Quantification of Total Flavonoid and Total Anthocyanin Content from Purple Potato

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In order to determine the total flavonoid and total anthocyanin contents were used crude extracts from purple potato (Blue-violet of Galanesti - Romania variety)). The amount of total flavonoid (TFC) was analysed using aluminium chloride colorimetric assay and the amount of total anthocyanin (TAC) was analysed using pH differential assay. For the flavonoids and anthocynins extraction were used different solvents (1% acidified methanol, 1% acidified ethanol and 1% acidified deionized water) and different contact time between solvent and sample (30, 45, 60 min). Methanolic extract showed highest amount also for TAC (140.703 mg/100gFW) and for TFC (105.305 mg/100gFW). Ethanolic extract showed lowest amount also for TAC (66.600 mg/100g FW) and for TFC (25.37 mg/100g FW).

Keywords: Purple potato, anthocyanin pigments, flavonoids

Processing of fruits and vegetables has increased considerably in the last 25 years mostly due to the fact that epidemiological studies have linked dietary consumption of fruit and vegetable fibre with a lower incidence of cancer and cardiovascular disease mortality.

Currently, there is a remarkable global interest to identify antioxidant compounds from plants, which may be a drug potential for use in preventive medicine and in animal and human feed [1]. Anthocyanin pigments are powerful antioxidants that protect cells from various forms of cancer. According to nutritionists, modern man who lives "assaulted" by pollution conditions and unhealthy foods needs to eat foods rich in antioxidant compounds (Anthocyanin pigments).

The only source for the production of anthocyanin pigments are plants. Anthocyanins are found in: blueberries, blackberries, raspberries, cherries, cranberries, black currant, elderberry, eggplant, purple corn, black beans, and purple potatoes.

Potatoes are significant source of natural antioxidants and exhibit antioxidant activity as demonstrated in recent time by many authors. Studies have indicated that these phytochemicals have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases and age-related neuronal degeneration [2]. Genotypes of potato with peel and pulp intensely colored (red, purple, blue) have antioxidant capacity 2-3 times higher than the white / yellow genotypes, and these aliments could help to supplement the required daily doses of antioxidants in the diet. As a result, in recent years, breeder efforts intensified to get new potato genotypes in different versions: blue peel and pulp [3 - 5].

Currently potato is the fourth food culture of the world, after corn, wheat and rice, with a production of 329 million tonnes per year. Worldwide, in terms of harvested area potato ranks seven after wheat, rice, corn, barley, sorghum and rapeseed. Potatoes are a source of valuable components such as carbohydrates, proteins, essential amino acids, vitamins (vitamin C), minerals, organic acids and phenolic compounds (with antioxidant effect).

Potatoes rich in antioxidants can be used in industry for obtaining natural dyes (acylated glucosides of pelargonidin). Assessments on the potential commercial value of pelargonidin derivatives are promising. These dyes have qualities similar to the synthetic ones: stability, attractiveness, satisfactory intensity of red colour in tested foods [6].

Experimental part

Materials and methods

Chemicals and reagents

Methanol, ethanol, deionised water, sodium nitrite, aluminium chloride, sodium hydroxide, sodium acetate, potassium chloride.

Extraction of sample

Purple potato in amount of 1g (fig. 1) were soaked in different solvents such as 1% acidified methanol, 1% acidified ethanol and 1% acidified deionised water for 30, 45 and 60 min. Extracts were centrifuged (10 000 rpm, 15 min) and concentrated at 45°C. The extraction procedure of flavonoids and anthocyanin pigments from Purple Potato is schematically presented in figure 2.

Determination of total flavonoid content

The total flavonoids content (TFC) of different extracts was determined using the aluminium chloride assay [7]. The extracts were taken in different test tubes and diluted

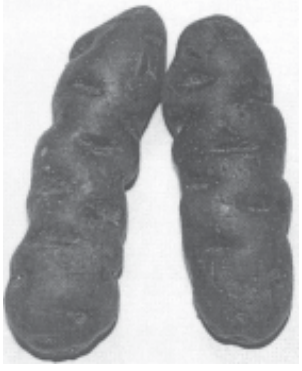

Fig. 1. Blue-violet of Galanesti - Romania variety

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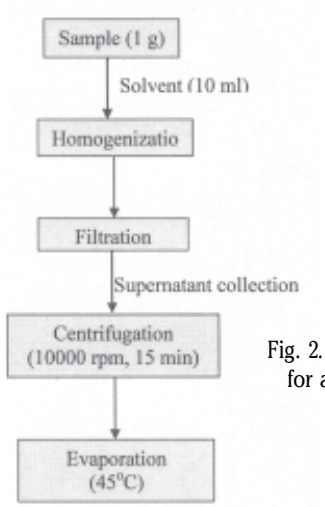

Fig. 2. Extraction procedure applied for anthocyanins and flavonoids

with distilled water followed by the addition of 150µL of sodium nitrite (5% $NaNO_2$, w/v). After 6 min the mixture was treated with 150µL of aluminium chloride (10% $AlCl_3$, w/v) and incubated for 6 min. Later 2 mL of sodium hydroxide (NAOH 1N) was added and volume was made up to 5 mL with distilled water. The absorbance was measured at 510nm after 15 min using a spectrophotometer. Distilled water was used as blank. The TFC was expressed in mg quercetine equivalents for 100g of fresh material (FW). For building the calibration curve , quarcetin is used as a standard materials. Various concentrations (table 1) of standard quercetin solution were used to make a standard calibration curve (fig. 3).

Concentration values of extracts were obtained from Quercetin standard curve, by interpolating to the X- axis. TFC was calculated by using the following formula:

$$TFC = \frac{R \cdot DF \cdot V \cdot 100}{W} \quad (1)$$

The semnifications of symbols used in this relation are:
R - Result obtained from the standard curve
D.F - Dilution factor
V - Volume of stock solution
W - Weight of plant used in the experiment

Determination of anthocyanin content

The total anthocyanins content were determined by the differential *p*H method [8] based on the property of anthocyanin pigments to change the color with *p*H. Two dilutions of the same sample were prepared, the first one in potassium chloride buffer (0.025 M, *p*H 1.0), and the second one in sodium acetate buffer (0.4 M, *p*H 4.5), *p*H being adjusted with HCl 0.2N. After equilibration at room temperature for 15 min, the absorbance of two dilutions was read at 510 nm and 700 nm. Total monomeric anthocyanins (mg cyanidin 3-glucoside equivalent/ 100 g Fresh Weight) were calculated as follows:

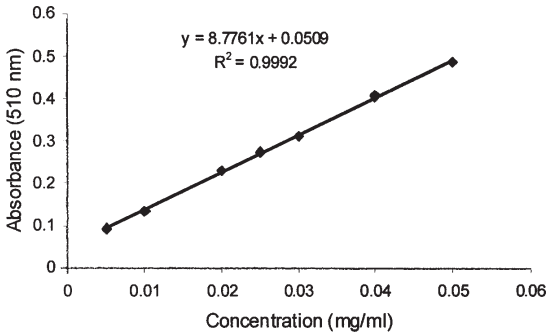

Fig. 3. Calibration curve of quercetine

Table 1
ABSORBANCE AT 510 NM OF DIFFERENT CONCENTRATIONS OF QUERCETINE

Concentration (mg/ml)	0.005	0.01	0.02	0.025	0.03	0.04	0.05
Absorbance (510nm)	0.093	0.135	0.231	0.274	0.312	0.406	0.485

$$\%\, w/w = \frac{A}{\varepsilon L}\, MW\ DF\ \frac{V}{W_t}\ 100 \quad (2)$$

$$A = (A_{510nm} - A_{700nm})_{pH=1} - (A_{510nm} - A_{700nm})_{pH=4.5} \quad (3)$$

(3)

The semnifications of symbols used in these relations are:

%w/w – percentage weight/weight (grams of solute in 100 grams of solution)
A – Absorbance
ε – Molar extinction coefficient (26900 L/mol cm)
L – Path length
MW – molecular weight (449.2 g/mol for cyanidin 3-glucoside)
DF – dilution factor
V – Volume
W_t – sample weight

Results and discussions

The present investigation has been carried out to determine the TFC and TAC present in various solvent extracts from purple potato tuber. The quantitative analysis of TFC and TAC revealed that the methanolic extract of purple potato contained highest amount of TFC (105.305 mg/100gFW) and TAC (140.703 mg/100gFW). Ethanolic extract from Albastru-Violet de Gălăneşti variety showed lowest amount also for TAC (66.600 mg/100g FW) and for TFC (25.37 mg/100g FW). In figure 4 and 5 is presented the influence of solvent and contact time on total flavonoid content and total antocyanin content.

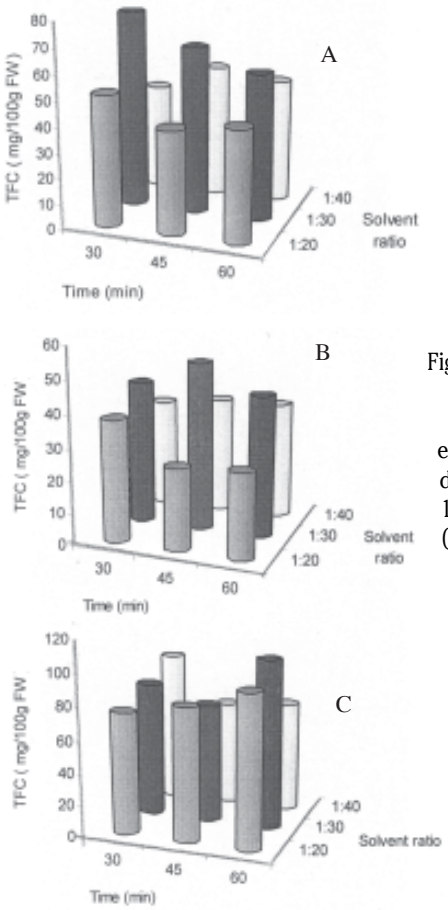

Fig. 4. Quantification of total flavonoids in different solvent extracts: 1% acidified deionized water (A), 1% acidified ethanol (B) and 1% acidified methanol (C)

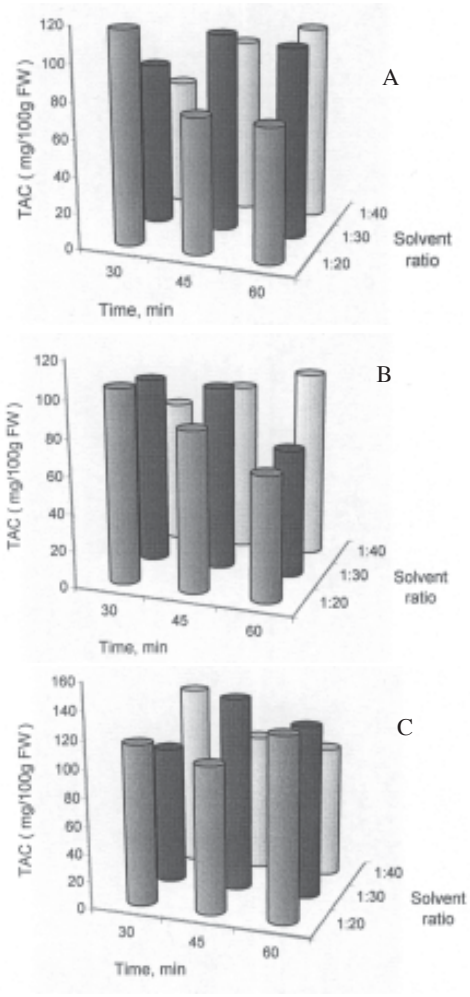

Fig. 5. Quantification of total anthocyanins in different solvent extracts: 1% acidified deionized water (A), 1% acidified ethanol (B) and 1% acidified methanol (C)

Conclusions

The results of the present investigation reports the quantitative analysis of total flavonoids and total antocyanins content of purple potato obtained in different conditions. The results are similar with literature reports about purple potato anthocyanin content and flavonoid content determined through presented method.

However further investigations are required to isolate, characterize the active constituents and to evaluate their therapeutic role.

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Manuscript received: 8.07.2014

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Factorul de impact pe anul 2013: 0,677
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Chemistry Citation Index

REVISTA DE CHIMIE

Bucharest - Romania
Chem. Abs.: RCBUAU 66 (2) (153-294)
ISSN 0034-7752
Vol. 66, no. 2, February, 2015

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Bucuresti - România
Chem. Abs.: RCBUAU 66 (2) (153 - 294)
ISSN 0034-7752
Vol. 66, nr. 2, februarie, 2015

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Journal ISSN: 0034-7752

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2008 Impact Factor : 0.389