Asian Journal of Chemistry Vol. 19, No. 7 (2007), 5659-5665

Detection of Free Sugars in Almond Genotypes from Eastern and Western Turkey by HPLC

M. FIKRET BALTA*, I.H. YORUK†, A. KAZANKAYA, P. BATTAL‡ and M.E.EREZ‡
Department of Horticulture, Faculty of Agriculture
University of Yüzüncü Yil, 65080 Van, Turkey
Tel/Fax: (90)(432)2251331; E-mail: fikret_balta@hotmail.com

This study evaluated the sugar contents in almond [*Prunus dulcis* (Mill.) D.A. Webb.] genotypes, depending on their kernel sizes and kernel colours. Based on small kernel (SK) and large kernels (LK), sugar contents were compared both in sweet kernelled almond genotypes from Balikesir (western Anatolia, Turkey) and in bitter kernelled almond genotypes from Tunceli (eastern Anatolia, Turkey). Sugar contents were detected by HPLC. In all sweet and bitter LK and SK genotypes, contents of sucrose, maltose, glucose and fructose did not differ statistically except for only fructose content in bitter almonds. The LK and SK sweet genotypes contained 2.14-2.79 g/100 g sucrose, 0.61-0.44 g/100 g maltose, 1.20-2.05 g/100 g glucose and 3.28-3.45 g/100 g fructose, respectively. The LK and SK bitter genotypes contained 3.08-1.80 g/100 g sucrose, 0.85-1.34 g/100 g maltose, 2.28-2.38 g/100 g glucose and 3.67-2.73 g/100 g fructose, respectively. On the other hand, sugar contents were also compared based on light coloured kernel (LCK) and dark coloured kernel (DCK) in sweet and bitter genotypes. In sweet genotypes, sucrose, maltose and glucose contents of LCK and DCK did not differ statistically. In bitter genotypes, sucrose and glucose contents of LCK and DCK differed statistically. The sweet LCK and DCK genotypes contained 2.36-2.10 g/100 g sucrose, 0.60-0.74 g/100 g maltose, 1.58-2.32 g/100 g glucose and 3.28-2.56 g/100 g fructose, respectively. The bitter LCK and DCK genotypes contained 3.73-2.05 g/100 g sucrose, 1.10-1.36 g/100 g maltose, 3.56-1.56 g/100 g glucose and 2.63-4.05 g/100 g fructose, respectively. In addition, the mean sugar was usually fructose in LK, SK, LCK and DCK.

Key Words: Almond, Kernel size, Kernel colour, Sugars, Balikesir, Tunceli.

†Department of Chemistry, Faculty of Art and Science, University of Yuzuncu Yil, Van, Turkey.

‡Department of Biology, Faculty of Art and Science, University of Yuzuncu Yil, Van, Turkey.

INTRODUCTION

Recently, almond is one of the most popular nuts. Its nutritional value is important for human health and human diets. The fact that California's almond production in USA has doubled in response to consumer awareness about the human healthfulness of almonds during last 20 years is remarkable[1]. In the fruit species, composition of sugars influences the taste and it can vary to varieties, ecological conditions, technical and cultural practices[2]. While sucrose, glucose and fructose are considered as the main sugars in fruits, fructose is sweeter than sucrose and sucrose is sweeter than glucose[3]. Almond's complex carbohydrates are widely used for weight reductions in human diets[1,4,5]. Sugar composition of almond kernel have been reported as mainly sucrose and raffinose and lower amounts of fructose, glucose and galactose[6-8] and it has vital value for good flavour and taste[9]. Almond kernel contains 20.4 % carbohydrate and sugar 3 % and the main sugar is sucrose[10]. Sugar composition of almond kernel is affected by irrigation, harvest time and storage conditions[9,11,12]. On the other hand, light kernel colour and large kernel are two important fruit characteristics for almond breeding efforts[13]. The light coloured and large kernelled almonds are more preferred. References show that researches on sugar composition in almond kernels are very limited. The objective of this study was to detect by HPLC sugar contents based on kernel sizes and kernel colours in sweet and bitter kernelled almond genotypes [*Prunus dulcis* (Mill.) D.A. Webb.] from Balikesir (western Anatolia, Turkey) and Tunceli (eastern Turkey).

EXPERIMENTAL

Plant materials and characteristics: The material of present studies constituted five small and large kernelled almond [*Prunus dulcis* (Miller) D.A. Webb.] genotypes with sweet and bitter kernels from Bigadic (Balikesir, western Turkey) and Tunceli (eastern Turkey) and ten sweet and bitter almond genotypes with light and dark coloured kernels from Bigadic (Balikesir, western Turkey) and Tunceli (eastern Turkey). Nuts of almond genotypes were collected at the harvest times which were late August-early September in Balikesir and early September in Tunceli. Many kernels within the 50 nuts for each genotype were randomly chosen for fruit and sugar analyses. Almond kernels were dried in a vacuum oven at 60°C for 3 d. Nut weight, kernel weight, kernel percentage and shell thickness were recorded for each almond genotype. Abbreviations were termed as LK for large kernel, SK for small kernel, LCK for light coloured kernel and DCK for dark coloured kernels.

Determination of sugars: Sugars (fructose, sucrose, glucose and maltose) were analyzed according to the modified methods of Torije *et al.*[14] and Karkacier *et al.*[15]. 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. After removal of methanol by rotary evaporator, 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. It was filtrated by 0.45 µm membrane filter and injected into HPLC. The column was calibrated by using fructose, sucrose, glucose and maltose standards. Sugar contents were expressed as g/100 g.

Statistical analysis: A completely randomized design with three replications was used for statistical analysis of sugar compositional data. The means were separated using Duncan's multiple range test. Significant differences were found at $p < 0.01$. 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.

RESULTS AND DISCUSSION

In LK and SK almond genotypes from Balikesir, the mean values of nut weight, kernel weight, kernel percentage and shell thickness were recorded 5.81-3.41 g, 1.08-0.74 g, 18.96-21.63 % and 3.90-3.18 mm, respectively. Nut and kernel weights of LK and SK differed statistically (Table-1).

In sweet kernelled genotypes from Balikesir, sucrose, maltose, glucose and fructose of LK and SK did not differ statistically. In bitter kernelled genotypes from Tunceli, only fructose contents of LK and SK differed statistically and there were no statistical differences in other sugar contents. The LK and SK sweet genotypes from Balikesir contained 2.14-2.79 g/100 g sucrose, 0.61-0.44 g/100 g maltose, 1.20-2.05 g/100 g glucose and 3.28-3.45 g/100 g fructose, respectively. The LK and SK bitter genotypes from Tunceli contained 3.08-1.80 g/100 g sucrose, 0.85-1.34 g/100 g maltose, 2.28-2.38 g/100 g glucose and 3.67-2.73 g/100 g fructose, respectively (Table-2).

On the other hand, sugar contents were also compared based on light (LCK) and dark coloured kernels (DCK) both in six sweet genotypes from Balikesir and in four bitter genotypes from Tunceli. In sweet genotypes from Balikesir, contents of sucrose, maltose and glucose did not differ statistically by LCK and DCK. Their only fructose contents differed statistically by LCK and DCK. The sweet LCK and DCK genotypes from

TABLE-1
IN ALMOND SELECTIONS FROM BALIKESIR (WESTERN TURKEY) AND TUNCELI (EASTERN TURKEY), VALUES OF NUT WEIGHT, KERNEL WEIGHT, KERNEL PERCENTAGE AND SHELL THICKNESS BASED ON SMALL AND LARGE KERNELS OF THE SAME GENOTYPE

Accessions	Genotypes	Nut weight (g)		Kernel weight (g)		Kernel percentage (%)		Shell thickness (mm)	
		LK	SK	LK	SK	LK	SK	LK	SK
Balikesir, Western Turkey	B-01	4.80	3.90	1.10	0.78	22.9	20.0	3.25	2.85
	B-14	6.50	3.30	1.07	0.75	16.5	22.7	4.05	3.60
	B-40	6.14	3.15	1.07	0.70	17.5	22.2	4.40	3.10
Mean		5.81a	3.45b	1.08a	0.74b	18.96	21.63	3.90	3.18
Significance		***		***		NS		NS	
Tunceli (Easter Turkey)	T-06	5.26	3.40	1.07	0.83	203	24.4	4.10	3.30
	T-97	5.02	3.71	1.19	0.76	23.7	20.5	3.90	3.30
Mean		5.14a	3.55b	1.13a	0.80b	22.0	22.5	4.00	3.30
Significance		***		***		NS		NS	

LK: large kernel, SK: small kernel, NS: Non-significant.

TABLE-2
IN ALMOND SELECTIONS FROM BALIKESIR (WESTERN TURKEY) AND TUNCELI (EASTERN TURKEY), SUGAR COMPOSITION (AS mg/g IN DRY WEIGHT) BASED ON KERNEL SIZE WITHIN THE SAME GENOTYPE

Accession	Genotype	Sucrose (g/100 g)		Maltose (g/100 g)		Glucose (g/100 g)		Fructose (g/100 g)		Kernel taste
		LK	SK	LK	SK	LK	SK	LK	SK	
Balikesir, Western Turkey	B-01	2.00	2.47	0.70	0.25	1.49	0.96	3.78	2.40	Sweet
	B-14	3.00	2.80	1.00	0.56	1.21	4.40	2.18	3.16	Sweet
	B-40	1.43	3.10	0.15	0.51	0.91	0.80	3.88	4.80	Sweet
Mean		2.14	2.79	0.61	0.44	1.20	2.05	3.28	3.45	
Significance		NS		NS		NS		NS		
LSD (0.01)		–		–		–		–		
Tunceli (Eastern Turkey)	T-06	2.30	1.09	1.00	0.99	1.20	1.20	4.04	2.90	Bitter
	T-97	3.85	2.51	0.70	1.70	3.35	3.56	3.30	2.57	Bitter
Mean		3.08	1.80	0.85	1.34	2.28	2.38	3.67	2.73	
Significance		NS		NS		NS		NS		
LSD (0.01)		–		–		–		–		

LK: large kernel, SK: small kernel, NS: Non-significant.

Balikesir contained 2.36-2.10 g/100 g sucrose, 0.60-0.74 g/100 g maltose, 1.58-2.32 g/100 g glucose and 3.28-2.56 g/100 g fructose, respectively. The bitter LCK and DCK genotypes from Tunceli contained 3.73-2.05 g/100 g sucrose, 1.10-1.36 g/100 g maltose, 3.56-1.56 g/100 g glucose and 2.63-4.05 g/100 g fructose, respectively (Table-3).

TABLE-3
SUGAR COMPOSITION (AS mg/g IN DRY WEIGHT) BASED ON KERNEL COLOUR IN SWEET AND BITTER KERNELLED ALMOND SELECTIONS FROM BALIKESIR (WESTERN TURKEY) AND TUNCELI (EASTERN TURKEY)

Accessions	Genotypes		Sucrose (g/100 g)		Maltose (g/100 g)		Glucose (g/100 g)		Fructose (g/100 g)		Kernel taste
	LCK	DCK	LCK	DCK	LCK	DCK	LCK	DCK	LCK	DCK	
Balikesir, Western Turkey	B-16	B-25	2.84	3.00	0.26	1.14	1.10	2.70	2.91	2.79	Sweet
	B-21	B-32	1.89	1.40	0.74	0.50	1.65	2.65	3.23	2.80	Sweet
	B-38	B-41	2.37	1.90	0.80	0.60	2.00	1.60	3.69	2.10	Sweet
Mean			2.36	2.10	0.60	0.74	1.58	2.32	3.28	2.56	
Significance			NS		NS		NS		NS		
LSD (0.01)			–		–		–		0.58		
Tunceli (Eastern Turkey)	T-43	T-21	4.35	1.65	0.89	1.80	2.71	1.21	3.66	5.20	Bitter
	T-118	T-62	3.10	2.45	1.30	0.91	4.40	1.89	1.60	2.90	Bitter
Mean			3.73	2.05	1.10	1.36	3.56	1.56	2.63	4.05	
Significance			**		NS		**		NS		
LSD (0.01)			1.52		–		1.87		–		

LCK: Genotypes with light coloured kernels, DCK: Genotypes with dark coloured kernels, NS: Non-significant.

Schirra *et al.*[16] determined 3.9 % sugar content in Texas almond cultivar in Italy. Nieddu *et al.*[12] investigated almond genetic resources of Sardinia (Italy) and they reported that almond genotypes contain the total sugar content between 0.44 and 5.33 % and reducing sugar content is lower than 2 % in the majority of genotypes. Kader[10] reported 3 % total sugar content in almond kernel. Saura-Calixto *et al.*[17] determined as 5.5 %. total sugar content in almond kernel. Barbera *et al.*[18] recorded that Ferragnes and Tuono kernels contain 3.47 and 3.19 % of total sugar, respectively. Barbera *et al.*[19] detected 4.15 and 5.29 % reducing sugar for Ferragnes and for Tuono kernels depending on the effect of rootstock. Ellis *et al.*[20] recorded 30.1-26.0 µg/mg galactose and 147.6-157.7 µg/mg glucose in seeds and skins of

raw almonds, respectively. Assessing almond genetic resources from Kemaliye (Erzincan, Turkey), Aslantas[21] determined total sugar content between 2.64 % (Ke-170) and 4.17 % (Ke-130) for promising genotypes. On the other hand, sugar composition of almond kernel is influenced by irrigation, harvest time and storage conditions[9,11,12]. Nanos *et al.*[9] reported that kernel soluble sugar content varied from 1.7 to 4.3 % under irrigated and non-irigated conditions in early and late harvested Texas and Ferragnes almond cultivars and kernel sucrose content was between 68.1 and 91.0 %. Kazantzis *et al.*[11] recorded that sucrose content ranges 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.

The data with respect to sucrose contents of all genotypes in this study had similarities to those reported by Nanos *et al.*[9] and Kazantzis *et al.*[11] when we turn into as g/100 g the values regarding sucrose contents reported in some references. In almond kernels, the main sugar has been reported as sucrose[6-8,10]. Whereas, the mean sugar was usually fructose in LK, SK, LCK and DCK according to findings of this study.

The findings of this study might contribute to nutritional breeding efforts of almond and to the related knowledges because almond's sugars are widely used for weight reductions in human diets[1,4,5], nutritional improvement of nut crops through breeding efforts will gain importance for more healthful life style[22-24], almond genetic materials should be nutritional identification[25], findings with regard to sugar composition of almond varieties or genotypes are very limited in the related references.

REFERENCES

1. C. Yen-Chen, K. Lapsley and J. Blumberg, *J. Sci. Food Agric*., **86**, 2245 (2006).
2. I. Karacali, Storage and Marketing of Horticultural Crops, Ege Univ. Press, No:494, ISBN 975-483-048-7, Bornova-Izmir, p. 413 (In Turkish) (1990).
3. A.A. Kader, in ed.: M. Knee, Fruits in the Global Market, Fruit Quality and its Biological Basis, Sheffield Academic Press, CRC, pp. 1-14 (2002).
4. G.E. Fraser, H.W. Bennett, K.B. Jaceldo and J. Sabate, *J. Am. Coll. Nutr.*, **21**, 275 (2002).
5. K. McManus, L. Antinoro and F. Sacks, *Int. J. Obes. Relat. Metab. Disord*., **25**, 1503 (2001).
6. P.C. Fourie and D.S. Basson, *J. Agric. Food Chem.*, **38**, 101 (1990).
7. F. Saura-Calixto, J. Canellas and G.A. Raso, *J. Agric. Food. Chem.*, **32**, 1018 (1984).
8. L. Soler, J. Canellas and F. Saura-Calixto, *J. Agric. Food Chem.*, **37**, 1400 (1989).
9. G.D. Nanos, I. Kazantzis, P. Kefalas, C. Petrakis and G.G. Stavroulakis, *Sci. Hort.*, **96**, 249 (2002).
10. A.A. Kader, in ed.: C.W. Micke, In-plant Storage, Almond Production Manuel, University of California, Division of Agric. and National Res., CA, pp. 274-277 (1996).
11. I. Kazantzis, G.D. Nanos and G.G. Stavroulakis, *J. Sci. Food Agric*., **83**, 354 (2003).
12. G. Nieddu, I. Chessa, M. Pala and G. Lovicu, *Acta Hort*., **373**, 135 (1994).

13. D.E. Kester and R. Asay, in eds.: J. Janick and J.N. Moore, Almonds, Advanced in Fruit Breeding, John Wiley & Sons, Inc. Vol. 3, pp. 387-419 (1979).
14. E. Torije, C.M.C. Diez, M. Camara, E. Camacho and P. Mazario, *J. Sci. Food Agric.*, **76**, 315 (1998).
15. M. Karkacier, M. Erbas, M.K. Uslu and M. Aksu, *J. Chromatgr. Sci.*, **41**, 331 (2003).
16. M. Schirra, M. Mulas and M. Mura, *Agric. Mediterranean*, **123**, 200 (1993).
17. F. Saura-Calixto, M. Bauza, T.F. Martinez and A. Argamenteria, *J. Agr. Food Chem.*, **29**, 509 (1981).
18. G. Barbera, T.L. Mantia, L.D. Palma, F. Monastra and M. Schirra, *Acta Hort.*, **373**, 125 (1994a).
19. G. Barbera, L.D. Marco and M. Schirra, *Acta Hort.*, **373**, 129 (1994b).
20. P.R. Ellis, C.W.C. Kendall, Y. Ren, C. Parker, J.F. Pacy, K.W. Waldron and D.J.A. Jenkins, *Am. J. Clin. Nutr.*, **80**, 604 (2004).
21. R. Aslantas, A Research on Breeding by Selection of Native Almonds (*Amygdalus communis* L.) Grown in Kemaliye District (Erzincan), (Master thesis), Ataturk University, Agric. Fac., Hort. Sci., Erzurum (In Turkish) (1993).
22. F.A. Bliss, *Hortscience*, **34**, 1163 (1999).
23. H.M. Munger, *The Hortscience*, **14**, 247 (1979).
24. B.H.M. Quebedeaux, *Hortscience*, **25**, 1473 (1990).
25. M.A. Askin, M.F. Balta, F.E. Tekintas, A. Kazankaya and F. Balta, *J. Food Comp. Anal.*, **20**, 7 (2007).

(*Received*: 6 January 2007; *Accepted*: 19 June 2007) AJC-5752