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Chemical Composition, Metabolizable Energy and Digestibility in Pea Seeds of Differing Testa and Flower Colors

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ABSTRACT

Chemical composition, tannin content, metabolizable energy, organic matter digestibility and their relations with gas production were assessed in pea seeds of differing testa and flower color in 2003 and 2004. The pea genotypes were classified in five testa colors (white, light green, dark green, dark green-brown and brown-black) or in two flower colors (white and purple). Wide variation existed in the chemical composition of the investigated individual pea genotypes between testa color or flower color groups. Crude protein content for pea seed samples varied from 192.3 to 262.1 g kg^{-1} with average N-free extract of 667.4 g kg^{-1}, crude fiber of 65.1 g kg^{-1} and ether extract of 21.4 g kg^{-1}. In general, crude protein content was higher in dark green-brown and dark brown-black seeded and in purple flowered genotypes. Dark testa colored pea genotypes had higher tannin content in both years; however this variation was not statistically significant. Purple flowered genotypes had significantly higher tannin content than the white flowered genotypes. White and green seeded peas with white flowers had consistently higher metabolizable energy than dark brown or black seeds with purple flowers, with an average of 12.6 MJ/kg DM for white seeds and 11.3 MJ/kg DM for dark brown black seeds. Organic matter digestibility showed a similar pattern to metabolizable energy. Gas production profiles showed variations in both rate and extent of gas production among the pea groups. Gas production from white and green seeded or white flowered genotypes was always higher than those of dark seeded or purple flowered genotypes. The correlation coefficients between tannin content and metabolizable energy, organic matter digestibility and gas production were weak and mostly non-significant.

Keywords: ***Pisum sativum*** **L., Tannin Content, Energy, Gas Production, Organic Matter Digestibility**

INTRODUCTION

Pea (*Pisum sativum* L.) seeds are rich in crude protein content and most mineral elements (Acikgoz et al 1985). Peas have been increasingly grown in Europe during the last 15 years to improve self sufficiency in protein rich feedstuff. Also, pea seeds are used as alternative source of crude protein to soybeans in particular for pigs and poultry diets in the European Union (Gatel 1994; Bourdillon 1999).

Several laboratory methods are available to determine the nutritional value of forages to aid formulation of rations for livestock. The proximate analysis has been widely used in the evaluation of feedstuffs. Particularly crude protein (CP) and crude fiber (CF) has been widely used to classify feeds (Fisher et al 1995). *In vitro* gas production is a rapid, simple and less time consuming method, and mostly correlated with *in vitro* digestibility (IVD) and forage quality (Sollenberger and Cherney 1995) Therefore, gas production method has been successfully used to evaluate the dry matter (DM) degradability, organic matter digestibility (OMD) or metabolizable energy (ME) of roughages (Lee et al 2000), hay and grain samples (Getachew et al 2004; Abas et al 2005), browse species (Khazaal and Ørskov 1994; Kamalak et al 2005).

White, yellow, green round and dark colored dried peas are used in the developed countries as a protein rich dietary component for animal feed. It is noteworthy that their digestible energy value is similar to soybean (*Glycine max* L. Merr.) meal or grains for white flowered peas and is slightly lower for purple flowered peas. Also, metabolizable energy values were higher for white and yellow peas than colored peas (Grosjean et al 1998; Smulikowska et al 2001). Seeds of purple flowered cultivars, which are rich in tannins, are less effectively utilized by monogastric animals than white flowered ones (Bastianelli et al 1998).

Polyphenolic compounds including tannins in legume seeds are considered as antinutritient because this group of compounds was shown to adversely affect the protein absorbsion in animals, particularly in non-ruminants (Griffiths 1981). A significant negative correlation was observed between *in vitro* digestibility (IVD) and condensed tannins in the seed coat and whole seed in cowpeas (*Vigna unguiculata* L.Walp.) (Laurena et al 1984). The IVD of the cotyledone portion of the two groups of faba beans (*Vicia faba* L.) were the same whereas testa portion of tannin free cultivars was much higher than that of tannin containing cultivars (Marquardt et al 1978). Tannins are localized in the seed coat or testa in grain legumes and the amount of tannins in the grain was directly related to seed color. The cultivars with white flowers, testa and hilums were found to contain no condensed tannin in their seed coats in peas (Griffiths 1981), in

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faba bean (Bond 1976), in beans (*Phaseolus vulgaris* L.) (Ma and Bliss 1978), in cowpeas (Laurena et al 1984). However, Price et al (1980) did not find any association between the seed color and tannin content (TC) in some grain legumes.

Little information is published on the TC, ME and OMD and their mutual relationships among those characteristics in pea seeds with different testa and flower colors. The main aim of this study was to determine these values in several pea genotypes differing testa and flower colors and to estimate nutritive value of pea seeds using the gas production technique, and to establish correlations between those characteristics.

MATERIALS AND METHODS

Pea samples were selected from a corresponding author's pea breeding nursery in Bursa, Turkey consisting of several cultivars, pure lines and ecotypes. The 31 genotypes in 2003 and 35 genotypes in 2004 were sampled in differing testa and flower color. The genotypes selected in testa color were 8 white, 16 light green, 2 dark green, 1 dark green-brown and 4 dark brown-black in 2003, and 11 white, 4 light green, 6 dark green, 9 dark green-brown and 5 dark brown-black in 2004. All of the white, light and dark green seeded genotypes were white flowered while dark green-brown and brown-black seeded genotypes were purple flowered.

Healthy whole seeds of each genotype were sampled for chemical analysis. All the chemical analyses were carried out in triplicate in the laboratories of Animal Science and Food Science Departments, Faculty of Agriculture, Uludag University, Bursa, Turkey.

Ash, nitrogen (N) crude fiber (CF) and ether extract (EE) was determined by the methods of AOAC (1990). Ash was determined by igniting the dried samples in muffle furnace at 525^{o}C for 8 h. N content was measured by the Kjeldahl method. Crude protein was calculated as N x 6.25.

TC of seed samples were determined by the Folin-Denis reagent according to the AOAC (1990). One g pea flour was put into volumetric flask containing 75 ml H_2O. After shaking, it was left overnight. 5 ml Folin-Denis reagent and 10 ml saturated Na_2CO_3 solution were added and diluted to volume with H_2O. It was mixed well, after 30 minutes it was filtered through glass wool. Absorbance values were determined at 760 nm. Tannins data were expressed as mg tannic acid per 100 g.

In order to determine ME and OMD, samples milled through a 1mm sieve were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (600 g kg^{-1}) and concentrate (400 g kg^{-1}). 0.2 gram dry weight of samples was weighed in triplicate into calibrated glass syringes of 100 ml. The syringes were prewarmed at 39^{o}C before the injection of 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39^{o}C. Readings of gas production were recorded before incubation (0) and 4, 8, 16, 24, 48 and 72 h after incubation. Total gas values were corrected for blank incubation. Cumulative gas production data were fitted to the exponential equation $p=a+b(1-e^{-ct})$ (Ørskov and McDonald 1979). The OMD and ME of the samples was calculated using equations of Menke and Steingass (1988).

Analysis of variance was performed using General Linear Model, MINITAB (University of Texas at Austin) and MSTAT-C (Version 2.1 Michigan State University, 1991) programs. Firstly, analysis of variance was done on the data of all pea genotypes. Later the pea genotypes were classified according to their testa and flower color. Analysis of variance was also performed on these classified data. The significance of main effects was determined by the Duncan test at 0.01 levels. Only the data classified for testa color and flower color were reported in the text. The correlations between chemical constituents were also computed using above mentioned programs.

RESULTS AND DISCUSSION

Chemical composition

Despite significant differences in chemical composition components between testa color or flower color groups, the proximate compositions of field pea genotypes were quite similar with high CP and N-free extract (NFE) contents, and low CF and EE content (Table 1).

CP content expressed as dry matter basis for pea seed samples varied from 196.2 to 262.1 g kg^{-1} in 2003, and from 192.3 to 226.3 g kg^{-1} in 2004. N-free matter content averaged 667.4 g kg^{-1}. Peas tested were low in CF and EE having an average of 65.1 and 21.4 g kg^{-1}, respectively. Chemical composition of pea seeds in this study was consistent with table values reported by Morrison (1956) and Ensminger et al (1990).

Table 1. Chemical composition (g kg^{-1}) of pea seeds of different testa and flower color

	Chemical Constituents*				
	CA	**CP**	**EE**	**CF**	**NFE**
Testa color	**2003**				
White	31.7^{b1}	202.3^{b}	19.3^{b}	63.8^{b}	682.8^{a}
Light Green	31.6^{b}	192.6^{b}	20.3^{b}	64.1^{ab}	691.3^{a}
Dark Green	33.5^{ab}	195.5^{b}	20.5^{ab}	64.2^{ab}	686.2^{a}
Dark Green-Brown	35.5^{a}	262.6^{a}	20.9^{ab}	65.9^{a}	615.1^{b}
Dark Brown-Black	33.6^{ab}	243.4^{a}	22.2^{a}	65.7^{a}	635.0^{b}
Flower color					
White	31.9^{b}	195.8^{b}	19.9^{b}	64.1^{b}	688.3^{a}
Purple	33.9^{a}	247.2^{a}	21.9^{a}	65.7^{a}	631.2^{b}
Testa color	**2004**				
White	32.2^{b}	194.1^{c}	21.3^{a}	64.1^{b}	688.3^{a}
Light Green	33.0^{ba}	191.7^{c}	20.3^{a}	64.8^{b}	690.3^{a}
Dark Green	31.6^{b}	210.7^{ab}	20.0^{a}	63.9^{b}	673.8^{a}
Dark Green-Brown	33.6^{ab}	207.2^{bc}	20.3^{a}	66.1^{b}	672.9^{a}
Dark Brown-Black	34.2^{a}	226.2^{a}	20.4^{a}	69.8^{a}	649.4^{b}
Flower color					
White	32.2^{b}	197.6^{b}	20.7^{a}	64.2^{b}	685.2^{a}
Purple	33.7^{a}	220.6^{a}	20.4^{a}	67.3^{a}	665.1^{b}

*: CA: Crude Ash, CP: Crude protein, EE: Ether Extract, CF: Crude Fiber, NFE: Nitrogen Free Extract
[1]: Means of the same column followed by the same letter was not significantly different at 0.01 level using Duncan test.

Although Morrison (1956) stated that there is no appreciable difference in the chemical composition of green, yellow or black seeded peas, CP content was higher in dark green-brown and dark brown-black seeded genotypes than white and green seeded genotypes and NFM content lower in this study. Similar pattern was observed in purple and white flowered genotypes. Purple flowered genotypes exhibited significant superiority in CP (234.3 vs 197.1 g kg^{-1}), ash (34.2 vs 32.4 g kg^{-1}) and CF (67.3 vs 64.4 g kg^{-1}) in both experimental years. Our findings were supported by Bastianelli et al (1998) who reported that dark colored testa peas had higher CP and CF than white colored feed and garden peas.

Tannin

Variability existed among pea genotypes tested for TC which was in the range of 2335-2810 mg kg^{-1} in 2003 and of 2162-2503 mg kg^{-1} in 2004 in testa color groups. Although dark testa colored pea genotypes had higher TC in both years, this variation was not statistically significant. Contrarily, the TC was significantly higher in the purple flowered genotypes than in the white flowered genotypes in 2003 (2407 vs 2798 mg kg^{-1}) and in 2004 (2247 vs 2496 mg kg^{-1}). These values are similar to earlier reports. For example, Habiba (2002) found 2060 mg kg^{-1} tannic acid equivalent tannin content in white flowered pea cultivar Progress 9. Shahidi and Naczk (1995) found that the tannin content as expressed in tannic acid equivalent varied from 5000 to 10500 mg kg^{-1} in whole pea seeds which is higher than those of our results.

In general, white flowered and lighter seeded faba bean lines had a lower levels of tannin content than colored flowered and darker seed coated ones (Marquardt 1989; Gatel and Grosjean 1990). The zero values for tannin content in white flowered faba bean cultivars were also recorded by several researchers (Martin Tanguy et al 1977; Duc et al 1995). Similarly, Deshpande and Campbell (1992) found that white or cream colored seeds of peavine (*Latyrus sativus* L.) were associated with low tannin levels, whereas seed with darker seed coats generally had high tannin levels. In this present study, the trends in relations between tannin content with testa and flower color were similar to those of earlier data. However, small variation in tannin content was determined in pea samples of differing testa and flower color. Furthermore, pea genotype without tannin or negligible level of tannin was not detected. The lowest tannin content was determined as 1702 mg kg^{-1} light green testa color pea genotype in 2003 and as 1462 mg kg^{-1} in dark green-brown seeded pea genotype in 2004. Our data showed that the selection of white flower or lighter testa color may be used to reduce tannin contents in pea breeding with some success.

Energy

White and green seeded peas had consistently higher ME than dark brown or black seeds. ME gradually decreased from white seeds (12.74 MJ/kg DM) to dark brown black seeds (11.36 MJ/kg DM) in 2003, and corresponding values 12.40 and 11.20 (MJ/kg DM) in 2004, respectively. This range is comparable to those reported by Morrison (1956) as 10.94 MJ/kg DM, Kearl et al (1979) as 12.80 MJ/kg DM, and NRC, (1989) 10.75 MJ/kg DM in peas. In close agreement with our findings, it was found that colored peas had significantly lowered ME values than feed peas. For example, Grosjean et al (1998) reported that mean values for digestible energy were 16.34, 15.06 and 15.60 MJ/kg DM in white-feed peas, purple flowered peas, and wrinkled white flowered peas. Later they also indicated mean ME values were 12.02, 11.35 and 10.50 MJ/kg DM for feed peas, colored peas and wrinkled peas, respectively in mash diets and 13.18, 12.72 and 11.63 MJ/kg DM for the same categories in pelleted diets (Grosjean et al 1999).

Organic matter digestibility

OMD of pea seeds appeared variable between both within testa and flower color groups. In general, OMD gradually decreased from white seeds to brown-black seeds in both experimental years. White seeded peas exhibited significantly higher OMD (921.1 g kg^{-1}) than brown-black colored peas (876.7 g kg^{-1}) in 2003, and 903.7 g kg^{-1} and 862.6 g kg^{-1} in 2004, correspondingly. On average, OMD of white flowered genotypes were 894.8 g kg^{-1}, and purple flowered genotypes registered slightly lower value of 880.6 g kg^{-1}. Mean OMD in pea seeds of different testa and flower color suggested that both testa and flower color was an indicator of digestibility.

An important determinant of nutritional quality of seed legumes is the digestibility of the organic matter. However, little has been done on OMD of pea and other legume seeds. Our findings were broadly similar to other commonly used grain legumes. For example, though the difference was not so great, white flowered faba bean cultivars had a mean digestibility of 879 g kg^{-1} compared with 832 g kg^{-1} for all color flowered faba beans (Bond 1976). Gurumoorthi et al (2003) also reported that protein digestibility varied from 749 to 769 g kg^{-1} in white seeds and from 724 to 729 g kg^{-1} in black seeds of *Macuna pruriens.*

Gas production

Gas production increased during the 72 h of incubation in all testa and flower color groups. The pea genotypes with white and green testa produced more gas compared to dark green-brown or brown-black seeds at all incubation recording points in both years. In contrast, gas produced from fermentation of white and green genotypes or green-brown or brown-black seeds was similar, and the differences were mostly non significant. Total gas production at 72 h of incubation ranged from 85.4 ml for white seeded peas to 75.4 ml for dark brown-black seeded genotypes. Volume of gas produced by white flowered pea genotypes was significantly higher than that of purple flowered genotypes (Table 2).

Table 2. Tannin content, organic matter digestibility and metabolizable energy in pea seeds of different testa and flower color

	Parameters*		
	2003		
Testa color	**TC**	**OMD**	**ME**
White	2335^{a}	921.1^{a}	12.74^{a1}
Light Green	2508^{a}	901.1^{ba}	12.47^{ab}
Dark Green	1888^{a}	889.2^{ba}	12.20^{bc}
Dark Green-Brown	2750^{a}	910.0^{ba}	11.80^{cd}
Dark Brown-Black	2810^{a}	876.7^{b}	11.36^{d}
Flower color			
White	2407^{b}	906.4^{a}	12.53^{a}
Purple	2798^{a}	883.3^{b}	11.45^{b}
Testa color	**2004**		
White	2162^{a}	903.7^{a}	12.40^{a}
Light Green	2349^{a}	874.6^{ab}	11.86^{ab}
Dark Green	2315^{a}	882.7^{ab}	11.77^{ab}
Dark Green-Brown	2491^{a}	873.7^{ab}	11.65^{b}
Dark Brown-Black	2503^{a}	862.6^{b}	11.20^{b}
Flower color			
White	2247^{b}	891.2^{a}	12.11^{a}
Purple	2496^{a}	870.0^{b}	11.50^{b}

*: TC: Tannin content (mg kg^{-1}), ME: Metabolizable energy (MJ/kg DM), OMD: Organic Matter Digestibility (g kg^{-1}),
[1]: Means of the same column followed by the same letter was not significantly different at 0.01 level using Duncan test.

It is well known that tannins depress rumen fermentation of feeds and reduced gas formation (Khazaal et al 1993; Getachew et al 2004). In this study, effects of tannins on rumen fermentation are reflected in the gas production, i.e. gas production gradually decreased with increasing tannin content of the seeds (Table 2, 3).

Table 3. Cumulative gas production (ml/200 mg DM) during incubation in pea seeds of different testa and flower color

	Incubation (hours)					
	3	6	12	24	48	**72**
Testa Color	**2003**					
White	30.79[a1]	48.53[a]	60.36[a]	72.69[a]	81.84[a]	85.03[a]
Light Green	29.83[ab]	47.40[a]	58.48[a]	70.91[ab]	79.39[ab]	83.19[a]
Dark Green	28.20[b]	44.47[b]	54.60[b]	69.00[bc]	76.95[bc]	79.75[b]
Dark Green-Brown	25.00[c]	43.17[bc]	52.67[b]	66.33[cd]	76.23[cd]	77.83[bc]
Dark Brown-Black	25.62[c]	41.28[c]	51.04[b]	63.62[d]	73.38[d]	75.88[c]
Flower color						
White	30.00[a]	47.52[a]	58.76[a]	71.31[a]	79.96[a]	83.49[a]
Purple	25.50[b]	41.66[b]	51.37[b]	64.16[b]	73.95[b]	76.27[b]
Testa Color	**2004**					
White	33.68[a]	48.40[a]	61.07[a]	71.12[a]	80.72[a]	85.80[a]
Light Green	29.70[a]	45.43[ab]	56.47[ab]	67.59[ab]	76.71[ab]	80.87[b]
Dark Green	30.43[a]	45.37[ab]	55.63[ab]	67.07[ab]	75.87[ab]	78.83[bc]
Dark Green-Brown	29.75[a]	41.17[bc]	53.90[b]	66.22[b]	72.07[bc]	77.75[bc]
Dark Brown-Black	24.27[b]	37.13[c]	49.70[b]	63.23[b]	68.83[c]	74.77[c]
Flower color						
White	31.87[a]	46.90[a]	58.56[a]	69.22[a]	78.50[a]	82.73[a]
Purple	27.92[b]	39.82[b]	52.50[b]	65.22[b]	70.99[b]	76.76[b]

[1]: Means of the same column followed by the same letter was not significantly different at 0.01 level using Duncan test.

Correlations

High levels of phenolic compounds including tannins in several forages limited utilization by ruminants through impaired feed digestibility and nutrient utilization (McGraw and Hoveland 1995; Puchala et al 2005). Several previous workers have reported that tannin content was negatively correlated with OMD and gas production (Khazaal et al 1993; Kamalak et al 2005). In the current study, the negative correlations between TC and ME, OMD and gas production were obtained in 2003, 2004 and combined years. However, most of the correlations were weak and statistically non significant (Table 4). Our results were not fully consistent with those previous reports. It seems that tannin content did not greatly reduced ME and OMD values in pea seeds. It is well known that tannins may be divided in two classes: low molecular weight hydrolysable and higher molecular weight non-hydrolysable (or condensed) tannins. Condensed tannins bind to proteins in the digestive tract and form complexes which are frequently indigestible in non ruminants. The hydrolysable tannins are often found to have little effect on digestibility (Marquardt 1989). It is possible that, the condensed tannins in pea genotypes may be closely related to OMD and ME.

Table 4. The correlations between tannin content and metabolizable energy, organic matter digestibility, and gas production at 24 h incubation. Upper line in 2003 (df: 29), middle line in 2004 (df: 33) and bottom line overall (df: 64)

	ME	OMD	Gas Production
2003	-0.30	-0.11	-0.32
2004	-0.35*	-0.32*	-0.25
2003-2004	-0.22	-0.14	-0.19

*: significant at $P \leq 0.05$

CONCLUSION

In summary, our study clearly showed that white and green seeded peas consistently had higher ME, OMD values and produced more gas than dark brown or black seeds. Dark colored testas and purple flowered genotypes had higher TC, than white and green seeded and the white flowered genotypes in both years, but showed only small variations. There were weak and mostly non-significant negative correlations between TC and ME, OMD values and gas production in pea seeds.

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