Summary of the SCIENTIFIC REPORT

Perioad of implementation: January 1 – December 31, 2013

During the 2013 project stage, specific activities were carried out within the objectives of the project PCE-2011-3-0474: assessment of total anthocyanins content from selected plants, in fresh state and under storage conditions for a determined period of time (freezing, drying), *in vitro* evaluation of the antimicrobial activity of anthocyanins extracts, addition of anthocyanins extract in (poly)unsaturated lipid systems, and *in vitro* evaluation of the inhibitory potential of extracts on the peroxidation in order to produce food products with improved qualities. Dissemination and evaluation of the research results were also done.

Physical-chemical, microbiological characterization of natural extracts

Microbiologically, the prepared bioextracts were according to normatives. Regarding the physical-chemical characterization, values of determined parameters for red onions, blackberries, cherries and red raspberries are presented below:

Sample	**Red onion (*Allium cepa* L.)/cultivation area**	**Moisture (g 100g^{-1})**	**pH**	**Total anthocyanins (mg 100g^{-1} FM)**	**Total phenolics (mg GAE 100g^{-1} FM)**
1	Turda	86.5	6.16	2.30	174.2
2	Turda	89.1	5.84	0.12	141.5
3	Turda	90.2	6.15	7.93	197.5
4	Făgăraş	90.4	5.99	2.36	159.2
5	Făgăraş	90.0	6.08	2.36	158.2
6	Făgăraş	87.0	6.18	1.35	*n.d.**
7	Făgăraş	89.5	6.05	1.01	*n.d.**
8	Buzău	90.1	6.00	2.05	147.5
9	Drăgăşani	90.0	6.15	6.19	185.7
10	Sibiu	14.9	3.80	99.66	1345.74

*n.d. = not determined

Sample	**Physical-chemical characterization**				**Extraction solvents**	**pH**
blackberry cv. Thornfree (*Rubus fruticosus* L.)	*Moisture (%)*	*TSS (^{o}Brix)*	*Refractive index (n)*	*Total phenolics (mg GAE 100g^{-1} FM)*		
	84.4	10.4	1.3477	257.18	0.1 % HCl in 60 % EtOH (v/v)	2.17
cherry cv. Black Gold (*Prunus avium* L.)	78.9	19.4	1.3621	184.85	0.1 % HCl in 60 % EtOH (v/v)	1.72

Sample		**Physical-chemical characterization**				
		Moisture (%)	*TSS (^{o}Brix)*	*Refractive index (n)*	*Total phenolics (mg GAE 100g^{-1} FM)*	*Total anthocyanins (mg 100g^{-1} FM)*
Red raspberry (*Rubus idaeus* L.)	wild	86.1	11.7	1.3495	340.00	71.26
	garden	87.6	10.9	1.3495	198.00	41.52
	garden	88.2	7.7	1.3445	215.00	40.84
	cv. Latham	84.4	10.8	1.3483	189.71	27.03
	cv. Heritage	82.7	11.2	1.3489	164.76	24.53

The prepared bioextracts were analyzed for the anthocyanins profiling using MS. The results were compared to samples of different origins (Fig. 1).

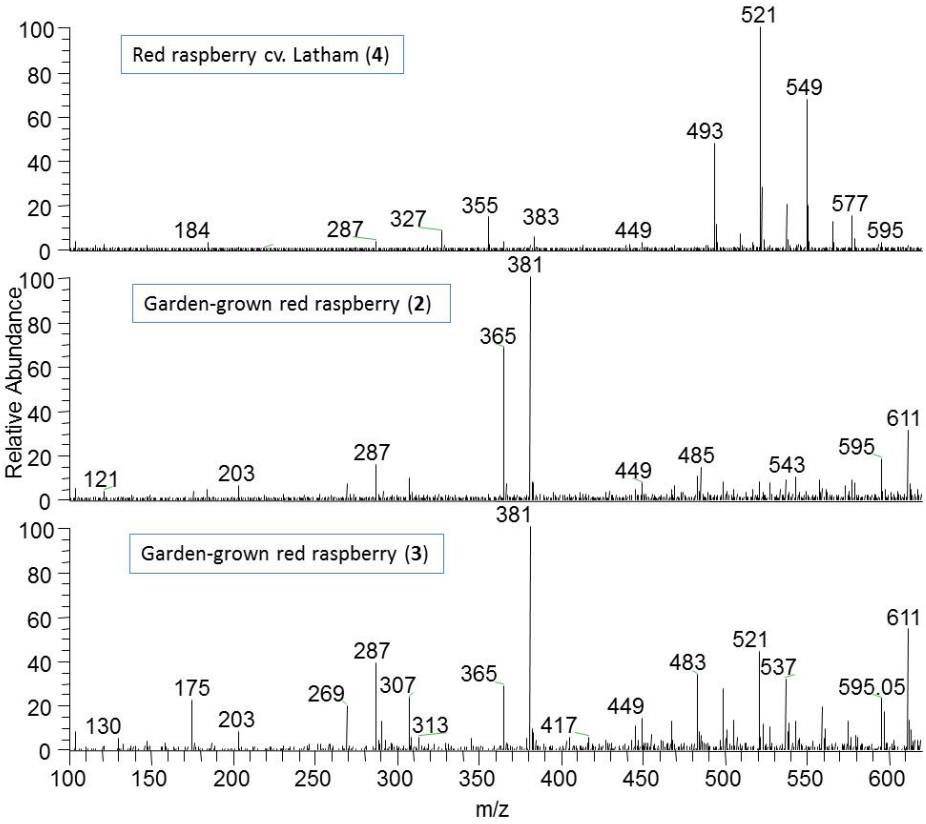

Fig. 1. The comparative anthocyanins profile performed by ESI/MS of red raspberry extracts.

Evaluation of the anthocyanins level of relevant extracts after storage for a determined period of time (freezing, drying)

The obtained results of the investigation of the influence of fruits freezing indicate a good recovery of anthocyanins in wild red raspberry compared to garden-grown which showed a decrease of 7-24%; cultivated samples showed a decrease of 47 – 69% in anthocyanins content after storage at – 18°C (Fig. 2).

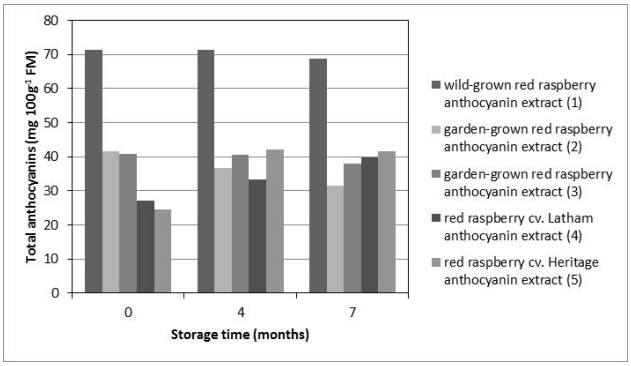

Fig. 2. Anthocyanisn content in *Rubus idaeus* L. during 7 months of freezed storage.

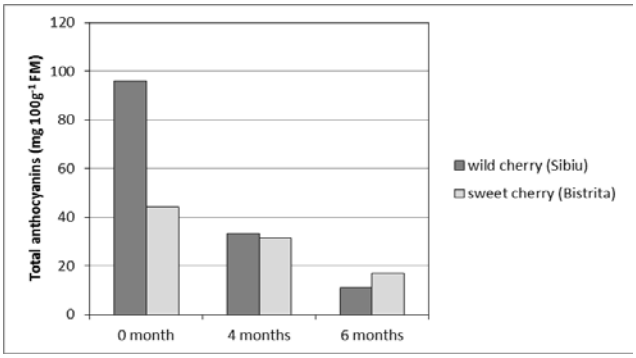

Fig. 3. Anthocyanins content in *Prunus avium* L. during 6 months of freezed storage.

Cherry samples showed low stability in time by freezing, in terms of the anthocyanins content which drastically decreased to 88.5% in the case of wild cherries and to 61.5% for cv. Black Gold after 6 months. Regarding blackberries, similar results were obtained in relation to the species frozen at -18°C up to 6 months.

The effect of drying of the raw material (oven and rapid infra-red IR methods) on the amount of anthocyanins was also investigated.

For raspberry samples, a drying time interval of 0-13 hours and temperatures of 60°C and 80°C was studied. The results showed that during the first 5 hours of oven drying no significant changes in the content of anthocyanins from 60°C to 80°C were registered, but a long period of drying strongly affect the content of anthocyanins (45% loss after 7 hours of fruits drying). The results of the IR drying showed a 38% decrease in the content of anthocyanins by drying at 60°C and a decrease of 20% at 80°C. These indicate the generation of chemical reactions which accelerate the degradation of anthocyanins, further investigation being required to establish the mechanism of action. The quality of dried fruits in terms of nutritional value and the economic issues (in particular energy consumption) are important factors to be considered for the decision of industrial application of different drying technologies, conventional or modern.

For blackberry samples, the content of anthocyanins show great variations after oven drying at 60°C with an initial increase of 18.5% to a final increase of 1.5% compared to initial state, suggesting the occurrence of chemical reactions of co-pigments formation, while at 80°C there was registered a steady decrease in the anthocyanins content for 2-6 hours.

For cherry samples, there was an initial increase in anthocyanins content (19-26%) by drying at 60°C, 70°C and 80°C respectively, followed by a steady decrease of 3.7% at 60°C, 55.5% at 70°C and 74% at 80°C for 8 hours. Through IR drying, after an initial increase of the anthocyanins content in the first 4 hours at high temperatures (70°C, 80°C), the content of anthocyanins reached 48-59% of the initial state after 8 hours of drying, showing large variations throughout the drying process.

Selection and isolation of bacterial strains. Evaluation of antimicrobial activity of natural extracts.

Our studies aimed at evaluation of total antioxidant activity by FRAP assay and of *in vitro* antimicrobial activity of 11 anthocyanins extracts obtained from wild and cultivated fruits. The highest total antioxidant activity was registered for blackberries and bilberries (26.95 mg ascorbic acid g^{-1} DM, respectively 18.00 mg ascorbic acid g^{-1} DM). The results of antimicrobial activity investigated by diffusimetric method on Gram-positive, Gram-negative and *Candida albicans* strains, showed an inhibitory effect of bilberry extract on *Bacillus cereus*, while red onion extract showed good antibacterial activity against *Streptococcus pyogenes*.

Testing the inhibitory potential of anthocyanins extracts on the peroxidation of (poly)unsaturated lipid systems.

Following the actual trend of replacing synthetic antioxidants used in the additives market, we investigated the antioxidant potential of anthocyanin extracts for oxidative stabilization of edible or dietary oils. We evaluated micelles systems obtained by adding the bilberry anthocyanins extract in cod oil. The results showed a very good efficiency of this extract compared to tocopherol-enriched cod oil sample and to control sample. Inhibition of the peroxides generation in the cod oil increased from 20 to 50.7% with the addition of anthocyanins extract and from 3 to 30.4% with the addition of a mixture of tocopherols, in the first 4 days at 30°C. During storage of the oil samples for 42 days at 15-17°C, an improvement in the oil oxidative stability was registered. Similar results were obtained with red onion anthocyanins extract which stabilized sunflower oil during storage at 40°C, investigation done by assessing the peroxide value and the thiobarbituric acid reactive substances (TBARS) assay.

Also in this period was done experimental research for textile applications by using the natural extract of red onion. Experimental tests of dying flax fabrics with red onion extracts were conducted, aiming to improve the dyeability of such substrates. Preparation of the textile substrate was made using the grafting procedure with a cyclodextrin derivative and the bath dyeing by using two different concentrations of 1-2% relative to the lignocellulosic fibre support at a 1:30 liquor ratio, at 80°C. The morphology of the surface was characterized by scanning electron microscopy SEM (Fig. 4), the chemical structure by FTIR spectroscopy, investigations which were completed with the determination of the color coordinates and the color fastness to washing and rubbing. For the samples subjected to such experiments, relevant results was obtained regarding the dyeing potential of natural extracts and the strength and intensity of color of dyed fabrics achieved by pre-treatment with an inclusion compound (cyclodextrin derivative). The surface of samples treated with cyclodextrin was

stiffer than that of untreated samples. Due to the inclusion of the dye into cyclodextrin molecule, IR spectra showed a significant shift of the band at 530 cm^{-1} to 890 cm^{-1}. The FTIR spectra of anthocyanins show intense bands at 3400 cm^{-1} (hydroxyl group) and at 1710 cm^{-1} (carbonyl group). The results showed that the dying becomes more resistant when using red onion extract at higher concentrations. The cyclodextrin functionalization of the flax substrate significantly lead to the increase and stabilization of color.

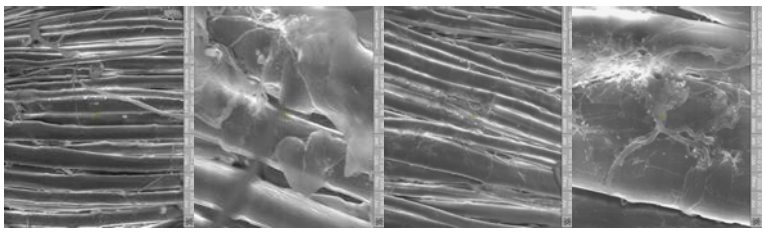

Fig. 4. SEM images of samples dyed with 1% red onion extract.

Color measurements and color fastness values for samples dyed with red onion extract

Textile sample	**L***	**a***	**b***	**C***	**H***	**ΔE***	**Washing fastnes**	**Dry and wet rubbing fastnes**
Flax untreated	92.57	-0.45	4.12	4.14	96.29	-	3	3
Flax/cyclodextrin	88.08	0.03	6.55	6.55	89.77	5.13	3-4	3-4
1	56.88	15.42	13.17	20.28	40.48	3.86	5	5
2	40.78	21.51	11.09	24.20	27.28	56.69	3-4	4-5
3	62.84	13.27	15.41	20.34	49.28	18.19	4-5	3-4
4	56.29	15.88	13.04	20.55	39.39	40.78	3-4	3-4

Dissemination and evaluation of research results by publication of articles, participation at scientific conferences

The research results obtained during the reported implementation period were disseminated through published/in press scientific articles, as follow:

- 5 articles in ISI quoted journals with total impact factor = 2.989 and relative influence score = 1.6118,
- 1 article under evaluation for publication in ISI quoted journal
- 3 articles published in journals indexed BDI
- 1 patent proposal registered at OSIM Bucharest, Romania
- 10 scientific papers presented at international conferences.

Training young researchers: three undergraduate theses were developed in the field. A Master student was involved in documentation and testing activities. Four scientific papers were presented by students at Student's Scientific Symposia.

Project director,
Professor OANCEA Simona, PhD