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HPLC Determination of Sugar Contents Based on Harvest Season in Almonds [*Prunus dulcis* (Mill.) D.A. Webb.]

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This study presents in comparison with harvest seasons sugar contents in 18 promising almond selections [*Prunus dulcis* (Mill.) D.A. Webb.] from Balikesir (western Anatolia, Turkey). Their majority had kernel weight over one gram since large kernel is a desired fruit characteristics for almond breeding efforts. Nuts of almond genotypes were collected at two seasons based on their harvest times. The harvest seasons were late August for 8 promising genotypes and early September for other 8 genotypes. Sugar contents were detected by HPLC. Selections harvested in late August and early September averagely contained 2.19-2.31 g/100 g sucrose, 0.68-0.92 g/100 g maltose, 2.09-1.82 g/100 g glucose and 4.08-3.41 g/100 g fructose. Sugar contents of selections did not differ by harvest season statistically. Whereas, significant differences were found among selections for each harvest season. In addition, the mean sugar was fructose in 12 genotypes, sucrose in 4 genotypes and glucose in one genotype.

Key Words: Almond, Kernel, Sugars, Selection, Balikesir, Relationship.

INTRODUCTION

Today, almonds are considered among popular nuts for human health. In USA which is the leading country in the world's almond production, California's almond production has doubled in response to consumer awareness about the human healthfulness of almonds during last 20 years[1]. In addition to morphological identification[2,3], promising almond genetic resources for modern breeding objectives should also be described in their nutritional values. Sucrose, glucose and fructose are the main sugars in fruits[4]. The amount and composition of sugars varying to fruit species,

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cultivars, ecological conditions, technical and cultural practices affect the taste[5]. With respect to fruit taste, fructose is sweeter than sucrose and sucrose is sweeter than glucose[4]. In almonds, complex carbohydrates are widely utilized for weight reductions in human diets[1,6,7]. Sugar composition of almond kernel constitutes mainly sucrose and raffinose and lower amounts of fructose, glucose and galactose (reducing sugars)[8-10] and it has vital value for good flavour and taste[11]. Almond cultivars with large kernels are highly desired for breeding studies and more preferred for commercial uses[12]. In almond, kernel quality is closely related to kernel taste. Protein, oil, sugar contents and other components of almond kernel influence its industrial use. Sugar content of almond kernels can vary to cultivars, genotypes, ecological conditions, technical and cultural practices. Kader[13] reported that kernel contains 20.4 % carbonhydrate and sugar 3 % and the main sugar is sucrose. Also, irrigation, harvest time and storage conditions affects sugar composition of almond kernel[11,14,15]. On the other hand, HPLC is more powerful technique in analyzing sugar composition of almond kernels than gas-chromatography method[11], although sugar compositional analyses were conducted by gas-chromatography method. Studies on sugar composition in almonds are very limited. This study aims at determining by HPLC sugar contents based on harvest season in 18 promising almond selections [*Prunus dulcis* (Mill.) D.A. Webb.] from Balikesir (western Anatolia, Turkey).

EXPERIMENTAL

The plant material of this study constituted kernels of 16 promising genotypes selected for future breeding efforts from almond [*Prunus dulcis* (Miller) D.A. Webb.] genetic resources of Bigadic district (Balikesir city, western Turkey). The majority of promising almond selections had kernel weight over 1 g since large kernel is a desired fruit characteristics for almond breeding efforts. Nuts of almond genotypes were collected at two seasons based on their harvest times. The harvest season was late August (24-31 August) for 8 promising genotypes and early September (7-12 September) for other 8 genotypes. Many kernels within the 50 nuts of each genotype were randomly chosen for analyses and they were removed from their shells. Then, they were dried in a vacuum oven at 60°C for 3 d.

Determination of sugars: The analysis of sugars (fructose, sucrose, glucose and maltose) was applied by the modified methods of Torije *et al.*[16] and Karkacier *et al.*[17]. 2 g of sample was ground into powder in liquid nitrogen and 40 mL of methanol was added. The mixture was incubated on a magnetic stirrer at 65°C for 0.5 h. It was centrifuged at 4°C, 1300 rpm for 40 min. The supernatant was transferred in clean tube and made up to 50 mL with methanol. Methanol was removed by rotary evaporator and the residue was dissolved in 25 mL double distilled water. Extract was passed through Sep-Pak C_{18} cartridge and 2.5 mL of filtrate was mixed with 7.5 mL acetonitrile. Then it was filtrated by 0.45 μm membrane filter and injected into HPLC. The column was calibrated by fructose, sucrose, glucose and maltose standards. Sugar contents were expressed as g/100 g.

Statistical analysis: A completely randomized design with 3 replications was used for statistical analysis of sugar compositional data. The means were compared with t-test. Statistical package program Minitab release 10.2 for Windows was used for the analysis of variance (Anova). The LSD values were computed for multiple comparisons of the means. Significant differences were recorded at $p < 0.01$. In addition, the analyses of correlation and regression were done to conclude relationships among sugar contents of almond kernels using Minitab release 10.2 for Windows and Excel package program.

RESULTS AND DISCUSSION

All almonds selections had sweet kernels. Except for two genotypes, all selections had kernel weight over 1 g. Almond cultivars having large kernels are valuable for breeding studies and more preferred for commercial uses[12]. Almond selections harvested in late August statistically differed by sugar contents. They had a range of 1.41-3.19 g/100 g sucrose, 0.13-1.02 g/100 g maltose, 0.91-4.50 g/100 g glucose and 2.20-7.20 g/100 g fructose contents. They also averagely contained 2.19 g/100 g sucrose, 0.68 g/100 g maltose, 2.09 g/100 g glucose and 4.08 g/100 g fructose. In addition, genotypes harvested in early September also differed by sugar contents statistically. They had a range of 1.40-3.26 g/100 g sucrose, 0.13-2.34 g/100 g maltose, 0.81-2.70 g/100 g glucose and 1.99-4.20 g/100 g fructose contents. They contained 2.31 g/100 g sucrose, 0.92 g/100 g maltose, 1.82 g/100 g glucose and 3.41 g/100 g fructose. When sugar contents are compared based on harvest seasons, sugar contents of selections did not differ by harvest season statistically (Table-1).

In addition, relationships among sugar contents of almond genotypes were also studied based on harvest seasons. In almond genotypes harvested in late August, all correlation coefficients among sugar contents were positive except for the relationship of sucrose and fructose ($r = -0.209$) (Table-2). Whereas, in almond genotypes harvested in early September, the relationships with other three sugars of fructose were negative (Table-3). The highest positive (Fig. 2) and negative (Fig. 1) relationships were computed between sucrose and maltose ($r = 0.771$) and between glucose and fructose ($r = -0.585$), respectively. Therefore, the relationships with other three sugars of fructose were influenced by harvest season.

TABLE-1
SUCROSE, MALTOSE, GLUCOSE AND FRUCTOSE CONTENTS (AS g/100 g IN DRY WEIGHT) BASED ON HARVEST SEASONS IN SWEET KERNELLED ALMOND SELECTIONS FROM BALIKESIR (WESTERN TURKEY)

Accessions	Almond genotype	Kernel weight (g)	Sucrose (g/100 g)	Maltose (g/100 g)	Glucose (g/100 g)	Fructose (g/100 g)	Kernel taste	Harvest time
Balikesir (Western Turkey)	B-1	1.10	2.00	0.70	1.49	3.78	Sweet	Late August
	B-3	1.01	2.50	0.13	4.50	3.50	Sweet	Late August
	B-4	1.02	3.19	1.30	3.10	3.13	Sweet	Late August
	B-7	1.00	1.60	0.13	1.20	2.20	Sweet	Late August
	B-14	1.07	3.00	1.00	1.21	2.18	Sweet	Late August
	B-23	1.17	2.25	1.02	1.90	5.53	Sweet	Late August
	B-28	1.02	2.33	0.89	2.23	7.20	Sweet	Late August
	B-33	1.00	1.41	0.80	2.25	5.30	Sweet	Late August
	B-40	1.07	1.43	0.15	0.91	3.88	Sweet	Late August
Mean		1.05	2.19	0.68	2.09	4.08		
Significance		NS	**	**	***	***		
LSD (0.01)		–	0.87	0.55	0.83	1.45		
	B-34	1.00	2.10	0.62	1.30	4.20	Sweet	Early September
	B-10	1.04	3.26	2.34	2.53	2.26	Sweet	Early September
	B-18	1.00	2.82	0.72	1.29	1.99	Sweet	Early September
	B-41	0.95	1.90	0.60	1.60	2.10	Sweet	Early September
	B-24	1.01	2.10	0.70	0.81	7.90	Sweet	Early September
	B-25	0.90	3.00	1.14	2.70	2.79	Sweet	Early September
	B-21	1.00	1.89	0.74	1.65	3.23	Sweet	Early September
	B-32	1.00	1.40	0.50	2.65	2.80	Sweet	Early September
Mean		0.99	2.31	0.92	1.82	3.41		
Significance		NS	**	***	**	***		
LSD (0.01)		–	0.79	0.26	0.81	0.94		
Mean of genotypes harvested in late August		1.05	2.19	0.68	2.09	4.08		
Mean of genotypes harvested in early September		0.99	2.31	0.92	1.82	3.41		
Significance		NS	NS	NS	NS	NS		

TABLE-2
RELATIONSHIPS AMOND SUGAR CONTENTS IN EARLY HARVESTED ALMOND GENOTYPES

Correlation coefficients (r)	Maltose	Glucose	Fructose
Sucrose	0.610	0.421	-0.209
Maltose	–	0.026	0.267
Glucose	–	–	0.118

TABLE-3
RELATIONSHIPS AMOND SUGAR CONTENTS IN LATE HARVESTED ALMOND GENOTYPES

Correlation coefficients (r)	Maltose	Glucose	Fructose
Sucrose	0.771	0.228	-0.249
Maltose	–	0.473	-0.243
Glucose	–	–	-0.585

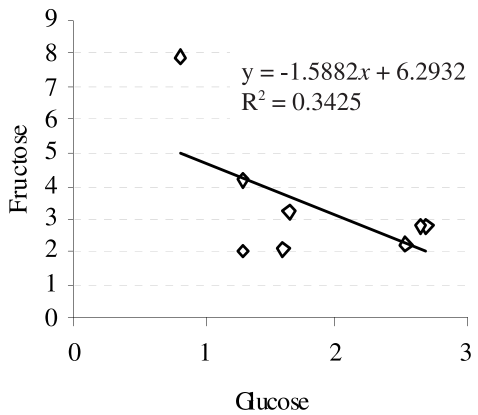

Fig. 1. Relationship of fructose and glucose contents in almond genotypes harvested in early September

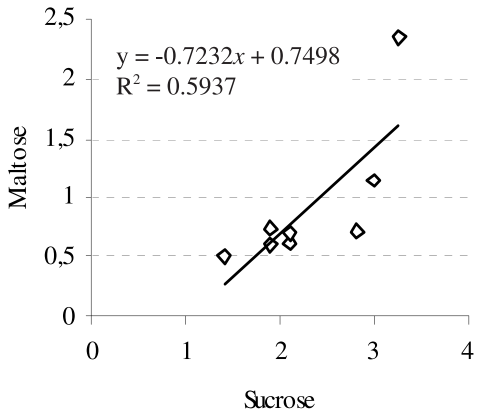

Fig. 2. Relationship of sucrose and maltose contents in almond genotypes harvested in early September

Kader[13] reported that total sugar content in almond kernel is 3 % and the main sugar is sucrose. According to Saura-Calixto *et al.*[18], total sugar content of almond kernel is 5.5%. Barbera *et al.*[19] recorded that Ferragnes and Tuono kernels contain 3.47 and 3.19 % of total sugar, respectively.

Barbera *et al.*[20] determined as 4.15 and 5.29 % reducing sugar for Ferragnes and for Tuono kernels depending on the effect of rootstock. Studying on almond genetic resources of Sardinia (Italy), Nieddu *et al.*[15] reported that almond genotypes had the total sugar content between 0.44 and 5.33 % and reducing sugar content is lower than 2 % in the majority of genotypes. Aslantas[21] determined total sugar content between 2.64 % (Ke-170) and 4.17 % (Ke-130) for promising almond genotypes selected from Kemaliye (Erzincan, Turkey). Schirra *et al.*[22] determined as 3.9 % sugar content of Texas almond cultivar in Italy. Ellis *et al.*[23] detected 30.1-26.0 μg/mg galactose and 147.6-157.7 μg/mg glucose in seeds and skins of raw almonds, respectively. In addition, irrigation, harvest time and storage conditions affects sugar composition of almond kernel[11,14,15]. Under irrigated and non-irigated conditions for early and late harvested Texas and Ferragnes almond cultivars, it was reported that kernel soluble sugar content varied from 1.7 to 4.3 % and kernel sucrose content was between 68.1 and 91.0 %[11]. Kazantzis *et al.*[14] recorded that sucrose content varies from 70.4 to 85.3 % at early and late harvest of Ferragnes cultivar, depending on storage conditions as shelled kernels and in-shelled almonds. When sucrose contents reported in some references are expressed as g/100 g, it seems that data regarding sucrose contents of all selections investigated in this study were in harmony with those reported by Nanos *et al.*[11] and Kazantzis *et al.*[14]. Also, according to findings of this study, the mean sugar was fructose in 12 genotypes, sucrose in 4 genotypes and glucose in one genotype. Whereas, the limited references in number reported that the main sugar is sucrose for almond kernels[8-10,13].

On the other hand, almond's complex carbohydrates are widely used for weight reductions in human diets[1,6,7]. It has been stated that nutritional improvement of nut crops through breeding efforts will gain importance for more healtful life style[24-27]. Also, almond genetic materials need to nutritional identification as well. Compositional findings regarding sugar contents of almond varieties or genotypes are very limited in the references. Therefore, findings of this paper will contribute to nutritional breeding efforts of almond.

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