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Differences in chemical composition of field pea (*Pisum sativum*) cultivars: Effects of cultivation area and year

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Abstract

The influence of the meteorological conditions during the cultivation year, along with the soil characteristics of the cultivation area, on nutrient and antinutrient compositions were evaluated for three field pea (*Pisum sativum*) cultivars. All varieties were cultivated in three different cultivation areas for two subsequent years. The location of the cultivation area significantly affected the proximate composition, sucrose, starch and non-starch polysaccharide contents, as well as the total tannin and phytic acid contents of peas. The cultivation year also affected all traits with the exception of starch. The major constituents of the pea seeds were significantly affected by the interaction between the cultivation area and the cultivation year. These results indicate that the composition of peas is highly dependent on the climate conditions, as well as on the soil characteristics of the cultivation area during the growing season.
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1. Introduction

Legumes are an important food source and play a significant role in traditional diets in many regions of the world. In Greece, pulses such as lentils (*Lens culinaris* Medikus), faba beans (*Lathyrus sativus* L.), broad beans (*Vicia faba* L.), chickpeas (*Cicer arietinum* L.) and peas (*Pisum sativum* L.) have been traditionally cultivated since ancient years.

Legumes are important sources of protein, complex carbohydrates, dietary fibre, vitamins and minerals in the diet. However, their contents include certain antinutritional factors, such as protease inhibitors, lectins, raffinose-series oligosaccharides, tannins and phytic acid (Hickling, 2003; Wang & Daun, 2004).

Studies have been done on differences in chemical composition between various pea cultivars. Kosson, Czuchajowska, and Pomeranz (1994), reported that seed type, wrinkled or smooth, affected the gross composition (protein, lipid, ash) of whole pea seeds. This was in agreement with the findings of Bishnoi and Khetarpaul (1993) who also studied the differences in proximate compositions between pea cultivars. Review data reported by Castell, Guenter, and Igbasan (1996), showed the gross and fibre compositional differences between whole and dehulled peas, as well as between two different pea cultivars, harvested from two subsequent years at various locations.

However, a very little knowledge exists on the influence of environmental factors, such as temperature, water availability and solarity of the cultivation area, on the composition of field peas. Reichert and MacKenzie (1982), found correlation between the protein contents of field peas and other compositional factors. Wang and Daun (2004), studied the combined effect of variety and seed protein content in the composition of field peas. Both authors indirectly related compositional factors with environmental conditions as the latter are mirrored in seed protein content differences. Al-Karaki and Ereifej (1999), studied the effects

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of cultivar in field peas over two subsequent years and related composition to rainfall and temperature.

Soil characteristics seem also to be important for other legumes (Berger, Loss, & Siddique, 1999; Kigel, 1999) but no data exist on field peas.

Therefore, the purpose of this work was to evaluate the impact of the climatic conditions of two consecutive years and of the soil characteristics, as well as their interaction, on the nutrient and antinutrient contents of different pea varieties. These data will be valuable for producers and industry in their effort to enhance the nutritional quality of peas, both for human consumption and animal feed, and to identify needs for future work.

2. Material and methods

2.1. Sample preparation

Seeds of three different white-flowered pea cultivars were provided from the Fodder Crops and Pasture Institute (FCPI, Larissa, Greece). All varieties were early middle season varieties, low in tannin content with light beige seed colour. The average weight of 1000 seeds was 169 g. The peas were cultivated in three cultivation areas and collected for two subsequent cultivation years, 2002–2003 and 2003–2004. The exact locations of the cultivation areas were: Palamas (39°27′43″N, 22°04′22″E), Fytorio (39°45′15″N, 22°51′40″E), FCPI (39°45′15″N, 51°45′22″E) and the total field sizes were 15, 43, and 53 ha, respectively. Allotment dimensions were 2×10 m. Each plot consisted of 7 rows, with 25 cm distance between rows, while each plot had 75 cm distance. Field peas were cultivated without any specific experimental design (simple arrangement of the plots) and sowing was done in different field positions for the two years to avoid soil exhaustion.

Environmental conditions (i.e. annual rainfall, relative humidity, solarization), as well as the soil characteristics of the three studied locations, have been presented by Nikolopoulou, Grigorakis, Stasini, Alexis, and Iliadis (2006). Annual average temperature for FCPI and Fytorio was 15.2 and 15.1 °C in the years 2003 and 2004, respectively, while, for Palamas cultivation area, the annual average temperatures were 11.8 and 14.2 °C, respectively. The Directorate of Agro-meteorology of the Ministry of Agriculture provided the meteorological data.

Seed samples were ground to pass a 0.5 mm sieve, using a ball-mill in order to obtain fine flour, and analysed for their chemical composition.

2.2. Chemical analysis

Proximate compositions of the pea seeds (protein, fat, moisture and ash) were determined according to AOAC (1998).

Total starch was determined enzymatically using a Megazyme Total Starch Assay kit (AA/AMG) (Megazyme International, Ireland) (McCleary, Gibson, & Mugford, 1992). A slight modification of the dimethylsulphoxide methodology was followed in order to achieve better solubilisation of starch. This included incubation of the pea samples for 15 min in dimethylsulphoxide in a boiling water bath under continuous stirring.

Oligosaccharides of the raffinose family, consisting of raffinose, stachyose and verbascose, as well as the sucrose and glucose contents were measured spectrophotometrically (Megazyme Raffinose/D-Glucose Assay Kit, Megazyme International, Ireland).

Total non-starch polysaccharides (NSP) were determined spectrophotometrically according to the method described by Englyst, Quigley, and Hudson (1994) with a modification in the calculation of the total NSP content. The content was calculated using a standard curve derived from a standard sugar solution, consisting of 4.01 g/l of arabinose, 9.92 g/l of glucose and 3.56 g/l of galacturonic acid. The modified standard solution was based on the actual sugar proportions found in peas from literature (Knudsen, 1997).

Total tannins were extracted by the method of Budini, Tonelli, and Girotti (1980) and determined by a modification of Prussian blue photometric method (Grahsm, 1992) Phytic acid content was determined in defatted material by a slight modification of the AOAC (1998) anion-exchange methodology. Specifically, heating of the extract following the anion-exchange was conducted in heat-mantles instead of the proposed Kjehldahl tubes, in order to achieve gradual and uniform heating and to avoid loss of sample due to fluxing.

2.3. Statistical analysis

The statistical analysis was done by two-way analysis of variance (MANOVA) with cultivation area and cultivation year considered as the independent factors. The Tukey–HSD test, with confident levels of 95%, was used for comparison of the means. The different parameters were correlated with each other by a Pearson 2-tailed significance correlation. SPSS 11.0 computer software for Windows was used for the calculations.

3. Results and discussion

The location of the cultivation area, as well as the climatic conditions prevailing each year, affected most of the chemical components of the field pea varieties tested in this study. Interaction between these variables also strongly affected the major constituents of the pea seeds.

The major components of the field peas are shown in Table 1. Protein contents of the studied peas varied from 24.3% to 32.6%, in accordance the values previously reported in the literature (Black, Brouwer, Meares, & Iyer, 1998; Wang & Daun, 2004). The present wide variations can be due to a combination of genetic factors and environmental conditions during the growing season (Monti & Grillo, 1983; Reichert & MacKenzie, 1982; Vidal-

Table 1
Composition (% dry matter) of the major constituents of field pea varieties from different locations in years 2003 and 2004

Cultivation area	Variety	Ash	Protein	Fat	Starch	NSP[a]
Year 2003						
FCPI	K-268	3.10 ± 0.0	25.6 ± 0.2	1.78 ± 0.1	47.5 ± 0.1	14.8 ± 0.3
	K-270	3.05 ± 0.0	25.8 ± 0.1	1.78 ± 0.1	47.3 ± 0.0	14.8 ± 0.3
	K-299	3.06 ± 0.0	26.3 ± 0.0	1.74 ± 0.1	45.8 ± 0.2	14.4 ± 0.2
Fytorio	K-268	3.39 ± 0.0	24.8 ± 0.1	2.05 ± 0.1	45.5 ± 0.2	15.0 ± 0.1
	K-270	3.29 ± 0.0	24.3 ± 0.1	2.20 ± 0.4	46.3 ± 0.2	14.8 ± 0.2
	K-299	3.34 ± 0.0	24.4 ± 0.2	2.13 ± 0.2	43.3 ± 0.0	15.4 ± 0.2
Palamas	K-268	4.06 ± 0.0	32.5 ± 0.2	3.91 ± 0.0	34.2 ± 0.2	18.0 ± 0.5
	K-270	3.85 ± 0.0	32.5 ± 0.2	3.92 ± 0.3	35.9 ± 0.0	18.4 ± 0.2
	K-299	3.81 ± 0.0	31.0 ± 0.0	3.95 ± 0.2	33.4 ± 0.0	17.5 ± 0.0
Year 2004						
FCPI	K-268	3.81 ± 0.0	30.4 ± 0.3	0.76 ± 0.1	41.1 ± 0.3	14.5 ± 0.1
	K-270	3.63 ± 0.0	27.5 ± 0.0	1.80 ± 0.1	40.8 ± 0.4	16.1 ± 0.2
	K-299	3.74 ± 0.0	29.3 ± 0.3	0.95 ± 0.1	40.3 ± 0.3	15.7 ± 0.0
Fytorio	K-268	3.56 ± 0.0	29.6 ± 0.4	2.67 ± 0.2	39.6 ± 0.2	17.0 ± 0.4
	K-270	3.50 ± 0.0	31.1 ± 0.1	2.69 ± 0.0	38.9 ± 0.0	18.0 ± 0.5
	K-299	3.47 ± 0.0	31.0 ± 0.0	2.42 ± 0.3	36.3 ± 0.5	19.5 ± 0.5
Palamas	K-268	3.55 ± 0.0	26.7 ± 0.0	2.63 ± 0.0	41.7 ± 0.5	18.0 ± 0.0
	K-270	3.38 ± 0.0	27.2 ± 0.1	2.22 ± 0.3	41.3 ± 0.1	16.3 ± 0.3
	K-299	3.43 ± 0.0	29.1 ± 0.1	2.76 ± 0.0	42.9 ± 0.2	17.2 ± 0.0

[a] NSP: Non-starch polysaccharides.

Valverde et al., 2003). The highest protein levels of the pea cultivars were observed at the location 'Palamas' for the year 2003. The average level was 24–30.5% higher than the average values found in the other two locations for the same year. This can be attributed to less rainfall in the Palamas cultivation area during the year 2003 (Nikolopoulou et al., 2006). It has been reported that water deficit (relative humidity) and high temperature are often responsible for elevated protein content. (Al-Karaki & Ereifej, 1999). This also confirms the decrease in the average protein level of Palamas in the year 2004 when rainfall was higher and the slightly lower protein level compared to the FCPI and Fytorio cultivation areas, which had less rainfall. However, in another study conducted under the same conditions, meteorological changes between the years 2003 and 2004 in the same cultivation area (Palamas) had no affect on the protein content of chickpeas (Nikolopoulou et al., 2006). This possibly indicates that different legumes respond differently to environmental changes during growth.

Total fat and ash contents were in agreement with data presented in other studies (Costa, Queiroz-Monici, Reis, & Oliviera, 2006; Perez-Maldonado, Mannion, & Farrell, 1999) Similar fat and ash contents have been also reported for other legumes, such as faba beans, cowpeas and vetch. (Allan et al., 2000; Hickling, 2003) The highest levels of total fat were observed for all three varieties, which were cultivated in the area of Palamas for the year 2003. On the other hand, the lowest levels were observed for the FCPI cultivation area for both cultivation years, and in particular in year 2004.

Total starch content of the pea seeds varied from 33.4 to 47.5%, which is similar to the range previously reported (Knudsen, 1997; Kosson et al., 1994). Starch content was negatively correlated ($P < 0.01$) with protein content (Table 4) since these are the major constituents in inverse proportions in the bulk of pea (Black et al., 1998; Castell et al., 1996).

The mean values of the total amount of non-starch polysaccharides (NSP) for the years 2003 and 2004 were 15.9 and 16.9%, respectively, similar to the results obtained by other workers (Englyst et al., 1994; Englyst & Cummings, 1984; Knudsen, 1997). NSP content was positively related to protein content but negatively correlated with starch. Wang and Daun (2004) also reported a positive correlation of fibre with protein. The lowest levels of NSP contents were observed for the varieties cultivated at the FCPI location for both years tested. The major characteristic of the FCPI cultivation area is that it consists of soil containing three times more clay than the other two cultivation areas. Since the meteorological conditions of the FCPI location were similar in both years, clay content in the soil must account for the lowest values of NSP and total fat mentioned above.

Analysis of variance given in Table 3 shows that location of the cultivation area and the cultivation year, as well as their interaction ($L \times Y$), significantly affected all major nutrients of pea seeds, with the exception of starch, where year had no significant effect.

Table 2 shows the total tannin and phytic acid contents, as well as the major soluble carbohydrate content, sucrose and oligosaccharides, found in peas. These raffinose series

Table 2
Composition (% dry matter) of the soluble carbohydrates, tannins and phytic acid of field pea varieties from different locations in years 2003 and 2004

Cultivation area	Variety	Sucrose	RSO[a]	Total tannins	Phytic acid
Year 2003					
FCPI	K-268	1.53 ± 0.0	6.54 ± 0.2	0.49 ± 0.3	1.12 ± 0.1
	K-270	1.52 ± 0.0	6.37 ± 0.5	0.45 ± 0.2	0.94 ± 0.0
	K-299	1.34 ± 0.2	6.47 ± 0.4	0.47 ± 0.2	0.87 ± 0.0
Fytorio	K-268	1.53 ± 0.0	6.61 ± 0.3	0.54 ± 0.3	0.76 ± 0.0
	K-270	1.13 ± 0.0	6.74 ± 0.1	0.49 ± 0.4	0.79 ± 0.1
	K-299	1.47 ± 0.0	5.88 ± 0.3	0.53 ± 0.0	1.01 ± 0.0
Palamas	K-268	1.44 ± 0.0	5.98 ± 0.4	0.70 ± 0.1	0.57 ± 0.0
	K-270	1.04 ± 0.0	6.07 ± 0.3	0.74 ± 0.7	0.89 ± 0.0
	K-299	1.15 ± 0.0	5.77 ± 0.1	0.75 ± 0.2	0.77 ± 0.0
Year 2004					
FCPI	K-268	1.66 ± 0.0	7.28 ± 0.3	0.56 ± 0.3	1.26 ± 0.1
	K-270	2.00 ± 0.0	6.88 ± 0.3	0.68 ± 0.8	1.10 ± 0.0
	K-299	1.65 ± 0.0	7.14 ± 0.3	0.61 ± 0.2	0.66 ± 0.0
Fytorio	K-268	1.55 ± 0.0	6.79 ± 0.3	0.78 ± 0.0	0.55 ± 0.0
	K-270	1.65 ± 0.0	6.28 ± 0.4	0.62 ± 0.0	0.54 ± 0.0
	K-299	1.39 ± 0.0	6.30 ± 0.3	0.73 ± 0.0	0.76 ± 0.2
Palamas	K-268	1.36 ± 0.1	6.72 ± 0.3	0.92 ± 0.1	0.21 ± 0.0
	K-270	1.58 ± 0.0	6.62 ± 0.3	0.91 ± 0.1	0.61 ± 0.0
	K-299	1.42 ± 0.0	6.62 ± 0.0	0.90 ± 0.0	0.79 ± 0.2

[a] RSO : Raffinose series oligosaccharides.

oligosaccharides (RSO) consist of raffinose, stachyose and verbascose and it has been reported that they cause flatulence in humans and animals. (Reichert & MacKenzie, 1982). Sucrose and RSO values were in the range of 1.04–2% and 5.77–7.28%, respectively, similar to the range found in other reports (Kosson et al., 1994; Vidal-Valverde et al., 2003). Glucose was found to be negligible in all cases. Regarding the effect of the environmental factors on the soluble sugar contents of peas, the cultivated area, the year and their interactions significantly affected the sucrose content (Table 2). The total RSO content was influenced only by the year of cultivation. This indicates that the RSO content in peas is affected only by changes in the weather conditions of a certain cultivation area and not by its soil characteristics.

Total tannins levels shown in Table 2 were similar to those reported by Castell et al. (1996) but lower than those reported by Perez-Maldonado et al. (1999). Low tannin contents were expected in the present study since the studied peas were initially selected as low-tannin varieties. Higher levels of tannins are usually found in brown peas (11.5–41.0 g kg^{-1}) (Hickling, 2003; Igbasan, Guenter, & Slominski, 1997).

In particular, the highest tannin values were observed for all three varieties grown at the Palamas cultivation area for both years, the highest being, in particular, in year 2004. This can possibly be attributed to the lower clay content of Palamas in comparison to the FCPI cultivation area. The total tannin content has been negatively correlated with soil clay content for another grain legume, *Vicia ervilia*, grown in the Mediterranean environment, thus confirming our results (Berger et al., 1999). Furthermore, the higher tannin levels observed in 2004, compared to those in 2003, can be attributed the higher solarity average observed in 2004, the highest being in Palamas (Nikolopoulou et al., 2006). It has been previously reported that the tannin content in plants increases in response to greater light (Kraus, Dahlgren, & Zacoski, 2003).

Total tannin content was positively correlated ($P < 0.01$) with protein content, indicating that high protein seeds are accompanied with high tannin and NSP contents (Table 4).

Levels of phytic acid varied but were similar to the range of 3–13 g kg^{-1} dry matter reported by Vidal-Valverde et al. (2003); Wang and Daun (2004). Similar phytic acid levels

Table 3
Analysis of variance of the effect of location, year and location × year on the nutrient and antinutrient compositions of field peas

	Location (L)	Year (Y)	L × Y
Chemical composition (% dry matter)			
Ash	**	**	**
Protein	**	*	**
Fat	**	*	**
Sucrose	**	**	*
RSO[a]	–	**	–
Starch	**	–	**
NSP[b]	**	*	**
Total tannins	**	**	–
Phytic acid	**	*	–

*, **Significant at $P < 0.05$, and $P < 0.01$, respectively.
– (Blank spaces): no significance.
[a] RSO: Raffinose series oligosaccharides.
[b] NSP: Non-starch polysaccharides.

Table 4
Correlation coefficients among proximate composition, sugars, tannins and phytic acid in field peas

	Ash	Protein	Fat	Sucrose	RSO	Starch	NSP	Total tannins
Composition (% dry matter)								
Protein	0.77**							
Fat	0.42**	0.50**						
Sucrose	–	–	−0.62**					
RSO[a]	−0.36	−0.46	–	−0.41				
Starch	−0.84**	−0.88**	−0.64**	–	0.42**			
NSP[b]	0.53**	0.71**	0.68**	–	−0.46**	−0.78**		
Total tannins	0.43**	0.43**	0.47**	–	−0.52**	−0.52**	−0.68**	
Phytic acid	–	–	−0.41**	–	–	0.29*	−0.54	−0.56**

*, **Significant at $P < 0.05$, and $P < 0.01$, respectively.
– (Blank spaces): no significance.
[a] RSO: Raffinose series oligosaccharides.
[b] NSP: Non-starch polysaccharides.

have also been reported for other legumes, such as chickpeas, cowpeas, lentils and pigeon peas (Ravindran, Ravindran, & Sivalogan, 1994; Nikolopoulou et al., 2006; Rehman & Shah, 2005).

The highest phytic acid levels were observed in the FCPI cultivation area for both years. This can be attributed to the highest soil phosphorus (P) content of this area, since the major portion of total P in plant tissues is stored in the form of phytate. (Ravindran et al., 1994). Higher phytic acid levels, due to higher P content in the soil of the cultivation area, have also been observed for chickpeas (Nikolopoulou et al., 2006).

As shown in Table 4, the phytic acid content of the pea seeds was positively correlated with starch but negatively correlated with fat, NSP and total tannin content. A negative correlation between phytic acid and fat has also been reported for lentils (Wang & Daun, 2006).

Consequently, the location of the cultivation area and the year of cultivation had significant effects on both tannin and phytic acid contents, in agreement with the results shown in Table 3. However the interaction of these two factors showed no effect on these antinutrients.

4. Conclusions

In this study, it has been shown that the cultivation area characteristics, as well as the cultivation year, affect the nutrient and antinutrient compositions of field pea seeds, giving the mature seeds a considerable range in their chemical composition. The interaction of the location of the cultivation area with the cultivation year significantly affected the major constituents of the pea seeds. The climate conditions, which occur during the growing season, were found to strongly affect the protein composition and total tannin content of the pea varieties.

Furthermore, the mechanical composition, as well as the phosphorus content of the soil at the cultivation area, seemed to affect the total fat, non-starch polysaccharides, total tannins and phytic acid contents.

These findings also indicate that selection of a field pea variety with the highest composition in nutritional compounds and reduced levels of antinutrients must take into consideration, not only the genetic factors, but also the environmental parameters, namely climate conditions and edaphic characteristics dominant during the growing season.

Knowledge of which quality traits of the field peas are influenced by these abiotic environmental conditions and the variation in the composition of different cultivars would be useful in efforts to improve the nutritional composition of peas.

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