

## CHARACTERIZATION OF PROTEIN FRACTIONS AND ANTIOXIDANT ACTIVITY OF CHIA SEEDS (*Salvia Hispanica* L.)

*Kvetoslava Kačmárová, Blažena Lavová, Peter Socha, Dana Urminská*

### ABSTRACT

Chia seed (*Salvia hispanica* L.) is an annual herbaceous plant categorized under *Lamiaceae* family. Chia seeds were investigated as a source of proteins and natural antioxidants. It is a potential alternative source of high quality protein, fats, carbohydrates, high dietary fibre, vitamins and mineral elements. The objective of this study was to evaluate chia seed from protein content and antioxidant activity and highlight the quality of this pseudocereal. A crude protein, moisture content, content of protein fractions, total antioxidant capacity (TAC) and superoxide dismutase activity of chia seeds and food products containing chia seeds were determined. The protein content of chia seeds ranged from 2.9% to 4.6% dry matter from that albumins and globulins ranged from 54.6% to 62.8%. Chia is poor in prolamin ( $<15\%$ ). Various chia seeds showed differences in their SOD activity and exhibited the high antiradical activity against 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The highest antioxidant capacity was found in sample chia seeds from Bolivia (1.46 mM TEAC.g<sup>-1</sup> in the dry matter) and the lowest values of antioxidant activity was estimated in sample chia seeds from Argentina (1.05 mM TEAC.g<sup>-1</sup> in the dry matter). The highest SOD activity was determined in sample chia from Argentina (2191.8 U.g<sup>-1</sup> in the dry matter). The lowest SOD activity was found in sample chia-bio from Argentina (754.0 U.g<sup>-1</sup> in the dry matter). It makes them potentially suitable for use in the gluten-free diet of coeliac people and it can be used as a potential ingredient in health food because of its high antioxidant activity.

**Keywords:** chia seed; protein; protein fraction; antioxidant activity

### INTRODUCTION

In recent years, demand for food with multiple health benefits has increased. There is an interest to introduce a new food to prevent various disorders (Mohd Ali et al., 2012).

During recent decades, it has been demonstrated worldwide increase in allergies and intolerances to certain foods, which is associated with nutrition, lifestyle, economic growth and urbanization (Gilissen et al., 2014). For example, the most common food-induced enteropathy, caused by intolerance to cereal proteins (gluten), is coeliac disease, also termed coeliac sprue. Large number of coeliac patients is also lactose-intolerant. It leads to mineral, vitamin and protein deficiencies in the diet suitable for them (Arendt et al., 2011).

Chia seeds (*Salvia hispanica* L.) are one of the potential alternative sources of high quality protein, fats, carbohydrates, high dietary fibre, vitamins and mineral elements. They also contain a high amount of antioxidants, and therefore are reintroduced to diets to provide health benefits for patients and healthy persons (Segura-Campos et al., 2014).

*Salvia hispanica* L. is a plant of the *Lamiaceae* or *Labiatae* family native of central and southern America, and grows in arid climates. It can grow up to 1 meter tall, has opposite arranged leaves and small flowers (Figure 1). It produces a small white and dark seeds (Mohd Ali et al., 2012), which are considered a pseudocereals and an oilseeds (Figure 2) (Sandoval-Oliveros and Paredes-López, 2012).

Due to the composition of seeds, they present a good alternative source of proteins for humans. Chia seeds contain a higher amount of proteins (19-23%) than other traditionally used grains, such as wheat (14%), barley (9.2%), oats (15.3%), corn (14%) and rice (8.5%) (Monroy-Torres et al., 2008; Sandoval-Oliveros and Paredes-López, 2012).

Determinant of quality proteins is digestibility. It is the amount of protein absorbed into the body relative to the amount that was consumed. Protein digestibility of chia flour is 79.8%, according to Monroy-Torres et al., (2008), as well as cereals processed for direct consumption (corn, wheat, oats, etc.).

Chia seeds are also rich in natural antioxidants, especially phenolic compounds such as chlorogenic acids, caffeic acids, kaempferol and quercetin. All of the mentioned characteristics may reduce cardiovascular diseases, regulate an intestinal transit or prevent of some diseases such as type II diabetes and some types of cancer (Sandoval-Oliveros and Paredes-López, 2012).

The main objective of the present study is to characterize and evaluate a content of proteins, protein fractions and antioxidant activity in Slovakia commercially available chia seeds.

### MATERIAL AND METHODOLOGY

Chia seeds (*Salvia hispanica* L.) were obtained from local markets in Nitra, Slovak Republic. Three types of chia seeds from different producers were used for analysis. The first sample of chia seeds is originating in Bolivia, the



**Figure 1** *Salvia hispanica* L. plant, (Mohd Ali et al., 2012).



**Figure 2** Chia seed, (Mohd Ali et al., 2012).

second sample of chia was originating in Argentina and as produced by ecological farming (bio). The last sample of chia was harvested also in Argentina (conventional farming). For analysis, we used a bio-raw apricot flapjack (containing 4% of chia seeds) and chia spelled biscuits (containing 3% of chia seeds). Flours and food products for analyses were prepared by milling (BOSCH, MKM 6000).

Moisture content was determined according to the ICC Standard Method No. 110/1 for cereals and cereal products. Approximately 8 g of each sample of seeds were weighed into special aluminium dishes and dried until constant weight, using a moisture analyzer KERN DBS 60-3.

For determination of crude protein content was used 500 mg of each sample of milled chia flours and products with chia seeds. Nitrogen content was measured by the Kjeldahl method according to the ICC Standard Method No. 105/2 (1994). The samples were digested in a Kjeldahl Digestion Unit type DK6 (Velp Scientifica), using cupric sulfate and potassium sulfate as catalysts. The digested samples were then distilled using UDK 127 Distillation Unit (Velp Scientifica) and the distillates were titrated with  $H_2SO_4$  ( $c = 0,1 M$ ). The protein content was calculated as nitrogen x conversion factor  $f$  ( $N \times 6,25$ ).

For extraction of protein fractions was used 2500 mg of each sample of chia flours and milled food products. Fractionation of proteins (albumin, globulins, prolamins and glutelins) was carried out according to the Golenkov,

using modification of the method reported by Michalik (2002). The protein content of the isolated fractions was assessed by Kjeldahl method.

In this study the QUENCHER procedure was used to measure the total antioxidant capacity (TAC) using ABTS<sup>+</sup> assay (Serpen et al., 2012). All three samples of chia seeds needed to be diluted at 1:1 (w/w) with cellulose. ABTS was dissolved in deionized water to a concentration of 7 mM. The radical cation of ABTS was obtained by reaction with 2.45 mM potassium persulfate and allowing the stock solution to stand in the dark at room temperature for at least 12 hours (Re et al., 1999). The working solution of ABTS<sup>+</sup> was prepared by diluting 10 mL of ABTS<sup>+</sup> stock solution with approximately 800 mL of a water/ethanol (50:50, v/v) mixture. The working solution absorbance was 0.750 – 0.800 at 734 nm (Sargi et al., 2013). Ten ( $\pm 1.0$ ) mg of powdered sample was weighed into a centrifuge tube having 15 mL capacity. The reaction was started by adding 10 mL of ABTS<sup>+</sup> working solution. The tube was shaken rigorously for 1 minute and placed on shaker in the dark. The mixture was shaken at 350 rpm at room temperature on the shaker (ThermoMixer C, Eppendorf) to facilitate the surface reaction between the solid samples and ABTS<sup>+</sup> solution. After exactly 30 minutes for ABTS probe from the first introduction of radical/oxidant solution onto solid samples, centrifugation (Avanti J-25, Beckman Coulter) was performed at 9,200 x g for 2 minutes. Optically clear supernatants were transferred into spectrophotometric cuvette and the absorbance values were measured at 734 nm for ABTS assay (6705 UV/VIS spectrophotometer, JENWAY). The TAC of samples determined with ABTS assay were calculated in mmol of Trolox equivalent antioxidant capacity (TEAC) per g of sample using the calibration curves (Serpen et al., 2012).

In this study the diagnostic Ransod set (RANDOX, Great Britain) was used for the determination of superoxide dismutase activity. The principle of the method was based on the xanthine and xanthine oxidase that produce superoxide radicals reacting with tetrazolium salt to red formasan. SOD activity is determined as a degree of inhibition of this reaction which occurs at 37 °C. Following preparation was identical both for the prepared yeast samples and standards from which the calibration curve was constructed (Březinová Belcredi et al., 2010).

The chia seeds were homogenized in chilled 0.1 M sodium phosphate buffer (pH 7.4) to prepare a 10% homogenate. The homogenate was centrifuged at 10,000 x g at 4 °C for 10 minutes and the supernatant was used for assays (Sangeetha, 2010). The sample (0.05 mL) and the substrate (1.7 mL) were added into a cuvette and the mixture was carefully blended. Reaction was started by addition of xanthine oxidase (0.25 mL). The cuvette was placed into the spectrophotometer and an absorbance of 505 nm was measured. The first absorbance was measured after 30 seconds ( $A_1$ ) and the second after 3 minutes ( $A_2$ ). The result was converting to SOD units/g of sample.

## RESULTS AND DISCUSSION

The moisture content, the total protein content and the proportion of the protein fractions of chia seeds and products with chia are summarized in the Table 1.

The moisture content of chia samples ranged from 5.8 to 6.72%. These results are within the range of 4.5% to 6.8% reported by numerous authors (Monroy-Torres et al. 2008; Coorey et al. 2012; Sandoval-Oliveros and Paredes-Lopéz 2012; Segura-Campos et al. 2014).

Chia seeds are characterized by a high protein content. Studies according to Monroy-Torres et al., (2008), Sandoval-Oliveros and Paredes-Lopéz (2012) and Segura-Campos et al., (2014), describe the value of protein content of 15 – 23%. In the present study, protein content of all samples of chia flour was very low and ranged from 2.9 to 4.6%. It could be caused by using unmodified chia flours. Sandoval-Oliveros and Paredes-Lopéz (2012) used defatted and dried flours of mucilage-free chia seeds for the same analysis, with the result 23% of proteins in dry solids.

After protein extraction and fractionation by solubility, all fractions were quantified by Kjeldahl method. The proportion obtained from chia 1 (Bolivia) was 55.8% of crude albumins and globulins, 13.8% of prolamins, 9.5% of glutelins, whereas 20.9% of the protein wasn't recovered. The proportion obtained from chia 2 (bio-chia, Argentina) was 62.8% of crude albumins and globulins, 14.2% of prolamins, 15.1% of glutelins, whereas 7.9% of insoluble residue. In the chia 3 (Argentina), the content of albumins was 54.6%, 12.5% were prolamins, 15.2% were glutelins and the content of insoluble residue was 17.7%. Albumins and globulins were the most abundant (from 55.8% to 62.8%) followed by glutelins (9.5% – 15.2%), and prolamins (12.5 – 14.2%). The values are similar to those reported by Sandoval-Oliveros and Paredes-López (2012), excluding the values of the insoluble residues (7.9 – 20.9%), which were higher.

Palenčárová and Gálová (2009) investigated the proportion of each protein fraction in selected cereals. Compared to their results, chia seeds contained double amount of nutritionally valuable fraction of storage proteins, albumins and globulins, compared to the common used cereals (wheat 25.4%, barley 27.12%, rye 41.34% and oats 20.22%). The content of the celiac active prolamin fraction was twice lower, compared to wheat (36.7%), barley (32.57%) and rye (28.75%). The prolamin content in chia was also lower than that of oats (16.65%). The glutelins content was determined lower, and content of insoluble residues was detected higher in chia seeds to

that of above-mentioned cereals. The values of mentioned protein fractions are also consistent with those reported by Gálová et al., (2011).

The dominance of albumin and globulin fractions was proved in pseudocereals such as amaranth (Hricová et al., 2011) and buckwheat (Guo and Yao, 2006). The only difference found between amaranth (*Amaranthus cruentus*) and chia was a higher proportion of glutelins and lower proportion of prolamins in chia seeds. On the other hand, the protein fractions of chia and buckwheat (*Fagopyrum tataricum*) were very similar, except insoluble residues that were not mentioned by authors Guo and Yao (2006).

From the results shown in Table 1 it follows that a content of crude protein detected in chia biscuits and apricot chia flapjack was 1.3% and 1.5%, respectively. Both types of chia meals presented a various composition of protein fractions. The main protein fraction corresponded to glutelins (41.9%) and fractions of albumins and globulins (39.3%) in spelt chia biscuits and apricot flapjack, respectively. The food products contain low level of prolamins, 11.9% in spelt biscuits and 17.3% in apricot flapjack. The differences were caused by various compositions of these meals, which represent a complex food composed of several different nutritionally valuable constituents. Spelt and oat, the two main components of the products, were also different in the nutritional value, thus in the composition of protein fractions (Socha et al., 2010).

The values of a crude protein in products were also different than those found for chia seed samples. From the nutrition point of view, chia seeds are better source of valuable proteins, compared to food products containing insignificant amount of chia seeds.

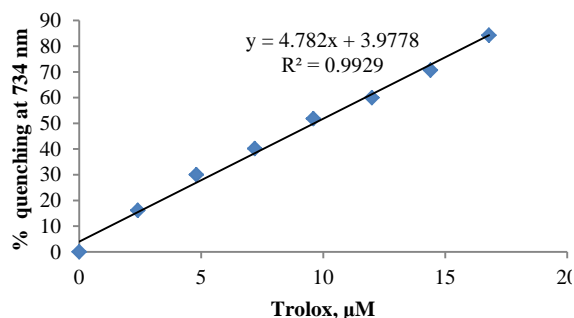
In the present study was used TAC measurement by QUENCHER method. The QUENCHER procedure eliminates time-consuming extraction steps, which assists to build a unique database and ease of comparison for the TAC of different food types. The solvent composition of probe radical solution had a significant influence on TAC measured by direct QUENCHER. In this procedure, the solvent not only acted as a reactant carrier but also a food matrix solubilizer (Serpen et al., 2012).

Based on the results of trolox standard curve (Figure 3) it was possible to calculate the trolox equivalent antioxidant capacity for chia seeds.

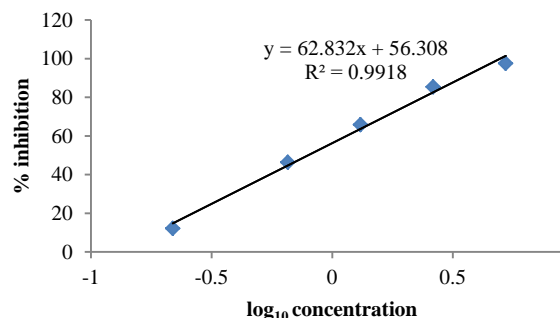
**Table 1** The total protein content and the proportion of the protein fractions of chia seeds and products containing chia.

Sample (Country of Origin)	Content of Chia seeds (%)	Crude Protein (%)	Albumins and Globulins (%)	Glutelins (%)	Insoluble Residue (%)
Chia (Bolivia)	100	2.9	55.8	9.5	20.9
Chia, bio (Argentina)	100	3.6	62.8	15.1	7.9
Chia (Argentina)	100	4.6	54.6	15.2	17.7
Apricot flapjack (United Kingdom)	4	1.5	39.3	26.2	17.2
Chia spelted biscuits (Slovakia)	3	1.3	26.9	41.9	19.3
n*	-	3	3	3	3

**Figure 3** Percentage quenching of absorbance at 734 nm as a function of Trolox concentrations built by using ABTS<sup>+</sup> working solution.



**Figure 4** Calibration curve using for calculation of SOD activity.



**Table 2** Antioxidant capacity (ABT<sup>+</sup>) and superoxide dismutase activity of chia seeds in the dry matter.

Samples (Country of Origin)	ATBS (mM TEAC.g <sup>-1</sup> d.w.)	SOD (U.g <sup>-1</sup> d.w.)	Moisture (%)
Chia (Bolivia)	1.46	1231.4	5.80
Chia, bio (Argentina)	1.13	754.0	6.72
Chia (Argentina)	1.05	2191.8	6.62
n*	3	3	3

\* The number of repetitions, TEAC = trolox Equivalent Antioxidant Capacity, d.w. = dry weight.

Based on results (Table 2) it can be concluded that the highest antioxidant capacity was found in sample chia seeds from Bolivia (1.46 mM TEAC.g<sup>-1</sup> in the dry matter) and the lowest values of antioxidant activity was estimated in sample chia seeds from Argentina (1.05 mM TEAC.g<sup>-1</sup> in the dry matter). As seen from the Table 2, obtained data showed that there was a difference between TAC of tested samples which can be caused by a different variety or growing conditions.

Serpen et al., (2012) used the QUENCHER procedure for the ABTS<sup>+</sup> assay and they determined the antioxidant capacity of some seeds such as wheat (17.0 mM TEAC.kg<sup>-1</sup> in the dry matter.), rice (14.9 mM TEAC.kg<sup>-1</sup> in the dry matter) and rye (32.7 mM TEAC.kg<sup>-1</sup> in the dry matter). These results are lower than those found for all seeds in present study for the ABTS<sup>+</sup> assay.

Sargi et al., (2013) determined the antioxidant capacity of chia seeds 2.56 mM TEAC.g<sup>-1</sup> in the dry matter. These results are higher than those found for seeds in present study. Vázquez-Ovando et al., (2009) found that antioxidant activity in the fiber-rich fraction of chia was 488 μM TEAC.g<sup>-1</sup> in the dry matter, Marineli et al., (2014) reported for Chilean chia seeds 436 μM TEAC.g<sup>-1</sup> and for Argentina chia meals Capitani et al., (2012) reported 557.2 μM TEAC.g<sup>-1</sup> in the dry matter.

Chia is considered a seed with high antioxidant capacity, because is loaded with high amount of phenolic compounds (Martínez-Cruz and Peredes-López, 2014). In this study was measured activity of superoxide dismutase, which protects the organism against the oxidative damage caused by active oxygen forms (Piterková et al., 2005). In this study was used a standard curve for determination of SOD activity (Figure 4).

The highest SOD activity was determined in sample chia from Argentina (2191.8 U.g<sup>-1</sup> in the dry matter). The

lowest SOD activity was found in sample chia-bio from Argentina (754.0 U.g<sup>-1</sup> in the dry matter.). There is no exact information about SOD activity in chia seed, so Kolahi-Ahari (2006) determined SOD activity in different species of kiwifruit on the level about 40 U.g<sup>-1</sup> fresh weight. Březinová-Belcredi et al., (2010) detected superoxide dismutase activity in grain samples of 12 varieties and lines of spring barley in the interval 62 – 147 U.g<sup>-1</sup> in the dry matter. In comparison with these results it can be concluded that SOD activity in chia seeds is very high. According to the results in Table 2 it is evident that SOD activity in chia tested samples was very high. Detection of high antioxidant activity and SOD activity in chia seeds indicate the chia seeds to be a potential ingredient in health food products such as nutrition bars or cookies.

## CONCLUSION

Based on the current research findings, chia seed is a good source of valuable protein fractions (albumins and globulins) and antioxidant compounds. The content of prolamins is low (<15%) what makes chia seeds potentially useful in the preparation of gluten-free products suitable for celiacs. The isolation and preparation of selected compounds from chia seeds could be used to produce potent natural antioxidants or ingredients with commercial applications in pharmacy, food industry or as a dietary supplements.

## REFERENCES

Arendt, E. K., Moroni, A., Zannini, E. 2011. Medical nutrition therapy: use of sourdough lactic acid bacteria as a cell factory for delivering functional biomolecules and food ingredients in gluten free bread. *Microbial Cell Factories*,

vol. 10, suppl. 1, p. 1-7. <http://dx.doi.org/10.1186/1475-2859-10-S1-S15> PMID:PMC3231922

Březinová Belcredi, N., Ehrenbergerová, J., Vaculová, K. 2010. Antioxidační aktivita enzymu superoxidodismutasy v zrna ječmene jarního. *Kvasný průmysl*, vol. 56, no. 3, p. 128.

Capitani, M. I., Spotorno, V., Nolasco, S. M., Tomás, M. C. 2012. Physicochemical and functional characterization of by-products from chia (*Salvia hispanica* L.) seeds of Argentina. *LWT - Food Science and Technology*, vol. 45, no. 1, p. 94-102.

Gilissen, L., Van Der Meer, I., Smulders, M. 2014. Reducing the incidence of allergy and intolerance to cereals. *Journal of Cereal Science*, vol. 59, no. 3, p. 337-345. <http://dx.doi.org/10.1016/j.jcs.2014.01.005>

ICC Standard Method No. 110/1. 1976. *Determination of moisture content of cereals and cereal products (Practical Method)*. International Association for Cereal Science and Technology, Vienna.

ICC Standard Method No. 105/2. 1994. *Determination of crude protein in cereals and cereal products for food and for feed*. International Association for Cereal Science and Technology, Vienna.

Kolahi-Ahari, A. 2006. *A study of superoxide dismutase activity and superoxide production in kiwifruit*. A thesis for degree of M.Sc., Canterbury : University of Canterbury, p. 39.

Marineli, Rd. S., Moraes, É. A., Ienquiste, S. A., Godoy, A. T., Eberlin, M. N., Maróstica, M. R. Jr. 2014. Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.). *LWT – Food Science and Technology*, vol. 59, no. 2, p. 1304-1340.

Martínez-Cruz, O., Peredes-López, O. 2014. Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica* L.) by ultra high performance liquid chromatography. *Journal of Chromatography A*, 1346, p. 43-48. <http://dx.doi.org/10.1016/j.chroma.2014.04.007> PMID:24811150

Michalík, I. 2002. Unifikovaná metóda diskontinuálnej frakcionácie bielkovinového komplexu zrna obilnín (Unified method of discontinuous fractionation of protein complex in grain cereals). *Polnohospodárstvo*, vol. 48, no. 7., p. 333-341.

Mohd Ali, N., Keong Yeap, S., Yong Ho, W., Kee Beh, B., Wei Tan, S., Guan Tan, S. 2012. The promising future of Chia, *Salvia hispanica* L. *Journal of Biomedicine and Biotechnology*, vol. 2012, no. 1, p. 1-9.

Monroy-Torres, R., Mancilla-Escobar, M. L., Gallaga-Solórzano, J. C., Medina-Godoy, S., Santiago-García, E. J. 2008. Protein digestibility of chia seed *Salvia hispanica* L. *Revista Salud Pública y Nutrición*, vol. 9, no. 1, p. 1-6.

Piterkova, J., Tomankova, K., Luhova, L., Petřivalsky, M., Peč, P. 2005. Oxidativní stres: Lokalizace tvorby aktivních forem kyslíku a jejich degradace v rostlinném organismu. *Chemické Listy*, vol. 99, p. 455-466.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, vol. 26, no. 9/10, p. 1231-1237. [http://dx.doi.org/10.1016/S0891-5849\(98\)00315-3](http://dx.doi.org/10.1016/S0891-5849(98)00315-3)

Sandoval-Oliveros, M. R., Paredes-López, O. 2012. Isolation and Characterization of Proteins from Chia Seeds (*Salvia hispanica* L.). *Journal of Agricultural and Food Chemistry*, vol. 61, no. 11, p. 193-201.

Sangeetha, R. 2010. Activity of superoxide dismutase and catalase in fenugreek (*Trigonella foenum-graecum*) in response to carbendazim. *Indian Journal of Pharmaceutical Sciences*, vol. 72, no. 1, p. 116-118.

<http://dx.doi.org/10.4103/0250-474X.62248> PMID:20582202

Sargi, S. C., Silva, B. C., Santos, H. M. C., Montanher, P. F., Boeing, J. S., Santos-Júnior, O. O., Souza, N. E., Visentainer, J. V. 2013. Antioxidant capacity and chemical composition in seeds rich in omega -3: chia, flax, and perilla. *Food Science and Technology (Campinas)*, vol. 33, no. 3. <http://dx.doi.org/10.1590/s0101-20612013005000057>

Segura-Campos, M. R., Ciau-Solis, N., Rosado-Rubio, G., Chel-Guerrero, L., Betancur-Ancona, D. 2014. Physicochemical characterizations of chia (*Salvia hispanica*) seed oil from Yucatán, Mexico. *Agricultural Sciences*, vol. 5, no. 3, p. 220-226. <http://dx.doi.org/10.4236/as.2014.53025>

Serpen, A., Gökmen, V., Fogliano, V. 2012. Solvent effects on total antioxidant capacity of foods measured by direct QUENCHER procedure. *Journal of Food Composition and Analysis*, vol. 26, no. 1-2, p. 52-57. <http://dx.doi.org/10.1016/j.jfca.2012.02.005>

Socha, P., Raždíková, A., Urmínská, D. 2010. Optimalizácia stanovenia prítomnosti celiakálne aktívnych bielkovín v cereáliách a pseudocereáliách (Optimization of celiac disease active proteins estimation in cereals and pseudocereals). *Potravinárstvo*, vol. 4, special no., p. 497-505. Available at:

[http://www.potravinarstvo.com/dokumenty/mc\\_februar\\_2010/pdf/5/Socha.pdf](http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/5/Socha.pdf)

Vazques-Ovando, A., Rosado-rubio, G., Chel-Guerrero, L., Betancur-Ancona, D. 2009. Physicochemical properties of a fibrous fraction from chia (*Salvia hispanica* L.). *LWT-Food Science and Technology*, vol. 42, no. 1, p. 168-173.

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#### Contact address:

Kvetoslava Kačmárová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: [vkacmarova@is.uniag.sk](mailto:vkacmarova@is.uniag.sk).

Blažena Lavová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: [blazena.lavova@uniag.sk](mailto:blazena.lavova@uniag.sk).

Peter Socha, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology and Center of AgroBioTech, Tr. A. Hlinku 2, 949 76 Nitra Slovakia, E-mail: [peter.socha@uniag.sk](mailto:peter.socha@uniag.sk).

Dana Urmínská, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra Slovakia and Institute of Chemistry, Slovak Academy of Sciences, Center for White-Green Biotechnology, Tr. A. Hlinku 2, SK-949 76 Nitra, E-mail: [dana.urminska@uniag.sk](mailto:dana.urminska@uniag.sk).