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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF INFUSIONS FROM TWO BY-PRODUCTS: WALNUT (*JUGLANS REGIA* L.) SHELL AND ONION (*ALLIUM CEPA* L.)

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Abstract: *Total phenolic content and antioxidant activity of two by-products of onion (Allium cepa* L.*) and walnut (Juglans regia* L.*) processing were studied in order to establish the possibility of capitalization as antioxidant sources. The by-products analyzed were walnut shell and onion peel. From these by-products, infusions were prepared using water as solvent. Total phenolic content was determined using Folin-Ciocalteu method, while the radical scavenging ability of the infusions was monitored using the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). Walnut shell infusion had a relatively low content in phenolic compounds (32.08 mg GAE/g), below the level reported for other walnut by-products. Given the low phenolic content, walnut shell infusion exhibited a reduced radical scavenging capacity (7.82%). On the contrary, the onion peel infusion had both high phenolic content (280.32 mg GAE/g) and antioxidant activity (69.38%). Based on these two results it was confirmed that onion peels resulted from bulb processing are suitable for reuse in antioxidants' extraction in both food and pharmaceutical industries.*

Keywords: *walnut, onion, antioxidants, DPPH, Folin-Ciocalteu*

1. Introduction

Official surveys indicate that the European food-processing industry produces vast volumes of wastes every year, and despite the efforts to reduce solid waste volume the impact of food industry on the environment remains unchanged. Apart from the environmental challenges posed, such streams represent considerable amounts of potentially reusable materials and energy [1]. Fruit and vegetable processing has the highest wastage rate of any food, rising up to 16.4% for fruits and to 25.8% in the case of vegetables [2]. According to Food and Agriculture Organization (FAO), nearly 50% of all fruits and vegetables in the EU go to waste, 5.5% only being lost during postharvest handling and processing [3]. Byproducts discharged from manufacturing of fruit or vegetable-based products depend on the fresh produce used and include peels, skin, hulls, pips, and cores. Fruit and vegetable waste can be capitalized in added-value products such as animal feed, single-cell protein (SCP) and other fermented products, baker's yeast, organic acids, amino acids, enzymes (e.g. lipases, amylases, and cellulases), flavors and pigments, gums and polysaccharides [4,5].
Walnut (*Juglans regia* L.) is a tree native to a region stretching from the Balkans eastward to the western Himalayan chain [6,7]. The walnut is traditionally cultivated throughout Europe for its valuable wood and seeds (walnuts). Seeds are of high

economic interest to the food industry and are valued globally for the nutritional, health and sensory attributes [8]. Romania is among the first ten world producers of walnut. In order to maximize the productivity and reduce the waste from processing, several by-products derived from the walnut tree, such as green walnuts, shells, kernels, bark, green walnut husks, and leaves have been used in both cosmetic and pharmaceutical industries [9,10]. Walnut shells and green walnut husk have various possible uses in the industry. Recent research has reported on the great potential of green walnut husk to remove Lanaset Red G from aqueous solution [11]. It was also investigated the ability of a powdered activated carbon (PAC) derived from walnut shell to adsorb mercury from aqueous solutions [12,13], and the reuse of walnut shell as a support material (biosorbent) for effective cesium adsorption from aqueous solution [14]. In addition to non-food applications, the central point of recent research is the phenolic composition and antioxidant activity of extracts from by-products. While several studies reported on the bioactive compounds of extracts from green walnut husk [10, 15-17], no data is available on the composition of aqueous extracts from walnut shells.

Onion (*Allium cepa* L.) is one of the major vegetable crops grown worldwide, after potato and tomato, with a production that was estimated to 84.75 Mt in 2013 [18] and increases constantly. Onions possess strong, characteristic aromas and flavours [19] that made the vegetable (bulb or scallion) an important ingredient of food. On account of the whopping production and use of onion, bulbs and scallions processing raises some concern over the large quantity of industrial waste produced and its disposal. Furthermore, the perishable nature make onion waste not suitable for fodder or landfill disposal due to the rapid growth of phytopathogens, e.g. *Sclerotium cepivorum* (white rot) [20].

In these conditions, the only suitable decision in onion waste management is the capitalization of by-products. The main onion by-products include onion skins generated during industrial peeling, and undersized, malformed or damaged bulbs [21]. Onion peel importance in bioactive compounds extraction is given by its high levels of two chemical groups with proven health benefits for humans, namely flavonoids and alk(en)yl cysteine sulfoxides (ACSO) [22]. The main flavonoid present in onion peel is quercetin aglycon [23], a compound known for its antioxidant and free radical scavenging activity, cardioprotective effect, anticancer, antiinflamatory and antiviral activity, as well as inhibitory activity against bacterial ureases [24-26]. Naturally occurring ASCOs in onion peel are trans-(+)-*S*-(1-propenyl)-*L*-cysteine sulphoxide, (+)-*S*-methyl-*L*-cysteine sulphoxide, and (+)-*S*-propyl-*L*-cysteine of sulphoxide [27].

Although Romania has a high production of walnuts and onion, the capitalization of waste resulted from processing has been less studied. The aim of this work was to analyze the composition in antioxidants of infusions from walnut shells, and onion peel in order to establish the possibility of reuse in Romanian industry according to the by-product composition. The solvent used in the extraction process was water. No alcoholic solvent was added to enhance the extraction. The parameters analyzed were total phenolic content and scavenging effect on DPPH (2,2-diphenyl-1-picrylhydrazyl).

2. Matherials and methods

2.1. Plant material

Onions were purchased from an organic farmer located in Suceava County, in north-eastern Romania. The bulbs were from the 2015 harvest. Walnuts were collected in September 2015 from a single tree that had not received any insecticide treatment.

The walnut shell was cracked and the kernel was removed, while the outer skin of the onion was sectioned, and then peeled. Both by-products were rinsed prior to being used. To enhance the extraction of bioactive compounds onion peels were cut into chunks and walnut shells were crushed.

2.2. Preparation of individual infusions

The infusion (Fig. 1) was prepared as follows: 80 g of freshly separated onion peels or walnut shells was placed in a stainless steel pot and 100 ml of water brought to the boiling point (100 °C) was added. The pot was quickly covered leaving the mix to infuse for about 15 minutes. The cover was then removed and the content was allowed to rest for about 4 hours. Finally, peels and shells were retained in a filter and the infusion was stored in a closed container until its composition was analyzed.

2.3. Total phenolic content determination

Total phenolic content of the obtained infusions was determined colorimetrically using the method described by Singleton and Rossi (1965) with some modifications [28]. Briefly, 50 µl of infusion was mixed with 250 µl of Folin-Ciocalteu reagent. After 3 minutes 500 µl of saturated sodium carbonate solution (20%, w/w) was added and mixed again. The mixture was allowed to stand for 30 minutes in the dark prior to measuring the absorbance at 765 nm in a single beam UV-Vis-NIR spectro-photometer (Shimadzu Corporation, Japan). Total phenolic content was expressed in mg gallic acid equivalents (GAE)/g. For gallic acid, absorbance is described by the following equation:

$$y = 0.0012x - 0.0345 \ (R^2 = 0.9997)$$

2.4. Antioxidant activity

The radical scavenging ability of the infusions was monitored using the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) following a procedure similar to those proposed by Mraicha et al. (2010) and Bouaziz et al. (2008) in previous studies [29,30]. Aqueous solutions of samples were prepared by mixing various concentration of infusion with ethanol solution, as follows: 60 µl onion peel infusion and 2940 µl solvent, and 30 µl walnut shell infusion in 2970 µl ethanol. The aliquots were added to 5 ml of a 0.004% (w/w) freshly prepared methanol solution of DPPH. The mixtures were left to stand at room temperature for 60 minutes in the dark (until stable absorbance values were obtained). The absorbance was measured against a blank at 517 nm in a single beam UV-Vis-NIR spectro-photometer (Shimadzu Corporation, Japan). Free radicals inhibition (I%) was calculated using the equation:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100,$$

Where: A_{blank} – absorbance of the control reaction, containing all the reagents except the analyzed infusion

A_{sample} – absorbance of the infusion

Fig. 1 Walnut shell infusion (left) and onion peel infusion (right)

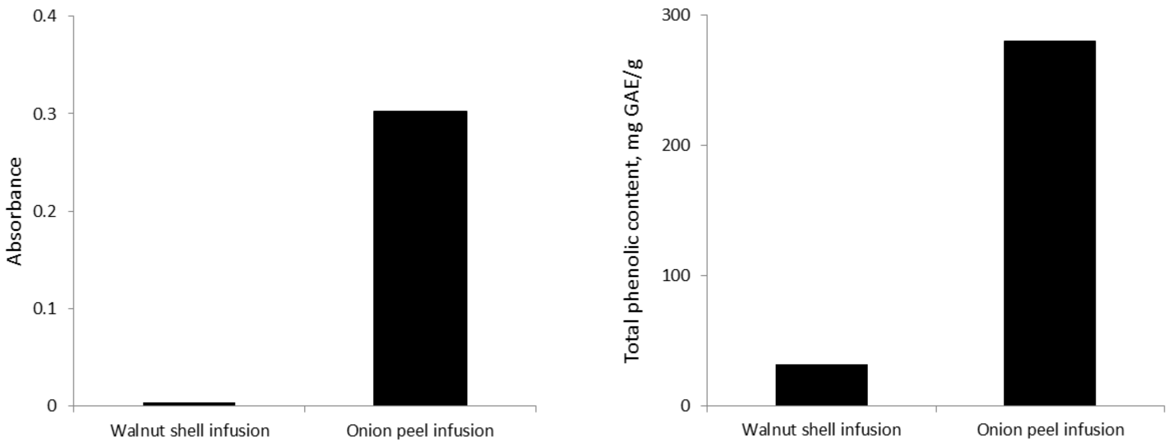

Fig. 2 Absorbance (left) and total phenolic content (right) of infusions

3. Results and discussion

3.1. Total phenolic content of infusions

The Folin-Ciocalteu assay, used for the determination of the total phenolic content of infusions from walnut shells and onion peel is a simple and widespread method that has been employed for a long time as a measure to evaluate total phenols of natural products. Extraction of phenolic compounds from plant material is influenced by the chemical nature of the compound, the extraction method employed, sample particle size, the solvent used, extraction conditions (time, temperature), as well as the presence of interfering substances [31]. Unlike the extraction with alcoholic solvents (methanol, ethanol), the use of water in the solubilization of bioactive compounds does not rise concern regarding the toxicity and recovery of the solvent from the extract. Recent studies on the extraction of anthocyanins, carried out on synthetic flavylium salts showed that the molecules require neutralizing their own electrostatic repulsions with water molecules so that the dimerisation can be carried out [32]. Therefore, water plays a fundamental role in phenolic compounds extraction.

Total phenolic content of walnut shell infusion and onion peel infusion, alongside the absorbance measured is presented in Fig. 2. As it was expected, total phenolic content of walnut shell infusion (32.08 mg GAE/g) was relatively low but exceeded the mean of 18.04 mg GAE/g reported for the phenolic content of methanolic extract from powdered walnut hull [33]. Higher phenolic content was determined for extracts from green walnut husk (50.18 mg GAE/g), leaves (94.39 mg GAE/g), and kernel (116.22 mg GAE/g) [34]. For onion peel infusion, total phenolic content measured was 280.32 mg GAE/g, a level that was close to the content obtained by Boo et al. (2012) for water extracts from powdered onion peel [35]. Regarding the influence of plant section and growth on the phenolic content of onion, it was noted that the greenish part of the onion had a higher phenolic content in comparison with the white part of the bulb [36].

3.2. Antioxidant activity

Antioxidants are essential to preserve the biological system from free radicals damage to biological molecules [37].

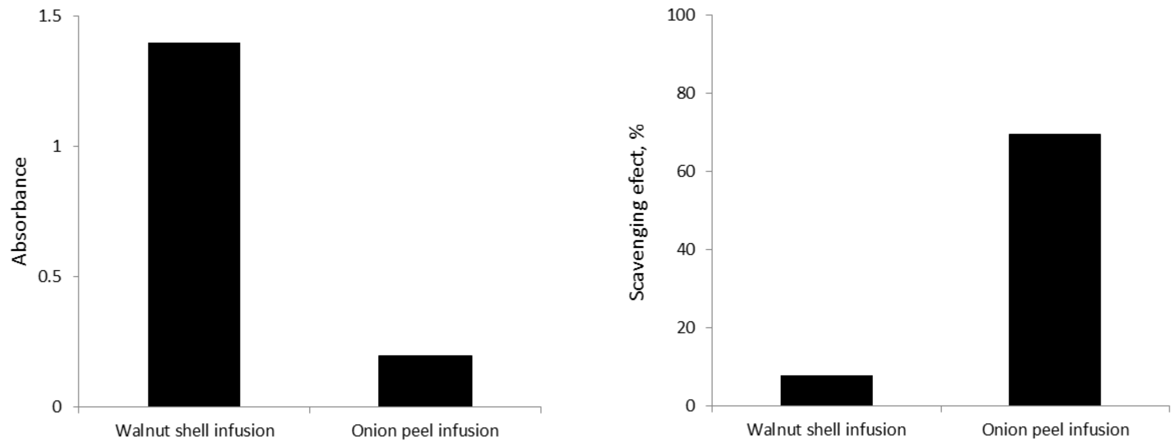

Fig. 3 Absorbance (left) and scavenging effect (right) of infusions

The capacity of antioxidants to protect a biological system against the harmful effects of oxidative processes is defined as antioxidant activity. In this study, the ability of the investigated walnut shell infusion and onion peel infusion to act as donors of hydrogen atoms or electrons in DPPH radical transformation into its reduced form of DPPH•-H was investigated in DPPH• assay. As Fig. 3 shows, both samples exhibited a radical scavenging activity below 70%. For walnut shell infusion the scavenging activity measured was 7.82%, a value that was greater than an inhibition of 7.19%, reported by other authors for the same by-product [33]. Compared to other by-products of walnut processing, the scavenging activity on DPPH radicals of infusions from shells was lower than the one obtained for water:ethanol extracts from green walnut husk [38].
Onion peel infusion, which had the highest content of phenols, exhibited a high DPPH free radical scavenging capacity, namely 69.38%. This value is close to the antioxidant activity of 69.7%, measured for methanolic extracts from red onion peel [39]. As in the case of polyphenols, the antioxidant activity is influenced by the distribution of the analyzed segment in vegetable material. It was reported that anthocyanins, a group of compounds with high antioxidant potential are heavily concentrated in onion skin [40]. Moreover, it was observed that peel shredding led to an increase in the content of quercetin 4'-*O*-β-glucopyranoside [41], a flavonoid with documented radical scavenging activity. These two last observations confirm the increase in antioxidant activity from the inside to the outer part (peel) of the bulb.

4. Conclusion

The present study reports on total phenolic content and antioxidant activity of two infusions prepared with by-products of onion and walnut processing. The by-products used were walnut shells and onion peels, and the solvent of extraction was water.
Walnut shell infusion had a low content of phenolic compounds, and therefore exhibited free radicals scavenging activity close to the levels reported in other similar studies. On the other hand, onion peel infusion presented a balanced phenolic content, and increased radical scavenging activity. Between the two by-products investigated, onion peel has a great potential for capitalization. Given the high

content of antioxidants, the reuse could imply both food and non-food applications.

5. References

[1]. KOSSEVA, M. R., Processing of food wastes, *Advances in Food and Nutrition Research*, 58:57-136, (2009)
[2]. WADHWA, M., BAKSHI, M. P. S., Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products, *RAP Publication*, 4, (2013)
[3]. PROKOPOV, T. V., Utilization of by-products from fruit and vegetable processing: A review, *Food and Packaging*, 49, (2014)
[4]. JOSHI, V. K., Food processing industries' waste: Opportunities, technologies, challenges and future strategies, *Biotechnology in Agriculture and Environment*, 129-148, (2002)
[5]. MARWAHA, S. S., ARORA, J. K. (Eds.), *Food Processing: Biotechnological Applications*, Asiatech Publishers, (2000)
[6]. TAHA, N. A., AL-WADAAN, M. A., Utility and importance of walnut, Juglans regia Linn: A review, *African Journal of Microbiology Research*, 5(32):5796-5805, (2011)
[7]. FERNANDEZ-LOPEZ, J., ALETA, N., ALIAS, R., *Forest genetic resources conservation of Juglans regia L.*, IPGRI, Rome, Italy, 20-25, (2000)
[8]. MARTÍNEZ, M. L., LABUCKAS, D. O., LAMARQUE, A. L., MAESTRI, D. M., Walnut (Juglans regia L.): genetic resources, chemistry, by-products, *Journal of the Science of Food and Agriculture*, 90(12):1959-1967, (2010)
[9]. OLIVEIRA, I., SOUSA, A., FERREIRA, I. C., BENTO, A., ESTEVINHO, L., PEREIRA, J. A., Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks, *Food and Chemical Toxicology*, 46(7):2326-2331, (2008)
[10]. STAMPAR, F., SOLAR, A., HUDINA, M., VEBERIC, R., COLARIC, M., Traditional walnut liqueur-cocktail of phenolics, *Food Chemistry*, 95(4):627-631, (2006)
[11]. ÇELEKLI, A., BIRECIKLIGIL, S. S., GEYIK, F., BOZKURT, H., Prediction of removal efficiency of Lanaset Red G on walnut husk using artificial neural network model, *Bioresource Technology*, 103(1):64-70, (2012)
[12]. ZABIHI, M., AHMADPOUR, A., ASL, A. H., Removal of mercury from water by carbonaceous sorbents derived from walnut shell, *Journal of Hazardous Materials*, 167(1):230-236, (2009)
[13]. ZABIHI, M., ASL, A. H., AHMADPOUR, A., Studies on adsorption of mercury from aqueous solution on activated carbons prepared from walnut shell, *Journal of Hazardous Materials*, 174(1):251-256, (2010)
[14]. DING, D., ZHAO, Y., YANG, S., SHI, W., ZHANG, Z., LEI, Z., YANG, Y., Adsorption of cesium from aqueous solution using agricultural residue - Walnut shell: Equilibrium, kinetic and thermodynamic modeling studies, *Water Research*, 47(7):2563-2571, (2013)
[15]. JAKOPIC, J., VEBERIC, R., Extraction of phenolic compounds from green walnut fruits in different solvents, *Acta Agriculturae Slovenica*, 93(1):11-15, (2009)
[16]. GHASEMI, K., GHASEMI, Y., EHTESHAMNIA, A., NABAVI, S. M., NABAVI, S. F., EBRAHIMZADEH, M. A., POURMORAD, F., Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (Juglans regia L.) green husks, *Journal of Medicinal Plants Research*, 5(7):1128-1133, (2011)
[17]. POPOVICI, C., GÎTIN, L., ALEXE, P., Characterization of walnut (Juglans regia L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science Technique and Technologies*, 1(1):5-9, (2012)
[18]. FAOSTAT, *Production/Crops for onions*, http://faostat3.fao.org/browse/Q/QC/E (2016)
[19]. YE, C. L., DAI, D. H., HU, W. L., Antimicrobial and antioxidant activities of the essential oil from onion (Allium cepa L.), *Food Control*, 30(1):48-53, (2013)
[20]. ROLDÁN, E., SÁNCHEZ-MORENO, C., DE ANCOS, B., CANO, M. P., Characterisation of onion (Allium cepa L.) by-products as food ingredients with antioxidant and antibrowning properties, *Food Chemistry*, 108(3):907-916, (2008)
[21]. BENÍTEZ, V., MOLLÁ, E., MARTÍN-CABREJAS, M. A., AGUILERA, Y., LÓPEZ-ANDRÉU, F. J., COOLS, K., DOWNES, K., TERRY, L. A., ESTEBAN, R. M., Characterization of industrial onion wastes (Allium cepa L.): dietary fibre and bioactive compounds, *Plant Foods for Human Nutrition*, 66(1):48-57, (2011)
[22]. PARK, J., KIM, J., KIM, M. K., Onion flesh and onion peel enhance antioxidant status in aged rats, *Journal of Nutritional Science and Vitaminology*, 53(1):21-29, (2007)
[23]. DOWNES, K., CHOPE, G. A., TERRY, L. A., Effect of curing at different temperatures on biochemical composition of onion (Allium cepa L.) skin from three freshly cured and cold stored UK-grown onion cultivars, *Postharvest Biology and Technology*, 54(2):80-86, (2009)
[24]. BONACCORSI, P., CARISTI, C., GARGIULLI, C., LEUZZI, U., Flavonol glucosides in Allium species: A comparative study by means of

HPLC–DAD–ESI-MS–MS, *Food Chemistry*, 107(4):1668-1673, (2008)

[25]. CARIDI, D., TRENERRY, V. C., ROCHFORT, S., DUONG, S., LAUGHER, D., JONES, R., Profiling and quantifying quercetin glucosides in onion (Allium cepa L.) varieties using capillary zone electrophoresis and high performance liquid chromatography, *Food Chemistry*, 105(2):691-699, (2007)

[26]. SHABANA, S., KAWAI, A., KAI, K., AKIYAMA, K., HAYASHI, H., Inhibitory activity against urease of quercetin glycosides isolated from Allium cepa and Psidium guajava, *Bioscience, Biotechnology, and Biochemistry*, 74(4):878-880, (2010)

[27]. MALLOR, C., THOMAS, B., Resource allocation and the origin of flavour precursors in onion bulbs, *The Journal of Horticultural Science and Biotechnology*, 83(2):191-198, (2008)

[28]. SINGLETON, V. L., ROSSI, J. A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American Journal of Enology and Viticulture*, 16(3):144-158, (1965)

[29]. MRAICHA, F., KSANTINI, M., ZOUCH, O., AYADI, M., SAYADI, S., BOUAZIZ, M., Effect of olive fruit fly infestation on the quality of olive oil from Chemlali cultivar during ripening, *Food and Chemical Toxicology*, 48(11): 3235-3241, (2010)

[30]. BOUAZIZ, M., LASSOUED, S., BOUALLAGUI, Z., SMAOUI, S., GARGOUBI, A., DHOUIB, A., SAYADI, S., Synthesis and recovery of high bioactive phenolics from table-olive brine process wastewater, *Bioorganic and Medicinal Chemistry*, 16(20): 9238-9246, (2008)

[31]. NACZK, M., SHAHIDI, F., Extraction and analysis of phenolics in food, *Journal of Chromatography A*, 1054(1):95-111, (2004)

[32]. CASTAÑEDA-OVANDO, A., DE LOURDES PACHECO-HERNÁNDEZ, M., PÁEZ-HERNÁNDEZ, M. E., RODRÍGUEZ, J. A., GALÁN-VIDAL, C. A., Chemical studies of anthocyanins: A review, *Food Chemistry*, 113(4):859-871, (2009)

[33]. AKBARI, V., JAMEI, R., HEIDARI, R., ESFAHLAN, A. J., Antiradical activity of different parts of Walnut (Juglans regia L.) fruit as a function of genotype, *Food Chemistry*, 135(4):2404-2410, (2012)

[34]. CARVALHO, M., FERREIRA, P. J., MENDES, V. S., SILVA, R., PEREIRA, J. A., JERÓNIMO, C., SILVA, B. M., Human cancer cell antiproliferative and antioxidant activities of Juglans regia L., *Food and Chemical Toxicology*, 48(1):441-447, (2010)

[35]. BOO, H. O., HWANG, S. J., BAE, C. S., PARK, S. H., HEO, B. G., GORINSTEIN, S., Extraction and characterization of some natural plant pigments, *Industrial Crops and Products*, 40:129-135, (2012)

[36]. MARINOVA, D., RIBAROVA, F., ATANASSOVA, M., Total phenolics and total flavonoids in Bulgarian fruits and vegetables, *Journal of the University of Chemical Technology and Metallurgy*, 40(3):255-260, (2005)

[37]. MISHRA, N., DUBEY, A., MISHRA, R., BARIK, N., Study on antioxidant activity of common dry fruits, *Food and Chemical Toxicology*, 48(12):3316-3320, (2010)

[38]. FERNÁNDEZ-AGULLÓ, A., PEREIRA, E., FREIRE, M. S., VALENTAO, P., ANDRADE, P. B., GONZALEZ-ALVAREZ, J., PEREIRA, J. A., Influence of solvent on the antioxidant and antimicrobial properties of walnut (Juglans regia L.) green husk extracts, *Industrial Crops and Products*, 42:126-132, (2013)

[39]. SINGH, B. N., SINGH, B. R., SINGH, R. L., PRAKASH, D., SINGH, D. P., SARMA, B. K., UPADHYAY, G., SINGH, H. B., Polyphenolics from various extracts/fractions of red onion (Allium cepa) peel with potent antioxidant and antimutagenic activities, *Food and Chemical Toxicology*, 47(6):1161-1167, (2009)

[40]. GENNARO, L., LEONARDI, C., ESPOSITO, F., SALUCCI, M., MAIANI, G., QUAGLIA, G., FOGLIANO, V., Flavonoid and carbohydrate contents in Tropea red onions: effects of homelike peeling and storage, *Journal of Agricultural and Food Chemistry*, 50(7):1904-1910, (2002)

[41]. ABOUZID, S. F., ELSHERBEINY, G. M., Increase in flavonoids content in red onion peel by mechanical shredding, *Journal of Medicinal Plants Research*, 2(9):258-260, (2013)