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Protein and mineral nutrient contents in kernels from 72 sweet almond cultivars and accessions grown in France, Greece and Italy

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Abstract

Almond protein and potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) contents were determined in 72 cultivars and accessions grown in France, Greece and Italy, as part of the networking of European SAFENUT AGRI GEN RES project, which aimed to explore and valorize the almond genetic resources in Europe. Great variation was found in the nutrient content and the amount of nutrient supplied when consuming the recommended daily amount of one serving of almond, among the different genotypes assayed. The variation among the different genotypes was greatest for Ca, followed by the protein content; the latter also exhibited the lowest variation considering the harvest year. Results from a principal component analysis showed that P and Mg were the most discriminant elements for categorizing samples. Cluster analysis showed groups of samples with interesting characteristics for breeding. There was no clear distinction among the different origins of samples. Correlation analyses between weather conditions and the nutrients assayed showed that the mean temperature recorded in the period between March and September was positively correlated with Ca and P only in France, a place where the greatest climatic difference between years was observed.

Keywords: *environment, genetic resources, mineral nutrient, protein, sweet almond*

Introduction

Sweet almond (*Prunus dulcis* M.) is one of the oldest cultivated fruit species and was appraised for its culinary uses and its numerous medicinal properties. The ancient Greek doctors Hippocrates and Galen ascribed to almond characterizations such as 'hot', 'heating', 'cleansing', 'nourishing' and 'strengthening mental functions' (Albala 2009). Recent medical research has shown that almond consumption has been associated with a wide range of health benefits, including reduced levels of coronary heart disease as a result of reduction in the low density lipoprotein (LDL)-cholesterol, hypertension, type 2 diabetes, obesity and reduction in oxidative stress (Chen et al. 2006). The nutritional properties of almonds are due

to their components such as lipids and unique fatty acids (containing mostly unsaturated fat, little saturated fat and no cholesterol), total fibre (containing small amounts of viscous fibre), protein, arginine, α -tocopherol, magnesium, copper, manganese, calcium, phosphorus and potassium (Chen et al. 2006; Yada et al. 2011). The high contents of macronutrients make almond an important dietary source for these essential elements. Almond is considered as a 'nutrient-dense' nut, meaning that it provides a variety of nutrients in high amounts per serving and because it is minimally processed and contains minimal solid fat, sugars, starches or sodium (United States Department of Agriculture (USDA) 2005). Moreover, approxi-

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mately 15% of energy in almonds is from proteins, which are of high quality, because of their unusually high content in arginine that positively affects digestibility (Ahrens et al. 2005).

The composition and characterization of macro- and micronutrients present in cultivated almond cultivars have been reviewed by Yada and colleagues (2011). The almond genotype was documented to have an important role in almond protein and mineral nutrient content in cultivars grown in California (Ahrens et al. 2005; Venkatachalam and Sathe 2006), Italy (Ruggeri et al. 1998), Spain (Saura-Calixto et al. 1981; Saura-Calixto and Cañellas 1982; Riquelme et al. 1985; Prats-Moya et al. 1997), India (Kumar and Sharma 2005), Tunisia (Ayadi et al. 2006) and Turkey (Aslantas et al. 2001). However, the majority of studies refer to widely cultivated cultivars, and little information is available for local almond cultivars (García-López et al. 1996; Aslantas et al. 2001). Nowadays, although a large number of cultivars are available, only few are widely cultivated because they possess superior agronomic characteristics such as late flowering, self-compatibility and good physical traits. Information on the nutritional characteristics of local almond varieties would be interesting from a nutritional aspect in order to depict the natural variability and elaborate the most nutrient-dense almond varieties. In the frame of the networking SAFENUT AGRI GEN RES Action activities morphological, molecular, lipid, phenolic, protein and mineral content characterization were made on the almond genetic resources in European countries with the aim to increase the knowledge and to improve its utilization by stakeholders and breeders.

The aims of this study were to (i) determine the variability and check for possible differences in protein and mineral nutrient content in various commercial cultivars and local accessions of almond which were sampled from *ex situ* collection orchards, or *in situ*, in France, Greece and Italy; (ii) select genotypes with high quality characteristics with the use of principal component analysis (PCA) and cluster analysis and (iii) evaluate the influence of harvesting year and growing region in the nutrient contents.

Materials and methods

The protein, K, P, Ca and Mg contents were analysed in almond kernel from 72 accessions/cultivars grown in France, Greece and Italy during 2008 and 2009. Thirty cultivars/accessions were analysed during both years, and another 42 accessions were analysed only in the second experimental year. Samples from France were the cultivars 'Ferraduel', 'Ferragnes', 'Ferrastar' and 'Lauranne', and were collected from a commercial orchard in Nîmes. Samples from Greece were 11 ecotypes or improved cultivars located in Northern Greece ('Alkion', 'Drepanoto', 'Truuito', 'Opsi-

manthis Volou' and 'Raptopoulou' in a collection orchard at NAGREF, Naoussa; 'Siatista 1, -2, -3 and -4' in Siatista; 'Rachia' in Imathia; 'Sfendami' in Katerini), four ecotypes located in central Greece ('Babatsiko', 'Bellou' and 'Retsou' in Magnisia; 'Afrata Chiou' in the island Chios) and 10 ecotypes located in the island Grete ('Athalia', 'Lakoniotika' and 'Sitia 2, -3' in Neapoli, east-Grete; 'Temen 1' and 'Kandanos 1, -2, -3, -4' in Kandanos, west-Grete; 'Stone Almond' in Vlatos, west-Grete). Samples from Italy were 12 cultivars located in a collection orchard at CRA-SCA, Bari ('Cristomorto', 'Ferrante', 'Filippo Ceo', 'Fragiulio', 'Gagiano', 'Giunco di Cozze di Alberobello', 'Montrone', 'Riviezze', 'Santoro', 'Senz'arte', 'Tenente' and 'Tuono'), and 31 local genotypes named with codes, located in two collection orchards in Abruzzo.

Almonds in shell were collected by hand at commercial maturity from four trees per cultivar, dried in an oven at 70°C, and a sample of about 1 kg was packed and transferred by courier to the Pomology Institute in Naoussa, Greece, where analyses were carried out. Immediately before analyses each sample was manually cracked and shelled. About 200 g of dehulled almonds was powdered using a household mill (Kenwood, 450 W, Kenwood Limited, Hampshire, UK) for homogeneity. Samples were stored in an airtight bag at 4°C until further use.

For the determination of the total protein content, the Kjeldahl method (titration with HCl) was used. Briefly, three replicates of 0.5 g of finely ground nut sample were digested with 25 ml of H₂SO₄ and one tablet (3.5 g) of CuSO₄. The total protein content was calculated as the amount of total N determined multiplied by the specific nitrogen-to-protein conversion factor of 5.18 (Association of Official Analytical Chemists [AOAC] 1995).

For the determination of the mineral contents, three replicates of about 0.5 g of finely ground nut were dried at 70°C for 2 days, and transferred to polytetrafluoroethylene vessels in which 10 ml of 65% nitric acid (HNO₃) was added. Digestion was performed in a microwave oven (model QWAVE 4000, Questron Technologies Corp.) using the following procedure: temperature was increased at 110°C in 5 min maintained at 110°C for 5 min and at 120°C for 5 min. The digest was diluted to 50 ml with deionized water prior to analyses.

Mineral contents were determined in Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (OPTIMA 2100 DV, Perkin Elmer, Wellesley-MA, USA), equipped with an autosampler and assisted by a computer for data acquisition and readout system. The instrumental operating parameters are shown in Table I. Minerals were quantified on the basis of peak areas and compared with a calibration curve obtained with corresponding standards.

Statistical analyses

Nutrient data were compared among genotypes and harvesting years by one-way analysis of variance (ANOVA) based upon genotype, or two-way multivariate analysis of variance (MANOVA) based upon genotype and year, and least significant difference (LSD) values were calculated in cases that significant variance was found at $p \leq 0.05$. Pearson's correlation analysis was carried out. PCA was applied to nutrient data that had been autoscaled to unit variance, and single linkage cluster analysis was carried out using the method of squared Euclidean distance on two factors produced after PCA (uncorrelated factor scores). Statistical analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results and discussion

The aim of this study was to find possible differences among a large and diverse almond source of genotypes from different sites, although climatological and growing conditions differ. The minerals examined in this study (K, P, Ca and Mg) were thought to be important minerals due to their abundance and perceived health effects (AOAC 1995). Next to Ca, P forms the structure of teeth, bones and cell membranes. Calcium, K and the absence of Na in almonds may favourably affect total dietary levels of these nutrients and work in coordination to decrease cardiovascular disease (CVD) risks, specifically hypertension (Ros 2010; Yada et al. 2011). Finally, Mg has been inversely associated with several cardiovascular risk factors, including hypertension, coronary artery disease and cardiac arrhythmias, as reviewed by Champagne (2008).

Significant differences were observed between protein and mineral composition of the 72 almond genotypes assayed ($p < 0.001$) (Table II). The protein content varied 3.0 times, ranging from 9.6 to 28.5 mg/100 g fresh weight (FW). The majority of samples (93%) were in the range of 15.1 and 25.9 mg/100 g, and greatest values were found in 'Siatista 4', 'Truquito' and 'Kandanos 2'. These data agree with previous studies obtained from other almond cultivars, ranging from 14 to 23 mg/100 g FW³. 'Ferragnes', which is a widely cultivated cultivar, which contained relatively low protein content

(11.4 mg/100 g FW) confirming the results reported by Cordeiro et al. (2001), when 12 Portuguese varieties were compared.

The most abundant minerals were K and P, followed by Ca and Mg. Similarly, in previous studies the nutrients present in high amounts in the kernel of almonds were K, P, Mg, S and Ca, and minor or trace elements are Na, Fe, Cu and Mn (Saura-Calixto and Cañellas 1982; Prats-Moya et al. 1997; Ayadi et al. 2006). Potassium content predominates with values ranging from 465 to 1235 mg/100 g dry weight (DW). Other values of K content in almond kernels from the literature varied from 390 to 940 mg/100 g in cultivars cultivated in Spain (Saura-Calixto et al. 1981; Saura-Calixto and Cañellas 1982; Prats-Moya et al. 1997), Tunisia (Ayadi et al. 2006) and Italy, where similar values for 'Ferragnes', 'Lauranne' and 'Tuono' were shown (Piscopo et al. 2010). In this study, only 7% of the samples studied had K content greater than 958 mg/100 g, and greatest values were found in the four almond accessions from Italy ('46D', '57D', '41D' and '72A').

Phosphorus content fluctuated from 119 to 748 mg/100 g DW, with greatest values being in the 'Bar vet 11 09-742', and 'Siatista 3' and 'Siatista 4'. The distribution of p values was normal with a skew value of only 0.060. Results herein reported agree with other published data on almond, as cultivars cultivated in Spain were in the range of 360–1050 mg/100 g (Saura-Calixto et al. 1981; Saura-Calixto and Cañellas 1982; Prats-Moya et al. 1997), in Tunisia 200–250 mg/100 g (Ayadi et al. 2006) and in California 440–510 mg/100 g (Hall et al. 1958).

Calcium content ranged from 160.0 to 663.0 mg/100 g DW. The 7% of samples had values greater than 485 mg/100 g DW, and greatest values were found in 'Truquito', 'Bar vet 4 09-736', 'Siatista 1', 'Drepanoto' and 'Lauranne'. Similar values were reported in studies in Spain (Saura-Calixto et al. 1981; Saura-Calixto and Cañellas 1982; Riquelme et al. 1985) and California (Hall 1958), while greater values were found in samples from Tunisia (390–1300 mg/100 g DW) (Ayadi et al. 2006). Piscopo et al. (2010) found considerably lower values of Ca in 11 almond cultivars grown in Italy, including cultivars 'Ferragnes', 'Lauranne' and 'Tuono', with the values ranging from 90 to 176 mg/100 g DW.

Magnesium content ranged from 100 to 333 mg/100 g DW, with greatest values found in the 'Bar vet 11 09-742', 'Bar pep 19 09-756' and 'Siatista 4'. In the literature, Mg content was found in the range of 154–276 mg/100 g DW in Italy (Piscopo et al. 2010), 90–180 mg/100 g in Tunisia (Ayadi et al. 2006) and 230–610 mg/100 g in Spain (Saura-Calixto et al. 1981; Saura-Calixto and Cañellas 1982; Prats-Moya et al. 1997).

With regard to nutritional aspects, percentage of recommended dietary allowances (RDA) or adequate intakes (AI) for those minerals for adult males (aged 19–50 years) was calculated. The RDA or AI for the

Table I. ICP-AES instrumental operating condition.

Inductively coupled plasma	Perkin-Elmer, Optima 2100
Operating power (W)	1300
Radiofrequency (MHz)	40
Coolant stream flow (l/min)	3.785
Torch type	Quartz torch with 2.0 mm i.d. injector tube
Nebulizer type	Crossflow
Nebulizer flow (ml/min)	0.8
Auxiliary flow (ml/min)	0.2
Flow rate (ml/min)	1.50

Table II. Protein (g/100 g FW), potassium, phosphorus, calcium and magnesium (mg/100 g DW) contents in 72 almond genotypes from France, Greece and Italy. Values are means of years 2008 and 2009 for 30 samples, or only the year 2009.

No.	Source ^a		Protein	Potassium	Phosphorus	Calcium	Magnesium
1	FR	Ferraduel	20	751	544	452	236
2	FR	Ferragnes	11	810	572	496	239
3	FR	Ferrastar	15	789	471	451	250
4	FR	Lauranne	17	811	632	542	283
5	GR-north	Alkion	17	566	415	324	172
6	GR-north	Drepanoto	22	681	521	561	240
7	GR-north	Opsimanthis Volou	19	881	681	481	260
8	GR-north	Rachia	18	681	581	481	241
9	GR-north	Raptopoulou	16	768	546	321	243
10	GR-north	Sfendami	11	822	671	321	241
11	GR-north	Siatista 1	10	561	661	581	220
12	GR-north	Siatista 2	13	708	677	243	253
13	GR-north	Siatista 3	24	663	713	261	271
14	GR-north	Siatista 4	29	641	721	241	291
15	GR-north	Truoto	26	844	603	663	261
16	GR-central	Afrata Chiou	20	666	472	447	241
17	GR-central	Babatsiko	23	813	614	244	257
18	GR-central	Bellou	18	689	603	242	218
19	GR-central	Retsou	21	510	355	458	238
20	GR-south	Athalia	19	499	401	466	241
21	GR-south	Kandanos 1	22	570	318	345	159
22	GR-south	Kandanos 2	23	544	344	304	167
23	GR-south	Kandanos 3	22	624	468	484	257
24	GR-south	Kandanos 4	22	558	470	368	232
25	GR-south	Lakoniotika	17	506	340	441	177
26	GR-south	Sitia 2	20	701	505	391	159
27	GR-south	Sitia 3	19	574	321	345	167
28	GR-south	Stone almond	21	587	463	383	257
29	GR-south	Temen 1	24	652	480	456	232
30	IT-Bari	Cristomorto	17	644	469	256	195
31	IT-Bari	Ferrante	24	846	601	302	214
32	IT-Bari	Filippo Ceo	18	691	416	243	180
33	IT-Bari	Fragiulio	22	666	351	341	173
34	IT-Bari	Gaglano	20	690	491	213	188
35	IT-Bari	Giunco di Cozze	20	779	395	300	162
36	IT-Bari	Montrone	22	683	310	322	174
37	IT-Bari	Riviezso	19	714	448	373	230
38	IT-Bari	Santore	23	657	474	318	200
39	IT-Bari	Semziarte	23	771	594	332	248
40	IT-Bari	Tenente	17	740	411	215	166
41	IT-Bari	Tuono	21	664	493	333	211
42	IT-Abruzzo	119 D6	21	751	500	334	256
43	IT-Abruzzo	14A 80	22	688	570	206	242
44	IT-Abruzzo	15A	21	732	423	463	210
45	IT-Abruzzo	16A	22	701	511	209	211
46	IT-Abruzzo	18D	25	789	529	311	256
47	IT-Abruzzo	1A BIS	20	789	413	293	246
48	IT-Abruzzo	21A	22	488	443	457	214
49	IT-Abruzzo	41D	23	959	521	291	239
50	IT-Abruzzo	46D	20	1235	488	317	211
51	IT-Abruzzo	50D	17	607	447	323	220
52	IT-Abruzzo	54A	22	691	480	365	232
53	IT-Abruzzo	57D	24	1102	647	260	258
54	IT-Abruzzo	71D	18	753	460	417	270
55	IT-Abruzzo	72A	23	905	465	252	213
56	IT-Abruzzo	74O	18	692	390	399	247
57	IT-Abruzzo	Bar att 1 09-725	21	721	631	160	260
58	IT-Abruzzo	Bar att 3 09-727	20	465	526	208	233
59	IT-Abruzzo	Bar att 6 09-730	18	561	521	247	251
60	IT-Abruzzo	Bar att 7 09-731	21	643	552	221	271
61	IT-Abruzzo	Bar pep 19 09-756	24	621	581	220	321
62	IT-Abruzzo	Bar vet 1 09-734	25	607	597	485	273
63	IT-Abruzzo	Bar vet 10 09-741	17	521	621	220	260
64	IT-Abruzzo	Bar vet 11 09-742	20	688	748	364	334
65	IT-Abruzzo	Bar vet 2 09-735	18	641	671	341	271

Table II – continued

No.	Source ^a		Protein	Potassium	Phosphorus	Calcium	Magnesium
66	IT-Abruzzo	Bar vet 4 09-736	17	607	607	586	283
67	IT-Abruzzo	Bar vet 5 09-737	21	890	688	188	283
68	IT-Abruzzo	Forme 1 09-715	22	721	611	234	231
69	IT-Abruzzo	Forme 11 09-724	17	521	501	300	220
70	IT-Abruzzo	Forme 6 09-720	17	581	621	340	250
71	IT-Abruzzo	Forme 7 09-721	16	623	542	221	271
72	IT-Abruzzo	Forme 8 09-722	18	502	432	362	281
	Mean (range)		20.0 (10–29)	691 (465–1235)	518 (310–748)	345 (160–663)	236 (159–334)
	<i>F</i> (<i>p</i> < 0.001)		8.2	6.5	6.4	10.5	6.4
	LSD		3.8	164.3	139.5	107.0	49.1
	% significant differences ^b		48.6%	40.5%	44.0%	55.3%	39.7%
	CV(%) among genotypes		17.1	19.6	20.5	31.5	15.6

^aFR, France; GR, Greece; IT, Italy; ^bPercent significant differences at $p < 0.050 = (N/M) \times 100$, where *N* is the number of significant differences found when samples were compared in pairs and *M* is the maximum number of significant differences that can be found.

USA and Canadian adult males (aged 19–50 years) for protein are 56 g/day, K 4700 mg/day, P 700 mg/day, calcium 1000 mg/day and magnesium 400–420 mg/day (Institute of Medicine 1999; Institute of Medicine 2004). Consuming the recommended daily amount of 28.3 g (1 oz) of almond for different genotypes supplies 6–17% of protein, 3–7% of K, 12–29% of P, 4–18% of Ca and 11–22% of Mg of the RDA or AI for adults. The great variations found above strengthen the importance of selecting almond cultivars characterized by high nutritional contents.

Results from the one-way ANOVA showed that Ca, followed by protein, had the greatest *F* value (Ca, 11;

protein, 8; K, P and Mg, 6) and percentage of samples with significant differences (Ca, 55%; protein, 49%; K, P and Mg, 40–44%) (Table II). Moreover, the variation coefficient among almond genotypes was greatest for Ca (Ca, 32%; protein, K, P and Mg, 16–21%). The above results suggest that nutrients such as Ca, followed by the protein content, could be used to mark differences among cultivars, since they exhibited the greatest variability in the studied almond genotypes. Similar results were reported for Ca when compared with other 18 elements in almond in the study by Prats-Moya et al. (1997).

A significant positive correlation was found between P and Mg ($r = 0.697$; $p < 0.001$), and a weak

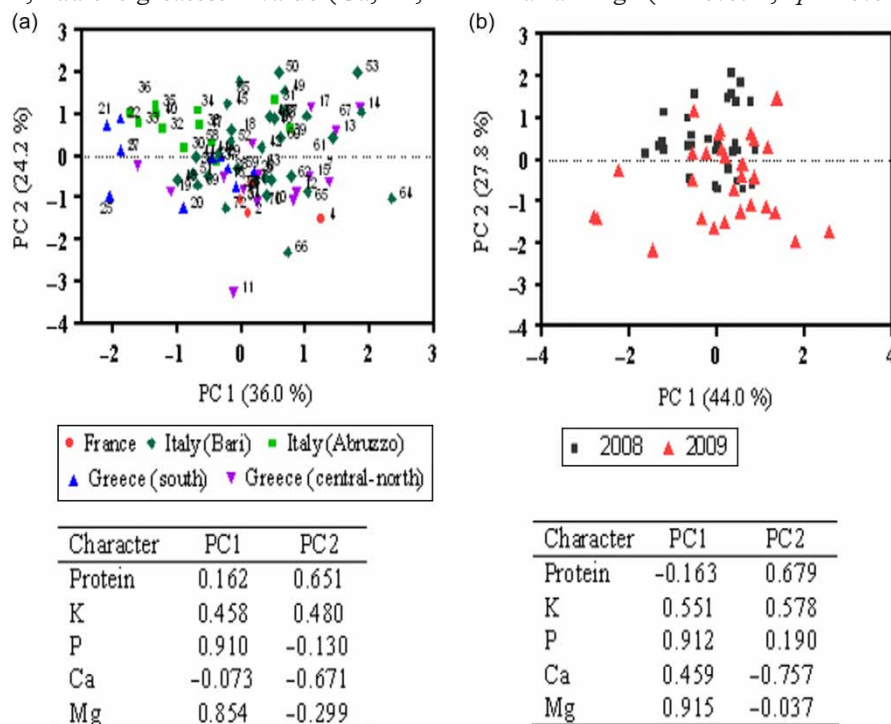


Figure 1. PCA plot and associated variables selected with factor loadings for the protein and mineral contents of (a) 72 almond kernels from France, Greece and Italy and (b) 30 almond genotypes, during years 2008 (squares) and 2009 (triangles). Numbers in PCA plot indicate the almond samples shown in Table II.

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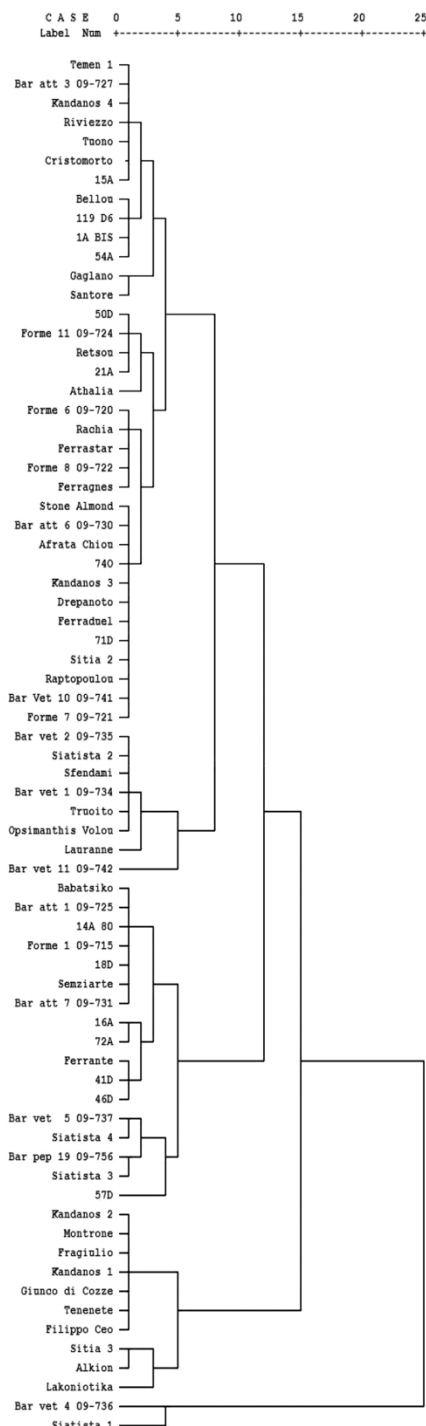


Figure 2. Cluster dendrogram of 72 almond cultivars and accessions.

correlation was found between P and K ($r = 0.278$; $p = 0.018$). The relationships among K, P and Mg can also be detected from their similar variation coefficients and F ratio when one-way ANOVA was applied, which differed from the greater values found for Ca (Table II). There was no other significant correlation found between the remaining studied parameters (data not shown). The strong correlation detected between P and Mg in the almond kernels may be due to the synergic effect of P on Mg accumulation in plants,

which is associated with the ionic balance related to cation and anion uptake into plants (Mulder 1952). Similar correlations among the studied macronutrients were also found in almonds by Prats-Moya et al. (1997), whereas Schirra (1997) reported negative correlation between P and Ca contents, which was not found in this study.

Calcium content was greater than Mg in the majority of samples (increased between 20% and 164% in 71% of samples), and this may be related to their ionic forms having two positive electrical charges which make them antagonistic during root absorption. Calcium and Mg contents were similar in 25% of samples, whereas they had an inverse relationship (decrease 31–38%) in only 4% of samples. An inverse relationship in the Ca and Mg contents was also reported in previous studies on almond (Saura-Calixto and Cañellas 1982; Cordeiro et al. 2001).

PCA and clustering analysis

PCA was used in order to establish the relationships and differentiation among the variables. Cluster linear analysis was also carried out to discover natural groupings of samples. PCA produced two components (PC1 and PC2) which accounted for a cumulative 60.2% of variation, and are presented as a scatter diagram in Figure 1. The parameters having an important correlation to PC1 (values > 0.30) (36.0% of variance) were P and Mg and to a lesser extent K. In the second component (PC2) (24.2% of variance), the protein and K had a positive correlation, while the Ca content had a negative correlation. The dendrogram shows five clusters at a distance value of 5 (Figure 2). The smallest cluster included 'Bar vet 4 09-736' and 'Siatista 1', which had low protein and high Ca contents. A sample with high P, Mg, K and protein contents (increased values in PC1 and PC2) was the '57D'. There was no clear distinction among the different origins of samples.

Influence of harvesting year and growing region

The harvesting year, growing region and cultivation methods would be expected to at least partially influence the nutrient composition of almonds, since minerals are obtained by the plant from the soil in which it grows, the water applied in production and growth is influenced by weather conditions. The study of variation in the measured traits due to harvest year showed that the lowest variability coefficient was that for protein (13%). It increased in K (17%), followed by Mg (19%), Ca (24%) and P (27%), which suggests that the protein content may be the most stable trait in relation to year variation (Table III). Considering that the protein content also had a relatively great variation among genotypes, it is suggested that it may be

Table III. Coefficient variation CV(%) for 30 samples harvested in both years 2008 and 2009.

Source	Genotype	Protein	Potassium	Phosphorus	Calcium	Magnesium
FR	Ferraduel	1	6	12	34	3
FR	Ferragnes	23	2	19	24	13
FR	Ferrastar	18	4	22	17	13
FR	Lauranne	24	1	23	37	14
GR-central	Afrata Chiou	0	14	18	36	6
GR-central	Babatsiko	6	1	22	23	14
GR-central	Bellou	11	19	14	11	8
GR-central	Retsou	8	3	18	26	7
GR-south	Athalia	2	11	3	23	6
GR-south	Kandanos 1	15	87	82	4	48
GR-south	Kandanos 2	20	84	92	7	54
GR-south	Kandanos 3	23	18	8	6	2
GR-south	Kandanos 4	18	7	26	14	13
GR-south	Lakoniotika	12	28	36	14	36
GR-south	Sitia 2	2	4	13	18	48
GR-south	Sitia 3	19	38	40	18	54
GR-south	Stone Almond	0	0	4	45	2
GR-south	Temen 1	11	37	23	39	13
IT-Bari	Cristomorto	1	10	36	48	27
IT-Bari	Ferrante	6	14	10	37	5
IT-Bari	Filippo Ceo	2	10	12	9	0
IT-Bari	Fragiulio	7	10	56	17	55
IT-Bari	Gaglano	23	6	6	23	9
IT-Bari	Giunco di Cozze	20	25	53	1	51
IT-Bari	Montrone	17	29	37	8	21
IT-Bari	Riviezso	11	7	25	35	8
IT-Bari	Santore	2	11	18	29	16
IT-Bari	Semziarte	3	13	19	38	7
IT-Bari	Tenente	34	19	58	43	29
IT-Bari	Tuono	7	1	18	48	14
CV(%) due to year		12.5	17.2	27.4	24.4	19.2

indicative of differences among genotypes and almond origin identification.

In order to detect any possible effect of weather conditions on mineral nutrient and protein contents, PCA was applied to the mean values of measured traits of the 30 almond genotypes analysed during both years 2008 and 2009 (Figure 1b). The components produced by PCA were similar to those produced when all 72 genotypes were analysed (Figure 1a). Although there was not a clear separation between harvesting years, most values from the year 2009 were negative in the Y-axis which represent the PC2, suggesting that Ca contents tended to be greater in year 2009 compared to those in 2008.

In order to determine the impact of environment on almond nutrient contents, data from monitoring stations positioned nearby the experimental sampling points were collected during the 1st March until the 30th September in each harvesting year (Table IV). Greatest climatic differences between years were found in Nîmes, France, compared with the other measured sites; the minimum, maximum and mean temperatures were greater by 1.1, 2.8 and 2.0°C, respectively, in 2009 compared with those in 2008, whereas in other sites the mean temperature was decreased in 2009 compared with that in 2008 by 0.2–1°C. Correlation analyses were carried out using data separated for each country during the two studied

Table IV. Observed weather conditions across the sites where almonds were sampled, between March and September in 2008 and 2009.

	Temperature (°C)							
	Av min		Av max		Mean		Precipitation (mm)	
	2008	2009	2008	2009	2008	2009	2008	2009
France (Nîmes)	12.7	13.8	23.5	26.3	18.1	20.1	496.6	342.8
Greece-north (Naoussa)	14.1	14.6	26.0	25.0	20.0	19.8	287.5	329.0
Greece-central (Volos)	22.3	21.5	27.3	26.2	24.8	23.8	257.2	140.0
Greece- south west Crete (Kandanos)	15.4	14.6	24.8	23.6	20.1	19.1	148.6	162.4
Greece- south east Crete (Ierapetra)	20.0	19.4	27.7	26.6	23.8	23.1	62.1	143.0
Italy (Bari)	13.7	13.8	26.0	25.5	19.9	19.7	183.3	299.8

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years. In France, the mean temperature was positively correlated with Ca ($r = 0.893$, $p = 0.003$) and P ($r = 0.778$, $p = 0.023$), and precipitation was negatively correlated with Ca ($r = -0.893$, $p = 0.003$) and P ($r = -0.778$, $p = 0.023$). There were no other significant correlation and strong correlation found between mineral nutrients, protein and climatic conditions, in the other sites studied, or when all experimental sites and climatic data were analysed (data not shown). The higher temperatures observed in 2009 in Nîmes may have favoured growth and nutrient utilization, resulting in greater nutrient contents in almonds.

Conclusions

In this study, the mineral nutrient and protein contents were analysed in a relatively large number of almond accessions found in Greece, France and Italy. The results showed a great variation in the nutrient content and the amount of nutrient supplied when consuming the recommended daily amount of one serving of almond, which strengthens the importance of selecting cultivars with high nutrient content. The protein content was found to be the most appropriate parameter to use for marking differences among genotypes and for identifying the almond origin. Also, the almond mineral and protein contents depended on genotype rather than correlating with their origin.

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