Summary of the SCIENTIFIC REPORT

Period of implementation: April 1 – December 31, 2012

During the implementation period April-December 2012 (9 months) the following specific activities of the project PCE-2011-3-0474 were conducted: (1) technical and scientific documentation; (2) identification and selection of plant sources rich in anthocyanins (fruit, vegetables) but at the same time rich in other bioactives; (3) optimization of extraction of anthocyanin pigments; (4) determination of total monomeric anthocyanins from selected samples.

Implementation began with the realization of the documentation stage and technical-scientific analysis, continued with preliminary laboratory analysis (including also purchases) and performing research and experimentation in laboratory; initially the methodology and development time for each stage activity was settled. The project continued with dissemination stage focused to encourage interdisciplinary collaboration (biochemistry, food science, environmental-health) needed to ensure sustainability of the project.

Anthocyanins are considered among the most active biomolecules in terms of physiological and biological effects, thus providing nutraceutical properties. By interpreting the results, through correlation of all documentary materials studied to date and through consideration of all modern aspects regarding the role of antioxidants, distribution and content of anthocyanins in different plants, biosynthesis, analytical aspects, the biological role and potential applications of these bioactive molecules (natural antioxidants *vs.* synthetic antioxidants, textile dyeing) several scientific papers were prepared which further were published/presented at national and international scientific meetings.

Identification, selection and sampling of biological materials. Extraction of anthocyanin pigments under optimum conditions and determination of total monomeric anthocyanins content

Samples of blueberry (*Vaccinium* spp.), black and red currants (*Ribes* spp.), hawthorn (*Crataegus* sp.) and four varieties of food plants from Romanian areas were subjected to analytical investigations and preliminary studies.

Anthocyanins extraction was investigated both by conventional (maceration) and modern (ultrasound) methods to obtain the highest level of anthocyanins. The extracts prepared according to the set extractive technology were analyzed for total content of anthocyanins (TA) using the validated spectrophotometric method (pH differential) - a fast technique which does not require prior hydrolysis being adopted by many laboratories in the world (AOAC validated as official method).

The results of investigation of 10 samples of red onion from Romania showed the optimum extraction using 80% hydroethanolic at 4°C (Fig. 1). The total anthocyanins and phenolics content varies among samples, being in close correlation with the methods of plant production, post-harvest practices and pre- and genetic factors. The highest content of anthocyanins and phenolics was found in edible bulb of the Red Turda variety. Instead, outer peels of red onion proved to be a valuable source of antioxidants (anthocyanins, polyphenols) which may lead to exploitation of such materials for food, pharmaceuticals and textiles applications. There have been made also studies regarding the stability of onion extracts under various conditions (temperature, pH) for a storage period of 10 days at room temperature. The results showed a significant decrease in the content of anthocyanins at pH> 4.5 (Fig. 2). Thermal degradation of anthocyanins studied using DSC (Differential Scanning Calorimetry) started at 44.64^{o}C in case of the 80% ethanol extract at pH 9, and at 51.74^{o}C of the same extract at pH 4.5 (Fig. 3 -4).

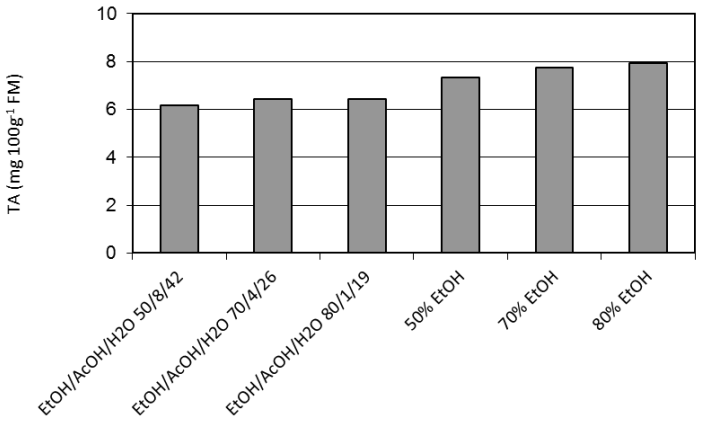

Fig. 1. Total anthocyanins (TA) of red onion (*Allium cepa* L. cv. Red of Turda) in different solvent systems, at 4 ^{o}C.

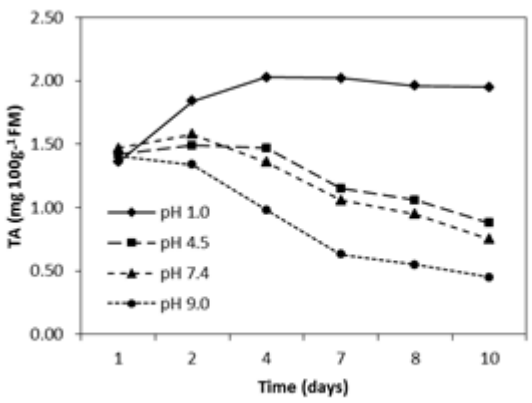

Fig. 2. The effect of pH on the stability of hydroethanolic extract from red onion (*Allium cepa* L. cv. Red of Turda).

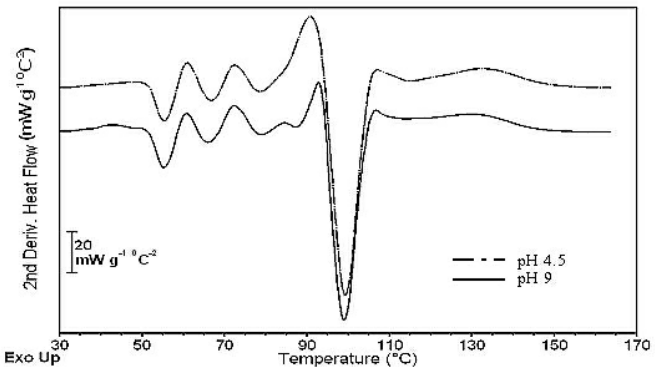

Fig. 3. DSC thermogram of anthocyanin extract from *Allium cepa* L. cv. Red of Turda, at different pH.

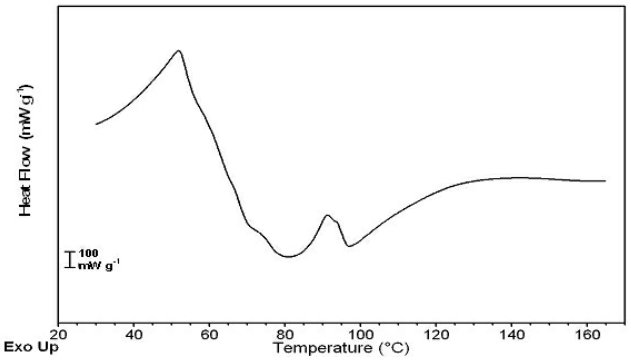

Fig. 4. DSC thermogram of anthocyanin extract from outer peel of red onion (*Allium cepa* L. , Sibiu).

The results of the investigation of three cherry samples from Romania (wild cherry, cherry grown in Sibiu region and cultivated variety (Black Gold), showed that conventional extraction with hydroethanolic acidified solution lead to a higher amount of anthocyanins. Of the analyzed samples, wild cherries have the highest content of anthocyanins (95.93 mg/100 g FM) and total phenolics (275.94 mg GAE/100 g FM). For the samples grown in Sibiu and those cultivated, the optimizitation of extraction was performed using ultrasounds and using the optimum extraction system resulted from the extraction by conventional methods (60% EtOH acidified 0.1% HCl), temperature 30°C. The extraction time and the ratio solvent/sample were studied. The optimum ratio has been obtained for the ultrasound-assisted extraction for 5 min. at a ratio of solvent/sample 15.

In the investigation of five samples of red raspberries from Romania, of which 2 are cultivated varieties (Heritage, The Lathan) and three are from spontaneous flora (Sibiu), the results showed a higher level of anthocyanins in samples grown in garden compared to cultivated varieties. Regarding the garden-grown sample of raspberry (Sibiu) optimization of extraction was performed using ultrasounds and EtOH 70%. Extraction temperature (20^{o}C, 30^{o}C) and ratio solvent/sample were evaluated. At a temperature of 30°C and ratio solvent/sample of 20/1, the results of 4 experiments have shown an efficient extraction time of 15 minutes. At a temperature of 20°C and extraction time of 15 minutes, the ratio of solvent/sample of 15/1 showed efficient extraction by ultrasound-assisted

extraction. At a temperature of 20°C and a ratio of solvent/sample of 10/1, the extraction time of 20 minutes showed a higher content of anthocyanins obtained by ultrasound-assisted extraction.

Regarding the investigation of 3 samples of blackberries from Romania (garden-grown in Sibiu, wild from Poiana Marului/Fagaras and cultivated variety Thornfree), the results showed a higher content in Thornfree variety (198.25 mg/100 g FM) followed by wild blackberries (177.21 mg/100 g FM) and then the garden-grown blackberries (101.46 mg/100 g FM). For the Thronfree variety, optimization of extraction was performed using ultrasounds and 80% EtOH acidified with HCl 0.1% at 30^{o}C. The impact of the extraction time and the ratio solvent/sample was studied. At a temperature of 30°C, the results indicate an optimum time of 5 minutes at an optimum solvent/sample ratio of 10/1.

The bioextracts were analyzed for the evaluation of the anthocyanins profile using mass spectrometry MS. The results indicate the potential of using such technique to distinguish the biological source of a product containing anthocyanins.

Dissemination and evaluation of research results

All research results obtained in the reported implementation period were disseminated through publication in scientific journals and by participation at international scientific conferences/ workshops, as follow:

- 3 articles published in journals indexed in International Databases (BDI)
- 12 scientific papers published and presented at international conferences
- 2 undergraduate theses
- participation of students at scientific sessions/symposia in the field

Also, we have achieved activities aimed to promote the partial results of the project (Power Point oral presentation) to the Exhibition/fair organized in Bucharest by the Romanian Employers' Federation of Food Industry, during the period October 30 - November 3, 2012.

We have also initiated an international collaboration in the field of anthocyanins profiling using mass spectrometry, with experts from the Research Institute CNR of Padova, Italy.

Project director,
Professor OANCEA Simona, PhD