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*Short communication*

SHORT COMMUNICATION

# Chemical composition of selected winter green pea (*Pisum sativum* L.) genotypes

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*Abstract*: Breeding and selection of winter pea for seed quality is a serious challenge to every breeder. The result of breeding mainly depends on good knowledge of the genetic material. Chemical and technological analysis is necessary for an accurate determination of the following traits of technologically mature seed of the winter pea collection: protein content, total nitrogen content, total sugars content, starch content, fatty oil content, cellulose content, and ash content (g (100 g)$^{-1}$). The protein content in the tested lines of pea was in the range 22.86–28.04 g (100 g)$^{-1}$, the total nitrogen content 3.66–4.49 g (100 g)$^{-1}$, total sugars content 10.30–14.67 g (100 g)$^{-1}$, starch content 39.44––46.23 g (100 g)$^{-1}$, fatty oil content 1.48–1.89 g (100 g)$^{-1}$, cellulose content 8.79–10.28 g (100 g)$^{-1}$ and ash content 3.08–3.67 g (100 g)$^{-1}$. PCA analysis was used to identify the three components that collectively explained 81.59 % of the total variation. The first component was mainly defined by the ash and the total nitrogen, protein and cellulose contents. The second one, independent from the first one, was mainly correlated to the fatty oil and starch contents, while the third was defined by the content of total sugars.

*Keywords*: technologically mature seed, winter varieties, nutritional composition, tolerance to low temperatures.

## INTRODUCTION

Pea is a widely cultivated crop species and the second most important food legume worldwide after common bean.[1] It is also one of the first domesticated crops in the Old World and one of the first genetic research materials.

Garden pea (*Pisum sativum* L.) is a valuable nutritive product for human consumption. It is characterized with numerous breeding types, such as grain, vegetable, forage, canning, non-food use, *etc*. The vegetable varieties are widely planted in both private gardens and for field production.[2] Pea occupies a prominent place among vegetables due to its high nutritive value, particularly pro-

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teins and other health building substances, such as carbohydrates, vitamin A, vitamin C, calcium and phosphorus.[3] Pea seeds are rich in protein (23–25 %), slowly digestible starch (50 %), soluble sugars (5 %), fibbers, minerals and vitamins.[4–6] It is well known that the chemical content of pea seeds can vary. Genetic (variety) and environmental factors (location of cultivation area, soil characteristics, exchangeable cations, trace elements, cropping year, total rainfall, relative humidity, solarisation, temperature) are of importance,[7–9] as well as technological treatments (dehulling, cooking, soaking, germination, extrusion),[10–12] Unfavourable weather conditions may negatively influence crop yield and protein content in pea seeds. Due to differences in climate, soil, varieties and agronomic practices, field peas may have different chemical components when grown in various parts of the world.[13]

The results of some authors suggest that proteins must have high solubility for optimal functionality in food processing applications.[14]

AS pea is a major cool season legume crop for human consumption as dry seeds or as vegetable and for feeding livestock, the most important tasks for pea breeding are the development of high yielding varieties with stable productivity, and sufficiently good resistance to disease and unfavourable environmental conditions, and increased protein content, essential amino acids and favourable rations among them.[15]

Other authors suggest that winter protein pea cultivars with a great potential for both tolerance to low temperatures and high and stable grain yields could increase the growing area of pea under the prevailing conditions of some regions in general, and create novel prospects in their utilisation.[16]

The aim of the present study was to determine important chemical and technological traits of selected winter pea lines and analyse the main components in order to perceive the structure of the variability in the traits of experimental pea genotypes, as well as to assess the contribution of specific traits in the total variability. Principal component analysis (PCA) and cluster analysis was used to visualize the association among different traits. The PCA and cluster analysis performed on the chemical traits divided the local pea genotypes into clusters. In addition, based on cluster analyses, pea genotypes are expected to be grouped into individual groups.

## MATERIALS AND METHODS

A small-plot trial was performed on chernozem soil at the experiment field of the Institute of Field and Vegetable Crops at Rimski Šančevi, Serbia, including twenty-one winter pea genotypes and one winter field pea variety (NS-Mraz). The cultivar trial with some of the winter pea collection was implemented in 2016 following the methodology for examining cultivars of leguminous vegetables. It was set up as a complete random block system in five replications with the length of the basic plot of 5 m, and width of 1 m. The distance between the rows was 25 cm and 5 cm between the plants in a row. All twenty-two genotypes were sown by hand in early October.

The date of harvest was according to the technological maturity of each genotype. After manual harvesting of pods and seed removal, the seeds of the analysed pea lines were dried to 11 % moisture content. All seed traits, the contents of ash, proteins, oil, cellulose, starch, total and reducing sugars, were expressed as g per 100 g of t dry weight (d.w.). The ash and moisture contents were determined gravimetrically, drying at 105 °C for 4 h in the first[17] and drying at 600 °C for 2 h in the second determination.[18] The nitrogen content in pea seed was determined using the Kjehdahl method,[19] and total protein content was calculated by multiplying the obtained value with the factor for pea protein, 6.25. The oil content was determined by Soxhlet extraction (with petroleum ether) of the oils for 8 h at 70 °C.[20] The content of crude fibre was determined by a modified Scharrer method.[21] The starch content was determined by a polarimetric method.[22] The content of total and reducing sugars was determined by the Luff–Schoorl method.[23]

Principal component analysis (PCA) has the ability to recognize and eliminate redundant data from experimental results. Using PCA, a large number of available data is reduced, which results in a different number of the variables, the so-called principal components (PC). A principal component (PC) is in fact a linear combination of original variables. In practice, it is usually sufficient to retain only a few principal components, the sum of which includes a large percentage of the total variable.

Cluster analysis is used for the reduction of extensive data; the objects are combined into groups of relatively homogenous composition. It could help to group parameters into clusters based on similarity or difference between them. The challenge for many studies in which extensive data is used is the identification and grouping of the elements into smaller groups based on specific relations.

All statistical analyses were realised using Statistica 12 (StatSoft Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

Pea proteins have high biological value because they contain all the essential amino acids in very favourable ratio for the human diet. The protein content in the tested material had an average value of 23.89 and varied between 22.86 to 28.04 g (100 g)$^{-1}$ as shown in Table I. The obtained results for the protein content in botanically ripe pea seed are in agreement with the results of most authors. A literature review showed that the protein content in pea seed ranged from 15.8 to 34.7 g (100 g)$^{-1}$.[24–32]

The content of total nitrogen in the seed of the tested pea genotypes ranged from 3.66 to 4.49 g (100 g)$^{-1}$ as could be seen in Table I. The obtained values are in agreement with the results of Urbano.[33] Studies of Gvozdanović[34] showed that content of total nitrogen varied between 3.3 and 3.5 g (100 g)$^{-1}$.

Chemical analysis of the ripe seed of the pea lines showed that the content of total sugars varied from 10.3 to 14.67 g (100 g)$^{-1}$, given in Table I. A literature review showed varying total sugars contents in pea seed ranging from 0.7 to 19.5 g (100 g)$^{-1}$.[24,26,28,29,32,33]

The starch content in the seed of the analysed lines ranged from 39.44 to 46.23 g (100 g)$^{-1}$, given in Table I. A literature review showed that the starch content in the seed varied from 18.6 to 54.5 g (100 g)$^{-1}$.[24,26,28–30,33]

Analysis of the seeds of the selected winter pea lines showed that fatty oil ranged between 1.48 and 1.89 g (100 g)$^{-1}$, given in Table I. Literature sources showed that this property of pea varied from 0.6 to 3.95 g (100 g)$^{-1}$.[24,26–32]

TABLE I. Values of chemically and technologically analysed seed traits expressed as g per 100 g of the dry weight (g 100 g$^{-1}$ d.w.) in the tested pea lines

| Pea line, genotype | Proteins | Total nitrogen | Total sugars | Starch | Fatty oil | Cellulose/fibre | Ash |
|---|---|---|---|---|---|---|---|
| L-1 | 22.98 | 3.68 | 14.65 | 41.83 | 1.58 | 8.79 | 3.33 |
| L-2 | 23.28 | 3.72 | 14.67 | 41.89 | 1.67 | 8.83 | 3.33 |
| L-3 | 23.01 | 3.68 | 12.55 | 42.69 | 1.59 | 8.91 | 3.32 |
| L-4 | 23.50 | 3.76 | 14.56 | 42.78 | 1.65 | 9.10 | 3.42 |
| L-5 | 23.74 | 3.80 | 10.30 | 46.23 | 1.61 | 9.50 | 3.29 |
| L-6 | 25.19 | 4.03 | 13.13 | 43.23 | 1.72 | 10.28 | 3.46 |
| L-7 | 23.50 | 3.76 | 13.53 | 45.46 | 1.72 | 8.98 | 3.38 |
| L-8 | 24.64 | 3.94 | 10.87 | 44.46 | 1.57 | 9.88 | 3.44 |
| L-9 | 24.61 | 3.94 | 13.94 | 42.92 | 1.69 | 8.98 | 3.31 |
| L-10 | 23.60 | 3.78 | 10.77 | 42.86 | 1.75 | 9.24 | 3.11 |
| L-11 | 23.14 | 3.70 | 13.09 | 43.01 | 1.62 | 8.97 | 3.23 |
| L-12 | 23.34 | 3.73 | 11.27 | 44.25 | 1.74 | 9.24 | 3.22 |
| L-13 | 23.47 | 3.75 | 13.98 | 43.06 | 1.64 | 8.84 | 3.20 |
| L-14 | 24.13 | 3.86 | 11.07 | 45.25 | 1.66 | 9.11 | 3.25 |
| L-15 | 24.50 | 3.92 | 12.36 | 44.10 | 1.48 | 9.30 | 3.17 |
| L-16 | 23.35 | 3.74 | 12.37 | 45.32 | 1.56 | 9.82 | 3.21 |
| L-17 | 23.42 | 3.75 | 13.99 | 40.69 | 1.71 | 9.93 | 3.34 |
| L-18 | 23.06 | 3.69 | 13.12 | 43.19 | 1.77 | 8.85 | 3.08 |
| L-19 | 23.37 | 3.74 | 10.37 | 39.44 | 1.89 | 9.07 | 3.37 |
| NS-Mraz | 28.04 | 4.49 | 11.39 | 45.93 | 1.66 | 10.01 | 3.67 |
| L-21 | 22.86 | 3.66 | 12.30 | 44.30 | 1.72 | 9.47 | 3.34 |
| L-22 | 24.95 | 3.99 | 14.47 | 42.91 | 1.83 | 9.19 | 3.40 |
| Average | 23.89 | 3.82 | 12.67 | 43.44 | 1.67 | 9.28 | 3.31 |
| Minimum | 22.85 | 3.65 | 10.30 | 39.44 | 1.48 | 8.78 | 3.08 |
| Maximum | 28.04 | 4.48 | 14.66 | 46.23 | 1.89 | 10.28 | 3.66 |

The cellulose (fibre) content of the tested pea lines had values from 8.79 to 10.28 g (100 g)$^{-1}$, given in Table I. Other authors stated that this property varied from 2.0 to 15.0 g (100 g)$^{-1}$.[24,25,27–29,31,32]

The ash content in the tested pea lines ranged between 3.08 to 3.67 g (100 g)$^{-1}$, given in Table I. Other authors recorded values between 2.3 and 5.2 g (100 g)$^{-1}$.[24,26,27,29,30–32]

The diversity between the seeds of the winter pea genotypes was shown by applying factorial analysis by the method of principal components (PCA – principal component analysis) based on the seven studied quality parameters (Table II).

The analysis of the principal components reduced the initial number of variables from seven to three artificial, mutually uncorrelated variables, *i.e.*, principal components.

This method concentrates the variability to the first principal component. The first principal component explains the larger part of the variability of all tested traits as much as possible. A value of the factor loading > 0.70 served as the criterion for the separation of the variables, which largely define the separated components. Factor loading represents the correlation between each variable and the factor that indicates the degree of correspondence between the variables and the factors.

TABLE II. Principal component analysis of the studied traits

| Variable | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| Ash | –0.72738781 | 0.45590397 | 0.17998147 |
| Fatty oil | 0.12548382 | 0.68397250 | –0.62419305 |
| Total nitrogen | –0.93548073 | 0.16377385 | 0.07319258 |
| Proteins | –0.93548073 | 0.16377385 | 0.07319258 |
| Total sugars | 0.32079302 | 0.45853488 | 0.77215989 |
| Starch | –0.51349714 | –0.70251502 | 0.06219246 |
| Cellulose | –0.74126518 | –0.05985957 | –0.17593581 |

The first component is related mainly to the performance of the parameters ash, total nitrogen, proteins and cellulose. Parameters fatty oil and starch also participate in the formation of the second component. The amount of total sugars is the characteristic of significance in shaping the third component.

The answer to the question concerning the number of principal components retained in the analysis was obtained based on the significance of the eigenvalue. A graphic display of the eigenvalues according to their ordinal numbers was chosen for this purpose. This diagram is called the “Scree test”, which was suggested by Cattell[35] (Fig. 1). It was used to choose principal components up to an eigenvalue of one (eigenvalue = 1).

When the variance of the principal component is below one, then the eigenvalue is also below one, which means that the component explains less than originally observed trait. Removal of all components that have eigenvalues below one from the system is one of the methods of choosing the number of appropriate observed principal components. Sometimes it is necessary to choose for observation as many principal components as needed in order to explain a satisfactory percentage of variability set.[36]

Three eigenvalues are higher than one and they determine the choice of four principal components. Three separated components showed cumulatively 81.59 % of total variability. The first one accounted for 45.87 %, the second one 20.52 % and the third 15.19 % of all the variations (Table III). The principal components PC1 and PC2 account for 66.39 % of all variations of genotype×characteristics.

Genetic similarity/dissimilarity evaluated by combination of qualitative traits using cluster analysis showed the presence of similarity and distances between

the analysed pea lines. The squared euclidean distance is used as a measure of the genetic distance.

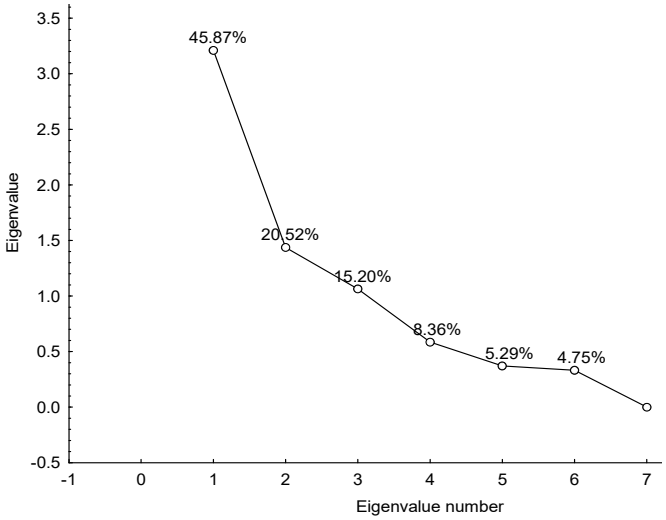


Fig. 1. Display of the Scree test of principal components for the tested pea traits.

TABLE III. Eigenvalue and % of total variance using three principal components (PC)

| PC | Eigenvalue | Total variance, % | Cumulative Eigenvalue | Cumulative, % |
|---|---|---|---|---|
| 1 | 3.211149 | 45.87356 | 3.211149 | 45.8736 |
| 2 | 1.436675 | 20.52393 | 4.647824 | 66.3975 |
| 3 | 1.063777 | 15.19681 | 5.711601 | 81.5943 |

Comparisons of data and cluster analysis generated a dendrogram in which 22 pea genotypes were grouped into clusters. The results presented as a dendrogram (Fig. 2) show different groupings of genotypes by similarity and difference. These genotypes are divided in one “independent group”. The other main group includes all others genotypes. This group is divided into next sub-groups.

Cluster analysis was used to classify the observed winter genotypes of pea into two main groups. The first group I included winter field pea cultivar NS Mraz. Seclusion of this cultivar from the rest was expected, since it is the cultivar with the highest values of protein, total nitrogen and ash content. This cultivar is also distinguished from the other groups by its high content of starch and cellulose. The other analyzed genotypes represent the line material and, according to the dendrogram, are classified into eight subgroups (Fig. 2).

Subgroup H included lines L6 and L8 with sufficiently similar values of the tested quality traits with small differences between them. The largest similarities were indicated in the content of total nitrogen, protein, ash and cellulose. The following subgroup G included four lines: L5, L14, L15 and L16. This subgroup had only the starch content values above the average of the observed genotypes.

On the other hand, this group was defined by lower contents of total sugars, fatty oil and ash. Line 19 from subgroup F had the highest oil content. Lines from subgroup E had high contents of oil, while the contents of the other observed traits were lower than average. Subgroups C and D included lines L9, L22 and L17, which exhibited higher sugar contents and lower starch contents than the others. Subgroup B included lines with higher content of starch, fatty oil and ash. In the first and largest subgroup A, lines L1, L2 and L4 had the highest values of the total sugar content at technological maturity. Lines L3, L11 and L13 had mostly lower values of all observed quality traits (Fig. 2).

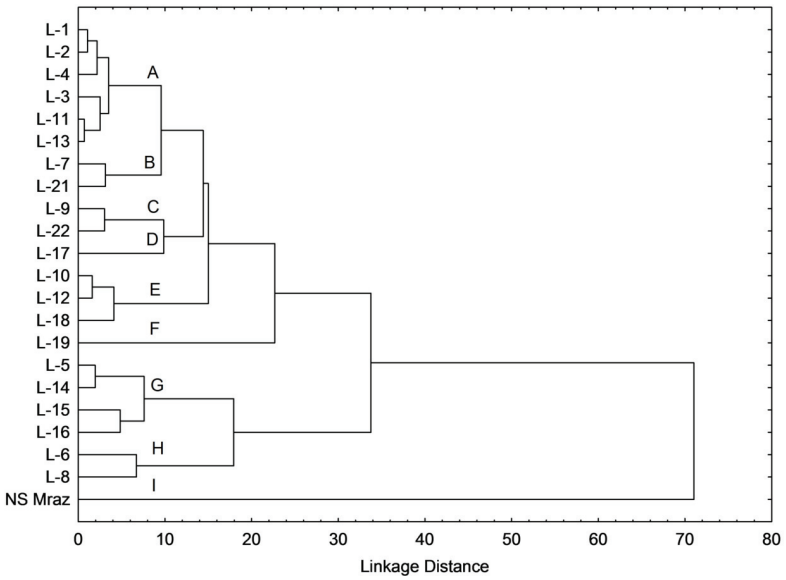


Fig. 2. Dendrogram of studied winter pea genotypes.

Previously, the chemical composition in the spring and winter forms of pea were analyzed by principal component analysis in which four eigenvalues had the following values: the first had 44.58 %; the second 26.81 %; the third 18.06 % and the fourth 7.38 %.[37] The principal components (PC1 and PC2) accounted for 71.39 % of all the variations of genotype×characteristics. In the present study, three similar eigenvalues were found, *i.e.*, for first 45.87 %, for the second 20.52 % and for the third 15.19 %. In the work of Esposito *et al.*[1] on pea genotypes, the two first components explained 67.7 % of variability in the first season of experiment and 69.8 % in the second. The principal components (PC1 and PC2) in the present investigation accounted for 66.39 %. Different values could be the result of the large number of traits analyzed by the cited authors. The results of the quality parameters presented in their dendrogram showed the different groupings

of the varieties by similarity and difference. These varieties were divided between an independent group A and the other main group B, which was divided into two sub-groups. Similar groupings of the values in dendrogram were calculated using the present results.

By analyzing the differences in the chemical composition of field pea cultivars, it was previously shown that the characteristics of the cultivation area, as well as the cultivation year, could affect the nutrient and anti-nutrient compositions of field pea seeds, giving the mature seeds a considerable range in their chemical composition.[38,39]

To obtain more prominent transgressive forms in hybrid combinations, genotypes from the different groups must be included to expect a good combination of favourable genes be obtained in one genotype. Hierarchical cluster analysis could be used in the selection process for planning the initial parent combinations.[40]

Dendrogram analysis showed that the classification of genotypes into nine subgroups was mostly the consequence of differences in the sugar and protein contents. The majority of the tested genotypes are good starting material when the breeding process is aimed at increasing the seed quality. Taking into account the goals of pea breeding, the divergence of the selection material is evident. The differences found in this study could be useful in the selection of potential parental pairs for crossing.

## CONCLUSIONS

Chemical and technological analysis of ripened seed of winter pea lines was used to study the following traits: content of ash, protein, oil, cellulose, starch, total and reducing sugars. The highest values of protein and total nitrogen content in studied pea lines were found in genotypes 6, 20 and 22. The content of total sugars was the highest in genotypes 6, 8 and 20. Starch content had maximum values in genotypes 3, 9 and 14. Maximum values of fatty oil content were measured in genotypes 18, 19 and 22. Cellulose content had its highest values in genotypes 2, 3 and 15. The ash content achieved highest values in genotypes 6, 8 and 20. The obtained results will be used in defining the direction and correct selection of lines for the future new cultivars of winter pea. In addition, the selection of parental components for crossing with the aim of content improvement for specific chemical composition of the seed would be easier to determine. However, in the breeding of winter pea, the commenced studies should continue with the aim of constant discovery of new parent lines and cultivars that would have an even better nutritional composition positively incorporated with other morphological traits of pea.

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ИЗВОД

## ХЕМИЈСКО-ТЕХНОЛОШКА СВОЈСТВА ЗРНА ОЗИМОГ ГРАШКА (*Pisum sativum* L.)

ЈАНКО ЧЕРВЕНСКИ, ДАРИО ДАНОЈЕВИЋ и АЛЕКСАНДРА САВИЋ

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Оплемењивање и селекција грашка на квалитет зрна представља озбиљан изазов сваког оплемењивача. Резултат оплемењивања у многоме зависи од доброг познавања генетског материјала на коме са ради. Хемијско-технолошком анализом потребно је било тачно одредити следећа својстава технолошки зрелог зрна дела колекције озимог грашка: садржај протеина, садржај укупног азота, садржај укупних шећера, садржај скроба, садржај масног уља, садржај целулозе и садржај пепела. Садржај протеина у испитиваним линијама грашка се кретао од 22,86 до 28,04 g $(100\ g)^{-1}$, садржај укупног азота од 3,66 до 4,49 g $(100\ g)^{-1}$, садржај укупних шећера од 10,30 до 14,67 g $(100\ g)^{-1}$, садржај скроба од 39,44 до 46,23 g $(100\ g)^{-1}$, садржај масног уља од 1,48 до 1,89 g $(100\ g)^{-1}$, садржај целулозе од 8,79 до 10,28 g $(100\ g)^{-1}$ и садржај пепела од 3,08 до 3,67 g $(100\ g)^{-1}$. PCA анализом смо издвојили три компоненте које су збирно објасниле 81,59 % почетне варијабилности. Прва компонента је у највећој мери дефинисана садржајем пепела, укупног азота, беланчевина и целулозе. Друга главна компонента, независна од прве, у највећој мери је била у корелацији са садржајем масног уља и скроба, док је трећу компоненту дефинисао садржај укупних шећера.

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