



Variability of phenolics, α -tocopherol and amygdalin contents of selected almond (*Prunus amygdalus* Batsch.) genotypes

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Abstract

Phenolic compounds may be regarded as one factor contributing to quality of fruits and juice because of high antioxidative effects. Limited information is available concerning the qualitative and quantitative composition of polyphenolic compounds in almonds. The phenolic acids, α -tocopherol and amygdalin contents of the 14 selected almond genotypes were investigated in the study. Catechin was the major phenolic acid ranging from 11.1 to 227.2 $\mu\text{g/g}$, followed by caffeic acid (2.9-32.1 $\mu\text{g/g}$), epicatechin (2.0-23.5 $\mu\text{g/g}$) and gallic acid (2.4-16.1 $\mu\text{g/g}$). α -Tocopherol contents of genotypes were between 143.97 and 462.78 $\mu\text{g/g}$, and significant differences were observed amongst genotypes. The highest amygdalin content was detected in slightly bitter almond genotype Isp-9 (22.53 mg/g). Amygdalin contents of sweet almond genotypes ranged from 1.53 to 11.56 mg/g. The results showed large variability amongst genotypes in phenolic acids, α -tocopherol and amygdalin contents.

Key words: Almond, phenolics, tocopherol, amygdalin.

Introduction

Almond (*Prunus amygdalus* Batsch.) is a member of the Rosaceae family and yields fruit of great commercial value. The annual inshell almond production in the world is 2,035,489 tons from an area of 1,797,927 hectares. Turkey has about 2.3% of world production producing 46,408 tons almond from an area of 17,000 ha¹. Turkey has a wide genetic variation for this species in some of its regions due to open pollination conditions and seed propagation since thousands of years. Almond kernels have concentrated source of energy, containing significant amounts of fats, protein, fiber and minerals^{2,3}. The fat is primarily unsaturated, mostly oleic and linoleic fatty acids maintaining lower blood cholesterol levels^{4,5}. Almond kernels contain high total tocopherol and total phenol⁶. Besides, almond kernel has significant levels of antioxidant activity and scavenging effect on free radicals⁷⁻⁹.

Phenolic compounds may be regarded as one factor contributing to quality of fruits and juice because of high antioxidative effects¹⁰. Phenolics also contribute to disease resistance of plants¹¹. Limited information is available concerning the qualitative and quantitative composition of polyphenolic compounds in almonds^{9,12}. Milbury *et al.*¹² reported that flavonoids and phenolics content of some almond cultivars (Nonpareil, Mission, Butte, Carmel, Fritz, Monterey, Padre and Price) were high. Amygdalin is a cyanogenic glycoside found in bitter almond, apricot kernels and seeds of other *Prunus* species. It was reported that amygdalin is used to cure for all types of cancer¹³, although its effects of treatment was not proven by scientific researches. Nevertheless, there are some studies reported that amygdalin may be hazardous for human health^{14,15}. Newton *et al.*¹⁴

determined that lethal dose of amygdalin (LD₅₀) was 522 mg per body weight for rats. Nut fruits have high tocopherol content and were recommended constituents of the daily diet.

In a previous paper, some genotypes, which have high yield and better fruit quality, were selected based on the desired characteristics from Isparta region of Turkey¹⁶. In this study, the objective was to determine the phenolics, α -tocopherol and amygdalin content of kernel of the 14 selected almond genotypes.

Material and Methods

In the study, fruits of 14 almond genotypes, selected by Yıldırım *et al.*¹⁶, were used. One genotype (Isp-9) was slightly bitter and the others were sweet. About 1 kg almond fruits were taken from different parts of trees. The almond fruits were dried for 20 days at room temperature and cracked out by hand. Five hundred grams almond kernel per genotype was ground and powder samples were placed in plastic bags. Samples were frozen and kept at -80°C until extraction.

Analysis of phenolic compounds: After 5 g of ground kernel was extracted in 100 ml diethylether for 5 hours at room temperature, each sample was placed in erlenmeyer flask and 25 ml of acetone-water solution (80% acetone and 20% water v/v) was added. Samples were incubated in a water bath at 50°C for 30 min and the upper phase was taken. The extraction was repeated two times using 25 ml of acetone-water solution each time. The upper phase was centrifuged at 10,000 rpm for 5 min. The solvent was evaporated at 40°C under vacuum and samples were re-dissolved in 2 ml methanol. Solutions were filtered by a 0.45 μm pore size

membrane filters and then 20 µl of the solutions was injected into HPLC.

HPLC analysis was performed using a Shimadzu HPLC system with a diode array detector (DAD). Phenolics were analyzed by the modified procedure of Caponio *et al.*¹⁷. The phenolics were analyzed at 280 nm. The column used was an Agilent EclipseXDB-C₁₈ (250 mm x 4.60 mm x 5 µm) operated at 30°C. The elution solvents were 3% acetic acid (A) and 100% methanol (B). Samples were extracted according to the following gradient: 5% in B as initial condition, 30% in B for 35 min, 50% in B for 35 min, 80% in B for 5 min and finally 100% in B for 5 min. The flow rate was 0.8 ml/min. Peak identification was done according to the standards (gallic acid, catechin hydrate, chlorogenic acid hemihydrate, caffeic acid, epicatechin, p-coumaric acid, quercetin and kaempherol). The quantities of phenolics were assessed from their peak areas. Concentrations were expressed as µg g⁻¹.

α-Tocopherol and amygdalin analysis: Lipids were extracted using methanol as a solvent with a Soxhlet equipment. For this purpose, 2 g of homogenized sample was placed in an extraction cell and then incubated overnight. Thereafter methanol was evaporated.

α-Tocopherol was analyzed by HPLC system with a diode array detector (DAD) at 295 nm¹⁸. One hundred milligrams of extracted almond oil was dissolved in 1 ml of mobile phase and 10 µl injected into HPLC. The column used was a Luna silica (150 mm x 4.60 mm x 5 µm) operated at 25°C. The elution solvents were 95% heptan (C₇H₁₆) (A) and 5% tetrahydrofuran ((CH₂)₄O) (B). Samples were extracted according to the following gradient: 5% in B as initial condition, 25% in B for 10 min, 80% in B for 10 min, 90% in B for 10 min and finally, 100% in B for 5 min. The flow rate was 1.2 ml/min. The amounts of α-tocopherol were assessed from peak areas of samples. Concentrations were expressed as µg g⁻¹.

Amygdalin was analyzed by HPLC system with a diode array detector (DAD) at 210 nm¹⁵. The almond oil was solved in 10 ml of mobile phase (75% acetonitrile (ACN)) and passed through a Sep-Pak C₁₈ cartridge. The resulting solution was diluted 500 times and 10 µl of sample was injected into the HPLC. The column used was a ACE 5-C₁₈ (250 mm x 4.60 mm x 5 µm) operated at 25°C. The elution solvents were 75% ACN. The flow rate was 0.9 ml/min. Concentrations of amygdalin were determined by correlation of their HPLC peak areas to their calibration curves. Concentrations were expressed as mg g⁻¹.

All chemical analyses were carried out in triplicates. Data were subjected to ANOVA using Minitab software (Minitab Inc.). The means were separated by Duncan's Multiple Range Test (P<0.05).

Results and Discussion

In Table 1, the amounts of polyphenols of almond kernel were reported. Generally, the same phenolic compounds were present in each almond genotype but there were differences in relative levels. Catechin was the major phenolic acid ranging from 11.1 to 227.2 µg/g, followed by caffeic acid (2.9-32.1 µg/g), epicatechin (2.0-23.5 µg/g) and gallic acid (2.4-16.1 µg/g). While Isp-52 sample had the highest value for catechin (227.2 µg/g), Isp-80 sample had the highest value for quercetin, gallic acid and α-coumaric acid (35.82, 16.1 and 3.44 µg/g, respectively). Isp-228 sample had the highest content of epicatechin and a high content of caffeic acid. The highest content of caffeic acid was obtained from Isp-

68 almond genotype (32.1 µg/g). The highest content of chlorogenic acid was obtained from Isp-298 genotype (22.9 µg/g). Kaempherol was detected from only three almond genotypes (Isp-66, Isp-231 and Isp-298) in low values. The contents of catechin, quercetin and epicatechin are similar but kaempherol content is lower than those of Milbury *et al.*¹². Our results showed that almond kernel also contains gallic acid, chlorogenic acid, caffeic acid and α-coumaric acid as well. Kornsteiner *et al.*⁶ reported that almond kernels have high total phenol content. Phenolic compounds may be regarded as one factor contributing to the so-called "inner quality" of fruits and juice because of high antioxidative effects¹⁰. Flavonoids and phenolics play a major physiological role in fruit, especially in resistance to various stress factors and diseases^{11,19}. Besides, phenolics have significant levels of antioxidant activity and scavenging effect on free radicals⁷⁻⁹. Antioxidative compounds such as phenolics have been widely used as food additives to provide protection against oxidative deterioration of food. So phenolics play an important role for human nutrition and should be included in human diet.

α-Tocopherol contents of almond genotypes were between 143.97 and 462.78 µg/g (Table 2). Significant differences were observed for α-tocopherol content amongst almond genotypes. Among almond genotypes, Isp-231, Isp-59 and Isp-241 had higher α-tocopherol contents (462.78, 401.69 and 399.70 µg/g, respectively) than the other selected genotypes. The lowest α-tocopherol (143.97 µg/g) was obtained from Isp-80 in the study. Kornsteiner *et al.*⁶ reported that α-tocopherol content of almond was 242 µg/g. The α-tocopherol contents of our almond genotypes were generally higher than those reported by Kornsteiner *et al.*⁶, although there are genotypes having higher or lower α-tocopherol content than those reported by the same study. Maguire *et al.*²⁰ mentioned that α-tocopherol was the most dominant tocopherol in almonds. They found lower α-tocopherol content of almond (186.4 µg/g oil) compared to our genotypes. Kodad and Socias i Company²¹ found that α-tocopherol content of almond cultivars were between 214.0 and 453.3 µg/g as similar to our results. Similar results were reported in some previous studies as well^{22,23}. Lopez-Ortiz *et al.*²⁴ found that α-tocopherol contents of almond were between 85.0 and 194.0 µg/g.

Amygdalin was detected with variable values (between 1.53 and 22.53 mg/g) depending on the genotypes (Table 2). The highest amygdalin content was detected in slightly bitter almond genotype Isp-9 (22.53 mg/g). Amygdalin contents of sweet almond genotypes ranged from 1.53 and 11.56 mg/g. Among the sweet kernelled almond genotypes, Isp-80, Isp-231 and Isp-298 had significantly higher amygdalin contents (11.25, 10.58 and 11.56 mg/g, respectively) than the other selected genotypes. Ohtsubo and Ikeda²⁵ found that amygdalin content of bitter almond was 47 mg/g in maximum, obtaining higher values than our results. They reported that amygdalin content of almond was changed according to different almond genotypes and their status of bitterness. Bitterness in almond is determined by the content of the cyanogenic diglucoside amygdalin^{26,27}. Dicenta *et al.*²⁸ reported that bitter almond genotypes had higher amygdalin content than sweet almond genotypes. Amygdalin content of bitter and sweet almond seeds are between 0.0 and 411.5 mg/100 g in some previous studies²⁸⁻³⁰. The amount of amygdalin in

Table 1. Phenolic composition of selected almond genotypes ($\mu\text{g/g}$).

Selected genotypes	Gallic acid	Catechin	Chlorogenic acid	Caffeic acid	Epicatechin	α -Coumaric acid	Quercetin	Kaempferol
Isp-9	10.2 \pm 0.6 bc	35.4 \pm 3.1 cde	1.0 \pm 0.2 de	9.3 \pm 0.4 cd	5.1 \pm 0.2 ef	0.15 \pm 0.01 def	5.38 \pm 0.24 d	nd
Isp-52	7.8 \pm 0.4 bcd	227.2 \pm 86.8 a	8.9 \pm 0.9 b	17.0 \pm 1.9 b	15.2 \pm 5.9 bc	0.57 \pm 0.01 b	1.51 \pm 0.52 ef	nd
Isp-57	7.1 \pm 0.3 cd	46.5 \pm 0.8 cde	1.3 \pm 0.02 de	9.6 \pm 0.8 cd	5.9 \pm 0.3 def	0.25 \pm 0.00 cd	4.15 \pm 0.08 de	nd
Isp-59	5.9 \pm 0.2 d	31.3 \pm 1.0 de	0.5 \pm 0.01 e	5.9 \pm 0.2 de	2.8 \pm 0.2 ef	0.14 \pm 0.01 def	0.82 \pm 0.06 f	nd
Isp-66	6.6 \pm 0.4 d	28.0 \pm 1.6 de	0.3 \pm 0.03 e	5.9 \pm 0.2 de	2.0 \pm 0.08 f	0.07 \pm 0.01 f	12.89 \pm 0.27 c	0.09 \pm 0.00 b
Isp-68	8.5 \pm 0.7 bcd	95.7 \pm 8.7 bc	4.6 \pm 0.6 cd	32.1 \pm 3.5 a	14.1 \pm 1.2 bc	0.49 \pm 0.05 b	0.93 \pm 0.08 f	nd
Isp-80	16.1 \pm 2.7 a	37.0 \pm 6.0 cde	nd	5.4 \pm 1.8 e	2.5 \pm 0.4 ef	3.44 \pm 0.15 a	35.82 \pm 2.30 a	nd
Isp-127	10.0 \pm 0.8 bc	41.2 \pm 1.5 cde	8.2 \pm 0.4 bc	10.7 \pm 0.8 c	9.0 \pm 0.4 cde	0.49 \pm 0.06 b	0.78 \pm 0.06 f	nd
Isp-129	10.3 \pm 0.1 b	11.1 \pm 0.5 e	1.9 \pm 0.03 de	4.2 \pm 0.03 e	2.1 \pm 0.02 f	0.19 \pm 0.00 cde	2.49 \pm 0.18 ef	nd
Isp-196	2.4 \pm 0.5 e	83.4 \pm 15.0 bcd	8.6 \pm 4.0 b	9.3 \pm 1.8 cd	11.5 \pm 2.9 bcd	0.18 \pm 0.01 cdef	17.31 \pm 3.05 b	nd
Isp-228	6.3 \pm 1.5 d	132.0 \pm 24.7 b	9.7 \pm 3.2 b	30.5 \pm 2.3 a	23.5 \pm 3.7 a	0.29 \pm 0.04 c	2.21 \pm 0.32 ef	nd
Isp-231	7.3 \pm 3.5 bcd	44.5 \pm 18.5 cde	8.3 \pm 2.9 bc	16.0 \pm 2.3 b	15.9 \pm 6.7 b	0.28 \pm 0.06 c	6.95 \pm 2.60 d	0.07 \pm 0.03 b
Isp-241	9.1 \pm 0.6 bcd	32.9 \pm 2.9 de	2.7 \pm 0.1 de	2.9 \pm 0.1 e	3.6 \pm 0.5 ef	0.19 \pm 0.01 cde	0.95 \pm 0.01 f	nd
Isp-298	7.8 \pm 0.4 bcd	19.1 \pm 0.8 e	22.9 \pm 0.8 a	6.3 \pm 0.6 de	4.1 \pm 0.3 ef	0.12 \pm 0.01 ef	10.68 \pm 0.65 c	0.35 \pm 0.01 a
Average	8.24	61.81	6.07	11.79	8.38	0.49	7.35	0.17

Each value is expressed as mean \pm standard deviation, means followed by different letters in the same column are significantly different ($P<0.05$), nd = not detected.

Table 2. α -Tocopherol and amygdalin contents of selected almond genotypes.

Genotype	α -Tocopherol ($\mu\text{g/g}$ oil)	Amygdalin (mg/g oil)
Isp-9	299.89 \pm 3.07 de	22.53 \pm 2.37 a
Isp-52	224.27 \pm 59.79 fg	7.36 \pm 0.18 c
Isp-57	306.83 \pm 28.19 cde	3.31 \pm 0.06 d
Isp-59	401.69 \pm 4.65 ab	1.53 \pm 0.18 d
Isp-66	377.04 \pm 0.93 bc	2.81 \pm 0.16 d
Isp-68	272.32 \pm 35.59 ef	3.70 \pm 0.00 d
Isp-80	143.97 \pm 23.08 h	11.25 \pm 2.33 b
Isp-127	340.13 \pm 34.80 bcde	2.28 \pm 0.31 d
Isp-129	326.08 \pm 22.34 bcde	3.03 \pm 0.32 d
Isp-196	358.98 \pm 19.61 bcd	3.44 \pm 0.47 d
Isp-228	180.43 \pm 24.32 gh	1.55 \pm 0.32 d
Isp-231	462.78 \pm 27.90 a	10.58 \pm 2.41 b
Isp-241	399.70 \pm 51.00 ab	4.29 \pm 0.04 d
Isp-298	378.50 \pm 44.55 bc	11.56 \pm 2.18 b
Average	319.47	6.37

Each value is expressed as mean \pm standard deviation, means followed by different letters in the same column are significantly different ($P<0.05$).

almond seeds significantly changes among genotypes due to their genetic differences.

Conclusions

In this study, eight phenolic acids were isolated from almond kernel. Our results suggested that catechin is the major phenolic acid, followed by caffeic acid, epicatechin and gallic acid in almond kernel. In this investigation, Isp-231, Isp-59 and Isp-241 contained the highest α -tocopherol content among all almond genotypes. The results showed large variability among genotypes in phenolic acids and α -tocopherol contents. Among the genotypes, slightly bitter almond genotype Isp-9 had higher amygdalin content than the sweet ones.

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