weight carbohydrates do not vary greatly in these populations of *L. officinalis.* This is interesting because it has been reported that soluble sugar content varies considerably within and among species ac-

cording to age, maturity and environmental conditions (17). This could be explained by similarities in living habitats, since the plants naturally grow with a similar distribution.

According to these results, cherry laurel fruits are rich in both glucose and fructose. This plant may be of potential importance as a source of food and may also be of industrial significance. However plants cultivated from wild forms should be examined further using similar analysis.

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