Dragičević et al. Chemical and Biological Technologies in Agriculture (2015) 2:2
DOI 10.1186/s40538-015-0031-7

Chemical and Biological Technologies in Agriculture
a SpringerOpen Journal

RESEARCH Open Access

Variations in level of oil, protein, and some antioxidants in chickpea and peanut seeds

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Abstract

Background: Chickpea and peanut are two legume species not frequently used in human diets. Chickpea is rich in starch and proteins, while peanut is mainly a source of oils and proteins and they could be successfully used as protein sources in vegetarian diets.
Seeds of 19 chickpea and 13 peanut landraces were colorimetrically analyzed in respect to antioxidant content (i.e., free soluble phenolics, total glutathione, and phytate). Oil and protein contents in grain were also determined.

Results: Free soluble phenolics content varied in range from 520 to1,050 mg kg^{-1} in peanut and from 720 to 1,370 mg kg^{-1} in chickpea. Total glutathione content ranged from 1,495 to 2,365 mmol kg^{-1} in peanut and from 955 to 1,232 mmol kg^{-1} in chickpea. Relatively low content of phytic phosphorus was found in grain of both species, ranging from 2.5 to 4.5 g kg^{-1} in peanut and from 1.4 to 3.0 g kg^{-1} in chickpea, respectively. Considering the lack of data for phytate variability in Macedonian chickpea and peanut local landraces up to date, the observed high variation in phytic phosphorus content could represent the great basis for further breeding programs for phytate decrease in seeds of those genotypes. This is significant, since phytate is an important antinutrient which affects availability of mineral elements. Regression analysis revealed positive and highly significant interdependence between oil content and total glutathione in chickpea seeds, as well as between oil content and phytic phosphorus in peanut seeds. In chickpea, significant and negative correlation between oil and phytic phosphorus content was also observed.

Conclusions: Results obtained indicated that chickpea genotypes with higher oil content could have increased nutritional value due to higher glutathione and lower phytate content observed. However, lower level of phytate content, along with higher level of soluble phenolics and total glutathione found in peanut seeds with lower oil content, indicated higher digestibility and increased antioxidant activity of those genotypes.

Keywords: Antioxidants; Nutritive value; Phenolics; Phytic phosphorus; Total glutathione

Background

Chickpea is a valued legume in Afro-Asian countries due to its nutritive seed composition high in protein content and of better protein quality compared to other legumes, thus increasingly used as a substitute for animal protein. Except of sulfur-containing amino acids, chickpea is rich in all the essential amino acids, being with a balanced content [1]. It has been shown that *in vitro* protein digestibility from protein isolates ranged between 95.6% and 96.1% [2]. Besides, it is also important to emphasize the high antioxidant activity of protein hydrolysates in chickpea [3]. Chickpea seeds contain less than 7% of oil, with linoleic and oleic acid as predominant [4]. According to Chitra et al. [5], chickpea contains relatively low phytic acid content, compared to other legumes. Significant and negative correlation between phytic acid and *in vitro* digestibility made chickpea seeds necessary in human diet. Relatively high genotypic variability is present in chickpea seed composition, including phytic acid and other antioxidants. Rincón et al. [6] found that Desi biotypes revealed lower fat and phytic acid contents, whereas Kabuli biotypes showed lower total dietary fiber, insoluble dietary fiber, and tannin content. Leading role in protection of chickpea seeds against fungal attack was given to phenolic substances [7], which could also contribute to increased nutritional value of chickpea seeds. Beside of particular nutritive parameter, larger seed with light color are considered as desirable traits for chickpea breeding programs [8].

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Since peanut flour is rich in oil and proteins, it is widely used in different foods: as a replacement for animal source proteins, in breakfast snack foods and cereals, as an improver of cereal flours, and it can be used to produce textured vegetable proteins or can be used directly in ground meats to provide adequate moisture and fat binding characteristics [9]. As an oilseed crop, peanut is characterized with proteins of high quantity and quality, as well as with high caloric value. It is also high in phytic acid and contains fibers and perhaps other binding agents which reduce mineral bioavailability from the seeds [10]. Chemical properties of grain are under high genotypic and environmental impact, reflected in induced variations in oil content, individual fatty acid contents, and derived oil quality parameters [11]. Dwivedi et al. [12] reported significant and negative correlation between oil and protein contents, as well as significant linear increase in oil content followed by seed mass increase. However, no such relationship was observed for protein content. Similarly to chickpea, phenolics from peanut seeds and particularly peanut skin have high antifungal and antioxidative activity [13-15]. Positive effect of peanut on human health can be confirmed by the studies of Emekli-Alturfan et al. [16], who ascertained that addition of peanut to the diet did not significantly change blood lipids, protrombin time, activated partial thromboplastin time, or fibrinogen levels, both in control and in hyperlipidemic groups. Peanut consumption improved glutathione (GSH) and high-density lipoprotein (HDL-C) levels and decreased thiobarbituric acid reactive substances (TBARS), without increasing other blood lipids in experimental hyperlipidemia.

As previously mentioned, peanut seeds are rich in phytic acid which is a strong chelating agent that can bind mono- and divalent metal ions, inducing poor bioavailability of minerals such as zinc, calcium, magnesium, iron, and phosphorus [10]. On the other hand, Chung and Champagne [17] found that phytic acid formed insoluble complexes with the major peanut allergens, resulting in peanut extract with reduced allergenic potency and suggested that phytic acid may find its use in the development of hypoallergenic peanut-based products.

According to findings reported, some of the antinutrients may play important beneficial roles in human diets by acting as anticancerogens or by promoting health in other ways such as in decreasing the risk of heart disease or diabetes. Thus, plant breeders and molecular biologists should be aware of the possible negative consequences of changing antinutrients in major plant foods [18]. In parallel, polyphenols, as well-known classes of phytochemicals, are considered to be important components in human diet. Several studies on cancer cell lines and animal models of carcinogenesis have shown that a wide range of polyphenols possess anticancerogenous and apoptosis-inducing properties [19]. GSH, as protein antioxidant, has important role in free radical scavenging, prevention from stress [20] and could reduce activity of trypsin inhibitors (e.g., Kunitz trypsin inhibitor) [21].

Caloric value of grain, rich in proteins and phytochemicals, enables chickpea and peanut to be broadly and successfully used in vegetarian diets. Hence, to achieve the lower antinutrient content and adequate level of nutritive factors in grain is of great importance.

Those findings prompted us to evaluate a set of 19 chickpea and 13 peanut local landraces in order to determine the content of the main seed constituents (i.e., oil and proteins), as well as seed antioxidant content (i.e., phytate, free soluble phenolics, and GSH). The aim of this investigation was to select the most promising genotypes as sources for further breeding programs for grain quality increase.

Methods

Average sample of each landrace was presented with 100 uniform seeds. Samples were milled on Perten 120 - Sweden (particle size <500 μm). Oil content was determined as subtraction after extraction with petroleum ether. For protein determination, samples (4 × of 0.20 g) were digested with 5 ml of mixture $H_2SO_4 + H_3PO_4$ (50:1) with addition of 2.5 ml H_2O_2 on 420°C. After that, micro-Kjeldahl procedure [22] was applied for protein determination. Phytic P (P_{phy}) and total GSH content were determined after extraction: four replicates of each sample (0.25 g) were treated with 10 mL of 5% trichloroacetic acid for 1 h at room temperature in a rotary shaker. The extract was centrifuged on 14,000 rpm for 15 min, and the supernatant was decanted and diluted. Phytic P was determined colorimetrically by the method of Dragičević et al. [23], based on the pink colour of the Wade reagent ($FeCl_3$ + 5-sulfosalycilic acid), formed upon the reaction of ferric ion and sulfosalicylic acid. The absorbance of reaction product was determined at 500 nm. GSH was determined from the same extract as P_{phy}, by adding 0.2 M potassium phosphate buffer (pH = 8.0) and 10 mM DTNB (5.5′-dithio(2-nitrobenzoic acid)) and measuring the absorbance at 415 nm [24]. Free soluble phenolics were determined after 1 h extraction with bi-distilled water by method of Simić et al. [25], based on a slightly modified Prussian blue method where 0.05M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_3Fe(CN)_6$ were added to sample solution. After 25 min, the absorbance of the reaction product was determined at 722 nm.

Statistical analysis

All analyses were performed in four measurements ($n = 4$), and the results were presented as mean ± standard deviation (SD). The differences among chickpea and peanut local landraces, based on mean values of observed parameters, were evaluated using regression analysis and principle component analysis (PCA). Statistical analysis was performed by SPSS 15.0 for Windows Evaluation version.

Results and discussion

Results presented in Table 1 showed that 1,000 seed weight varied in wide range for both species: from 211.7 to 363.6 g for chickpea landraces and from 420.5 to 661.4 g for peanut genotypes. According to Toker and Cagirgan [26], seed weight of chickpea is negatively correlated with yield, thus hindering the breeding for high yielding plants, particularly those with larger seeds. However, weight of peanut seeds is a trait, highly influenced by genotypic effect in compare to other factors, including stress [27].

Variations in oil content were insignificant for chickpea seed, ranging from 4.44% to 5.16%, while for peanut seeds, those variations were higher (i.e., from 43.5% to 52.4%). Our findings were in line with Zia-Ul-Haq et al. [4], who also reported less than 7% of oil content in chickpea seeds. Besides the differences in oil content between chickpea and peanut, there were also the differences in protein content: in peanut seed, protein content was higher, varying in wider range (i.e., from 18.4% to 29.1%) compared to chickpea seeds, where it varied from 11.3% to 17.6%. Obtained results indicated that seeds of examined peanut landraces could be considered as high oil and protein food, consisting of about 66.6% to 74.2% oil + protein content. Dwivedi et al. [12] ascertained that oil and protein content negatively correlate in peanut seeds, which was confirmed with our results, where high oil genotypes, like P1, P7, P8, P9, and P10 have also the lowest protein content. Compared to genotypic variations, environmental factors have shown to have more pronounced effect on variations in oil content of peanut seeds [11].

Group of biomolecules such as antioxidants could additionally improve the nutritional value of produced seeds. Phytic acid, as important antioxidant, could be also considered as antinutrient. According to the results presented in Table 2, examined chickpea landraces have P_{phy} in wide range from 2.39 to 4.46 mg g^{-1}, while in peanut seeds, P_{phy} content ranged from 1.44 to 2.96 mg g^{-1}, being in average by 27% lower compared to chickpea. Since Duhan et al. [28] also determined that chickpea seeds are rich in phytic acid, they recommended soaking, cooking, autoclaving, or sprouting as methods for successful phytate degradation. However, Chung and Champagne [17] found that phytic acid formed complexes with main allergenic proteins from peanut seeds and even suggested that addition of phytic acid to meals that contain peanut could reduce its allergenic properties. Nevertheless, relatively lower P_{phy} implied potentially higher quality of peanut seeds. According to results of our investigation, chickpea genotypes with $P_{\mathrm{phy}} < 2.5$ mg g^{-1}, such as C2, C3, C9, and C10 landraces, could be used for breeding program for further phytate decrease, i.e., increased bioavailability of mineral elements.

Table 1 Weight of 1000 seed, oil and protein content in investigated chickpea and peanut local landraces

Local landraces	1,000 seed weight (g)	Oil (%)	Protein (%)
Chickpea			
C1	330.1 ± 29.9*	4.69 ± 0.43	13.68 ± 0.32
C2	270.3 ± 28.7	4.85 ± 0.49	12.36 ± 0.46
C3	286.6 ± 28.0	4.99 ± 0.43	12.60 ± 0.23
C4	325.7 ± 28.2	4.89 ± 0.46	12.39 ± 0.12
C5	293.2 ± 30.2	4.72 ± 0.46	13.70 ± 0.39
C6	339.5 ± 35.9	4.66 ± 0.66	15.19 ± 0.30
C7	227.5 ± 30.7	4.44 ± 0.74	13.22 ± 0.33
C8	237.8 ± 28.8	5.16 ± 0.48	13.02 ± 0.36
C9	302.1 ± 32.1	4.95 ± 0.43	14.61 ± 0.29
C10	278.3 ± 26.5	4.67 ± 0.43	12.18 ± 0.82
C11	211.7 ± 29.1	4.78 ± 0.64	12.81 ± 0.44
C12	310.1 ± 25.8	4.81 ± 0.53	11.26 ± 0.15
C13	280.1 ± 31.4	5.14 ± 0.76	12.82 ± 0.26
C14	296.0 ± 33.8	4.44 ± 0.82	14.60 ± 0.31
C15	286.4 ± 35.5	4.91 ± 0.56	15.17 ± 0.38
C16	341.3 ± 33.9	4.74 ± 0.45	14.80 ± 0.29
C17	311.1 ± 37.2	4.65 ± 0.70	16.43 ± 0.23
C18	363.6 ± 39.6	4.64 ± 0.50	17.63 ± 0.12
C19	320.8 ± 32.6	4.71 ± 0.46	14.88 ± 0.31
Peanut			
P1	460.0 ± 40.6	52.39 ± 0.40	18.56 ± 0.14
P2	505.7 ± 53.2	45.29 ± 0.29	24.51 ± 0.20
P3	487.8 ± 51.0	47.44 ± 0.30	23.96 ± 0.35
P4	426.1 ± 58.0	43.50 ± 0.31	26.77 ± 0.13
P5	529.4 ± 59.3	45.22 ± 0.51	26.84 ± 0.15
P6	420.5 ± 61.8	45.03 ± 0.41	28.67 ± 0.17
P7	532.3 ± 44.0	48.89 ± 0.51	19.71 ± 0.08
P8	463.3 ± 46.6	48.52 ± 0.48	21.21 ± 0.15
P9	504.1 ± 17.9	48.18 ± 0.44	18.37 ± 7.19
P10	498.1 ± 46.2	48.54 ± 0.43	20.97 ± 0.04
P11	482.8 ± 47.8	46.83 ± 0.43	21.94 ± 0.14
P12	661.4 ± 63.3	44.32 ± 0.35	29.10 ± 0.17
P13	523.2 ± 58.9	47.33 ± 0.42	26.91 ± 0.14

*The results are represented as mean ± SD (standard deviation) in four measurements.

GSH is protein, but it is not obligatory that seeds rich in proteins have high content of thiolic groups and GSH. Dragičević et al. [29] underlined the importance of GSH

Table 2 Phytic P, total glutathione, and phenolics content in seeds of chickpea and peanut local landraces

Local landraces	P_{phy} (mg g^{-1})	GSH (nmol g^{-1})	Phenolics (μg g^{-1})
Chickpea			
C1	2.57 ± 0.09*	1,496.8 ± 21.47	522.7 ± 12.8
C2	2.43 ± 0.08	1,687.3 ± 8.27	571.2 ± 25.9
C3	2.45 ± 0.08	2,197.5 ± 14.34	670.8 ± 3.2
C4	2.77 ± 0.04	1,652.1 ± 13.21	670.6 ± 16.8
C5	2.86 ± 0.02	1,614.2 ± 757.64	678.5 ± 26.9
C6	3.85 ± 0.10	2,008.6 ± 8.25	686.7 ± 7.4
C7	4.04 ± 0.08	1,820.2 ± 10.61	648.5 ± 7.4
C8	2.72 ± 0.07	2,354.8 ± 54.99	962.6 ± 34.8
C9	2.39 ± 0.03	2,364.3 ± 12.60	1,026.6 ± 42.0
C10	2.45 ± 0.02	2,302.2 ± 22.79	1,075.1 ± 25.6
C11	3.48 ± 0.05	2,035.3 ± 4.87	951.0 ± 14.1
C12	3.02 ± 0.12	1,936.2 ± 18.18	851.0 ± 10.2
C13	4.12 ± 0.10	2,099.9 ± 14.58	789.2 ± 15.7
C14	4.46 ± 0.05	1,976.5 ± 762.23	932.0 ± 27.7
C15	3.68 ± 0.05	2,314.0 ± 7.69	1,005.8 ± 52.3
C16	3.01 ± 0.05	2,066.6 ± 20.62	1,051.1 ± 28.7
C17	4.16 ± 0.04	2,210.5 ± 15.15	909.6 ± 30.5
C18	3.39 ± 0.09	1,953.9 ± 6.97	984.4 ± 39.8
C19	3.08 ± 0.02	1,891.4 ± 11.68	992.3 ± 26.4
Peanut			
P1	2.75 ± 0.11	996.5 ± 4.58	801.1 ± 16.8
P2	1.47 ± 0.11	1,002.0 ± 19.88	812.7 ± 553.8
P3	1.44 ± 0.20	1,034.4 ± 8.43	727.3 ± 26.0
P4	1.62 ± 0.23	1,078.4 ± 5.99	993.2 ± 15.7
P5	2.45 ± 0.14	968.3 ± 8.16	916.2 ± 14.8
P6	2.68 ± 0.11	988.6 ± 3.72	751.2 ± 28.6
P7	2.32 ± 0.15	907.5 ± 5.82	1,036.1 ± 26.8
P8	2.95 ± 0.10	1,042.8 ± 5.75	1,369.6 ± 6.2
P9	2.81 ± 0.19	956.5 ± 5.91	1,286.9 ± 27.1
P10	2.68 ± 0.09	1,008.7 ± 3.31	1,020.8 ± 41.5
P11	2.73 ± 0.13	1,016.4 ± 8.10	983.9 ± 17.9
P12	1.75 ± 0.13	1,006.5 ± 2.94	1,214.2 ± 7.7
P13	2.96 ± 0.10	1,232.5 ± 4.12	941.4 ± 20.2

*The results are represented as mean ± SD (standard deviation) in four measurements. P_{phy}, phytic P; GSH, total glutathione.

and other thiolic proteins in soybean grain. In this study, GSH from peanut seeds ranged from 727.3 to 1,369.6 nmol g^{-1} (Table 2), while in chickpea seeds, it was in range from 1,496.8 to 2,364.3 nmol g^{-1}, contributing to higher antioxidant potential of chickpea proteins. Obtained results could be partly supported by the finding of Li et al. [3], who ascertained high antioxidant activity of chickpea protein hydrolysates. Also, relatively high GSH level in examined chickpea landraces (particularly in C8, C9, C10, and C15) could be considered as a good source of thiolic amino acids, contrary to Jukanti et al. [1], who established poor sourcing of chickpea grains with thiolics.

Soluble phenolics content varied in high range in seeds of both species, with slightly higher values observed in peanut landraces. In chickpea seeds, phenolics varied in range from 522.7 to 1,075.1 μg g^{-1} and in peanut seeds in range from 727.3 to 1,369.6 μg g^{-1}. Chérif et al. [7] underlined the importance of phenolics as antifungal factor for chickpea, as well as Yu et al. [14] and Nepote et al. [15] for peanut. The same authors found that peanut skin rich in phenolics content also have high antioxidative activity.

Interactions between main seed constituents, such as oil, proteins, and antioxidants in chickpea seeds, revealed in significant and negative correlation between P_{phy} and oil ($R^2 = 0.159$; Figure 1), as well as in significant and positive correlation between P_{phy} and proteins ($R^2 = 0.165$; Figure 2). Chitra et al. [5] also observed positive correlation between phytate and proteins, but with lower significance compared to other legume seed. This could mean that irrespectively to relatively low oil and protein content in chickpea seed (Table 1), its nutritional quality could be increased by slight oil increase in parallel with phytate decrease. It is also important to underline that this trend was supported by significant and positive interdependence between oil and GSH content. Oppositely, in peanut seeds, highly positive interdependence was found between phytate and oil ($R^2 = 0.273$; Figure 1), as well as between proteins and GSH ($R^2 = 0.149$; Figure 2). In addition, negative interdependence was observed between phytate and protein content ($R^2 = 0.145$). This could indicate that in genotypes with slightly reduced oil and increased protein content, nutritive value of seeds could be higher, due to lower phytate and increased GSH content. This was supported by the findings of Dwivedi et al. [12], who also observed negative correlation between oil and protein content in peanut seeds.

Projection of variables in PCA revealed that in chickpea seeds, GSH and phenolics contributed to PCA1, which explained 39.40% of the total variability (Table 3). The second axis (PCA2), which explained 34.60% of the variation, was defined only with oil content. This means that traits, such as GSH and phenolics, vary simultaneously. In peanut seeds, oil and proteins contributed to PCA1, which explained 46.60% of the total variability while P_{phy} and GSH contributed to PCA2, which explained 23.10% of the variation. According to the results presented, oil and proteins in peanut seeds vary simultaneously, but in opposite directions. Independent to

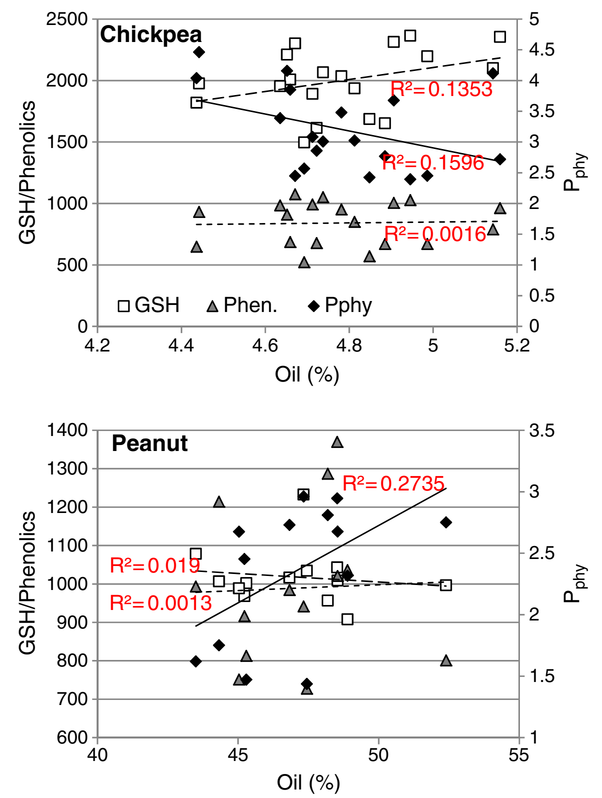

Figure 1 Regression between oil content, phytic P, glutathione, and phenolics in seeds of chickpea and peanut landraces.

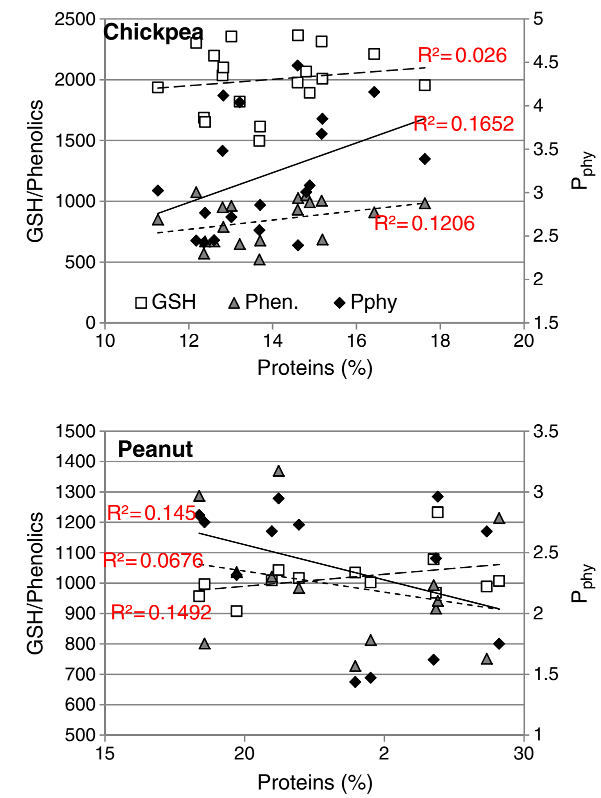

Figure 2 Regression between protein content, phytic P, glutathione, and phenolics in seeds of chickpea and peanut landraces.

main constituents of peanut seed, antioxidants such as phytate and GSH vary simultaneously with higher significance of GSH. This could be important in further breeding programs considering investigated genotypes, since increase in GSH implies P_{phy} increase to some extent.

Experimental

Plant material

Nineteen chickpea and 13 peanut local landraces from Macedonian gene bank (harvested in 2011) were the objectives of the present study.

Conclusions

Based on the results obtained, it could be concluded that investigated chickpea and peanut genotypes could be considered as highly valuable foods, particularly in vegetarian diets. This could underline the necessity for further breeding, especially if increase in the level of antioxidants is taken into account.

Chickpea landraces, with relatively low oil and protein content could be additionally improved by breeding with slight oil increase, which is related to phytate decrease. It is important to observe that protein composition could be enhanced by GSH increase, along with increase of phenolics, which could reflect in increased antioxidative capacity. Obtained results indicate that C8, CP9, C10, and C15 local landraces could be used as potential source of increased thiolic content in further breeding programs.

Guidelines for further peanut breeding could be associated with oil and/or protein content increase. Parallel increase in proteins and GSH is connected with phytate reduction, which could have positive impact on the availability of mineral elements. However, if positive impact of

Table 3 Principal component analysis for seed chemical composition of chickpea and peanut local landraces

	Chickpea		Peanut	
Variable	PCA1*	PCA2	PCA1	PCA2
Oil	0.097	0.647	0.579	−0.024
Proteins	−0.485	−0.336	−0.600	0.220
P_{phy}**	−0.356	−0.450	0.443	0.522
GSH	−0.522	0.449	−0.213	0.763
Phenolics	−0.597	0.254	0.250	0.313
Explained variance	1.971	1.732	2.329	1.157
Proportion of total variance (%)	39.40	34.60	46.60	23.10

*Synthetic variables: PCA1, principal component axis 1 and PCA2, principal component axis 2. PCA, principal component analysis; **P_{phy}, phytic P; GSH, glutathione.

phytate on lowering of allergenic properties of peanut seeds was taken into consideration, it's increase, along with GSH and phenolic increase, could additionally raise antioxidative properties of peanut seeds.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
VD and SK designed the research. SK and ZD (Dimov) provided seeds of chosen chickpea and peanut local landraces for the analyses. VD performed chemical analyses. ZD (Dumanović) analyzed the data. VD and NK wrote the paper. All authors read and approved the final manuscript.

Acknowledgements
This study was supported by Project TR31068 'Improving the quality of maize and soybean by conventional and molecular breeding' from the Ministry of Education, Science and Technological Development, Republic of Serbia and by COST Action FA 0905 'Mineral Improved Crop Production for Healthy Food and Feed'.

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Received: 22 September 2014 Accepted: 6 January 2015
Published online: 01 February 2015

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