SYNTHETIC SCIENTIFIC REPORT

Period of implementation: April 1, 2012 – December 31, 2016

The main goal of the project is valorisation of plant resources for extraction of anthocyanins, quantitative assessment of these phytochemicals in certain species (primary screening for supporting food plant in the Romanian territory) and application of bioextracts for the purpose of multifunctionality added to a dietary supplement - oil (contribution to the prevention of major diseases of the population) subject to investigation of the *in vitro* antioxidant potential. The specific objectives of the project are: Determining the optimal conditions for extraction of anthocyanins from 4 types of vegetable raw materials; determination of monomeric anthocyanin concentration of the plants investigated, under fresh and storage conditions for a determined period of time (freeze drying) and processing (jams); The *in vitro* antimicrobial activity of the anthocyanin extracts from plants investigated; Incorporation of anthocyanin extracts to edible oils and *in vitro* evaluation of potential inhibition of peroxidation processes in order to achieve improved quality of foodstuffs; Application of the prepared natural extracts in the dyeing of textile materials, in the context of new environmental requirements including eco-compatibility of textile wastewater as an alternative to using synthetic dyes; Dissemination and evaluation of research results.

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.

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.

Anthocyanins are considered among the most active biomolecules in terms of physiological and biological effects, thus providing nutraceutical properties.

During 2012 project stage, identification, selection and sampling of biological materials and Extraction of anthocyanin pigments under optimum conditions and determination of total monomeric anthocyanins content were performed.

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).

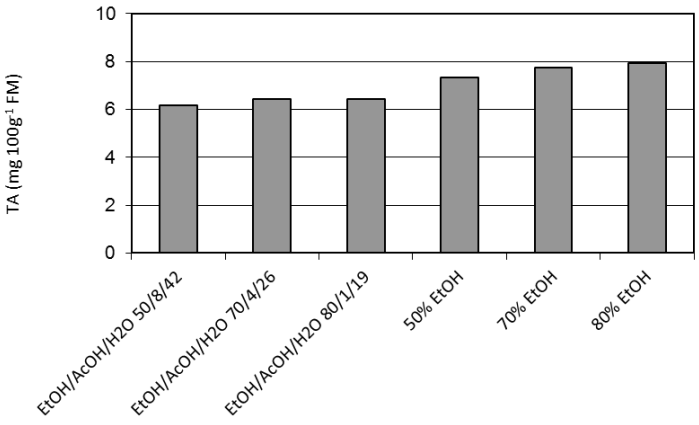


Fig. 1. Total anthocyanins (TA) of red onion (*Allium cepa* L. cv. Red of Turda) in different solvent systems, at 4 ^{o}C.

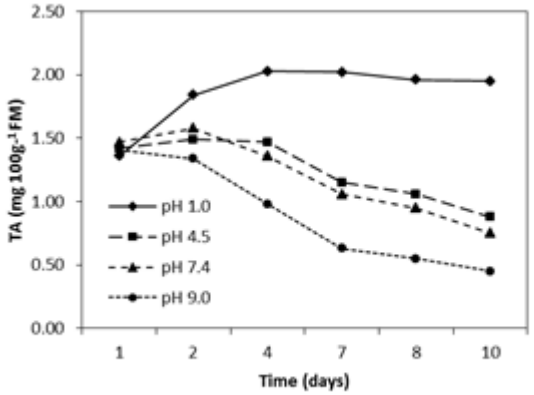


Fig. 2. The effect of pH on the stability of hydroethanolic extract from red onion (*Allium cepa* L. cv. Red of Turda).

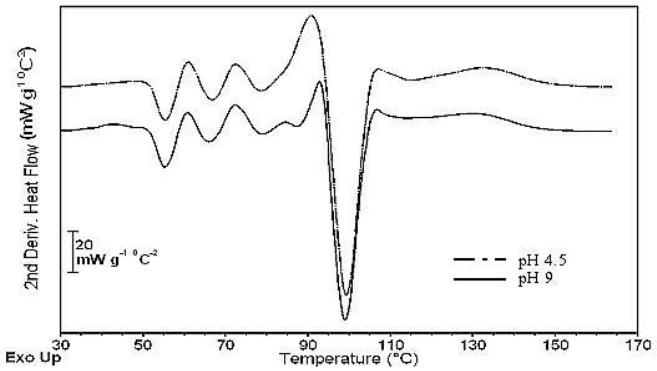


Fig. 3. DSC thermogram of anthocyanin extract from *Allium cepa* L. cv. Red of Turda, at different pH.

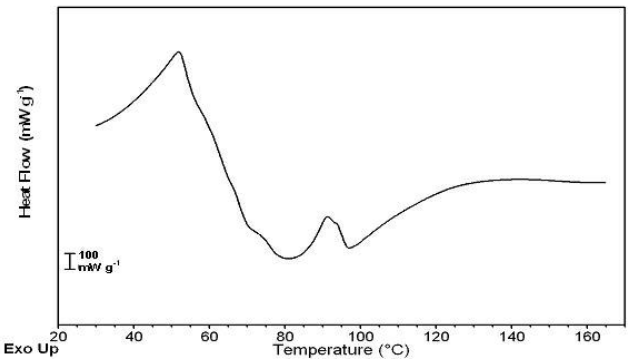


Fig. 4. DSC thermogram of anthocyanin extract from outer peel of red onion (*Allium cepa* L. , Sibiu).

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.

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.

It has been done the physical-chemical and microbiological characterization of natural extracts. Microbiologically, the prepared bioextracts were according to normatives.

The prepared bioextracts were analyzed for the anthocyanins profiling using MS. The results were compared to samples of different origins (Fig. 5).

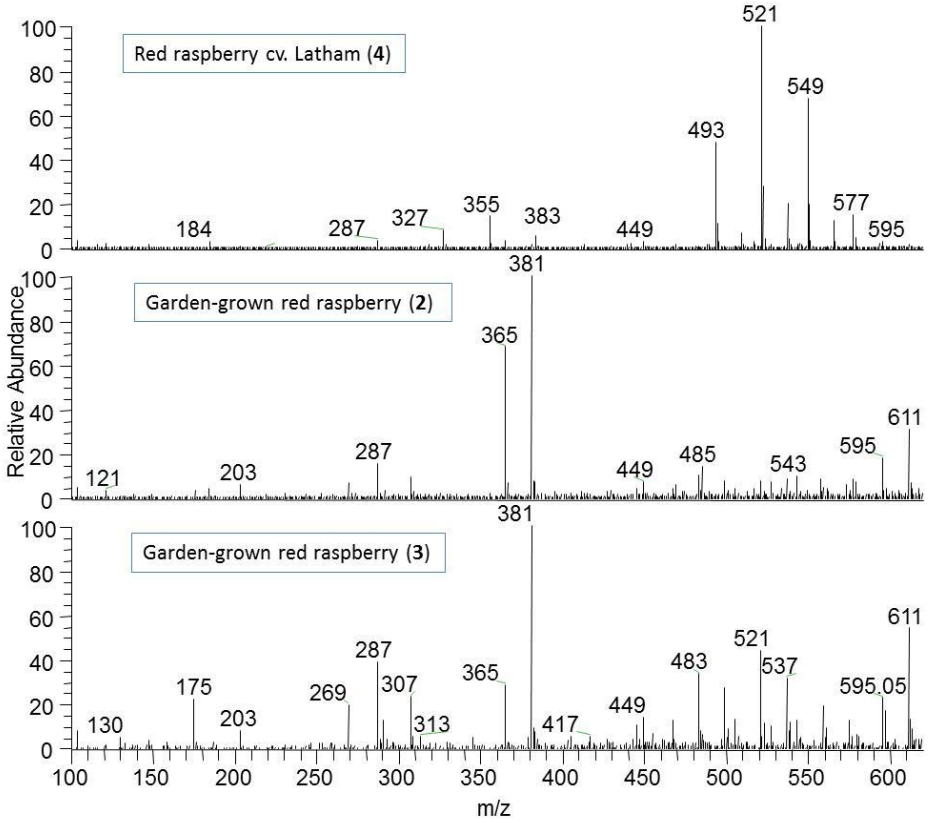


Fig. 5. The comparative anthocyanins profile performed by ESI/MS of red raspberry extracts.

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^{o}C (Fig. 2).

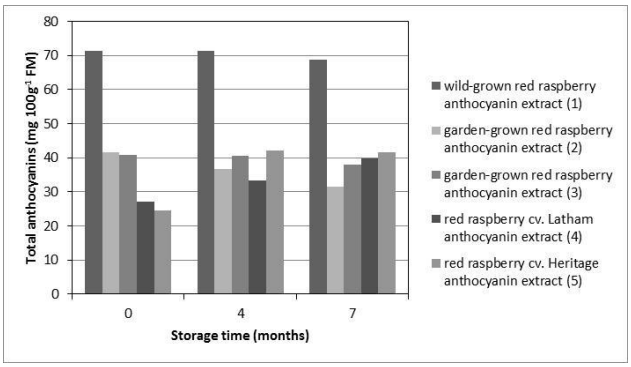


Fig. 6. Anthocyanisn content in *Rubus idaeus* L. during 7 months of freezed storage.

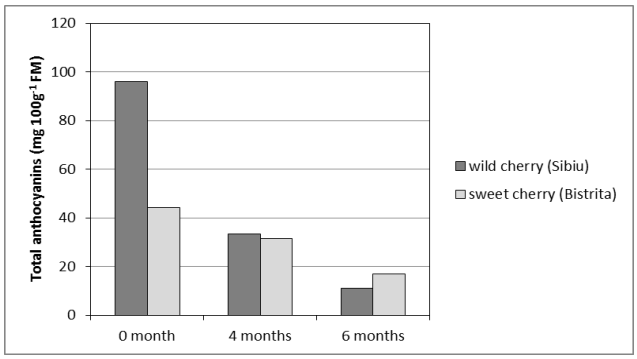


Fig. 7. Anthocyanins content in *Prunus avium* L. during 6 months of freezed storage.

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^{o}C and 80^{o}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^{o}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^{o}C, 70^{o}C and 80^{o}C respectively, followed by a steady decrease of 3.7% at 60^{o}C, 55.5% at 70^{o}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.

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*.

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. 8), 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.

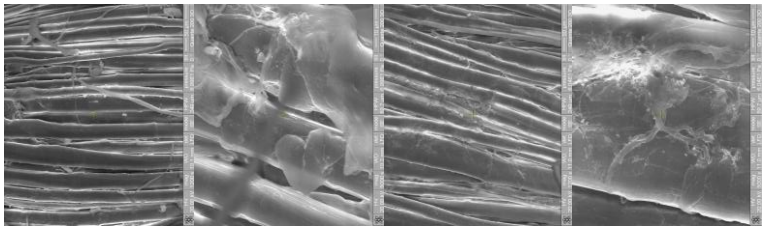


Fig. 8. SEM images of samples dyed with 1% red onion extract.

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

All objectives were achieved and all planned results were obtained during the 2014 stage. Scientific results show that fruit processing in jams by traditional method lead to significant changes in overall levels of antioxidant compounds, particularly anthocyanins compared to that of phenolics. Instead, the antioxidant compounds degradation during storage of jams at 18°C for 5 months occurs at very slow rates. However, fruit jams from various sources still contain important amounts of bioactive compounds, optimization of the process for less content losses being required. Scientific results obtained through addition of anthocyanin crude extracts in (poly)unsaturated lipids in reverse micellar systems, highlights the potential of these bioactive compounds to act as inhibitors of lipid peroxidation with possible applications in the food industry.

The visibility of the scientific production of the project team is guaranteed by the publication of 3 articles in international journals, acceptance for publication of 1 article in ISI journal, 2 articles published in journals indexed BDI, 1 book published in Lambert Academic Publishing and 5 papers presented/published at international conferences in 2014. Also, 2 articles have been evaluated for publication in ISI journal. 2 undergraduate theses were developed in the field. International visibility is proved by getting 3 citations in 2014 of articles already published in the project (according to ISI Web of Science). Project web page has been updated.

Given the fact that fruits rich in antioxidant compounds are seasonal with limited availability throughout the year, we studied the influence not only of the preservation methods (freezing, drying) previously investigated but also the form of food processing. Thus, we studied the effect of fruits processing – jams, on the content of bioactive compounds (anthocyanins and phenolics) compared to fresh fruits. To this end, different phases of experimentation were involved, such as the extraction of anthocyanins and polyphenols using optimized extractive technologies described during previous phases of the project (2012, 2013), centrifugation and determination of the total content using standard spectrophotometric pH differential method. Polyphenols were analyzed quantitatively using the Folin-Ciocalteu method.

In the next phase, it was experimented and established a method of producing fruit jams with low added sugar (<40%) and no added additives, colorants or pectin.

The obtained results showed a decrease of more than 60% of the total content of phenolics, particularly for red raspberry samples. Instead, the study on the evolution of phenolics level of jams at 18^{o}C showed that there was a significant decrease during 5 months of storage except cherry jam which registered a final decrease of 21% of total phenolics.

Regarding the content of anthocyanins, it was found a drastic decrease through in jams: more than 80% in blackberry and wild cherry jams, over 60% in red raspberry and cherry jams, compared with the anthocyanins content in fresh fruits. This is due to low stability of these molecules under the influence of different environmental factors.

The study on evolution of anthocyanins level during storage of jams at 18^{o}C, showed a decrease of 36-62% over 5 months in all samples, depending on the fruit. The lowest value was recorded for wild cherry jam, and the highest value for red raspberry jam.

We conducted studies on the degradation of anthocyanins, considering that this process is a reaction following first order kinetics. We calculated the rate constant (k) and half-life ($t_{1/2}$) from the following equations:

$$\ln \frac{[TA]}{[TA_0]} = -kt \qquad t_{1/2} = -\frac{\ln 0.5}{k}$$

The highest stability was registered for wild cherry jam, followed by cherry, blackberry and red raspberry jams.

Following the trend of replacing synthetic antioxidants from the additives market, we investigated the antioxidant potential of anthocyanin extracts for oxidative stabilization of edible or dietary oils. Such systems have been tested and evaluated in reverse micelles systems obtained by the addition of the anthocyanin extract to polyunsaturated lipids systems by extending the previously developed model for obtaining micellar systems.

The bilberry bioextract was tested for the *in vitro* antioxidant activity in rapeseed oil consisting of 62% monounsaturated fatty acids and 30% polyunsaturated fatty acids. The hydroethanolic extract was characterized by a total anthocyanins content of 111.14% and a total phenolics content 195.76%.

The oxidative stability was monitored by incubating the samples at 40°C for 14 days. After determination of two characteristic parameters (peroxide and TBARS values) very good results were obtained compared to the control sample and to the sample treated with a reference antioxidant, α-tocopherol.

The peroxidation process was shown to evolve much more slowly in the sample treated with anthocyanins extract (Figure 9). Statistical studies have shown a positive correlation of the peroxide value (PV) with the time of storage, the correlation coefficient being 0.98343. Instead, α-tocopherol proved a pro-oxidant effect after 3 days of storage.

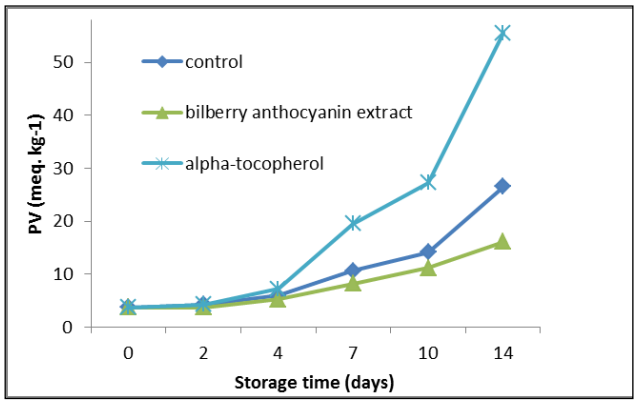


Fig. 9. The oxidative stability of rapeseed oil with added anthocyanins extract and α-tocopherol, monitored by peroxid value.

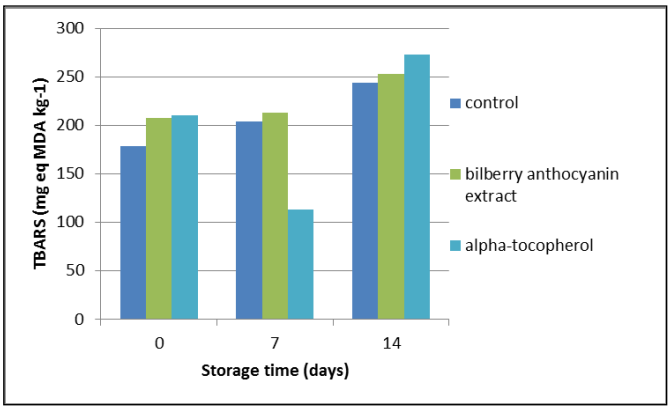


Fig. 10. The oxidative stability of rapeseed oil with added anthocyanins extract and α-tocopherol, monitored by TBARS.

Research on evolution of oxidation byproducts during storage of samples at 40°C for 14 days showed similar trend for the control sample and for the sample with added anthocyanin extract (Figure 10).

The results achieved by addition of crude anthocyanin extracts to polyunsaturated lipids in reverse micelles systems, highlights the potential of these bioactive compounds to act as inhibitors of lipid peroxidation leading to possible applications in the food industry.

Throughout the implementation of the project, acquisition of materials, chemical reagents and instruments necessary to conduct the planned research activities was also conducted.

All these activities prove the project contributions to the development of human resources for research. Such activities led to increased collaboration and encouraged interdisciplinary needed to ensure the project sustainability.

In the coming period, in order to complete all the project objectives, activities regarding applications of natural extracts in textile dyings will be planned by testing the optimal conditions for dyeing of cellulosic materials with natural extracts rich in anthocyanins. These results will lead to opportunities for innovative products and processes, in the framework of the new environmental requirements including eco-compatibility of textile wastewater as an alternative to the use of synthetic dyes.

All objectives were achieved and all planned results were obtained during the 2015 stage. The obtained scientific results show that anthocyanins extracts from bilberries and blackberries optimized by previously described methodology can be successfully applied for dyeing of textile supports (flax and flax functionalized with MCT-β-CD) by two methodologies - exhaustion and ultrasonication, respectively. The results obtained from characterization of textiles dyed using analytical techniques such as SEM, FT-IR spectroscopy and BET analysis, showed that the ultrasonication technique is useful in optimization of dyeing capacity, expressed through the K/S values, compared to the conventional method through exhaustion. By comparing the color fastness values and the behavior of dyed substrates to abrasion, the results indicate low to satisfactory resistance, for both functionalized samples and dyed samples by the investigated methods of coating. The obtained results show that the natural dyeing procedure with bilberry and blackberry anthocyanin extracts of flax fabrics grafted with a cyclodextrin derivative might be a good alternative for the optimization of the resistance to washing and rubbing.

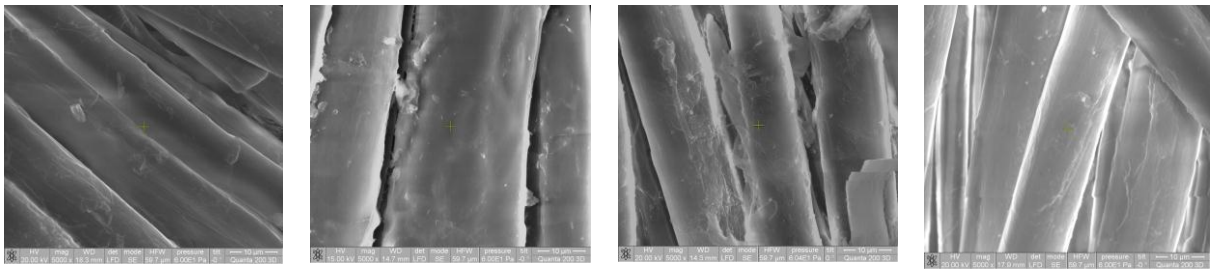
The visibility of the scientific production of the project team is guaranteed by the publication in 2015 of 2 articles in ISI journals, acceptance for publication of 1 article in ISI journal, 2 articles published in journals indexed BDI, 3 participations at Patent/inventions events and 5 papers presented/published at international conferences in 2015. One undergraduate thesis was developed in the field. International visibility is proved by getting 7 citations of articles already published. Project web page has been updated.

The presence of natural extracts in textile fabrics is important, as it contributes to consumer's hygiene and also to clean processes. Application of anthocyanin extracts obtained from fruits on textile substrates could further improve certain properties such as antimicrobial, anti-inflammatory and anticancer.

Conventional dyeing process involves the use of chemical agents and heat. By using ultrasonication, dyeing baths can have lower temperature because of improved dispersion and inclusion of dyes.

In terms of methodology, our experimental studies primarily aimed at preliminary improving the flax substrate by using β-cyclodextrin-monochlorotriazinyl (MCT-β-CD). Secondly, in the experimental protocol, bilberry or blackberry extracts were applied to the material by two methods of the dyeing process: exhaustion and ultrasonication. The motivation of such research is to improve the fastness properties of dyed textile substrates by use of the capacity of the hydrophobic cavity of MCT-β-CD to form inclusion complexes with the pigments based on their selectivity. Moreover, it highlighted the inclusion complex formation between anthocyanin dye and MCT-β-CD.

Morphological, structural characterization and strength properties of coated materials was obtained using a SEM investigation, FT-IR, analysis of specific surface area by the BET method and fastness resistance. The results of the analysis of the samples showed homogeneity of samples through dyeing by US procedure. In addition, optimization of resistance to washing and rubbing was quantified by values increased by 0.5-1 points. The morphology of the flax fibers after the grafting and dyeing by the two methods was assessed by SEM. The results showed that the fine structure of the samples is quite different from the fibers of non-functionalized flax and the absorption properties of the natural extract is probably due to the micro-cavities created after functionalization with the derivative of ß-cyclodextrin MCT. By ultrasonication, the fiber morphology becomes very smooth.



Flax | Flax –MCT-ß-CD | Functionalized flax dyed with bilberry extract by exhaustion method | Functionalized flax dyed with bilberry extract by ultrasonication

Fig. 11: SEM images of flax functionalized substrates dyed by exhaustion and ultrasonication methods with 2% bilberry extract (*Vaccinium myrtillus*) at ×5000 magnification

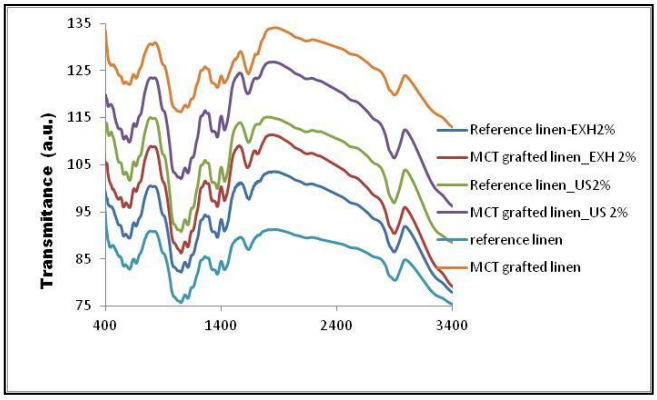


Fig. 12: FT-IR spectra of flax substrates dyed by exhaustion and ultrasonication methods with 2% bilberry extract.

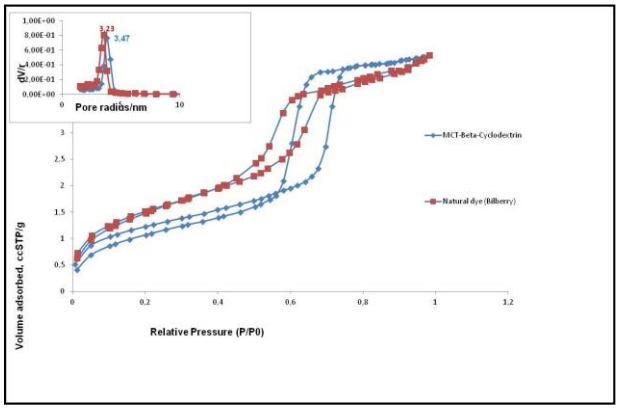


Fig. 13: Nitrogen adsorption/desorption isotherm of inclusion compound (MCT-β-cyclodextrin and pigments)

Spectroscopic investigations demonstrate the formation of the inclusion compound. In addition, there are changes in IR spectra, which can be noticed at 1634, 1180, 1127, and 900 cm^{-1}, due to binding of ß-CD MCT. This is well supported by BET analysis, revealing entrapping of dye molecule inside the MCT-β-cyclodextrin.

Evaluation of dyed materials with extracts relevant for dyeing technology was completed by determination of color fastness and color attributes of the treated supports. The fastness intensities, respectively the K/S values of dyed lignocellulosic substrates were measured using the spectrophotometer Datacolor 110 LAV. Abrasion testing was performed using the instrument NU-Martindale for abrasion and pilling. The friction test was performed using the Crockmaster instrument.

Table 1 shows the results of measurements of resistance to washing and rubbing of flax fabrics dyed with bilberry extract, compared to reference samples (flax or flax grafted with MCT-ß-cyclodextrin), together with the values attributed to nanocavities of MCT-ß-cyclodextrin and of the dye molecule.

Table 1: Fastness values and ratio radius cyclodextrin/pigment of flax fabrics dyed with bilberry extract.

Sample	**Dry rubbing fastness**	**Wet rubbing fastness**	**Washing fastness**	**Ratio CD ray /dye ray, nm**
Flax	2	1-2	2-3	
Flax / MCT-ß-cyclodextrin	3-4	2	4-5	
Flax dyed with bilberry extract by exhaustion method	3	3	2-3	-
Functionalized flax dyed with bilberry extract by exhaustion method	4-5	3-4	4-5	2.17 / 2.76
Flax dyed with bilberry extract by ultrasonication	3-4	3	4	-
Functionalized flax dyed with bilberry extract by ultrasonication	5	4	4-5	3.23 / 3.47

During the experiments, the color fastnes values of the samples dyed with anthocyanins extracts from bilberries and blackberries reveals moderate to good washing and rubbing fastness for most samples, in particular for those of functionalized flax and dyed using the ultrasonication procedure. Color attributes of naturally dyed samples show the sustainability through superior

resistance to washing, friction and abrasion due to the encapsulation process. These experiments are relevant for the use of (micro)encapsulation technology of a natural dye directly on the fabric treated with MCT-ß-CD *versus* conventional technique of dyeing by assisted mordants. This process has the potential to substitute the classic treatment, favoring future applications, in order to obtain new ecological textiles.

The resistances of the color of samples dyed with the blackberry anthocyanins extract have shown that the washing and rubbing fastness, in great majority of samples, are moderate to good, in particular for functionalized and dyed textile substrates.

Table 2: Values of color changes after the abrasion test of flax fabrics dyed with blackberry anthocyanins extract.

Sample	**Resistance to abrasion**			**Ratio CD radius /dye radius, nm**	
	ΔE		K/S		
	Reference	Abrading	Control	Abrading	Control
Flax	-	-	2.74	-	
Flax / MCT-ß-cyclodextrin	-	-	2.53	-	
Flax dyed with blackberry extract by exhaustion method	5.20	3.14	6.52	5.42	8.34
Functionalized flax dyed with blackberry extract by exhaustion method	8.74	6.34	7.89	6.47	7.15
Flax dyed with blackberry extract by ultrasonication	3.57	2.26	4.30	3.60	6.8
Functionalized flax dyed with blackberry extract by ultrasonication	3.78	2.11	4.60	3.59	4.7

Cumulative results of analytical techniques of SEM, FTIR and BET analysis showed that ultrasonication technique is useful for optimization of the dyeing capacity, expressed by K/S values compared with the conventional method of dyeing by exhaustion. By comparing the values of color fastness and the behavior to abrasion, the results indicate low to satisfactory resistance, both for functionalized samples, as for the dyed samples using both methods of coating. The results show that the natural dyeing with bilberries and blackberries of flax fabric grafted with cyclodextrin derivative might be a good alternative for the optimization of the washing and rubbing fastness.

All objectives were achieved and all planned results were obtained during the 2016 stage. During the stage 2016 we expanded our research using experimental methodology assisted by biomordants for application on various textile supports of natural extracts of black currants and nut shells obtaining encouraging results. As biomordants we tested citric acid, tannic acid by comparison with a classical one (copper sulfate).

The results showed a large difference in color attributed to wool compared to polyamide and bamboo and dyed with walnut shell extract in the presence of copper sulphate as mordant. The smallest color differences were observed in the samples coated by co-assisting tannic acid and citric acid, as biomordants in 3% concentration, compared to the reference sample. Wool samples possess higher color intensity, a fact attested by the specific surface area of the fibers, compared to polyamide fibers, therefore dye molecule being well protected by the wool fiber morphology. Values of color difference in samples of polyamide dyed with both extracts (nut shells, black currants) are well represented, highlighting the linear correlation attributed to the same mordant, in concentration of 3% and 5%, the color change diminishing progressively, from the samples dyed by co-assisting copper sulphate (3%, 5%) to a concentration of 5% tannic acid.

The color changes were made using natural dyeing extract, without mordant, as a reference sample. Experimental data were aquired by reflectance spectrophotometer with Datacolor 110 LAV operating in CIELab system parameters: ΔL *, Δa *, Δb *, ΔC *, Δh * color differences ΔE *. By making a comparison of the three textile supports dyed with extracts from blackcurrants and nut

shells, we conclude that major changes color are remarkable when dyeing process is assisted by citric acid as biomordant, while the classic agent mordant does not induce relevant color differences.

The investigation regarding the antimicrobial effects have also shown that dying with walnut shell extract co-assisted by biomordanţi expresses good antimicrobial activity. Testing was performed on two standard bacterial strains *Staphylococcus aureus* ATCC 25923 (Gram-positive) and *Pseudomonas aeruginosa* ATCC27853 (Gram negative). There has been an increase in the diameter of inhibition with increasing value CIE / DE, resulting in an approximately linear correlation.

The fastness to abrasion test was performed according to standard method EN ISO 105-X12 SR using a laboratory Crockmaster 760. For washing resistance, the SR EN ISO 105-C10 at 40˚C was applied. The results demonstrate that dyeing assissted by mordants in wool fibers, polyamide and bamboo with 3% concentration of mordant have poor strength properties, as compared with those dyed with biomordants in concentration of 5% (Fig. 14).

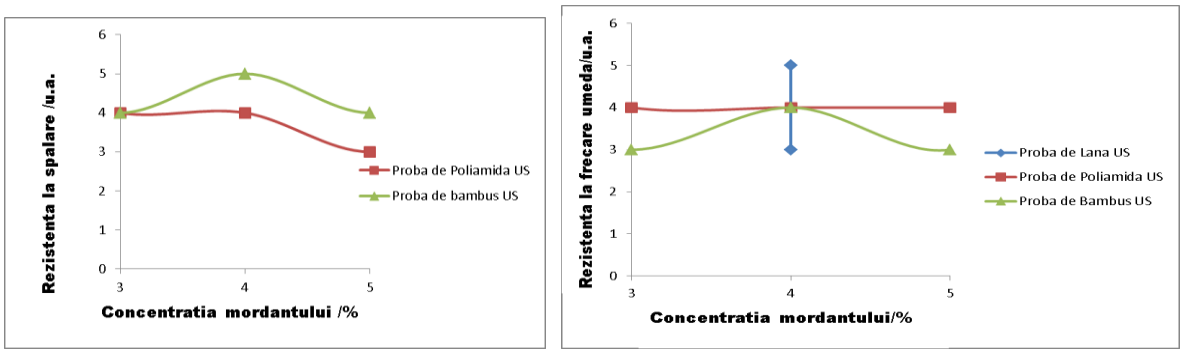


Figure 14. Plots of washing and wet scrub resistance of wool, polyamide and bamboo.

The conducted research during the last stage of the project constitutes a key point in identifying possible technological alternative applied to ecological textile finishing of natural and chemical textile supports, quantified and controlled by colorimetric response and antibacterial potential, aiming at developing hygienic textiles.

Dissemination and evaluation of research results

All research results achieved during 2012-2016 project implementation were disseminated through publication of scientific articles and participation with papers at international scientific meetings / workshops:

- 13 articles published in ISI journals of which 1 article in "red zone" and 1 article in the "yellow zone" with a cumulative impact factor of 6.062, influence score of 4.797 and 21 citations in ISI journals
- 13 articles published in journals indexed IDB
- 35 scientific papers published / presented at international conferences
- 1 book at LAP Publishing
- 1 Patent proposal and 3 participations in the Inventions events
- 6 undergraduate theses in the field
- 7 student participation in student scientific sessions

Promoting activities were also achieved under the Fair organized in Bucharest by the Romanian Employers' Federation of Food / 2012.

We have also initiated an international collaboration on anthocyanin profile studied using mass spectrometry, at the Research Institute CNR, Padova, Italy.

Project director,
Professor OANCEA Simona, PhD