Partially Defatted Pumpkin (*Cucurbita maxima*) Seeds - a Rich Source of Nutrients for Use in Food Products

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Pumpkin (Cucurbita maxima) seeds are a rich source of ingredients such as aminoacids, fatty acids, minerals, and phytochemicals exhibiting nutraceutical effects on human health. In this work, partially defatted pumpkin seeds flour, a by-product obtained during the manufacture of pumpkin seeds oil, was studied as an additive for common wheat flour. We explored the physico-chemical properties as well the content in amino acids of the partially defatted pumpkin seeds. The obtained results revealed that partially defatted pumpkin seeds are a good source of protein (42.75% d.m.), lipids (12.28% d.m.), total carbohydrates (37.4% d.m.), from which crude fiber (26.64% d.m.). This by-product presents a high mineral content (mg/100g): potassium (1290), magnesium (693), iron (87.8), zinc (11.5) and copper (2.49).The partially defatted pumpkin seeds proteins contain significant amounts of essential amino acids such as valine, histidine, isoleucine, leucine, threonine and methionine.

Key words: pumpkin seeds, nutritional quality, crude fiber, mineral content

Pumpkins belong to the *Cucurbitaceae* family. *Cucurbita maxima* is one of five species of *Cucurbita sp.* cultivated for pulp, flowers and seeds. This species originates in South America, where the wild form *(Andreea cucurbita)* has been known for over 4000 years [1]. It was introduced in Europe at the end of 19th century. Later, the culture expanded to the southern European countries neighboring the Mediterranean Sea, after which it also penetrated Balkan countries such as Turkey, Greece and Bulgaria.

On the territory of Romania the edible pumpkin culture appeared at the middle of 17th century, first in Wallachia, after which it spread in Moldova. Currently, in our country, edible pumpkin is grown on relatively large surfaces, especially in the southern, southwestern and partly south-eastern areas, both in basic crops and in crops combined with corn. In some countries, the pumpkin flower is consumed as a vegetable; the fruit is preserved, under suitable conditions of light, temperature and humidity, for up to 6 months under good conditions. It is rich in â-carotene and glucose.

A good pumpkin production, as in the case of all crops, can only be achieved by taking into account soil composition and structure, crop rotation and climatic conditions in the cultivated area (especially rainfall and temperature) [2-7]. Also, it should be considered the influence of pollutants (industrial, domestic, zootechnical, etc.) that reach the soil [8-11], which may harm plant development, thus reducing the quantity and quality (the content in active principles) of fruit or vegetables cultivated on that surface [12-16]. Preserved in inappropriate conditions after harvesting, pumpkins could be contaminated with aflatoxins and ochratoxins; these situations impose rapid and specific methods for detection of these harmful substances [17-21].

The seeds of this fruit are known as *pumpkin seeds* and therapeutic properties are attributed to them in natural medicine [22].

Pumpkin seeds are rich in proteins, unsaturated fatty acids, fibers, antioxidant vitamins such as carotenoids and tocopherols [23] and have an important content of minerals, especially zinc, which is why the World Health Organization recommends their daily intake.

C. maxima seeds have oil content of 11 to 31%, of which 73.1 to 80.5% are total unsaturated fatty acids [24]. Total tocopherol content in pumpkin seeds was measured to be 15.9 mg/100 g [25]. Furthermore, the oil extraction method plays decisive role in antioxidant content [26,27]. Microwave-assisted aqueous enzymatic [28, 29] and ultrasound - assisted [30] oil extraction methods have been found more effective than other existing methods in retaining of antioxidants in the oil.

They are a good source of vitamin K. β-carotene seed content reduces skin damage caused by sun exposure and acts as an anti-inflammatory agent. α-Carotene slows the aging process, reduces the risk of cataracts and prevents tumors. Vitamin E (tocopherol) protects the oxalate process tube by preventing oxidation of fatty acids from the cell membrane [24].

Various studies have reported that pumpkin seeds can benefit in the treatment of benign prostatic hyperplasia due to the high content in phytosterols [31, 32]. β-sitosterol is indicated for reducing the cholesterol in the blood and the risks of certain types of cancer [33]. Following the study of seed composition of various pumpkin species cultivated in Korea, it has been demonstrated that pumpkins have considerable variations in nutrient content, depending on the growing medium and the species [33]. Also in this study, general chemical compositions and some

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bioactive components (such as tocopherols, carotenoids, and â-sitosterols) were analyzed for three major pumpkin species (*Cucurbitaceae pepo*, *C. moschata* and *C. maxima*) cultivated in Korea, and also for three parts (skin, pulp, and seeds) of each pumpkin species. Authors concluded that *C. maxima* had a significantly higher content in carbohydrates, proteins, lipids, and fibers than *C. pepo* or *C. moschata* [33].

Habib et al. [34] analyzed the composition of pumpkin seeds cultivated in Bangladesh and obtained the following results: moisture 4.06%, ash 3.80%, crude cellulose 2.91%, total lipids 36.70%, total protein 34.56%, sugar 1.08%, and 2.15% starch. The oil extract content was 12% and the GLC analysis of the oil indicated that it contained 40.58% oleic acid, while the content of stearic acid, linoleic acid, and palmitic acid was 27.06, 17.39, and 14.97% respectively.

The objective of this work was to characterize the partially defatted pumpkin seeds through evaluation of their nutritional composition such as crude fibers, minerals and amino acids, which are main quality attributes of this by-product for use in food industry.

Experimental part

Materials and methods

Partially defatted pumpkin seed flour, a by-product obtained during manufacture of pumpkin seed oil, was obtained from pumpkin (*C. maxima*) seeds on a large scale through hulling, grinding and degreasing at low temperatures (below 40 ^{0}C). The degree of damage to the components of this material may be considered to be low because all steps were performed at low temperature.

Moisture was determined at 103^{0}C (±2^{0}C) until constant weight (ICC Standard No. 110/1).

Total fat was determined by extracting 10 g of sample with petroleum ether at 40-65 ^{0}C, using a Soxhlet apparatus.

Total nitrogen was analysed following Kjeldahl method (SR ISO 20483/2007). Ash content (SR ISO 2171/2009) in muffle furnace at 450-500 ^{0}C. Crude protein content was calculated by multiplying total nitrogen content with the factor 6.25. The crude fiber content of the samples was determined using a Fibertherm-Gerhardt apparatus.

The method for determination of crude fibers begins with treating the sample with an acid detergent solution (20 g N-cetyl-N,N,N-trimethylammonium bromide dissolved in 1 L sulfuric acid 0.5 M). In this solution, cellulose and lignin from the analysed material are insoluble, unlike all other components. Using special FiberBags, the dilution and filtration steps are simplified. The most important aspects of this method of analysis of fibers are adherence to strict boiling times and to weighing procedures.

After treatment with the acid detergent solution, the insoluble residue is dried, weighed and then burnt. The acid detergent fiber (ADF) content represents the insoluble part of the sample that is left after boiling in acid detergent solution from which the ash obtained upon calcination is subtracted:

$$\%ADF = \frac{((\chi-\alpha)-(\delta-\xi))*100}{\beta};$$

$$blank\ value\ (\xi) = \gamma - \psi$$

where:α - mass of FiberBag (g); β - sample mass (g); χ - mass of crucible and dried FiberBag, after digestion (g); δ- mass of crucible and and ash (g); ξ = blank value of empty FiberBag (g); γ - mass of crucible and ash of the empty FiberBag (g); ψ - mass of crucible (g).

Carbohydrate contents were calculated as the difference of 100-(ash+protein+fat+moisture).

Mineral content was determined using an atomic absorption spectrophotometer (ContrAA 700; Analytik Jena). Total ash was determined by incineration at 550°C, in an oven. Analysis was performed using an external standard (Merck, multi element standard solution) and calibration curves for all minerals were obtained using 6 different concentrations. Dried samples were digested in concentrated HCl.

For the analysis of amino acids content, samples were hydrolyzed at 100-120^{0}C in 6N hydrochloric acid for 22-24 h, under vacuum. After evaporation to dryness of hydrochloric acid, the dry residue was diluted using 4 mM stock solution of norleucine. For the separation of amino acids by gradient anion exchange with pulsed electrochemical detection (PED) was used an ICS300 (Dionex, USA) equipment with the following eluents: deionized water, 0.250 M NaOH and 1M CH_3COONa.

The chemical score of partially defatted pumpkin seeds flour was calculated according to FAO/WHO (1985) as being the ratio between mg/g essential amino acid in test protein and mg/g of essential amino acid in reference protein, multiplied by 100.

Using ^{1}H-NMR spectral technique, the fatty acids composition was determined, especially the concentrations of short-chain saturated fatty acids (C4-C8), di-unsaturated fatty acids, mono-unsaturated fatty acids and long-chain saturated fatty acids (>C8). ^{1}H-NMR spectra were recorded on a Bruker Ascend 400 MHz spectrometer, operating at 9.4 Tesla corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus.

Samples were analyzed in 5 mm NMR tubes (Wilmad 507). The NMR samples were prepared by dissolving 0.2 mL oil in 0.8 mL $CDCl_3$. The chemical shifts are reported in ppm, using the TMS as internal standard.

Statistical analysis

All the measurements were performed at least in triplicate. The values of different parameters were expressed as the mean ± standard deviation (s_r).

Results and discussions

Proximate composition of partially defatted pumpkin seeds flour are presented in table 1 and display that this by-product is a rich source of protein, lipids and crude fibers, since contents are 42.75, 12.28, and respectively 26.64%. Ash was 7.50% and total carbohydrate was 37.4%.

Table 1
CHEMICAL COMPOSITION OF PARTIALLY DEFATTED PUMPKIN SEEDS FLOUR

Components	Content (g/100 g based on dry weight bases)
Total fat	12.28 ± 0.19
Ash	7.50 ±0.11
Crude fibers	26.64 ± 0.45
Total protein (Nx6.25)	42.75 ± 0.22
Total carbohydrates	37.40 ± 0.05

** Results given as M ± SD (mean ± standard deviation) of triplicate trials*

These data confirm that partially defatted pumpkin seed flour is a good source of bio-compounds, especially crude fibers (26.64%, d.m). Partially defatted pumpkin seeds should be considered a source of added value carbohydrate compounds with potential known prebiotic properties,

Table 2
MINERALS CONTENTS OF PARTIALLY DEFATTED PUMPKIN SEEDS

Elements	Content (mg mineral/100 g sample)	RDI (FDA 2016)
Potassium	1290 ± 247	4700
Magnesium	697 ± 115	420
Calcium	127 ± 1.92	1300
Sodium	19.50 ± 4.4	2300
Iron	87.80 ± 1.80	18
Manganese	8.20 ± 1.89	2.3
Zinc	11.5 ± 2.5	11
Copper	2.30 ± 0.41	0.9

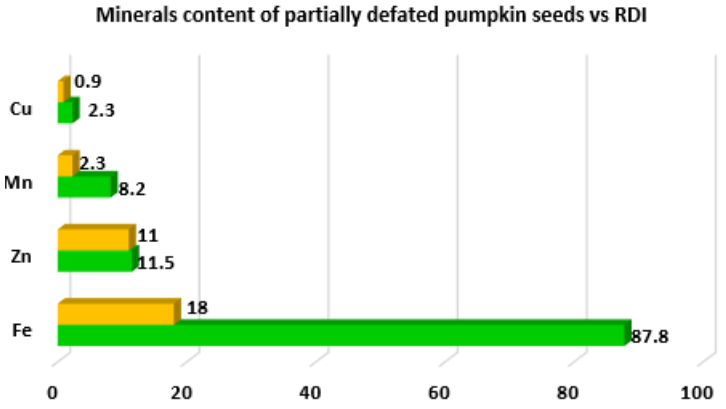

Fig.1.The significant minerals content of partially defatted pumpkin seeds (mg /100 g sample)

useful to formulate functional foods as well as nutraceuticals. In the present study, the contents of four biologically essential mineral elements were determined (table 2).

From performed analysis regarding minerals content, it can be observed that partially defatted pumpkin seeds represent a material having important minerals contents, 100 g assuring the daily intake for some of these elements according to The Reference Daily Intake (RDI) of macronutrients and micronutrients recommended [35]. Specifically, 100 g of this by-product contains more than the necessary daily intake of iron, magnesium, manganese, copper and zinc and quarter of the daily demands of potassium (fig. 1).

The amino acid composition of partially defatted pumpkin seeds flour is given in table 3. The results show that partially defatted pumpkin seeds contain high amounts of all essential amino acids.The essential amino acids are very important for human health as they cannot be biosynthesized *de novo* and serve as building blocks of certain proteins with major roles in the human body [36,37].

The chemical score procedure was developed as a direct evaluation of the ability of a protein source to meet human amino acid requirements [36]. The procedure consists in calculating the percent that each amino acid represents in the tested protein out of the amount of the respective amino acid in the standard protein, where egg protein was initially set as standard for evaluation of food proteins. Figure 2 shows the amino acids content of partially defatted pumpkin seeds in comparison with those of the standard protein set by FAO/WHO/UNU [35].

Table 4 presents the chemical scores of selected amino acids from the partially defatted pumpkin seeds calculated according to FAO/WHO [34].

Table 3
THE AMINO ACIDS CONTENT IN PARTIALLY DEFATTED PUMPKIN SEEDS

Indispensable Amino acid	Content (mg/100 g sample)	Dispensable Amino acid	Content (mg/100 g sample)
Arginine	11.55	Glutamic acid	18.11
Leucine	7.32	Glycine	4.98
Valine	5.69	Serine	5.48
Lysine	3.97	Proline	4.48
Phenylalanine	5.10	Aspartic acid	10.96
Isoleucine	3.93	Alanine	5.02
Threonine	3.76		
Histidine	2.84		
Tyrosin	3.30		
Methionine	2.26		
Cysteine	1.42		

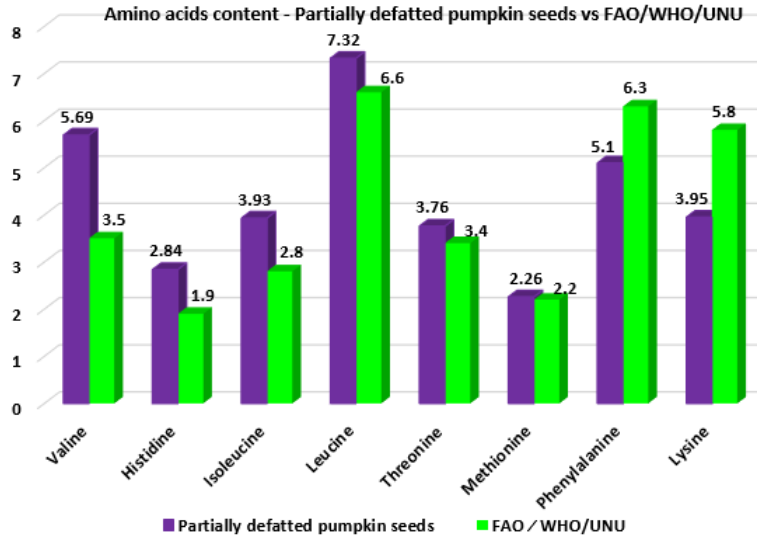

Fig.2. Percentages of amino acids in the partially defatted pumpkin seeds protein in comparison with the percentages of amino acids in the standard protein

Aminoacids	Content (mg/100 g sample)	FAO/WHO/UNU	Amino acid scores %
Valine	5.69	3.5	162.6
Histidine	2.84	1.9	149.5
Isoleucine	3.93	2.8	140.4
Leucine	7.32	6.6	110.9
Threonine	3.76	3.4	110.6
Methionine	2.26	2.2	102.7
Phenylalanine	5.1	6.3	81.0
Lysine	3.95	5.8	68.1

Table 4
CHEMICAL SCORES OF AMINO ACIDS IN THE PARTIALLY DEFATTED PUMPKIN SEEDS

Table 5
FATTY ACIDS CONTENT OF PARTIALLY DEFATTED PUMPKIN SEEDS

Type of Fatty Acids	Content g /100 g sample
Short-chain saturated	19.28
Mono-unsaturated	27.3
Di-unsaturated	49.12
Poli-unsaturated	4.3
Total unsaturated	80.72

A strong correlation was reported between chemical scores determined in this manner and values obtained through biological assays for protein quality. Data from table 4 indicates that the partially defatted pumpkin seeds proteins are a rich source of valine with a chemical score of 162.6%, histidine (149.5%), isoleucine with 140.4%, leucine (110.9%), threonine (110.6%) and respectively methionine (102.7%).

Essential amino acids are very important for health since they are building blocks of proteins, which carried functions of the human body [33]. Leucine has beneficial effects for skin, bone and tissue wound healing and promotes growth hormone synthesis. Valine is essential for muscle proteins. Isoleucine is necessary for the synthesis of hemoglobin in red blood cells. Furthermore, it can be noticed that lysine is the most limited amino acid. The fatty acids profile of samples is presented in table 5.

Analyzing the fatty acid profile expressed by the relative percentage concentration in the lipid sample contained in the partially defatted pumpkin seeds, it can be seen (table 5) that the total unsaturated fatty acids are about 80%, which denotes that partially defatted pumpkin seeds are a valuable material in terms of beneficial fats for the human body.

In short, studies of the food potential of partially defatted pumpkin seeds are not yet available in the scientific literature. Taking into accout that consumers are more and more aware about the food quality, especially from the nutritive point of view, the new food resources rich in bioactive compounds are necessary to be found. In this respect, partially defatted pumpkin seeds meet the expectations of such consumers.

According to the data obatined and presented above, it can be confirmed that partially defatted pumkin seeds are a good source of bio-compounds, especially the total fiber (26.64 %, d.m) and has to be considered a source of added value carbohydrate compounds with potential prebiotic known properties, useful to formulate functional foods as well as nutraceuticals.

Obviously, studies regarding the content in active substances of some parts of plants need to be deepened. These substances provide resources for the formulation of compounds with an extremely beneficial effect in the healing of various pathologies [37-39].

Conclusions

The purpose of the study was to evaluate the functional potential of flour obtained from partially defatted pumpkin seeds (for eventual use in the preparation of foods rich in valuable biocompounds) and to determine its chemical composition using both classical and spectral methods.

The measurements and results obtained have shown that partially defatted pumpkin seed flour is a valuable source of minerals, especially iron, magnesium, manganese, copper and zinc. The high nutritional value of these seeds, the complex physiological effect and the wide range of possible uses can be attributed to their substantial oil content and their favorable fatty acid composition. Increasing the content of partially defatted pumpkin seeds flour in various food products can lead to an increase in the content of dietary fibers, minerals, proteins and amino acids.

References

1.SANJUR, O.I., PIPERNO, D.R., ANDRES, T.C., WESSEL-BEAVER, L., Proceedings of the National Academy of Sciences, **99**, nr. 1, 2002, p.535.

2.POPA, M., POPA, D., Int. J. Agric. Inform. Environ. Syst., **8**, nr. 1, 2017, p. 63.

3.POPA, M., LATO, A., CORCHES, M., RADULOV, I., BERBECEA, A., CRISTA, F., NITA, L., LATO, K.I., POPA, D., Agrolife Sci. J., **5**, nr. 1, 2016, p. 174.

4.DOMUTA, C., SANDOR, M., CIOBANU, G., DOMUTA, C., BORZA, I., BREJEA, R., VUSCAN, A., J. Environ. Prot. Ecol., **13**, nr. 1, 2012, p. 135.

5.DOMUTA, C., SANDOR, M., CIOBANU, G., SAMUEL, A.D., CIOBANU, C., DOMUTA, A., BORZA, I., DOMUTA, C., BREJEA, R., GITEA, M., J. Environ. Prot. Ecol., **13**, nr. 2, 2012, p. 736.

6.SAMUEL, A.D., DOMUTA, C., SANDOR, M., VUSCAN A., BREJEA, R., Rom. Agric. Res., 28, 2011, p. 157.

7.SAMUEL, A.D., DOMUTA., C., CIOBANU, C., SANDOR, M., Rom. Agric. Res., **25**, 2008, p. 61.

8.TIT, D.M., BUNGAU, S., NISTOR CSEPPENTO, C., COPOLOVICI, D.M., BUHAS, C., J Environ. Prot. Ecol., **17,** nr. 4, 2016, p. 1425.

9.BUNGAU, S., BUNGAU, C., TIT, D.M., J. Environ. Prot. Ecol., **16**, nr. 1, 2015, p. 56.

10.COPOLOVICI, L., TIMIS, D., TASCHINA, M., COPOLOVICI, D., CIOCA, G., BUNGAU, S., Rev.Chim.(Bucharest), **68**, no. 9, 2017, p. 2076.

11.TASCHINA, M., COPOLOVICI, D. M., BUNGAU, S., LUPITU, A. I., COPOLOVICI, L., IOVAN, C., Farmacia, **65**, nr. 5, 2017, p. 709.

12.BUNGAU, S., BALDEA, I., COPOLOVICI, L., Rev. Chim.(Bucharest), **54**, no. 3., 2003, p. 213.

13.COPOLOVICI, D., BUNGAU, S., BOSCENCU, R, TIT, D.M., COPOLOVICI, L., Rev. Chim.(Bucharest), **68**, no. 3, 2017, p. 530.

14.PALLAG, A., BUNGAU, S.G., TIT, D.M., JURCA, T., SIRBU, V., HONIGES, A., HORHOGEA, C., Rev. Chim.(Bucharest), **67**, no. 3, 2016, p. 530.

15.GITEA, D., VICAS, S., GITEA, M.A., NEMETH, S., TIT, D.M., PASCA, B., PURZA, L., IOVAN, C., Rev. Chim.(Bucharest), **69**, no. 2, 2018, p. 305
16.OPREA, O.B., APOSTOL, L., BUNGAU, S., CIOCA, G., SAMUEL, A.D., BADEA, M., GACEU, L., Rev. Chim.(Bucharest), **69**, no. 1, 2018, p. 70
17.BADEA, M., FLOROIAN, L., RESTANI, P., MOGA, M., Int. J. Electrochem. Sci., **11**, 2016, p. 6719.
18.BADEA, M., FLOROIAN, L., RESTANI, P., COBZAC, S.C., MOGA, M., PLoS One., **11,** no. 7, 2016, p. e0160021.
19.BADEA, M., CHIPEREA, C., BALAN, M., FLOROIAN, L., RESTANI, P., MARTY, J.-L., IOVAN, C., TIT, D. M., BUNGAU, S., TAUS N., Farmacia, **66**, nr. 1, 2018, p. 83
20.MICCOLI, A., RESTANI, P., FLOROIAN, L., TAUS, N., BADEA, M., CIOCA, G., BUNGAU, S., Rev. Chim.(Bucharest), **69**, no. 4, 2018, p. 854.
21.BUNGAU, S., TIT, D.M., IOVAN, C., COPOLOVICI, L., BOSCENCU, R., CIOCA, G., COPOLOVICI, D., Rev. Chim.(Bucharest), **68**, nr. 11, 2017, p. 2495.
22.BARDAA, S., HALIMA, N.B., ALOUI, F., MANSOUR, R.B., JABEUR, H., BOUAZIZ, M., SAHNOUN, Z., J. Lipids Health Dis., **15**, nr. 1, 2016, p.73.
23.STEVENSON, F.J. ELLER, L. WANG, J.-L. JANE, T. WANG, G.E. INGLETT, J. Agric. Food Chem., **55,** nr. 10, 2007, p. 4005.
24.RYAN, E., GALVIN, K., O'CONNOR, T.P., MAGUIRE, A.R., O'BRIEN, N.M., Plant Foods Hum. Nutr., **62**, nr. 3, 2007, p. 85.
25.APPLEQUIST, W.L., AVULA, B., SCHANEBERG, B.T., WANG, Y.-H., KHAN, I.A.,J. Food Compos. Anal, **19**, nr. 6-7, 2006, p. 606.
26.MOSTEANU, D., BARSAN, G., OTRISAL, P ., GIURGIU, L ., OANCEA, R., Rev. Chim.(Bucharest), **68**, no. 11, 2017, p. 2499.
27.MOSTEANU, D., BARSAN, G., GIURGIU, L., EPURE G., Rev. Chim.(Bucharest), **60**, no. 3, 2009, p. 290.
28.JIAO, J., LI, Z.-G., GAI, Q.-Y., LI, X.-J., WEI, F.-Y., FU, Y.-J., MA, W., Food Chem., **147**, nr. 1, 2014, p. 17.
29.BANDICI, L., VICAS, S.I., TEUSDEA, A.C., BANDICI, G.E., POPA, D., J. Microw. Power Electromagn. Energy, **51**, nr. 3, 2017, p. 161.
30.HERNANDEZ-SANTOS, B., RODRGUEZ-MIRANDA, J., HERMAN-LARA, E., TORRUCO-UCO, J.G., CARMONA-GARCIA, R., JUAREZ-BARRIENTOS, J.M., CHAVEZ-ZAMUDIO, R., MARTINEZ-SANCHEZ, C.E., Ultrason. Sonochem., **31**, nr. 1, 2016, p. 429.
31.CARBIN BE, LARSSON B, LINDAHL O.,BJU Int., **66,** nr. 6, 1990, p. 639.
32.TSAI, Y.S., TONG, Y.C., CHENG, J.T., LEE, C.H., YANG, F.S., LEE, H.Y., Urol. Int., **77**, nr. 1, 2006, p. 269.
33.MI-YOUNG, K., EUN-JIN, K., YOUNG-NAM, K., CHANGSUN, C., BOG-HIEU, L., Nutr. Res. Pract., **6**, nr. 1, 2012, p. 21.
34.HABIB, A., BISWAS, S., SIDDIQUE, A.H., MANIRUJJAMAN, M., UDDIN, B., HASAN, S., KHAN, M.M.H., UDDIN, M., ISLAM, M., HASAN, M., RAHMAN, M., ASADUZZAMAN, M., SOHANUR-RAHMAN, M., ISLAM, M.A., RAHMAN, M., J. Nutr. Food Sci., **5**, 2015, p. 374.
35.REZIG, L., CHIBANI, F., CHOUAIB, M., DALGALARRONDO, M., HESSINI, K., GUEGUEN, J., HAMDI, S., J. Agric. Food Chem., **61**, nr. 32, 2013, p. 7715.
36.SUKMANOV, V., GACEU, L., ZAKHAREVYCH, V., MARYNIN, A., ROGOVYI, I., FARISIEIEV, A., J. EcoAgri. Tourism, **12**, no. 1, 2016, p. 83.
37.ZHO, J., Recent Pat. Biotechnol., **1**, nr. 1, 2007, p. 74.
38.ABDEL-DAIM, M.M., ZAKHARY, N.I., ALEYA, L., BUNGAU, S.G., BOHARA, R.A., SIDDIQI, N.J., Oxid. Med. Cell. Longev., **2018**, ID 2098123, 2018, 2 pages.
39.FODOR, K., TIT, D.M., PASCA, B., BUSTEA, C., UIVAROSAN, D., ENDRES, L., IOVAN, C., ABDEL-DAIM, M., BUNGAU, S., Oxid. Med. Cell. Longev., **2018**, ID 4147320, 2018, 10 pages.

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