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# POLYPHENOLS CONTENT AND ANTIOXIDANT CAPACITY OF GOJI FRUITS (*Lycium chinense*) AS AFFECTED BY THE EXTRACTION SOLVENTS

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***ABSTRACT.*** *Antioxidants are substances which, when present in low concentrations, compared with that of an oxidisable substrate, significantly delay or prevent oxidation of the substrate (Halliwell & Gutteridge 1989). The most abundant antioxidants in fruits and vegetables are polyphenols and vitamins, especially vitamin C. Vitamins A, B and E and carotenoids are present to lesser extent in some fruits. The aim of this paper was to study the antioxidant capacity and total polyphenols content of fresh and dried goji fruits (L. chinense) using various solvents for extraction from the samples. The examinations have been carried out both at the fresh goji fruits (L. chinense) harvested at maturity of consumption as well as at sun-dried fruits (dry matter content of 85.31%). We also determined the content of these fruits in different chemical compounds which affect the antioxidant capacity, such polyphenols and vitamin C. The nature and the concentration of the solvent used for extraction affects the total polyphenols content and the antioxidant activity of goji berries, which is in accordance with previous data. Extraction with alcohol 80% as well as with hydrochloric acid 2% have resulted in the highest values of the polyphenols content while the highest antioxidant activity was found by using as solvent the hydrochloric acid 2%, normally used for vitamin C determination.*

***KEYWORDS:*** *goji berries, antioxidant activity, polyphenols, vitamin C*

## INTRODUCTION

Horticultural products play a significant role in the human diet providing protection against oxidative cellular damages (Mahattanatawee et al. 2006). Fruits and vegetables are rich in antioxidants which help in lowering incidence of degenerative diseases, such cancer, arthritis, arteriosclerosis, heart disease, inflammations, brain dysfunction and acceleration of the ageing process (Feskanich et al. 2000, Lim et al. 2007). Fresh extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are of increasing interest in the food industry, because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food (Kähkönen et al. 1999, Rice-Evans et al. 1995).

In last decades, interest has increased considerably in finding naturally occurring antioxidants which can be used in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effect such as carcinogenicity (Gülçin et al. 2006, Kumaran & Karunakaran 2007). Several researchers have studied the relationship between total phenol and different methods of antioxidant capacity assay (Fattahi et al. 2011). Antioxidants are substances which, when present in low concentrations, compared with that of an oxidisable substrate, significantly delay or prevent oxidation of the substrate (Halliwell & Gutteridge 1989). The antioxidant components can be maintained due to low preservation temperatures (Balouchi et al. 2011). The most abundant antioxidants in fruits and vegetables are polyphenols and vitamins, especially vitamin C. Vitamins A, B and E and carotenoids are present to lesser extent in some fruits. These polyphenols, most of which are flavonoids are present mainly in ester and glycoside forms (Lim 2007). In last years a large range of researches have been carried out concerning the antioxidant properties of some tropical fruits, using different methods (Leong & Shui 2002, Lim 2007, Beltrán-Orozco et al., 2009, Mahattanatawee et al. 2006, Chemah et al. 2010). Wolfberry or goji berry is the name for the fruit of two related species of *Lycium* genus: *L. barbarum* and *L. chinense*. Fruit from *L. barbarum* L. is well-known in traditional Chinese herbal medicine for longevity, vision,

wellness, and headaches. Additionally, wolfberry has been noted for its multiple traditional health benefits, such as nourishing the yin, strengthening the liver and kidney, and sustaining the blood (Luo et al. 2004, Burke et al. 2005). In recent studies, it has been found that the flavonoids from wolfberries protect the blood cells and mitochondria against oxidative damages (Luo et al. 2004). Also, in animals and *in vitro*, wolfberry has been shown to have interesting antioxidant, immune-enhancing, radioprotective, anti-aging, and other health benefits. Nowadays, goji berries are known as being very rich in nutrients with high antioxidant capacity, fact for which they were included in the novel category of "super-fruits". Goji berries contain high amounts of antioxidants, carotenoids, vitamin A and zeaxanthin. They are also rich in vitamins B and C and polysaccharides. These fruits have high protein content (10%) and provide 18 different amino acids of which 8 essential ones. Also the goji fruits contain components rich in glutathione, which is among the most effective antioxidants, which helps the body cells to function optimally.

In order to determine the antioxidant capacity, beside the amount of various substances with antioxidant character, an important issue is their extraction from the sample, respectively the used solvent. Water, aqueous mixtures of ethanol, methanol, acetonitrile, acetone and hydrochloric acid are commonly used to extract plants substances (Sun & Ho 2005). The results are influenced by the degree of extraction of antioxidant substances as well as to their nature. Antioxidants may be carotenoids, vitamin C, polyphenols, each of them can influence the results because of their degree of solubility in various solvents. Also, instability of some antioxidant compounds such as ascorbic acid, which is rapidly oxidized in contact with oxygen from air, may influence the obtained results (Kayashima & Katayama 2002).

The aim of this paper was to study the antioxidant capacity and total polyphenols content of fresh and dried goji fruits (*L. chinense*) using various solvents for extraction from the samples.

## MATERIALS AND METHODS

**Plant material.** The examinations have been carried out both at the fresh goji fruits (*L. chinense*) harvested at maturity of consumption as well as at sun-dried fruits (dry matter content of 85.31%). Goji berries come from the plants that are in the second year of production, in a private plantation from Dragasani, Valcea county. Worth noting that the plants have not reached maximum production potential, because plantation is newly established and the plants are not adapted to the environmental conditions of the area. We have determined the antioxidant capacity using various solvents for the extraction from the samples. We also determined the content of these fruits in different chemical compounds which affect the antioxidant capacity, such polyphenols and vitamin C.

**Determination of total phenolics content.** Total phenolic compounds were determined using the Folin-Ciocalteu reagent (Obanda et al. 1997). A calibration curve of gallic acid was prepared and the results, determined from regression equation of the calibration curve were expressed as equivalents of mg gallic acid equivalents per 100 g of sample. Absorption has been recorded at a wavelength of 765 nm. For samples preparation we used more solvents in order to extract the polyphenols: methanol, ethylic alcohol 50%, ethylic alcohol 80%, acetone and hydrochloric acid 2%.

**Determination of antioxidant activity.** The antioxidant activity was measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method. The radical scavenging activity was determinated by measuring the absorbance at 516 nm in the spectrophotometer and expressed as RA (%) = (1–absorbance at 516 nm after 10 min/absorbance at 516 nm after 0 min) x 100. The samples preparation was made by using different solvents for extraction respectively: methanol, ethylic alcohol 50%, ethylic alcohol 80%, acetonitrile and hydrochloric acid 2%. Compounds not readly soluble in the solvent are usually excluded in their contribution to antioxidant activity (Howard et al 2000).

**Determination of vitamin C content.** Vitamin C content was measured by the iodometric method through oxidation until dehydroascorbic acid in the presence of starch as indicator. For extracting vitamin C we have been used hydrochloric acid 2 % as a solvent. The results were expressed as mg vitamin C per 100 g of fresh matter.

## RESULTS AND DISCUSSIONS

### Total phenolics content

The results regarding total phenolics content in function of various solvents are showed in table 1.

Table 1. Total phenolics content depending of various solvents*

| Variant | Total phenolics content (gallic acid equivalents /100 g) | | | | |
|---|---|---|---|---|---|
| | Methanol | Acetone | Ethanol 50% | Ethanol 80% | Hydrochloric acid 2% |
| Fresh goji fruits | 132.02±0.54 | 9.28±0.08 | 169.71±0.85 | 174.27±0.67 | 141.96 ±0.52 |
| Dried goji fruits | 259.54±0.57 | 18.25±0.09 | 333.64±0.72 | 342.59±0.73 | 414.1 ±0.59 |

* Data are expressed as mean ± SE triplicate experiments

The data presented in table 1 show that the highest content of phenols was found in dehydrated goji fruits because of the dry matter concentration during drying. Also the content of phenols found to dry fruits was lower than data reported in the literature (Medina 2011). This is due to the fact that fruits come from the young plants which have not reached the maximum of production, and, on the other hand, probably because of different climate conditions from the origins center of species. Also the data demonstrate the influence of the solvent used for the extraction of phenols on the values obtained for the phenolic content. It should be noted that extraction with alcohol 80%, as well as those with hydrochloric acid 2%, have resulted in the highest values of the phenolic content, results which are in accordance with the conclusions presented by Kayashima and Katayama (2002).

### Antioxidant capacity

The results regarding the antioxidant capacity are showed in table 2.

Table 2. Antioxidant capacity (RA %) depending of various solvents*

| Variant | Antioxidant capacity (RA) (%) | | | | | |
|---|---|---|---|---|---|---|
| | Methanol | Ethanol 50% | Ethanol 80% | Acetonitrile | Water | Hydrochloric acid 2% |
| Fresh goji fruits | 19.37±0.13 | 23.55±0.16 | 30.42±0.30 | 2.06±0.09 | 30.08±0.27 | 91.51±0.26 |
| Dried goji fruits | 27.06±0.28 | 32.90±0.24 | 42.50±0.53 | 2.88±0.07 | 42.03±0.34 | 91.75±0.28 |

* Data are expressed as mean ± SE triplicate experiments

Data presented in table 2 showed that the antioxidant capacity of dehydrated goji fruits was higher than that of the fresh fruits, values which correlate well with the results obtained for total phenolics content. Concerning the used solvents, from the data provided it is observed clearly their major influence on obtained results. In the same way as for the determination of phenols, the highest values have been recorded by using the ethylic alcohol 80% and hydrochloric acid 2 % as solvents for extraction. The solvent concentration affects the antioxidant capacity, fact which is according with the results presented by Turkmen et al. on mate tea (2006). The high values obtained by using hydrochloric acid 2%, solvent generally used for the determination of ascorbic acid, has led us to the conclusion that vitamin C is an unstable compound which oxidize rapidly under the influence of oxygen contained in the organic solvents, thus influencing the obtained results.

**Vitamin C content.**

Dried goji berries have shown much higher values of the content in vitamin C than the fresh fruits (table 3), which correlates well with the results obtained for the antioxidant capacity.

This comes in accordance with the data presented by some authors who have indicated that DPPH method is affected by the reduction of the ascorbic acid to dehydroascorbic acid and by some organic acids present in fruits (Kayashima & Katayama 2002, Scalzo et al. 2005).

Table 3. Content of vitamin C of fresh and dried goji fruits*

| Variant | Vit. C content (mg/100 g f. m.) |
|---|---|
| Fresh goji fruits | 29.92 ±0.73 |
| Dried goji fruits | 45.76 ± 0.89 |

* Data are expressed as mean ± SE triplicate experiments

## CONCLUSIONS

The nature and the concentration of the solvent used for extraction affects the total polyphenols content and the antioxidant activity of goji berries, which is in accordance with previous data. Dried goji fruits (*L. chinense*) had the highest content of total polyphenols and vitamin C based on the cellular juice concentration due to fruits dehydration. Extraction with alcohol 80% as well as with hydrochloric acid 2% have resulted in the highest values of the polyphenols content while the highest antioxidant activity was found by using as solvent the hydrochloric acid 2%, normally used for vitamin C determination. The DPPH method was affected by the content of vitamin C.

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