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Research Note

Soluble Sugar Composition of *Elaeagnus angustifolia* L. var. *orientalis* (L.) Kuntze (Russian olive) Fruits

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Abstract: Soluble sugar composition from ethanolic extract of *Elaeagnus angustifolia* L. (Russian olive) (*Elaeagnaceae*) collected from six different populations in the Gümüshane-Bayburt Valley was determined by gas chromatography. In the fruits, fructose and glucose were determined to be the major sugars. The analysis showed the occurrence of fructose and glucose in concentrations (w/w) of 32.62-34.60% and 23.37-24.10% (minimum-maximum), respectively. Sucrose was not detected in mature fruits while fructose and glucose were identified as the major sugars among these populations.

Key Words: Fructose, glucose, fruit, gas chromatography, *Elaeagnus angustifolia, Elaeagnaceae*.

Elaeagnus angustifolia L. var. *orientalis* (L.) Kuntze (İğde) Meyvelerin Çözünebilir Şeker İçeriği

Özet: Gümüşhane-Bayburt Vadisinde altı populasyona ait *Elaeagnus angustifolia* L. var. *orientalis* (L.) Kuntze (iğde) meyvalarının etanolde çözünebilir şeker içeriği gaz kromatografisi ile analiz edildi. Bu meyvalarda fruktoz ve glukoz ana şeker olarak belirlendi. Analizler fruktoz ve glukozun konsantrasyonlarının sırasıyla % 32,62-34,60 ve 23,37-24,10 (a/a) (maksimum-minimum) arasında olduğunu gösterdi. Bu populasyonlarda, glukoz ve fruktoz ana şekerler olarak aydınlatılırken sukrozun varlığına rastlanılmadı.

Anahtar Sözcükler: Fruktoz, glukoz, meyve, gaz kromatografisi, *Elaeagnus angustifolia, Elaeagnaceae*.

Introduction

Elaeagnus angustifolia L. (Russian olive) (fam: *Elaeagnaceae*) is a shrub or tree up to 7 m in height. Fruits are elliptic-oblong, 10-20 (-30) x 6-12 (-20) mm in diameter and reddish-brown (1, 2). The plant (var. *orientalis* (L.) Kuntze), doubtfully native to Turkey, is widely cultivated for its edible fruits in Central and Eastern Anatolia. The leaves and flowers of this plant are well-known for their use as diuretic and antipyretic in folk medicine, and also the fruits are eaten as an appetizer in Turkey (3).

Free sugars are one of the most important constitutents 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. Mono- and disaccharides such as fructose, glucose and sucrose are the most important sugars (4).

Fructose and glucose are considered to be the main sugars in most fruits contributing to the flavour of the fruits such as apples (5, 6), persimmon (7), berries (4, 8), strawberries (9), etc. In some fruits, however, such as highbush berries (8) both glucose and fructose appear to be the predominant sugars, which are present approximately in equal proportions (glucose:fructose ratio of about 1). Most fruits such as peach (10), mango (11), and certain melons (12) accumulate and store either sucrose or hexoses while citrus fruits (13) store relatively high levels of sucrose. It has been reported that blackberries such as boysenberries contain only glucose and fructose but no sucrose whereas others (highbush berries) contain sucrose at levels of up to 10 % of the total sugars by weight (14). Sakamura and Suga (15) identified glucose and fructose as the major sugars in oleaster fruits during ripening. They also found that the soluble sugars and polyphenol (mainly proanthocyanins) contents remained constant indicating that pulp ripening probably occurs after ripening of the stone. Kusova et al. (16) isolated isorhamnetin, isorhamnetin-3-*o*-β-D-galactopyranoside and caffeic acid from mature *E. angustifolia* fruit. In the three forms of *E. angustifolia* fruits, linoleic (C 18:0) and palmitic (C 16:0) acids in seeds, and palmitoleic (C 16:1) acid in pericarps were abundantly isolated (17). The fatty acid composition of phospholipids and glycolipids in *E. angustifolia* fruits were

also reported by Goncharova et al. (18). Potter (19) isolated 84 peaks of floral volaties by GC-MS from the steam distillate of *E. umbellata* Thunb. fruit. The principal constituents were palmitic acid (16.9 %), eugenol (11.1 %) and methyl palmitate (10.5 %). From the "purge and trap" analysis, 47 peaks were detected, 37 of which were assigned structures. Among the headspace volatiles, the most abundant compounds were 4-methyl anisole (33.0-42.7 %) and 4-methyl phenol (10.9-13.3 %). No detailed studies on the ethanol soluble sugar content of the fruits of *Elaeagnus angustifolia* have previously been reported.

The aim of this study was particularly to investigate the content of soluble sugars in the fruits of *Elaeagnus angustifolia* and to compare the contents among different populations collected from the Gümüşhane-Bayburt Valley.

Materials and Methods

Sampling

Well ripened fruits of *Elaeagnus angustifolia* were collected from the young trees along Gümüshane-Bayburt road from six populations in 1997. These populations were Torul, Mescitli, Eskibağlar, Kale, Akşar (Bayburt) and Uğrak (Bayburt). From each population, a half kg fruit was randomly collected and transported to the laboratory in cold conditions (below 4˚C). The fruits were harvested at a commercial maturity and stored in a deep freeze in the laboratory until use. For the extraction, 5 g mesocarps were used in triplicate for soluble sugar extraction.

Sugar extraction

The fine powdered mesocarps were defatted by extraction for 4 hours with a mixture of petroleum ether and chloroform (1:1, v/v) at room temperature and extracted twice with hot 80% ethanol by 4 times extraction with 70 % ethanol (20). The ethanolic extracts were concentrated at 40˚C under diminished pressure. The aqueous extract was de-proteinated with basic lead acetate, removing excess lead by addition of sodium oxalate crystals (21). After ethanol extraction, the sugar free-residue was air-dried, and used for gas chromatographic analysis.

TMS Derivatization of Sugars

Trimethylsilylated oximes of sugar extract were prepared according to the method of Biermann and McGinnis (22). A 50 mg portion of sample was weighed and dissolved in pyridine (2 ml) and 50 µl of the pyridine solution was transferred to a vial for pyridine stock solution containing 3% hydroxylamine hydrochloride, and a certain amount of methyl α-D-glucopyranoside as an internal standard (250 mg/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 ml of TMCS (trimethylchlorosilane) were added for silylation. The silylation was left to complete at room temperature for 30 min before analysis.

A mixture containing certain amounts of the internal standard and pure reference sugars (glucose, fructose, sucrose, mannose, sorbitol, inositol and raffinose) were further analyzed in order to determine correction factors (GC detection responses) using the internal standard for each analyzed sugar.

Gas Chromatography (GC)

GC conditions were selected according to the method of Ayaz et al. (23). Gas chromatography 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 mm film thickness), and the column oven was programmed to start at 100˚C rising to 280˚C at 6˚C /min. Hydrogen was used as the carrier gas at a flow rate of 1.8 ml/min. A Merck-Hitachi 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 identification. The GC-MS analyses were performed with a HP 5890-5970 instrument using a similar GC column operated at the same temperatures as in the GC-FID.

Statistical Analysis

All extractions and determinations were conducted three times independently. Analysis of variance of the data was evaluated with the Statistical Analysis System. Duncan's Multiple Range Test was employed to determine the statistical significance of differences among the means.

Results and Discussion

In the present study, we only investigated the soluble sugar composition from ethanolic extracts of *E. angustifolia* fruits by gas chromatography. The

Table 1. Contents of sugars in the fruits of *E. angustifolia* L. var. *orientalis* (L.) Kuntze collected from the Gümüşhane-Bayburt *Valley*.

	Sugar content (% dry weight)					
Sugars	Pop. 1 (Torul)	Pop. 2 (Mescitli)	Pop. 3 (Eskibağlar)	Pop. 4 (Kale)	Pop. 5 (Akşar)	Pop. 6 (Uğrak)
Fructose	32.62[a]	33.09[a]	33.23[a]	33.92[a]	34.60[a]	34.35[a]
Glucose	23.37[a]	23.86[a]	23.77[a]	23.75[a]	24.07[a]	24.10[a]
Sucrose	—*	—	—	—	—	—

Means of three different extraction and determinations. Values with the same letter are not significantly different at *p=0.05.* The means were compared within each row of the data, not columns

*not detected (< 0.1 %)

monosaccharide compositions of ethanol extracts are shown in Table 1. The GC chromatograms of reference sugars and ethanol soluble sugars are given in Figure 1.

Fructose and glucose were identified as principal monosaccharides in the fruit. Fructose was found to be in the greatest quantities and identified as the major sugar in the fruits collected from all populations. Identifications were confirmed with known standards. In the fruit extracts the minimum and maximum ranges of concentrations (w/w) found for the fruits from population 1 to 6 were as follows: fructose 32.62-34.60 and glucose 23.37-24.10%. There were no statistically significant differences among populations in the sugar constituents.

Similarly, glucose and fructose were characterized as the main sugars in oleaster fruits during ripening while sucrose was determined in lower amounts (15). The absence of sucrose can be explained by the decomposition effect of invertase during the ripening of some fruits (24, 25). Nielsen et al. (26) proposed that acid invertase is the main sucrose cleaving enzyme during early development, whereas sucrose synthetase is responsible for the cleavage of sucrose during the late phase of growth until the fruits start to ripen.

Sucrose synthesized in the leaves is thought to be enzymatically flesh of the fruit (7). Low quantities of sucrose were measured in mature and dried persimmon (*Diospyros kaki* L.) fruits. A remarkable decrease in the quantity of sucrose was also noted in some cultivars of the same plant as they ripened (27-30). In plums, apricot and peach fruits, sorbitol and sucrose are thought to be synthesized in leaves and then translocated to the developing fruit (31-33). Radiolabelling experiments also suggest that some of the sorbitol is converted into sucrose in peach mesocarp tissue (33).

It has been reported that soluble sugar content varies considerably within and among species according to age, maturity and environmental conditions (34). 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 (35).

The effects of growing area on the sugar composition were studied in fresh and stored apples. Both types of apples showed no significant difference except raffinose which was found to be significantly lower in the juice produced from stored apples in only one collecting site out of seven (36). Smocle and Neubert (37) reported that the sugar content of apples varied from one location to another among seven countries. In their work the sugar content was found to be significantly different between cultivars and locations as well as growing season.

To sum up, the results showed that, contrary to our expectations, there were no significant differences in the sugar content in *E. angustifolia* fruits collected from six different populations. However, it was observed that all analyzed fruits in each population reached the same developmental stage and commercial maturity. In

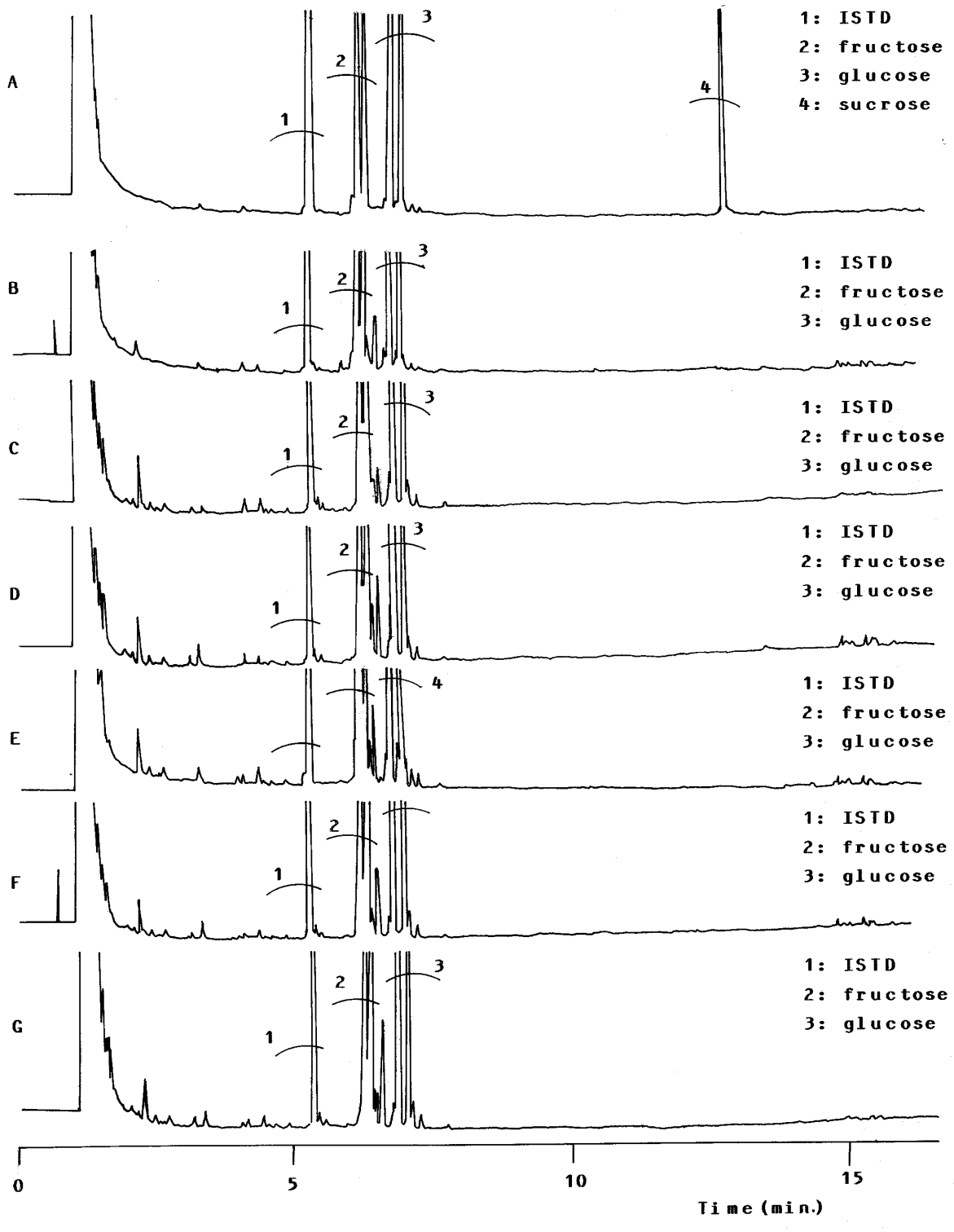

Figure 1. GC chromatograms of standard and ethanol soluble sugars in the fruits of *E. angustifolia* var. *orientalis* collected from 6 population in the Gümüşhane-Bayburt Valley. A; sugar standard, B; population 1 (Torul), C; population 2 (Mescitli), D; population 3 (Eskibağlar), E; population 4 (Kale), F; population 5 (Akşar), G; population 6 (Uğrak). (1. ISTD (α-D-glucoyranoside), 2. fructose, 3. glucose, 4. sucrose).

addition, fructose and glucose appeared to be the major sugars contributing to the sweet taste of *E. angustifolia.* The absence of sucrose in the fruit could be due to the decomposition effect of invertase during the further ripening, or it could be accounted for by the fact that sucrose may be synthesized in the fruits during the early weeks of development, but at latter stages it was enzymatically hydrolyzed to glucose and fructose when translocated to the flesh of the fruit.

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