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Variability of phenolic composition and tocopherol content of some commercial Almond cultivars

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Summary

The phenolic (gallic acid, catechin, caffeic acid, chlorogenic acid, epicatechin, ferulic acid, kaempferol, naringenin, and p-coumaric acid) and tocopherol contents (α, β, γ, and δ) of some commercially significant almond cultivars were determined in the research. Wide variations in phenolic and tocopherol contents were detected among the cultivars. The highest content among the phenolic substances was obtained for catechin, with the average values of 27.35 mg kg^{-1} in 2008 and 39.87 mg kg^{-1} in 2009. The highest catechin content was recorded in the cultivar 'Ferraduel' in both years, with the values of 117.59 mg kg^{-1} in 2008 and 145.86 mg kg^{-1} in 2009. The highest content among the tocopherols was obtained in α-tocopherol, and the average values were detected as 312.29 mg kg^{-1} in 2008 and 467.31 mg kg^{-1} in 2009. The highest α-tocopherol contents were determined as 899.49 mg kg^{-1} and 945.41 mg kg^{-1} in the cultivar 'Supernova' in both years, respectively. In the research, α-tocopherol turned out to be the major tocopherol.

Introduction

Almond is the species with the highest economic value among the nut fruits cultivated worldwide (Venkatachalam and Sathe, 2006). Its high contents of oil, proteins, minerals, fiber, sterols, phenolics and tocopherols (α, β, δ, and γ) (Beyhan et al., 2011; Kirbaşlar et al., 2012; Venkatachalam and Sathe, 2006; Yada et al., 2011) and its different ways of consumption and uses (e.g. uncooked, roasted, blanched, the chocolate and cream cake industry, and the pharmaceutical and paint industries) are among the features which highlight almond (Esfahlan and Jamei, 2012; Socias i Company, 2008; Vijeratne et al., 2006). In addition, it is effectively used to reduce the risk of cardiovascular and other chronic diseases (28 to 100 g day^{-1}); to decrease the level of LDL cholesterol in blood; to reduce the effect of many types of cancer such as lung, pancreas, stomach, and colon; to nourish the brain and nervous systems; and to fight obesity and diabetes for a better life (Ahmad, 2010; Bolling et al., 2010; Davis and Iwahashi, 2001; Esfahlan et al., 2010; Jenkins et al., 2002). Furthermore, the richness of almond oil in antioxidants also allows it to be utilized as a natural softener and a skin rejuvenator today by beauticians, massage specialists, and aromatherapists (Ahmad, 2010).

Natural antioxidant substances are compounds which are comprised of carotenoids, ascorbic acid, phenolics, and tocopherols that are found in the fruits, leaves, roots, seeds, and oils of plants and that provide them with their characteristics such as color, bitterness, and astringency (Isfahlan et al., 2010; Kasnak and Palamutoğlu, 2015; Nizamlioğlu and Nas, 2010). Phenolics and tocopherols are widely used in the food industry for the stability of oils, the preservation of the quality of food, the extension of their shelf life, and their healthy consumption (Kirbaşlar et al., 2012; Korekar et al., 2011; Sang et al., 2002; Takeoka and Dao, 2003). Moreover, they have a feature of scavenging the free radicals which induce the oxidative degradation of lipids, proteins, and nucleic acids (Esfahlan and Jamei, 2012; Isabelle et al., 2010; Izaddost et al., 2013; Kirbaşlar et al., 2012; Mishra et al., 2010). Phenolics play a significant role in such physiological events as growth and reproduction, and in developing the defense mechanism against pathogens and predators in plants (Balasundram et al., 2006).

Tocopherols (Vitamin E) are lipophilic antioxidants with isomers as α, β, γ, and δ that are synthesized particularly by the leaves and seeds of plants (Szymanska and Kruk, 2008). By inhibiting lipid peroxidation, they enhance the biological activity of the body and protect the skin against external effects like ultraviolet rays (Di Mambro et al., 2003).

Although almond is known to be a fruit which is rich in biochemical contents, no sufficient information is available about the potential of commercial almond cultivars in terms of these characteristics (Moayedi et al., 2011), for the genetic traits of species and cultivars directly affect biochemical contents. Besides, the maturity state of fruits, the growing season, soil properties, temperature and the duration of storage of products also have indirect effects on biochemical contents in plants (Balasundram et al., 2006; Garcia-Pascual et al., 2003; Habila et al., 2012; Ismail et al., 2004; Kodad et al., 2006; Kodad et al., 2011; Milbury et al., 2006; Piironen et al., 1986).

In this study, it was aimed to determine the variations of some commercial almond cultivars grown at Isparta, Turkey in terms of phenolic and tocopherol contents. In addition, the studied traits were subjected to principal component analysis for the classification and discrimination of almond cultivars.

Materials and methods

Plant material

The research was conducted at Eğirdir Fruit Research Institute in 2008 and 2009. The Cultivars 'Cristomorto', 'D. Largueta', 'Ferraduel', 'Ferragnes', 'Ferrastar', 'Glorieta', 'Lauranne', 'Masbovera', 'Nonpareil', 'Picantili', 'Sonora', 'Supernova', 'Texas', 'Tuono' and 'Yaltinski', grafted on almond seedling planted in 2003, were used in the study. The trees were irrigated by using a drip irrigation system and supported with the standard fertilizers ammonium nitrate, potassium sulphate, phosphoric acid, zinc sulphate, and borax. The study was set up according to a completely randomized design with 3 replications, and one tree was used in each replication. The mature fruits were collected at an approximate amount of 1 kg from different places of the trees, dried at room temperature for about 20 days, and then crushed by hand. Almond kernels obtained from each cultivar were ground into powder and placed in plastic bags. The samples were preserved at -80 °C until extraction.

HPLC analysis of phenolics

The samples (5 g) were extracted in 25 ml of methanol for 16 hours in shaker incubator operated at 40 °C. The samples were passed through membrane filters with 0.45 µm pore size. Extracts were then evaporated at 40 °C under vacuum until they dried. Extracts were re-

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dissolved in 2 ml of methanol, and then 20 µl of samples was injected into high performance liquid chromatography (HPLC- Shimadzu).
Phenolics were determined using HPLC device with a diode array detector (DAD, λ max = 278 nm) by the modified procedure of CAPONIO et al. (1999). 20 µl of the extracts were injected into HPLC device equipped with an Agilent Eclipse XDB-C 18 (250 × 4.60 mm 5 µm) column operated at 30 °C. The elution solvents were solvent A: 3% acetic acid and solvent B: 100% methanol. The gradient as follows: 7% in B as initial condition, 28% in B for 20 min, 25% in B for 8 min, 30% in B for 7 min, 30% in B for 15 min, 33% in B for 10 min, 42% in B for 2 min, 50% in B for 8 min, 70% in B for 3 min, 80% in B for 2 min, 100% in B for 5 min and 7% in B for 1 min. The flow rate was 0.8 ml min^{-1}. Peak identification was performed according to the standards (gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, o-coumaric acid, rutin, hesperidin, cinnamic acid, quercetin, naringenin, luteolin, kaempferol) (Fig. 1). Phenolic quantities were explained as mg kg^{-1} DW.

HPLC analysis of tocopherols

Two g from ground kernels were taken and oil was extracted in a soxhlet apparatus with 100 ml of hexane as a solvent. The solvent was evaporated at 40 °C under vacuum using a rotary evaporator and the oil was taken (BLIGH and DYER, 1959).
Tocopherols (α, γ, δ, and β) were analyzed by HPLC with a RF-10AXL fluorescence detector (295 nm). 10 µl of the oil was injected into the HPLC device equipped with Luna Silica column (250 × 4.6 mm, 5 µm particle size). The conditions were as follows: mobile phase flow rate, 1.2 ml/min; mobile phase mixture, heptane: THF (95:5) (v/v). Peak identification was performed according to standards (Fig. 2). The quantity of tocopherols was determined according to peak area and expressed in mg kg^{-1} oil.

Statistical analysis

All experiments were performed with three replications. The data were subjected to analyze of variance (ANOVA) using Minitab software (Minitab Inc.) at 5% significance level. The means were separated by Tukey's test.

Results and discussion

Phenolic contents

In the research, the phenolic contents of the almond cultivars are presented in Tab. 1. Wide variations were detected among the cultivars in terms of phenolic contents. The analysis of variance showed that the effects of cultivars, years and the cultivars × years interaction were significant for all phenolic compounds except p-coumaric acid ($p < 0.05$). The highest content among the phenolics was obtained for catechin, with 27.35 mg kg^{-1} in 2008 and 39.87 mg kg^{-1} in 2009. The highest catechin content was recorded in the cultivar 'Ferraduel' in both years, with 117.59 mg kg^{-1} in 2008 and 145.86 mg kg^{-1} in 2009. The gallic acid contents ranged from 1.22 mg kg^{-1} (Ferraduel) to 3.26 mg kg^{-1} (Nonpareil) in 2008 but from 0.67 mg kg^{-1} (Glorieta) to 3.03 mg kg^{-1} (Picantili) in 2009. The highest caffeic acid content was determined as 2.53 mg kg^{-1} in the cultivar 'Ferraduel' in 2008 but as 2.80 mg kg^{-1} in cultivar 'Sonora' in 2009. Although varying by year, no chlorogenic acid could be detected in some cultivars. The chlorogenic acid content was found to be in the range 0.47 mg kg^{-1} (Texas)-6.16 mg kg^{-1} (Ferraduel) in 2008 and in the range 0.92 mg kg^{-1} (Lauranne)-7.00 mg kg^{-1} (Sonora) in 2009. The highest epicatechin content was detected in the cultivar 'Ferraduel' in both years (21.07 mg kg^{-1} in 2008 and 27.57 mg kg^{-1} in 2009). Ferulic acid, kaempferol, naringenin and p-coumaric acid contents were found to be rationally less than the other phenolic contents. Additionally, it was established that these phenolics varied very slightly by year and were more stable. The highest ferulic acid content was recorded in the cultivar 'Ferraduel' (0.48 mg kg^{-1}) in 2008 and in the cultivar 'Yaltinski' (0.52 mg kg^{-1}) in 2009, whereas the lowest ferulic acid content was detected in the cultivar 'Texas' in both years. Kaempferol could not be determined in most cultivars in either year. It was found to be in the range 0.05 mg kg^{-1} (Sonora)-0.21 mg kg^{-1} (Yaltinski) in 2008 and in the range 0.04 mg kg^{-1} (Ferragnes)-0.12 mg kg^{-1} (Nonpareil and Picantili) in 2009. Naringenin ranged from 0.05 mg kg^{-1} (Supernova) to 1.82 mg kg^{-1} (Yaltinski) in 2008 and from 0.11 mg kg^{-1} (Glorieta) to 3.23 mg kg^{-1} (Ferrastar) in 2009 but could not be determined in the cultivar 'D. Largueta' in either year. No statistical difference in p-coumaric acid was found among the cultivars. P-coumaric acid was detected to range from 0.04 mg kg^{-1} (Masbovera, Glorieta, and Cristomorto) to 0.13 mg kg^{-1} (D. Largueta and

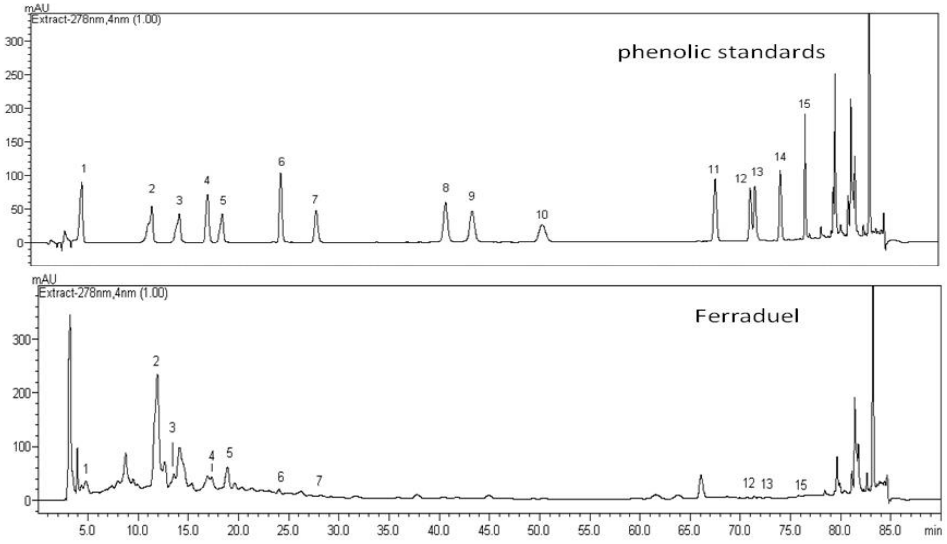

Fig. 1: HPLC chromatograms of phenolic standards and extracts of Ferraduel kernel. 1; Gallic acid 2; Catechin 3; Chlorogenic acid 4; Caffeic acid 5; Epicatechin, 6; p-Coumaric acid 7; Ferulic acid 8; o-Coumaric acid 9; Rutin 10; Hesperidin 11; Cinnamic acid 12; Quercetin 13; Naringenin 14; Luteolin 15; Kaempferol

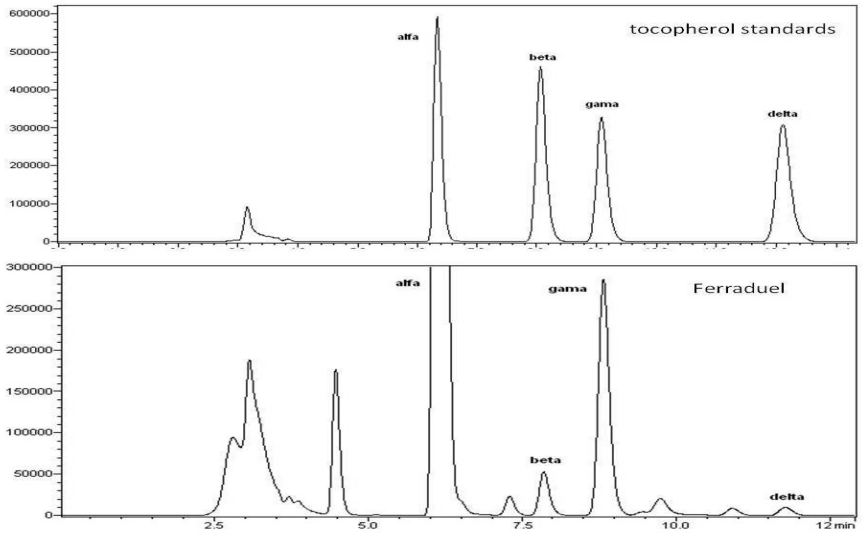

Fig. 2: HPLC chromatograms of tocopherols standards and extracts of Ferraduel kernel.

Ferraduel) in 2008 and from 0.03 mg kg^{-1} (Glorieta) to 0.38 mg kg^{-1} (D. Largueta) in 2009.

The phenolic contents were found to vary by year. This variation was significant in some cultivars but more stable in some of them. In previous studies, it was reported that such factors such as fungi, bacteria, pests, air and light might be effective on the occurrence of this difference, along with the environmental factors in the region of cultivation, cultivation techniques, the maturity state of fruits, the soil properties in the region, and the genetic traits of species and cultivars (Balasundram et al., 2006; Esfahlan and Jamei, 2012; Garcia-Pascual et al., 2003; Habila et al., 2012; Ismail et al., 2004; Kodad et al., 2006; Kodad et al., 2011; Piironen et al., 1986; Rice-Evans et al., 2006).

Bolling et al. (2010) reported that the phenolic contents varied by cultivar and that the catechin content ranged from 0.5 mg kg^{-1} to 3 mg kg^{-1}, the epicatechin content from 0.3 mg kg^{-1} to 0.7 mg kg^{-1}, the kaempferol content from 0.2 mg kg^{-1} to 1.4 mg kg^{-1}, and the naringenin content from 0.2 mg kg^{-1} to 0.8 mg kg^{-1}. Milbury et al. (2006) detected a large number of phenolics in many almond cultivars and determined lower contents of catechin (0.09-0.4 mg kg^{-1}), epicatechin (0.03-0.1 mg kg^{-1}), naringenin (0.002-0.02 mg kg^{-1}), and kaempferol (0-0.02 mg kg^{-1}) than the results presented in this paper. Similarly, Bartolome et al. (2010) obtained lower kaempferol and naringenin contents than our results.

Nut fruits are rich in phenolic compounds (Yada et al., 2011), and such phenolic compounds as catechin, p-coumaric acid, caffeic acid, and ferulic acid are also prevalent in kernels. It is reported that the catechin content of almond kernel is quite high, while its ferulic acid content is low (Rice-Evans et al., 2006; Sang et al., 2002). These results are also supported by the findings obtained from our study.

Tocopherol contents

In the research, the tocopherol contents of the almond cultivars are presented in Tab. 2. Statistically significant differences in tocopherol contents occurred among the cultivars ($p < 0.05$). The analysis of variance showed that the effects of cultivars, years and the cultivars × years interaction were significant for all tocopherols ($p < 0.01$).

The highest content among the tocopherols was obtained in α-tocopherol, and the average values were recorded as 312.29 mg kg^{-1} in 2008 and as 467.31 mg kg^{-1} in 2009. The highest α-tocopherol content was determined in cultivar 'Supernova' in both years (899.49 mg kg^{-1} and 945.41 mg kg^{-1}, respectively). The highest β-tocopherol content in the research was again found in cultivar 'Supernova' in both years (10.53 mg kg^{-1} and 7.64 mg kg^{-1}, respectively). When the γ-tocopherol content of the almond cultivars was evaluated, the highest content was recorded in cultivar 'Supernova' (57.24 mg kg^{-1}) in 2008 but in cultivar 'Picantili' (77.87 mg kg^{-1}) in 2009. In the research, the lowest values among the tocopherols were obtained in δ-tocopherol and varied between 0.10 mg kg^{-1} (Masbovera) and 2.29 mg kg^{-1} (Supernova) in 2008 and between 0.10 mg kg^{-1} (Masbovera) and 2.86 mg kg^{-1} (D. Largueta) in 2009. In the research, the average tocopherol concentrations were determined to be higher in 2009 than in 2008. It is thought that this difference might be due to the ecological factors which vary by years (Kodad et al., 2006; Korekar et al., 2011). Furthermore, it was established that the α-tocopherol was the predominating tocopherol which is in agreement with findings of Kodad et al. (2006) who reported that the α-tocopherol content was ten times higher than the δ- and γ-tocopherol contents and that this feature might be used in almond breeding studies. Kornsteiner et al. (2006) and Zacheo et al. (2000) reported that α-tocopherol was the dominant tocopherol in nut fruits, particularly almond.

Yang (2009) stated that the α-tocopherol content was 439.5 mg kg^{-1} and that the γ-tocopherol content was 12.5 mg kg^{-1}. Kodad et al. (2011) reported that the tocopherol contents varied by years and cultivars and that, in parallel with our results, the α-tocopherol content ranged from 210.9 mg kg^{-1} to 553.4 mg kg^{-1}, the γ-tocopherol content from 4.64 mg kg^{-1} to 14.92 mg kg^{-1}, and the δ-tocopherol content from 0.2 mg kg^{-1} to 1.02 mg kg^{-1}. Socias i Company et al. (2014) stated that the tocopherol contents varied by years and the region of cultivation and that the α-tocopherol content ranged from 437.83 mg kg^{-1} to 488.89 mg kg^{-1}, the γ-tocopherol content from 13.17 mg kg^{-1} to 26.23 mg kg^{-1}, and the δ-tocopherol content from 0.65 mg kg^{-1} to 1.39 mg kg^{-1}, which were lower than our results. Almond is the fruit with the highest tocopherol content among nut fruits (Kodad et al., 2011; Yang, 2009; Izaddost et al., 2013). This feature ensures that the fruits can be stored without becoming rotten for a long period of time, that the oils they contain remain stable for a long period of time, and that the nutritional content is not impaired without adding any synthetic additive (Sang et al., 2002; Miraliakbari and Shahidi, 2008). This is because tocopherols have a high antioxidant quality

Tab. 1: Phenolic compounds of almond cultivars (mg kg^{-1} DW).

Cultivars	Gallic Acid				Catechin				Caffeic acid				Chlorogenic acid			
	2008		2009		2008		2009		2008		2009		2008		2009	
Cristomorto	1.46±0.17	defA	0.73±0.15	cdB	12.58±2.40	fgB	43.81±7.26	dA	0.53±0.03	fB	2.13±0.51	bcA	Nd		1.12±0.01	e
D. Largueta	1.27±0.27	efB	1.43 ±0.27	bcA	31.18 ±2.82	bcA	21.08 ±4.18	hB	1.50 ±0.29	bcdA	0.97±0.19	efB	2.08±0.38	cA	1.19±0.13	deB
Ferraduel	1.22±0.40	fA	0.78±0.23	cdB	117.59 ±1.35	aB	145.86±2.17	aA	2.53 ±0.40	aB	2.57±0.69	abA	6.16±0.27	aA	4.67±0.02	bB
Ferragnes	2.34±0.21	bcA	1.53 ±0.26	bB	31.03±1.21	bcB	57.86 ±0.41	cA	1.23 ±0.29	cdeB	1.80±0.52	cdA	1.73±0.35	cdB	1.83±0.02	cdA
Ferrastar	2.10±0.40	cdA	1.70 ±0.23	bB	24.22 ±3.87	cdB	45.98±1.42	dA	0.43±0.02	fB	1.26±0.81	deA	0.88±0.09	efgB	0.93±0.08	eA
Glorieta	1.67±0.40	cdefA	0.67 ±0.29	dB	10.67 ±1.30	fgB	21.40±1.98	hA	0.73±0.03	efB	1.00±0.29	efA	1.30±0.14	def	Nd	
Lauranne	1.59±0.61	defA	1.33 ±0.52	bcdB	29.66 ±0.48	bcB	30.37±2.12	fgA	1.83±0.23	bcB	2.40 ±0.34	abcA	Nd		0.92±0.03	e
Masbovera	1.73±0.52	cdefA	1.05 ±0.54	bcdB	9.40±2.15	gA	5.19±1.54	jB	0.57±0.05	fA	0.50±0.04	fB	1.50±0.40	cde	Nd	
Nonpareil	3.26±0.70	aA	1.37 ±0.29	bcdB	6.77±2.75	gB	20.25±2.56	hA	0.57±0.07	fB	1.00±0.44	efA	Nd		Nd	
Picantili	2.83±0.52	abB	3.03±0.27	aA	8.15±1.80	gB	13.72 ±2.18	ıA	0.45±0.06	fB	0.87±0.08	efA	0.62±0.06	fghB	2.09±0.88	cA
Sonora	3.10±0.52	aA	1.70 ±0.37	bB	34.68 ±2.08	bB	41.87 ±1.70	deA	1.87±0.58	bB	2.80±0.52	aA	2.95±0.28	bB	7.00±0.01	aA
Supernova	1.60±0.58	cdefA	1.27 ±0.38	bcdB	19.46±2.91	deB	73.34±2.64	bA	0.47±0.08	fB	2.56±0.78	abA	1.23±0.46	def	Nd	
Texas	1.65±0.45	cdefA	1.60±0.23	bB	Nd		12.23±1.22	ı	0.43±0.07	fB	0.59 ±0.06	fA	0.47±0.04	gh	Nd	
Tuono	2.10±0.23	cdA	1.19 ±0.29	bcdB	16.38 ±1.51	efB	28.31±2.28	gA	1.00±0.11	defB	2.67 ±0.64	abA	1.63±0.71	cd	Nd	
Yaltinski	2.00±0.42	cdeA	0.99±0.29	bcdB	31.16 ±0.81	bcB	36.72±3.20	efA	1.03±0.23	defA	1.00±0.07	efB	1.46±0.65	cde	Nd	
Mean	1.99±0.43		1.36±0.31		27.35±1.96		39.87±2.46		1.01±0.17		1.61±0.39		1.69±0.26		2.47±0.08	
LSD	0.6597				6.458				0.5755				0.6470			

Each value is expressed as mean ±standart deviation, means followed by different capital letters (years) in the row are significantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are significantly different ($p<0.05$). Nd: Not detected.

Tab. 1: Phenolic compounds of almond cultivars (mg kg^{-1}).

Cultivars	Epicatechin				Ferulic acid				Kaempferol				Naringenin				P-coumaric acid	
	2008		2009		2008		2009		2008		2009		2008		2009		2008	2009
Cristomorto	2.53±0.52	deB	21.07±0.16	bA	0.16±0.07	eB	0.30±0.01	cA	Nd		Nd		0.14±0.03	cB	0.26±0.03	eA	0.04±0.02	Nd
D. Largueta	6.27±0.84	bA	6.23±0.81	fgB	0.47±0.08	aA	0.39±0.02	bB	Nd		Nd		Nd		Nd		0.13±0.01	0.38±0.04
Ferraduel	21.07±0.40	aB	27.57±0.81	aA	0.48±0.09	aA	0.41±0.10	bB	0.07±0.02	bcB	0.09±0.02	abA	0.15±0.04	cB	0.23±0.05	eA	0.13±0.06	Nd
Ferragnes	3.60±0.95	cB	10.20±0.69	dA	0.30±0.09	bA	0.25±0.07	dB	Nd		0.04±0.01	bc	0.12±0.04	cB	0.15±0.07	eA	0.06±0.02	Nd
Ferrastar	1.63±0.08	efgB	4.97±0.19	hA	0.23±0.01	cd	Nd		Nd		0.07±0.01	b	0.06±0.01	cB	3.23±0.09	aA	0.06±0.02	0.05±0.01
Glorieta	0.93±0.06	ghB	3.17±0.78	ıA	0.27±0.06	bcA	0.25±0.09	dB	Nd		Nd		0.14±0.03	cA	0.11±0.03	eB	0.04±0.02	0.03±0.01
Lauranne	2.60±0.40	deB	7.10±0.81	fA	0.25±0.05	cdB	0.37±0.05	bA	Nd		0.05±0.02	b	0.07±0.01	cB	0.17±0.05	eA	0.06±0.02	0.06±0.02
Masbovera	1.50±0.55	fghA	0.90±0.08	kB	0.22±0.06	dB	0.30±0.07	cA	Nd		Nd		0.15±0.05	cB	0.21±0.06	eA	0.04±0.01	0.04±0.02
Nonpareil	1.03±0.09	ghB	5.93±0.87	gA	0.30±0.04	bA	0.25±0.07	dB	0.11±0.01	bB	0.12±0.03	aA	1.45±0.03	aA	0.77±0.04	dB	0.06±0.01	0.05±0.02
Picantili	1.10±0.09	ghB	1.20±0.87	kA	0.22±0.06	dB	0.50±0.07	aA	0.08±0.03	bcB	0.12±0.04	aA	0.78±0.02	bB	2.07±0.06	bA	0.06±0.03	0.06±0.01
Sonora	3.37±1.19	cdB	8.10±1.04	hA	0.26±0.09	bcdB	0.39±0.08	bA	0.05±0.01	cB	0.06±0.01	bA	Nd		0.16±0.04	e	0.10±0.03	0.10±0.02
Supernova	2.10±0.95	efB	11.67±1.73	cA	0.21±0.07	d	Nd		Nd		Nd		0.05±0.01	c	Nd		0.05±0.01	Nd
Texas	0.53±0.07	hB	0.97±0.07	kA	0.11±0.09	fA	0.13±0.04	eB	Nd		Nd		1.74±0.39	aA	1.41±0.28	cB	0.11±0.01	0.07±0.03
Tuono	2.37±1.01	efA	1.30±0.09	kB	0.24±0.06	cdA	0.16±0.06	eB	Nd		0.06±0.01	b	0.11±0.02	cB	0.12±0.02	eA	0.10±0.03	Nd
Yaltinski	2.53±0.92	deA	2.20±0.07	jB	0.47±0.04	aB	0.52±0.06	aA	0.21±0.03	aA	Nd		1.82±0.04	aA	1.16±0.10	cB	0.10±0.02	0.05±0.01
Mean	3.54±0.54		7.51±0.60		0.28±0.06		0.32±0.05		0.10±0.007		0.08±0.01		0.52±0.04		0.77±0.06		0.08±0.02	0.09±0.02
LSD	0.8929				0.04461				0.04533				0.3849				0.139	

Each value is expressed as mean ±standard deviation, means followed by different capital letters (years) in the row are significantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are significantly different ($p<0.05$). Nd: Not detected.

Tab. 2: Tocopherol contents of almond cultivars (mg kg^{-1} oil).

Cultivars	Alpha-Tocopherol				Beta-Tocopherol				Gamma-Tocopherol				Delta-Tocopherol			
	2008		2009		2008		2009		2008		2009		2008		2009	
Cristomorto	195.67±11.16	fB	586.28±17.40	eA	1.80±0.98	fgB	5.99±2.05	bcA	11.26±4.14	hfB	43.03±7.27	dA	0.17±0.01	cdeB	1.30±0.42	bcA
D. Largueta	291.12±18.60	deB	352.58±17.70	hA	2.88±1.19	dB	5.44±1.22	cdA	15.32±1.79	fgB	42.97±2.14	dA	0.41±0.06	cdeB	2.86±0.21	aA
Ferraduel	303.07±19.30	dB	704.52±4.28	cA	2.42±0.38	defB	4.99±0.69	dA	15.80±0.21	fB	41.85±0.99	deA	0.23±0.03	cdeB	1.18±0.07	bcA
Ferragnes	300.94±1.40	dB	512.16±21.30	fA	3.06±0.72	cdB	4.01±0.64	eA	13.33±1.54	ghB	25.58±1.22	hA	0.39±0.04	cdeB	0.58±0.07	deA
Ferrastar	299.77±15.45	dA	231.64±11.20	dB	2.73±0.45	deB	2.89±0.47	fA	19.29±1.36	eB	27.98±1.66	gA	0.57±0.09	cdA	0.55±0.09	defB
Glorieta	267.81±8.06	eB	342.43±23.80	hA	2.24±0.64	defB	5.11±0.36	dA	5.87±1.78	kB	16.31±1.88	jA	0.40±0.03	cdeA	0.27±0.08	efB
Lauranne	260.23±18.40	eA	146.75±15.44	kB	3.74±0.67	cA	1.99±0.08	gB	16.79±0.91	fA	8.78±1.50	lB	1.17±0.06	cdeA	0.49±0.02	efB
Masbovera	191.31±6.91	fA	165.60±25.20	hB	2.87±0.40	dB	3.17±0.46	fA	8.00±0.89	jkB	14.04±0.88	kA	0.10±0.02	eB	0.10±0.01	fB
Nonpareil	376.89±25.50	cA	350.65±6.73	hB	2.52±0.72	defB	4.17±0.68	eA	27.45±1.30	cB	28.14±1.08	gA	0.29±0.02	cdeB	0.96±0.15	cdA
Picantili	193.69±24.80	fB	262.49±13.11	ıA	1.96±0.67	efgB	3.57±0.96	efA	40.14±2.29	bB	77.87±1.93	aA	0.60±0.03	cB	1.43±0.60	bA
Sonora	546.95±22.90	bA	402.24±25.10	gB	9.23±0.54	bA	6.25±0.58	bB	21.68±1.00	dA	20.80±2.46	ıB	1.28±0.05	bB	1.29±0.07	bcA
Supernova	899.49±24.10	aB	945.41±22.40	aA	10.53±0.62	aA	7.64±0.47	aB	57.24±2.01	aA	56.46±1.91	bB	2.29±0.12	aA	1.40±0.17	bcB
Texas	223.11±39.40	fB	793.51±41.60	bA	1.97±0.46	efgB	7.06±0.70	aA	9.73±2.19	ıjB	49.49±1.79	cA	0.12±0.04	deB	1.10±0.21	bcA
Tuono	136.33±2.85	gB	673.85±21.00	dA	0.73±0.09	hB	5.48±0.57	bcdA	6.42±1.80	kB	40.20±2.25	eA	0.45±0.08	cdeB	1.07±0.68	bcA
Yaltinski	198.07±9.92	fB	539.50±23.00	fA	1.27±0.52	ghB	2.78±0.67	fA	9.94±1.78	ıjB	31.41±1.73	fA	0.11±0.02	deB	0.33±0.07	efA
Mean	312.29±16.58		467.31±19.28		3.33±0.60		4.70±0.71		18.55±1.67		34.99±2.05		0.57±0.05		0.99±0.19	
Lsd	30.03				0.7368				2.18				0.4054			

Each value is expressed as mean ±standard deviation, means followed by different capital letters (years) in the row are significantly different ($p<0.05$). Means followed by different small letters in the columns (cultivars) are significantly different ($p<0.05$).

(Esfahlan et al., 2010; Izaddost et al., 2013; Yada et al., 2011).
To reveal the variables (phenolics and tocopherols) causing differences among the almond cultivars, multivariate analysis was used in the research. The characteristics with the eigenvalues greater than 1 are evaluated as descriptive in PCA (Shin et al., 2012). The results of the principal component analysis (Tab. 3) indicate that the first 4 components accounted for 71.72% of the total variability observed. The shares of components PC1, PC2, PC3, and PC4 in the total variance were 28.49%, 18.07%, 14.15%, and 11.02%, respectively. PC1 showed 8 variables with higher scores (values ≥ 0.50) with respect to phenolic components (catechin, chlorogenic acid, caffeic acid, and epicatechin) and tocopherols (α, β, γ and δ). The highest contribution of PC2 corresponded to chlorogenic acid and ferulic acid as well as to α, β, and γ tocopherols. The separation along PC3 was primarily due to variations in gallic acid, naringenin, and kaempferol. The highest contribution of PC4 corresponded to p-coumaric acid. These results support the relevance of catechin, caffeic acid, epicatechin, and p-coumaric acid as discriminant parameters to differentiate almond varieties. Similar results were reported in previous PCA applications to almond research (Garrido et al., 2010; Bartolome et al., 2010; Maestri et al., 2015; Barreira et al., 2012; Shin et al., 2010; Colic et al., 2012; Kodad et al., 2013).

Tab. 3: Principle component analysis of almond varieties.

Variable	Principle component			
	1	2	3	4
Gallic acid	-0.391	-0.071	0.544	0.034
Catechin	0.728	0.450	-0.181	-0.282
Chlorogenic acid	0.500	0.603	0.095	0.058
Caffeic acid	0.743	0.343	-0.003	0.024
Epicatechin	0.768	0.431	-0.162	-0.210
p-Coumaric acid	0.128	0.068	0.403	0.760
Ferulic acid	0.237	0.561	0.388	0.276
Naringenin	-0.304	0.022	0.635	-0.433
Kaempferol	0.079	0.383	0.728	-0.280
Alpha tocopherol	0.680	-0.554	-0.079	-0.297
Beta tocopherol	0.661	-0.588	0.100	-0.028
Gamma tocopherol	0.501	-0.540	0.449	-0.255
Delta tocopherol	0.649	-0.466	0.364	0.323
Eigenvalue	3.988	2.530	1.981	1.542
Percent variation	28.487	18.072	14.147	11.017
Cumulative percent variation	28.487	46.559	60.706	71.723

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