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### **President's** Allocution

We have the special pleasure to let you know that the Review of our University, "**Bulletin of Scientific Information**", having ten years of consecutive issue, it achieved the recognition of the N**ational Gouncil for Sciențific Research in Nigher Education** (NURG), beiņg comprised in the catęgory "N**ational Reviews –** C G**atęgory**".

So, the Bioterra University review **"Bulletin Of Sciențific Information**" works as a real plątform for the information and exhibition of the most recent and valuable research in the agricultual field and connected sciences (food industry, agro-tourism, ecology, agricultural economics etc.).

This way I express my gratitude the contributors to our review, authoritative academic and univeritary names of whose studies are found in the selection done by the scientific board of the review, co-workers with whom we have strong relations of partnership and mutual support in the development and course of some conjointed research projects.

I wish to the review many and consistent issues.

**Drof. Floarea Nicolae, DhD** Dresident of Senat Bioterra University Bucharest

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### Summary: » THE APPLICATION OF SOME COORDINATION COMPOUNDS IN REGULATION OF THE CONTENT OF ANTIOXIDANTS IN SPIRULINA PLATENSIS Valentina BULIMAGA<sup>2</sup>, Nadejda EFREMOVA<sup>1</sup>, Liliana ZOSIM<sup>2</sup>, Ludmila BATIR<sup>1</sup>, Daniela ELENCIUC<sup>3</sup> <sup>1</sup> Institute of Microbiology and Biotechnology of Moldova, Chishinau, Moldova <sup>2</sup> State University of Moldova, Laboratory of Phycobiotehnology, Chishinau, Moldova <sup>3</sup> University of Academy of Sciences of Moldova, Chishinau, Moldova Corresponding author: Nadejda Efremova, Institute of Microbiology and Biotechnology of Moldova, 1, Academiei, 2028 MD, Chishinau, Moldova, efremova.nadejda@gmail.com ----- 01 » THE CORRELATION "MELLIFEROUS PLANTS-BEES-HONEY OF BEES-MAN-SOCIETY" AND ITS ROLE IN THE STUDENT'S EDUCATION BY BIOLOGICAL DISCIPLINES Mariana Iancu Department of Specialized Psycho-Pedagogical Profile, BIOTERRA University of Bucharest iancummariana@gmail.com » IRESEARCHES ON THE IMPACT OF AGRICULTURAL PRODUCTS FROM MYCOTOXINS VEGETABLE ON CONSUMER HEALTH Roxana ANDRONE<sup>1</sup>, Ioan ROŞCA<sup>2</sup> <sup>1</sup> Bioterra University of Bucharest No. 81, Gârlei Street, sector 1, Bucharest (Ph.D student USAMV Bucharest) <sup>2</sup> University of Agronomical Sciences And Veterinary Medicine, Bucharest, 011464, Romania » RESEARCH ON QUANTITATIVE DETERMINATION OF BIOLOGICALLY-ACTIVE COMPOUNDS IN NATURAL JUICES OBTAINED BY COLD PRESSING Maria Pop Varsta Ph.D student USAMV Bucharest mariapophypericum@yahoo.com » RESEARCHES ON THE IDENTIFICATION OF MACROSCOPIC CHARACTERISTICS, MINERALS AND THE FOOD VALUE AND NUTRITIONAL USE OF THE SPECIES PORTULACA OLERACEA Aurora DOBRIN<sup>1</sup>, Cătălin GALAN<sup>2</sup> <sup>1</sup> U.S.A.M.V. Bucharest No. 59, Mărăști Street, sector 1, Bucharest auroradobrin@yahoo.com <sup>2</sup> Bioterra University of Bucharest No. 81, Gârlei Street, sector 1, Bucharest



### THE APPLICATION OF SOME COORDINATION COMPOUNDS IN REGULATION OF THE CONTENT OF ANTIOXIDANTS IN SPIRULINA PLATENSIS

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Abstract: Investigation of non-traditional sources of bioactive substances is one of the current directions of biotechnology development in many countries worldwide. This paper reports on the study of possibility of utilization of some coordination compounds of V(IV) and Co(III) as regulators of the content of bioactive substances with antioxidant properties: superoxiddismutase (SOD), phycobiliproteins, carotenoids and lipids in the biomass of cyanobacteria Spirulina platensis. Natural antioxidants possess a broad spectrum of biological activity due to the ability to neutralize the negative effects of free radicals in living organisms. The obtained experimental results demonstrate that utilization of some coordination compounds of V(IV) at the spirulina cultivation contribute to the accumulation of  $\beta$ -carotene and lipids in spirulina biomass, as well as metallocomplexes of Co(III) have the beneficial effect on the content of phycobiliproteins, lipids and activity of SOD. The present study reveals that obtained biomass of cyanobacteria Spirulina platensis with high content of bioactive substances with antioxidant properties can be used for the elaboration of medical remedies for prophylaxis and treatment of diseases, caused by the negative effect of oxidative stress on live organisms and, also, for the elaboration of cosmetic preparations for the prevention of premature skin aging, protection against solar radiation and treatment of skin diseases.

*Key words:* cyanobacteria Spirulina platensis, antioxidants, biologically active substances, coordination compounds of V(IV) and Co(III)

### Introduction

Investigation of non-traditional sources of bioactive substances is one of the current directions of biotechnology development in many countries worldwide. Cyanobacteria Spirulina platensis is widely explored and used in recent decades as a source of valuable biologically active substances [1, 2, 32.]. An important role in neutralizing the harmful effects of oxidative stress have the enzime superoxiddismutase (SOD), that it is



present, also, in the biomass of cyanobacteria Spirulina platensis [29, 35]. SOD is an important regulator of cell oxidation processes, acting for the conversion of O2- radicals with formation of hydrogen peroxide and triplet oxygen. Summarize the most powerful antioxidants in spirulina biomass can be mentioned water-soluble pigments - phycobiliproteins, possessing anti-inflammatory, immunostimulatory, neuroprotective, hematopoietic properties [11, 16, 30]. According to the literature data, phycocyanin is an efficient scavenger of oxygen free radicals, reacts with other oxidants of pathological relevance such as HOCl and ONOO- and, also, inhibits the processes of lipid peroxidation, mediated by ROS (reactive oxygen species) [7-9].

Another group of pigments - carotenoids are widely used as natural colorants and antioxidants [3]. Carotenoids possess immunostimulatory properties and inhibit the growth of cancer cells that present the perspective of their use in prophylaxis and complex treatment of cancer [2]. Due to the presence of conjugated double bonds, carotenoids bind singlet oxygen and inhibit the formation of free radicals [21, 24].

Cyanobacteria Spirulina platensis presents, also, a source of polyunsaturated fatty acids, which, have a cardioprotective role, possess immunostimulatory, antiviral, antibacterial properties [6, 17, 18]. Previously, it was established that coordination compounds of Zn(II) with aminoacids and halogenoacetates can be used as effective regulators of lipids accumulation process by cyanobacteria Spirulina platensis [31].

Researches have demonstrated the possibility of obtaining of spirulina biomass with high content of zinc, iron, chromium, germanium, selenium, iodine and other bioactive substances at the cultivation of spirulina in the presence of coordination or inorganic compounds of these elements by the controlled synthesis [32]. It was established the stimulatory effect of coordination compounds of Mn (II) and Zn (II) on the activity of SOD and content of phycobiliproteins in spirulina biomass [15]. The differential response of superoxiddismutase (SOD), nitrogenase, growth and physiological processes in the presence of four different metals ions at varying concentrations were investigated in the heterocystous cyanobacterium, Anabaena variabilis Kütz. Growth and enzyme activities were influenced by the metal ions. SOD activity was optimum at 100 mcM concentration of FeSO<sub>4</sub>. In the presence of ZnSO<sub>4</sub> SOD activity increased in direct proportion with metal concentration [28].

The influence of vanadium compounds on photosynthesis in cyanobacteria hasn't been yet studied completely. Vanadium is suggested to act as a redox catalyst in the electron transport from PS II to PS I. Despite of the importance of vanadium as human micro-nutrient, it is yet to be unequivocally accepted by biologists and biomedical scientists, because of its toxicity Pharmacological uses of vanadium include lowering of cholesterol, triglycerides and glucose levels [4, 19, 26]. Vanadium also possesses anti-carcinogenic and anti-diabetic properties [36]. The chemoprotective and anti-cancer effect of vanadium, a dietary micronutrient, against chemically induced hepatocarcinogenesis in rats was demonstrated [10] and, also, inhibition of growth of colon and lung tumors in human organism was established [20, 27].

Another trace element - cobalt has important biological significance, ions of which are actively involved in the reactions of oxidation and reduction, have a positive influence on the processes of cell respiration and metabolism, as well as on the biosynthesis of phycobiliproteins and nucleic acids [23, 37].



Thus, it can be assumed that synthesis of bioactive substances in spirulina biomass may be increased by the cultivation of this cyanobacteria in the presence of some compounds of Co (III).

The aim of this investigation presents a study of possibility of utilization of some coordination compounds of V(IV) and Co(III) as regulators of the content of bioactive substances with antioxidant properties (superoxide dismutase, phycobiliproteins, carotenoids, lipids) in the biomass of cyanobacteria Spirulina platensis.

### Materials and methods

Object of study is a strain of cyanobacteria Spirulina platensis CNM-CB-02, stored at the National Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Academy of Sciences of Moldova.

Cultivation of cyanobacteria Spirulina platensis was carried out on the nutritive medium SP - 1 (Table 1) [33].

With the certain ratio of macro-and micronutrients for normal growth and development of culture with the following composition:

Table 1.

The composition of nutritive medium SP-1 [33].

Reagents	at 1000 ml of nutritive medium
NaHCO <sub>3</sub>	16,8g
K <sub>2</sub> HPO <sub>4</sub>	0,1g
KNO3	3,75g
NaCl	1,0g
$K_2SO_4$	3,75g
CaCl <sub>2</sub>	0,04g
MgSO <sub>4</sub> •7H <sub>2</sub> O	0,70g
sol. Fe-EDTA	1 ml
sol. microelements	1 ml

Solution of microelements, mg/l: H<sub>3</sub>BO<sub>3</sub> – 2,86; MnCl<sub>2</sub>•4H<sub>2</sub>O - 1,81; ZnSO<sub>4</sub>•7H<sub>2</sub>O -0,22; MoO<sub>3</sub> – 0,015. Cultivation was carried out in Erlenmeyer flasks with a volume of a suspension of spirulina in the medium of cultivation 100 ml, for 144 hours at 30°C, the intensity of illumination 3000 - 4000 lux. As stimulators of growth processes of cyanobacteria Spirulina platensis and accumulation of bioactive substances with antioxidant properties in the biomass coordination compounds of V(IV) and Co(II) with different ligands of organic nature:

L1 - [(VO)<sub>2</sub>(2PyTCH)]SO<sub>4</sub>·4H<sub>2</sub>O;

L2 - [(VO)<sub>2</sub>(2PyCH)]SO<sub>4</sub>·4H<sub>2</sub>O;

L3 - [(VO)<sub>2</sub>(2PyFx)]SO<sub>4</sub>·4H<sub>2</sub>O;

L4 -  $[Co(L-H)En]\cdot 3H_2O;$ 

L5 -  $Na[Co(DH)_2(NO_2)_2]$  have been used.

Productivity of spirulina was determined according to photocolorimetrical method [34]. The determination of activity of superoxide dismutase in the spirulina biomass was carried out according to the method proposed by Bulimaga [12].

The content of phycobiliproteins was determined according to spectrophotometrical method, elaborated by Bousiba and Richmond [11].

The carotenoids content in the biomass of spirulina was measured according to spectrophotometrical method [13].

Determination of lipid contents was effectuated by spectrophotometrical method [34].

Regression and statistical analysis of data obtained in three series was carried out by the methods proposed by Maximov [39] and Dospehov [38].



# Results and discussions

The high degree of biological activity of the cyanobacteria Spirulina platensis is caused mainly by the presence of antioxidants in the composition of its biomass. Results of investigation of influence of coordination compounds of some transition metals on productivity of spirulina and content of bioactive compounds with antioxidant properties in the biomass are presented below (Fig. 1-6).

It was established a weak inhibitory effect of the two studied compounds:

 $[(VO)_2(2PyTCH)]SO_4 \cdot 4H_2O$ 

in concentrations within 5 to 25 mg/l and Na[Co(DH)<sup>2</sup>(NO<sub>2</sub>)<sub>2</sub>] (10-25mg/l) on the productivity of spirulina. In the case of utilization of the other compounds, productivity values are within the reference sample, except for compounds [Co(L-H) En]·3H<sub>2</sub>O and Na[Co(DH)<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>], which contribute to increase of productivity by 11-18% compared to the reference sample in the concentration of 5 mg/l (Fig. 1).



Fig. 1. Productivity of spirulina at 7th day at the cultivation in the presence of some coordinative compounds of vanadium and cobalt



Fig. 2. Content of phycobiliproteins in the biomass of spirulina cultivated in the presence of some coordinative compounds of vanadium and cobalt



The analysis of obtained results has demonstrated that phycobiliproteins content is increased by 45-74% compared to the reference sample in the case of utilization of compounds of V(IV) (Fig.2).

The administration of the coordinative compound of Co (III) -  $[Co(L-H)En] 3H_2O$  in the concentration of 20mg/l has recorded a maximum increase of phycobiliproteins contents (by 160% compared to the reference sample).

The beneficial effect is caused possibly by the fact that coordination compounds of Co (III) causes a stimulation of electron transport in photosynthetic apparatus of the cell, therefore cells possess a greater fluidity of the thylakoid membrane, and also there is an enhancement of heme oxygenase synthesis, which causes the increase of phycobiliproteins content in the biomass [5]. Ligands of coordination compounds have a great role in regulation of biosynthetic activity of cyanobacteria. Probably, acetate, one of the ligands of the metalocomplex



Fig. 3. Content of  $\beta$  – carotene in the biomass of spirulina cultivated in the presence of some coordinative compounds of vanadium and cobalt



Fig. 4. The influence of some coordinative compounds of vanadium and cobalt on the activity of SOD in the biomass of spirulina



[Co(L-H)En]·3H<sub>2</sub>O may be included in the structure of precursors of phycobiliproteins, such as protoporphyrin, uroporphyrinogen and thereby contribute to enhancement the processes of biosynthesis of tetrapyrroles [22]. The significant stimulatory effect was established in the case of study of influence of some of complex compounds of V(IV) and Co (III) on the content of  $\beta$  - carotene in spirulina biomass (Fig. 3).

Beta-carotene contents in the biomass of spirulina cultivated in the presence of complexes  $[(VO)_2(2PyTCH)]SO_4 \cdot 4 H_2O$  and  $[(VO)_2(2PyCH)]SO_4 \cdot 4H_2O$  reaches the values of 29 and 25mg%, respectively, at optimal concentration of compounds of 25 mg/l.

The determination of activity of superoxiddismutase (SOD) in obtained extracts from spirulina biomass (by the utilization of 0.1 M Na - phosphate buffer pH 7.8 - 8.0 (+10 mM EDTA)) allowed to establish a positive effect of coordination compounds of Co (III) on the activity of this enzyme.

Maximum increase of superoxiddismutase activity in the biomass of spirulina (by 38% compared to the reference sample) was established in the case of utilization of the compound  $[Co(L-H)En]\cdot 3H_2O$  in the concentration of 15mg/l. The significant increase of SOD activity (by 32-36% compared to the reference sample) is registered in the case of administration of the compound Na $[Co(DH)_2(NO_2)_2]$  in the concentration range of 15-25mg/l.

It was demonstrated that all tested compounds contribute to increased synthesis of lipids in biomass with a maximum of about 45-47% increase of their content compared to the reference sample in the case of utilization of coordination compounds of Co(III) - $[Co(L-H)En]\cdot 3H_2O$  and Na $[Co(DH)_2(NO_2)_2]$ in the optimal concentration of 20 and 15mg/l, respectively (Fig. 5).

Thus, can be indicated that the stimulatory effect of coordination compounds of V(IV) and Co(III) on the contents of bioactive substances with antioxidant properties is obvious and can be explained by the possible implications of these metals and ligands in metabolic processes cells.

The obtained experimental results reveal that utilization of coordination compounds of V(IV) at the spirulina cultivation contributes to the accumulation of  $\beta$  - carotene and lipids in spirulina biomass,



Fig. 5. Content of lipids in the biomass of spirulina cultivated in the presence of some coordinative compounds of vanadium and cobalt



as well as metallocomplexes of Co(III) have the beneficial effect on the content of phycobiliproteins, lipids and activity of SOD. So, the administration of studied coordination compounds of V(IV) to the medium of cultivation of cyanobacteria Spirulina platensis provides the maximum increase of  $\beta$  - carotene contents with 60% compared to the reference sample in the case of utilization of compound [(VO)<sub>2</sub>(2PyTCH)]SO<sub>4</sub>.4H<sub>2</sub>O in the optimal concentration of 25 mg/l. In another study similar results were observed, where some vanadium compounds (vanadate, vanadyl citrate) have beneficial effect on the photosynthesis in Chlorella fusca and algal chloroplasts both vanadate and vanadyl citrate enhance photosynthetic O<sub>2</sub> production in intact cells [25].

In the present investigation the maximal increase of phycobiliproteins content (160% compared to the reference sample) in the biomass of spirulina by the directed synthesis with the supplementation of metallocomplex of Co(III) -  $[Co(L-H)En]\cdot 3H_2O$  in the optimal concentration of 20 mg/l was reached. The data obtained is confirmed by other researchers, according to which some cobalt compounds are able to stimulate the processes of electron transport of PS II and phycobiliprotein synthesis in the biomass of cyanobacteria [5, 37].

According to the results of some researchers on the effect of inorganic forms of transition metals - sulfate of Zn(II) on SOD activity of spirulina the data are contradictory. Thus, the utilization of different concentrations of Zn<sup>2+</sup> (0.09 - 9,0 mg/l) in the form of sulphate had inhibitory effect on the activity of antioxidant enzymes: superoxiddismutase and catalase in spirulina biomass collected at the end of exponential phase [40]. In another study, the authors revealed that the metal ions(Mn <sup>2+</sup> and Zn <sup>2+</sup>) in the form of sulphate play an important role in regulating of the SOD activity [28]. The utilization of coordinative compounds of zinc and manganese contributes, also, to the significant increase of SOD activity in the biomass of cyanobacteria Spirulina platensis [15]. In the present investigation an increasing of activity of superoxiddismutase in biomass of spirulina (by 38% compared to the reference sample) in the case of utilization of coordinative compound of Co(III) -[Co(L-H)En]·3H<sub>2</sub>O in optimal concentration of 15mg/l has been established. In this research, for the first time, it was demonstrated the possibility of lipids directed synthesis in the biomass of spirulina by the coordinative compounds of V(IV) and Co(III). So, in the case of utilization of coordination compounds of Co(III) - Na[Co(DH)<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>] in the optimal concentration of 15mg/l an increase of lipid contents in the biomass of spirulina by 47% compared to the reference sample it was observed. Similar data was obtained at the utilization of the coordinative tribromoacetate compound of zinc [Zn(CBr<sub>2</sub>COO)<sub>2</sub> 4H<sub>2</sub>O] at concentration of 15.0 mg/l that contribute to the increase of lipid accumulation in spirulina biomass by 31% compared to the blank [31].

Probably, the assimilation of coordination compounds of transition metals with ligands of organic nature takes place due to the specific mechanism of transport of the metal-ligand complexes that lead to the increasing of metal concentration in the cell [14]. Thus, it becomes possible to use higher concentrations of metal, causing oxidative stress in cells that contributes to the enhacement of synthesis of bioactive substances with antioxidants properties in the biomass of the cyanobacteria Spirulina platensis.



## **C**onclusions

The present study reveals that obtained biomass of cyanobacteria Spirulina platensis with high content of bioactive substances with antioxidant properties (superoxiddismutase, phycobiliproteins, carotenoids, lipids) can be used for the elaboration of medical remedies for prophylaxis and treatment of diseases, caused by the negative effect of oxidative stress on live organisms and, also, for the elaboration of cosmetic preparations for the prevention of premature skin aging, protection against solar radiation and treatment of skin diseases.

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### THE CORRELATION "MELLIFEROUS PLANTS-BEES-HONEY OF BEES-MAN-SOCIETY" AND ITS ROLE IN THE STUDENT'S EDUCATION BY BIOLOGICAL DISCIPLINES

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**Abstract:** Scientific research of the correlation melliferous plants-bees-honey bees-man-society and its role in the student's education by biological disciplines for faculties of food domain at the BIOTERRA University of Bucharest, Romania, showed that honey qualitative impairment due to the depreciation of the natural environment in which man lives, it is required due to the reckless actions of nature's time to take measures to protect plants, animals, the whole natural environment is important the ecological education and environmental education during training in education and continue throughout life.

At the same time, it may be appropriate professional education, food education, training of the competence of the choice of the foodstuff, such as it is, honey of bees, based on the knowledge and awareness of the need to distinguish between a food product natural, quality, one counterfeit software, his choice of falsified and therefore, in such a way as to decrease the morbidity and mortality of the social environment, marketing natural products, not some fake, counterfeit, polluted, contaminated.

Key words: melliferous plants, honey of bees, professional and food education, ecological education

# Introduction

At level European Union is considered that it would be appropriate for education for the environment to be a part of basic education, and consumers and producers to be involved in sustainable use of natural resources.

Living matter environment is an integral part of the substrate abiotic environment. "Environmental health is a problem on which it is to think, to reflect, as well as to our own health, to create solutions, which then to put them into effect." (Ciobanu, I.M., 2009). We understand that our health and environmental health social health will depend on the natural environment.

Man (Homo sapiens sapiens) is a biological being which must be integrated into the natural environment, including other species of creatures, but a information being and a social being that must integrate into society, respectively, social environment. Man, especially with industrialization and urbanization initiated and promoted him to alter the natural environment in a reckless manner, mainly through forest clearing,



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overexploitation of pastures, overexploitation of species of plants and animals, pollution, which influenced beyond the deterioration of the natural environment and impaired social environment by increasing morbidity and mortality due to direct effects of peer damaged the natural environment, but also indirect effects such as obtaining raw materials and food polluted as the honey of bees obtained on nectar collected by bees from the flowers of herbaceous and woody plants that live in a polluted environment. Consumer must be informed correctly on essential characteristics of all the products they consume; both by label on the packaging of products, as well as by media.

An example of interaction between natural environment and social environment is illustrated by the correlation "melliferous plants-bees-honey of bees-man-society", which protected health as the environment, as well as to human health, social environment. Solutions envisaged at the disposal environment school or university education for health environment, for the protection of environmental education and education for the environment in their forms, further formal, by biological disciplines and borderline, but and non-formal and informal. Ecological education (in Greek oikos = house, household) is integrated environmental education for all biotic and abiotic, biotic environment for networking between links (alive) for networking between them and the abiotic (non-living factors) for the purpose of protection, care and their preservation. Environmental education of young people is education which directs and coordinates all actions taken by them on the ecosphere; maintain the ecological balance and stability of ecosystems, thus ensuring better living conditions for all present and future on Earth. Environmental education is based on ecological education, being an applied branch

of it, reflected in the action concentrated and require more practice, of course, learning the basic concepts of ecology, supporting practical activities. Ecological education and environmental education is based on the Biological Sciences, which are absolutely necessary in teaching Educational Sciences and Psychology, scientific basic disciplines in Teaching of Biology and therefore in harnessing courses, laboratories and specialized workshops in ecological education and environmental education plan. Can be done environmental education and ecological education and by carrying research projects by students, coordinated by professors.

Addressing the students at the Faculty of Control and Expertise of Food Product and Faculty of Food Engineering at the BIOTERRA University of **Bucharest** correlation "melliferous plants-bees-honey of bees-man-society" shall contribute both ecological education, environmental to education, especially in professional education and their food education.

"Professional education is ideal to prepare young people for at least a craft, utility social and need a good school and professional orientation even started preschool." (Iancu, M., 2011)

Food education is a component of health education and vocational education materializes students from food faculties and specialties, with the foundation sciences Biochemistry, Microbiology, Science Education, Psychology.

# $oldsymbol{M}$ aterials and methods

### 1. Overview of the research

Sixteen students in the 2nd year of study at Faculty of Control and Expertise of Food Products and from the Faculty of Food Engineering at the BIOTERRA University



of Bucharest, coordinated, guided closely by professor PhD Mariana Iancu have developed in Biochemistry lab, beyond the courses and work practical academic program, extracurricular scientific research about the quality of honey of bees, the quality of the natural environment, social quality and quality of life by contributing to their professional education, their food education, ecological education and environmental education for their study based on correlation "melliferous plants-bees-honey of bees-man-society". Some of the students were documented on melliferous plants, bees, honey bees, national and international environmental policy, environmental quality standards, quality standards of "honey bees" food, national and international legislation on environment, making the correlations with more biological disciplines, as "Botanic", Biology", "Biochemistry", "Vegetal "General Microbiology", "Protection and Conservation environmental biodiversity" or trans-disciplinary with "Education Science", "Pollutants and impact their environmental and consumer", "Food biotechnology" and so on. Other students actually participate in the development of practical and experimental work highlighting the counterfeiting honey. "The experiment-fundamental method in Lives Science education" (Cerghit, I., 2006). Others students have developed and colleagues administered a questionnaire on students mainly from how to purchase "honey bees" food. Then they participated at all the concrete action awareness of the need of environmental protection, maintaining a clean environment, unpolluted, imperative living world, including man, both directly and indirectly through food. For this last phase of the research, some students were concerned about the development of educational themed flyers, ecological education, environmental education and

food education, all these sides of education contributing to their professional education. Subsequently, the expanded team of students distributed flyers other colleagues from university, family, friends, their neighbors and other peers, spreading their scientific and exhortations environmental, consumer protection, ecological education and food education. Also, showed that students involved were aware, both from practical work and experiments, the analysis of questionnaires administered and the scientific documentation, documenting practice in the field, on the spot, in the supermarket, the need for continuous education and selfeducation, otherwise, are many dangers to life of people and life of the entire planet.

The period of the research was included in March, April and May 2013. Beyond human resources, material resources were necessary represented by didactic material of BIOTERRA University of Bucharest being purchased as research samples jars of honey and other products that were shown on the shelf "energizing honey".

Honey bees is a bio/organic food, naturally produced by bees, to which they use raw provided directly by plants (floral nectar or extra-floral) and, to a lesser extent, parts from other sources. This definition contained in the Codex Alimentarius shall specify the specific nature of this product. "Invert sugar is together with sucrose the main constituent of honey, strong sweet taste of which is given fructose." (Neguţ, L., E., 2000).

2. Objectives of scientific research by expertise and controlling bees honey

Students involved from Control and Expertise of Food Products Faculty and from the Faculty of Food Engineering at the BIOTERRA University of Bucharest, together with their professor, conducted in the present research more practical activities which have developed extracurricular power



to distinguish food, or honey of bees, which meet the standards of quality and pollutant food / or forged, and thus to be withdrawn from sale to the public. However, they continued environmental education on the need respect, preserve food chains in nature, nutrition-based correlations between melliferous plants, to be protected and cultivated and bees, between melliferous plants, insects producing manna and bees, one part, and on the other part, have developed specific activities the student community awareness of the need to protect the natural environment.

Monitoring and food expert called the ''honey bees" had the following objectives: 1-assessing the quality and purity of honey bees;

2-detection of counterfeiting honey of honey bees;

3-assessing the state of degradation or alteration of honey bees.

To achieve these objectives, the teacher and students involved in research conducted organoleptic examination, physicochemical examination and microbiological examination of a total of 100 different samples of honey bees.

# **3.** The practical work and experiments in the teaching-methods of research and hypotheses

# » Organoleptic examination (sensory analysis) of research

Organoleptic examination (sensory analysis) of research evidence consisted of more practical work have been referred to the following criteria for quality standards honey bees: color, consistency, flavor and taste, degree of contamination (Table 2, Table 3). Initially, fluid honey bees are organoleptic examined. We note if the foam and / or impurities exist. Honey bees was stirred

with a glass rod for uniform dispersion

throughout the mass of the impurities, after

which it was filtered through double gauze first use, mix and allow to stand for removal of air incorporated, to complete clarification, subjecting complete sensory examination (appearance, texture, color, smell and taste). Originally, honey bees are crystallized on the sample as organoleptic. We note if you have foam and/or impurities, kinds of crystallization (very early, partial or total) and characteristics of crystals (fine, suitable, coarse). Honey of bees jar tightly closed shall be thinning by heating to a temperature of 40...45°C to complete melting of crystals. After cooling, remove the lid, mix well using a glass rod for uniform dispersion of dirt all

The appearance is judged by the degree of transparency posed introduced a honey in a colorless glass tube with a diameter of 16 mm., examined the direct light of the day. Record in detail the various shades such as: transparent, clear, bright, cloudy, and other. On the shelf in stores, the food is in direct sunlight all day.

over the table.

The consistency is assessed by way of flow of honey from a glass rod or a wooden shovel, stating that state: aqueous, fluid-thin, fluid-viscous, and sticky.

Place a teaspoon of honey in jar and stand, and if honey bees flows continuously, then it is natural, and if it disrupts the flow in the form of drops, honey bees is adulterated and have chosen another supplier of honey. Generally, in trade, the jars are not completely filled and may be used to move the jar, turning his cap down to observe consistency, especially for observation of possible impurities.

The color is assessed by direct visual examination, in daylight, on a white background honey placed in a clear glass tube with a diameter of 16 mm. In stores before purchasing food called the "honey bees" is observed jar in direct sunlight.

The smell and taste are evaluated by smelling



and tasting the sample.

The dominant flavor notes hue (for poly-floral honey bees) and intensity (pronounced, well evidenced, moderate, and discrete). It also appreciates the sweetness intensity (strong, well highlighted, and moderate) and any secondary colors (sour, bitter, astringent, fad other). Typically, only sweet honey is forged

Table 1 PHYSICO-CHEMICAL PROPERTIES OF HONEY, SOME NORMAL GENERAL

Physico-chemical properties	Values
Density	1,0628
pH	pH < 4,5
Extract	84,12%
nitrogenous	0,5%
substances	
organic acids	0 1-0 2%

without any aroma and flavor of the natural one with which it is collected.

» Biochemical examination of honey beesmethods and hypotheses

Physico-chemical examination is to determine the pH, or free concentration of H ions by means of pH indicator paper (products resulting from the degradation determination of the normal components) in determining the actual forging agents to correct consistency (starch, flour, derived similar) to correct taste (addition of artificial invert sugar, sucrose), honey and potential dilution detecting them more likely to ferment and decay, given the biochemical, physico-chemical-normal general honey

Table 2

### ORGANOLEPTIC EXAMINATION OF SAMPLES OF HONEY BEES AND "ENERGIZING HONEY"-THE APPEARANCE, THE CONSISTENCY

Honeys bees	r.	The app	The consistency						
experienced	Tnfnvfm	Tb	0	Cwfb	Hf	Α	Ft	Fv	S
Locust honey	+	-	-	-	-	-	+	-	-
Lime honey	+	-	-	-	-	-	-	+	-
Polyfloral honey	+	-	-	-	-	-	-	+	-
"Energizing honey"	-	-	+	-	-	+	-	-	-

Tnfnvfm - Transparent, no foam, no visible foreign matter; Tb-There bright; O-Opalescent; Cwfb-Cloudy, with foreign bodies; Hf-Honey foam; A-Aqueous; Ft- Fluid-thin,; Fv- Fluid-viscous; S-sticky.

Table 3.

# ORGANOLEPTIC EXAMINATION OF SAMPLES OF HONEY BEES AND "ENERGIZING HONEY"-THE COLOR, THE SMELL, THE TASTE

Honeys bees	The color			The smell				The taste					
Experien-ced	At	Lpy	Yf	Lyg	Df	Ра	Fge	Fm	Fd	Pst	Stwh	Mst	Sht
Locust honey	+	-	-	-	-	-	+	-	-	-	+	-	-
Lime honey	-	+	-	-	-	-	+	-	-	-	-	+	-
Polyfloral honey	-	-	+	-	+	-	-	-	-	-	-	+	-
Energizing honey	-	-	+	-	-	-	-	-	-	+	-	-	-

At-almost transparent, Lpy-light pale yellow, Yf-yellow-fawn, Lyg.-light yellow-green, Df-dominant flavor, Pa-Pronounced aroma, Fge-Flavor good emphasized, Fm-Flavored moderate, Fd-Flavored discrete, Pst-Pronounced sweet taste, Stwh-Sweet taste well highlighted, Mst-Moderately sweet taste, Sht-Secondary hue taste (sour, bitter, astringent, fad and so on.), S-Sugar



(Table 1). Before commencing the test, the honey has to be stirred.

To achieve some honey analysis, sample preparation is required to be analyzed weighed 50 g. honey, previously is homogenized and make distilled water in a 250 milliliters flask distilled water is added to the mark. From this solution will be extracted samples taken for measurements. Many samples were prepared several days of investigation (100 samples in total) of acacia honey, honey poly-floral, linden honey, a product on the shelf in the store, it was shown that "honey energizing" but not about food honey, but one counterfeit, which emerged from the reading the label. Hence competence training students to study food labels, not only in terms of durability, but their biochemistry, the power to distinguish natural product original one counterfeit.

Experiment 1 (E1), determination of the pH, starting from the assumption that this is within normal limits 5 milliliters of each sample to be analyzed is put in a crystallizer, place one piece of paper pH indicator in each crystallizer, expect about 5 minutes, remove paper indicator of each crystallized honey is dripping, sits on indicator scale box cover pH indicators, the corresponding color right to read the pH indicated (Figure 1).

Experiment 2 (E2) - identifying the addition of starch, flour and derivatives thereof, starting from the assumption that honey samples do not contain such additives. The crystallized samples shall 5-10 milliliters of 1:1 honey boiled and cooled and then added by pipette a few drops of a solution of iodine in potassium iodide 1% (Figure 2). Blue coloration indicates the presence of starch.

Experiment 3 (E3) - based forgery detection density, starting from the assumption that all the products are natural and not fake.

Natural honey has a density of 1.4-fold higher than water and not dissolved. Adding honey

in water, honey should not fall to the bottom and spread rapidly in the water (Figure 3a). Most counterfeit has a lower density and is dissolved (Figure 3b).

Experiment 4 (E4) - identifying honey dilution hypothesis from which we started is that some samples could be diluted. One tablespoon of honey in each sample shall be placed for 1 minute a pencil tip. If color spreads, honey is diluted. If the honey is colored pencil, then it is not natural. If honey has not changed color, then it is natural.

Experiment 5 (E5) - identifying invert sugar, artificial and sucrose (Elser method). It is assumed that, "energizing honey" content is abnormally increased sucrose.

Determining the invert glucose and fructose in free state has the property of reducing copper sulfate in a warm alocaline and converts it into copper oxide in an amount proportional to the concentration of both the reducing sugars in solution studied. The direct reducing sugar determined before and after inversion by acid hydrolysis, sucrose calculating the difference between these measurements.

» Microbiological examination of honey bees Contamination by microorganisms occurs in the honey hive by bees during nectar collection and submission, that after harvesting and primary processing of honey. We performed microscopic preparations of honey samples were subjected to scientific research and viewed by optical microscopy.

**4. Questionnaire correlated with honey bees** Some of the students involved in scientific research ,,melliferous plants-bees-honey of bees-man-society" fellow students were administered a questionnaire regarding:

• how to purchase food "honey bees";

• read the information on the package;

• students considerations related to quality of honey bees;

• recognition of honey bees counterfeit;



• significance of the correlation "melliferous plants-bees-honey of bees-man-society";

• frequency and mode of consumption of honey bees;

• preference for a certain range of honey, and its considerations regarding the importance of human health.

Over 150 students from all faculties of the BIOTERRA University of Bucharest and all years of study completed the questionnaire.

# Results and discussions

# **1.** The practical work and experiments in the teaching-observations and results

» Organoleptic examination (sensory analysis) of research. Honey bees has a number of specific sensory characteristics: look no foam, no visible foreign color from colorless to faint yellow, yellow-gold, yellow-orange, dark yellow, ruby, yellowbrown, dark brown, smell and taste honey flavor less or more pronounced sweet taste, consistency is fluid, viscous, crystallized.

Notes. "Energizing honey" is a different honey food, no flavor, dye pronounced sweet taste of sugar aqueous opalescent, which by the way is presented in stores may induce mislead the purchaser, if not competent to document-label study, to observe the appearance, color, consistency, or those skills which must be formed from years of training and education.

No food was not observed researched as troubled with foreign bodies, dirt in it, but one called opalescent, "energizing honey", which in this respect, the products are not contaminated, but some are falsified education required youth for ecological education, environmental education, food education. Poly-floral honey being reddish yellow, the colors, normally it is pale yellow to yellow-amber. » *Biochemical examination of honey bees*. Experiment 1 (E 1). Determination of the pH-results.

Figure 1. Determination of pH of research evidence



Notes. Experimental data has indicated that all the analyzed samples has a pH of between 3.8 and 4.5, the normal range, the event is confirmed and the edible products in this regard, the upper limit of normal is achieved by energizing the product taken as evidence. Experiment 2 (E2). Identifying the addition of starch, flour and derivatives thereof-results.



Figure 2. Identification of the addition of starch, flour and derivatives



Notes. Experimental data indicated that 95% of the analyzed samples contain no starch, flour or derivatives thereof, being in this respect normal, while 5% had such additions slightly color, sometimes, in dark blue I2 + KI (last picture in Figure) assumption is invalidated, and the products are from this point of view, the overwhelming majority is not entirely natural, without additives.

Experiment 3 (E3). Detection density-results.



Figure 3. Berzelius vas with water and bees honey (a); Berzelius vas with water and "energizing honey" - forged (b)

Observation. The hypothesis was refuted, samples, "energizing honey" is a fake spills quickly into the water.

Experiment 4 (E4). Identifying honey dilution-results.

Observation. Product "energizing honey" is diluted, the hypothesis was confirmed.

Experiment 5 (E5). Identifying invert sugar, artificial and sucrose (Elser method)-results. Observation. Product "energizing honey" is a counterfeit product, with a high content of sucrose-30 %, the hypothesis was confirmed. So, some labeled products purchased from the supermarket honey substitutes are any dangerous kind of sugar syrup, hydrolyzed corn, safflower, as the product, "energizing honey" investigated or other hydrolyzed with dyes and flavor of rule, and sometimes molasses coming through the transformation of the "honey" of acacia, lime, poly-floral, sunflower other.

» Microbiological examination of honey bees-results. Microbiological examination of samples did not reveal the presence of microorganisms. Honey has the ability to inhibit microbial growth or destroy them (high sugar content and very low in free water, pH <4.5, very low protein content and C / N ratio greater honey, "inhibin" the lack of oxygen in honey other) for which microbiological examination of honey was not a concern for specialists in the field.

However, a risk, even if it is insignificant, there is, which is why some countries require that honey before being marketed, to be tested for its microbiological quality.

Dominant yeasts found in fermented honey are species of the genera Debaryomyces and Saccharomyces.

"Certain osmofile yeasts of the genus Zygosaccharomyces supports high levels



of sugar and can cause fermentation of concentrated sugar syrup and honey." (Bălăucă, N., Atudosiei, L., N., 2004)

Molds honey from intestinal confinutul bees in the hive and the bees they operate or in which the processed honey. Literature of speciality signals the presence black mold in forest honey. Frequently encountered species belonging to the genera: Aspergillus, Atrichia, Cephalosporium, Chetonium, Coniothecium, Penicillium, Triposporium, Uredinaceae, Ustilaginaceae and so on. By comparison with other raw foods thermally number of bacteria in honey is much smaller (tens-hundreds) and varies depending on the type of honey processing state (unbleached, finite, commercial), age, time of harvest. Common species belong to the genera: Flavobacterium. Bacillus. Enterobacter. Klebsiella, Micrococcus, Neisseria, Proteus, Pseudomonas, Xanthomonas and so on.

It requires ecological education, environmental education within the meaning of concrete actions against pollution of the natural environment, including pollution control biological pathogens producing disease that affects the health of melliferous plants, bees, human consumption of bees honey taking into account the correlation of scientific research "Melliferous plants-beeshoney of bees-man-society", affecting the natural and social environment globally.

# 2. Results of questionnaire correlated with honey bees

Data collected following administration of the questionnaire revealed, inter alia, that:

• most students eat honey, which is purchased by parent's supermarkets, when they buy, read the label, particularly durability, as well as their parents;

• students do not recognize counterfeit honey bees examination;

• students know the kinds of honey, but also

part of the honey quality standards, such as those related to organoleptic;

• few students know how to explain what the correlation "Melliferous plants-bees-honey of bees-man-society".

All this evidence emphasizes the need for increased ecological education, environmental education, education for health food and also their professional education which will be continued over the next year undergraduate or master.

# Conclusions

1. Natural environment, represented by biotic and abiotic factors, particularly important for life on earth perpetuation and social environment represented people should coexist in a mutually beneficial balance, quality of life on planet Earth, some solutions are also professional education, nutritional education, ecological education and environmental education in the context of appropriate environmental policies and legislation.

2. An analytical and comparative approach to ecological education and environmental education reveals correlations between them, meaning that ecological education is the foundation of environmental education, which in turn contributes to ecological education as only working towards environmental protection, contribute to balance stability and self-regulation of ecosystems within the ecosphere.

3. Man get and sells the honey with herbal and nutritional value, but not always a fair conduct of this foodstuff is sold without achieving the quality standards, in the analyzed samples the physico-chemical and organoleptic and counterfeit goods,



some other people not being able to be aware of the danger of illness, death and morbidity growth through the marketing of counterfeit products counterfeit, contaminated, morbidity and mortality by selling counterfeit products, counterfeit, contaminated.

4. Scientific research of the correlation melliferous plants-bees-honey of beesman-society and its role in student's education by biological disciplines, from food faculties at BIOTERRA University of Bucharest showed that honey qualitative impairment due to the depreciation of the natural environment in which man lives, it is required due to the reckless actions of nature's time to take measures to protect plants, animals, the whole natural environment is important the ecological education and environmental education during training in education and continue throughout life. First, however it performs by various biological disciplines, as "Botanic", "Vegetal "Biochemistry", Biology", "General Microbiology", "Protection and Conservation environmental biodiversity", between them being the correlations interor trans-disciplinary with disciplinary "Education Science", "Pollutants and impact their environmental and consumer", "Food biotechnology" and so on. At the same time, the necessary professional education, nutritional education, training has the power to choose food, such as the honey bees, the knowledge and awareness of the need to distinguish between a natural food product, quality and one counterfeit, forged and his election therefore to lower the degree of morbidity and mortality of the social environment, the marketing of natural products, not some fake, counterfeit, contaminated.

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### **RESEARCHES ON THE IMPACT OF AGRICULTURAL PRODUCTS FROM MYCOTOXINS VEGETABLE ON CONSUMER HEALTH**

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**Abstract:** It is extremely important to achieve optimum economic fertilizer management. WorldwideMycotoxins are substances toxic mushroom character with critical role in the pathological process. They act on the host cell protoplasm in extremely low concentrations by altering membrane permeability.Some toxins cause all or part of the symptoms. The most common toxin in many crop plants aflatoxin is produced by Aspergillus fungi genius. The basic structure of aflatoxins is the core cumatinic one condensed furan. Aflatoxins can exist in wheat, sesame, sweet potatoes, corn, rice, peanuts stored in improper conditions.

Key words: Mycotoxins, agricultural products, consumer, aflatoxins

# Introduction

Mycotoxins are substances toxic mushroom character with critical role in the pathological process (Figure 1).

They act on the host cell protoplast, in extremely low by altering membrane permeability, some toxins produce all or some of the symptoms. Also some toxins do not act on host plants but are particularly toxic to animals that eat plants. They can be seen and metabolite, toxic for both humans and animals.



Figure 1. Image microscopic fungi

Currently about 300-400 substances have been classified as mycotoxins, but only 20-30 mycotoxins are supervised by the authorities, which are considered the most toxic and most common, belonging to 24 chemical groups of toxins that may occur in of the different agricultural production in various foods made from them. Plant pathogens produce profound changes in the biochemical, functional, or structural cell [1, 7, 9].

# $oldsymbol{M}$ aterials and methods

In general, mycotoxins enter the body through contaminated food but may enter by air or by direct contact with skin . Most of mycotoxins are resistant to high temperatures (baking, broiling and frying some cases resist). Many toxins resist the industrial processing of food that had to be analyzed mycotoxins free food raw materials (wheat, milk, vegetables, meat, etc.). Because they are resistant to



processing can be found in bread, breakfast cereals, wine, beer, etc.. By processing only can reduce the amount of mycotoxins not total elimination. Mycotoxins can develop during cultivation, transport, storage or at other times during production [3, 6, 10, 13]. The end result is that they are found in many foods (especially those based on cereals). It is thought that mycotoxins are at risk greater than food additives, contaminants and synthetic pesticides. In for cereals and maize are known these very dangerous toxins:

**Fumonisin B1** (FB1) produced by Fusarium verticiloides (present especially corn), developing mainly in Canada, USA, South Africa, Nepal, Australia, unlike Fusarium graminearum is very present in our country, especially in difficult and rarely triticale and rye and barley. The action of these toxins act on the ribosome synthesizing cells in the majority of DNA and cellular RNA and therefore, they inhibit cell division, especially in children [1, 2, 6, 10].

**Patulin** is a mycotoxin produced by fungi that attack, this time mainly in grain storage, and other products especially fruits and among fruits especially apples. To this fungus, the Scientific Committee for Food, in the meeting of 23.03.2000 establishes limits NOEL (NO Effect Level) at 0.1 mg/kg and index PTWI (Provisional tolerable weekly intake) at 7 mg/kg [1, 2, 6, 10] (Figure 2).

Ochratoxin A (OTA) is a mycotoxin



Figure 2. Microscopic image patulin

produced by fungi other severe, Aspergillus and Penicillium which are situated in nature Fifer strains of cereals, beans, beans and dried fruits. It is a carcinogenic toxin nephrotoxic properties, various tumors developing in Romania along the urinary tract. Cancer induction in humans of Ochratoxin A has been confirmed by the International Agency for Research on Cancer (IARC). Induction of kidney cancer afost obtained in mice to low doses of only 70 mg / kg / body alive [4, 7, 10] (Figure 3).



Figure 3. Microscopic image Ochratoxin A (OTA)

In foods derived from plants of 7,000 samples, OTA was found in 57% of cases in concentration over limit. Research on animal reproduction have shown that they can contribute to both animals and humans with toxic effects such as diarrhea, bacterial infections or parasites favoring, reduced growth, kidney tumors, vascular and digestive system effects. An American study rate as mycotoxins are 10,000 times more harmful pesticide residues [2].

In Romania, wheat production stands at about 2,500 average kg/ha. It is therefore expected and is already confirmed, the grains contain an average of 100-500 mg/kg mycotoxins (1.0-5.0 ppm), which is 10-50 times the limit allowed by the rules and quite apropos NOEL the onset of chronic toxicity of 50-78 mg/kg/body alive. In milk aflatoxin



was found between 0.5-3%. In rural areas the degradation of improperly stored cereals contained in this makeshift warehouses, grain became a brown mass, toxic fungus impregnated hufele its consummation by animals, for man can not eat, make toxins to move quickly in milk, eggs and meat to enter the food chain and poison people. The liver is the first to receive toxins and trying to degrade. When large amounts of mycotoxins, mitosis ceases proliferating cells do not appear cancerous cells proliferate and secure individual becomes a client of hospital or dies without knowing why, neither he nor his doctor [1, 3, 4, 7, 8].

# Results and discussions

A study called "Food Safety", published in 2002 in French collection "Food Science and Technology" shows that Romania is among the countries with mycotoxin infected. The products analyzed by French native, half flies also have killer substances. Chapter "Romania" in this document is entitled "Studies Dutton", 1996, (studies have been made since then), and Western scientists place our country in the area affected by what they called 15 years ago "Balkan syndrome". Ever since then it was known that populations of Romania, Bulgaria and the former Yugoslav space consuming countries heavily infected grain mycotoxinproducing fungi, as "aflatoxin", especially those that attack the kidneys. While developed countries have created advanced technologies for food storage, in order to prevent disease mycotoxins population, poor countries, including Romania, have not invested anything in this area. The country also did not study and did not put a word about the connection between food and aflatoxin Romanian market [8, 9].

The only data about ourselves can give us Westerners who say that at least half of the food they put on the table containing daily ochratoxins were identified in many vegetable foods: corn, wheat, rice, oats, barley, sorghum, soy, legumes, coffee and salted fish, in concentrations up to 2800 mg/kg.

In Denmark for example, it was established that the cause ochratoxin contamination of rice with nephropathy in pigs. Cases of renal disease ochratoxin products have been identified in birds. Ochratoxin A can accumulate in tissues (kidney, liver, muscle) and remove the milk. In pigs who had renal disease, kidney ochratoxin was presented in high concentrations. Acute toxicity of ochratoxin is between 0.2 and 0.34 mg/kg. Ochratoxin A has been detected in pig blood and kidney as well as in human blood and

and kidney as well as in human blood and mother's milk. The highest accident with this toxin has been found in the blood of pigs 60% of the cases, the grain of 13%, 21% pig kidney. Using RIDASCREEN Ochratoxin A can cause these residues in grain, hay, beer and pig serum in a quick and safe. As producers of ochratoxin identified species of Aspergillus and Penicillium, namely:

- Aspergillus ochraceus;
- Aspergillus alliaceus;
- Melleus aspergillus;
- Aspergillus ostianus;
- Petrakii Aspergillus;
- Aspergillus sclerotiorum;
- Aspergillus sulphureus;
- Penicillium viridicatum;
- Penicillium commune;
- Penicillium cyclopium;
- Palitans penicillium;
- Penicillium purpurescent;
- Penicillium variable.

Strains of Aspergillus ochraceus, highly toxic, are commonly present in the natural environment.



There may be a synergism between different fungi to produce mycotoxins. Are contaminated with OTA, especially cereals. Patulin known by many names including more frequent and clavacina. Se accumulate in grains and many fruits and vegetables: apples, pears, peaches, apricots, cherries, grapes, bananas, tomatoes, etc. can pass and processing products, in particular in juice fruits. Patulina is very toxic to plants and animals. Action was revealed mutagenic and teratogenic mycotoxin carcinogen. Synthesis is performed by different molds depending on the environment [1, 3, 6, 7, 8, 9].

# **C**onclusions

The European Union considers that the residues of inputs, especially nitrates and pesticides, food safety of its citizens is at risk, the incidence of cancer, heart disease and food is too big and too expensive treatments. Therefore, it was decided to reduce harvest levels below biological limits forcing varieties and animal breeds.

This will be done through the introduction of good agricultural practices and reducing hazardous conventional inputs. In contrast, in Romania, the food quality is not affected by overproduction, underproduction but generated by lack of inputs and proliferation of diseases caused by fungi.

Appear, therefore, oversized amounts of mycotoxins in food, very dangerous to the public, starting the path towards underdevelopment disease, but of cancer.

Mycotoxins in Romania is the second reason of causing a cancer.

Troubleshooting is still by practicing good agricultural practices must place production at levels that mycotoxins do not place animals no longer transmitted and therefore no people.

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### RESEARCH ON QUANTITATIVE DETERMINATION OF BIOLOGICALLY-ACTIVE COMPOUNDS IN NATURAL JUICES OBTAINED BY COLD PRESSING

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**Abstract:** Research conducted on berries show that they can be used as sources antioxidant, antibacterial being rich in phytochemicals which are obtained through bioactive properties. Analysis of active substances was made in the 3 categories of plant products, namely fresh fruit juices and frozen sea buckthorn, black currant and blueberry.

Key words: natural surroundings juices, vitamin C, polyphenols, antioxidants

## Introduction

Lately consumer demand for products was safe, free from harmful agents that can maintain or improve human health. It is desirable to use products with high nutritional value and biological (Banu, C., 2002).

Consumers are beginning to realize that crop, unprocessed or minimally processed can improve health. There is evidence that shows that by eating many fruits, vegetables and other plant-derived consumer health benefit is due to the presence of biologically active substances. These secondary metabolites are biologically active substances derived from plants. They prevent microbial spoilage stress of UV radiation. In addition to plant products, it is desirable to obtain products not endanger health products without chemical preservatives and less processed. It is well known that berries are natural sources of nutrients were investigated due to nutritional and medicinal value. Are widely studied in both food and pharmaceutical industry and medicine.

Some biologically active compounds include phenolic compounds, including flavonoids, phenols and tannins. These compounds are important due to their antioxidant and antibacterial activity they develop (Niculita, P., Popa, M., Belc, N., 2006).

## $oldsymbol{M}$ aterials and methods

The material in this case study consists of three natural products as sea buckthorn juice (Hippophae rhamnoides), black currant (Ribes nigrum) and blueberry (Vaccinium myrtillus). Analyses of these products were made at specialized laboratory of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

Research methods used were (Banu C., 2009):

- Titrimetric method for determination of vitamin C;



- Method of Folin - Ciocalteu, for the determination of polyphenols.

- Determination of vitamin C

Vitamin C is the major vitamin synthesized by plants, which passes through the slow oxidation in a diketone. The reaction is reversible and lead oxide-reducing properties of vitamin C. It also has important physiological roles in the body, and at the same time a very effective antioxidant. Vitamin C is considered the main natural antioxidant natural primary, non-enzymatic. Chemical methods of vitamin C dosage is based on reducing property of ascorbic acid, which by oxidation turns dehydroascorbic acid. The dosage is made by the titrimetric method, the oxidizing solution: potassium iodate and the like.

Determination of total polyphenols Principle Folin - Ciocalteu:

Determination of polyphenols from plant sources was done by measuring the optical density of a primary extract by complexing with the Folin - Ciocalteu, absorb in the Vis wavelength l = 750 nm.

Polyphenols in fruit juices was taken up in 40% ethanol again. Alcoholic extracts were Vortex 1 minute, sonicated 15 minutes and centrifuged for 10 minutes at 3000 revolutions / minute, is then filtered through a qualitative filter paper. From the filtrate thus obtained and 1 ml was placed in a 100 ml volumetric flask, add 60-70 ml of distilled water and stirred. Add 5 ml Folin - Ciocalteu and mix. After 1 minute and 8 minutes before 15 ml of 7.5 % sodium carbonate solution.

Record this as the time of "0" and stirring. Make it to 100 ml with distilled water. After 2:00 absorbance read at l = 750 nm sample compared to the control (blank). From the calibration equation calculates the amount of total polyphenols (expressed in mg / ml plant extract).

### $oldsymbol{R}$ esults and discussions

The results obtained from the analysis of three juices are shown in the following table 1. Vitamin C is the center of the network of natural antioxidants are found in most plants. The results for the three samples of juice (blueberry, sea buckthorn and black currant) are presented in the following table 2.

As can be seen from the table, the greater the total amount of polyphenol was determined in sea buckthorn juice, which is not surprising, that the plant (in particular fruit) is known for its very high content of natural antioxidant substances.

Table 1

The amount of vitamin C in samples of blueberry juice, black currant and sea buckthorn

Current issue	Sample title	Quantity vitamin C (mg/l)
1	Blueberry juice	2340,0
2	Black currant juice	5947,5
3	Sea buckthorn juice	3575,0

Table 2

The amount of polyphenols in samples of blueberry juice, black currant and sea buckthorn

Current issue	Sample title	Polyphenols (mg/l)
1	Blueberry juice	1.517
2	Black currant juice	2.328
3	Sea buckthorn juice	2.649



# **C**onclusions

» Juices made from berries by pressing enter in the category of functional foods because they contain active substances with nutritional value, reduce the risk of a large number of diseases and helps maintain the overall health of the body;

» The content of natural vitamins, minerals and active substances cause the body's own natural extracts of sea buckthorn, black currant and blueberry have an important role in increasing the body's natural immunity and antioxidant activity against free radicals because most chronic and degenerative;

» Phenolic substances in berries influences sensory quality and reduce oxidative stress;

» Vitamin C present in large quantities in this fruit, white and black currants and blueberries and a little lower in juices obtained from these fruits has an immunostimulant and antioxidant value;

» The highest amount of vitamin C was recorded in black currant juice, 5947.5 mg/l;
» Highest amount of polyphenols was recorded in sea buckthorn juice, namely 2.649 mg/l.

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### RESEARCHES ON THE IDENTIFICATION OF MACROSCOPIC CHARACTERISTICS, MINERALS AND THE FOOD VALUE AND NUTRITIONAL USE OF THE SPECIES PORTULACA OLERACEA

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**Abstract:** In Romania, Portulaca oleracea grows starting from lowland areas up in the highlands and salty soils. Plant herbaceous, succulent, with a length of about 50 cm, is widespread in crops, gardens, ruderal areas, the edges of roads, etc. [3],[4]. It extracts a significant amount of salt from the soil, bringing the soil salinity at a reasonable level [7].

From the analysis it was found that the highest values of minerals recorded from 100 g of plant material were in Ca (mg/100g 2540.707), Mg (17964.7 mg/100g) and Na (1095.54 mg/100g) and the lowest values in Cr (0.169 mg/100g), Zn (17.54 mg/100g) and Fe (31.85 mg/100g). Thus, we can conclude that Portulaca oleracea, having a high content of essential minerals can be used in curative, therapeutic and food, in various forms: fresh, powder or oil.

Key words: : portulaca oleracea, minerals, medicinal herbs

### Introduction

# *»Portulaca oleracea importance for human, veterinary and agriculture uses.*

Following profound pharmacological and biochemical research has proven that herbs can become useful preventive drugs, especially curative in most various diseases in human and veterinary pathology. About the use of medicinal plants remained ancient Sumerian data (with 6000 years BC), Babylonians, Assyrians, Egyptians, Arabs, Chinese and also by Hippocrates (460-375 BC), the greatest physician antiquity, Pliny the Elder (24-79 AD), Galen (131-201 AD), thus proving the great importance they gave these plants. Chinese folk medicine has an ancient tradition. It was developed during the same period, but independent of Europe and Arab countries. The emperor Chen Noung who lived 3000 years BC was concerned with the study of medicinal plants, He is considered the founder of Chinese medicine. Also, in China has been discovered a book written 2600 years BC, which mentions several medicinal plants and their uses. Chinese herbs have emerged in Europe only in 1517. In our country the medicinal herbs have been known since ancient times, Dacians and Scythians having knowledges about their use. Herodotus (484-425 BC) described the



practice of smoking hemp to relieve pain and produce sleep. [10]

Dioscorides (40–90 BC) in "Pedacii Dioscoridis Anazarbaei De Materia Medica, Lib. V" reminds the use of Lax (Portulaca) by Dacians, for medicinal puropse. [18]

Portulaca oleracea is an annual succulent member of the Portulacaeae family. It is widely spread in Europe, North America, North Africa, Middle East, India, China, Malaysia, Australia. There is archaeological evidence that this species has existed since prehistoric times.[19]

Portulaca oleracea has a root system, and stems that are creeping, glabrous, herbaceous, cylindrical, with a diameter of 1-2 mm, canoeing and fiber nodes with many branches and presenting spaced intervals. The leaves are fleshy, entire, alternate, spatulate, narrowed towards the base, with obtuse tips, dark green on the upper surface and pale green on the underside (Fig. 1).

The flowers are small, axillary grouped by 1-3, calyx with 2 sepals and corolla with 5 petals golden yellow, 4 mm long, with 4-12 stamens unequal. The fruit is a pyxis capsule (Fig. 2), with numerous dark gray seeds (37-93). In one gram there are about 2.400 seeds. Blooming period is around 9 o'clock in the morning and closes at different times of day, depending on the heat. Maximum flowering is 3-4 hours (between 9.00 am -12.00 am). The plant blooms from June up until October.



Fig. 1 Flowering plant



Fig. 2 Pyxis seed capsule

In Romania, Portulaca oleracea grows in lowlands to highlands and in saline soils. The plant grows to a height of about 50 cm, It is widespread through, gardens, rural areas, roadsides, etc. [3],[4].

Portulaca oleracea, extracts a significant amount of salt from the soil, thus soil salinity should be at a reasonable level [7]. Portulaca oleracea is also used for food, and can be added in salads or in various dishes, as internal medicine administration for: digestive inflammation (mucilage), airway inflammation (asthma), inflammation (cystitis), urinary stones, bleeding, diabetes, or Lichen planus. The leaves are diuretic, the seeds are cited as having vermifuge effects. [1];[13].

Aqueous extracts of Portulaca oleracea shows antioxidant properties, helps reduce lipid peroxidation, reduces oxidative stress, is neuroprotective, provides protection of gastric mucosa, and is antifungal and antiviral [8]. Studies made in Kahramanmaras Sutcu Imam University, Turkey have concluded that the addition of Portulaca oleracea in the diet of chickens increased significantly eggs production and the omega 3 content of the eggs. [2]. Externally, the fresh plant extract produces faster healing of skin lesions. Crushed leaves are used in gum inflammations [11].

Directions for plant infusion: dosage of 20 - 30g / 1 of water, 3-4 cups / day or in the form



of capsules, Portulaca oleracea extract 10:1 ( 250 mg / cps ) -1 cps/day. [16] Portulaca oleracea extract has grassy odor, specific taste sour. The aqueous extract must have a concentration of 5% in dosage of 0.25 ml / kg bw, for bronchodilator effect. For type-2 diabetes the recomanded dose is 5 grams of seed powder / day for 2 months. For uterine metrorrhagia the recomanded dose is 5 grams of seed powder administered from 4 to 4 hours for 3 days. [9]. Portulaca oleracea is used for food, and can be added in salads or in various dishes.

# Materials and methods

Farmacognostics determination

Biotypes of Portulaca oleracea were collected from a soil clay illuviated, reddish brown in Bucharest in October 2012

»Macroscopic and organoleptic control of plant products:

The macroscopic control of plant products is determined by their characteristics that are visible to the naked eye and can be determined by smell and taste.

The macroscopic control has some particularities depending on the presentation of plant products (whole, pieces or powder). The macroscopic determinations were performed according to the European Pharmacopoeia [17].

Determination of the odor of whole plant products is performed by rubbing the vegetable between fingers.

Determination of taste of whole plant products carried out on a wetted fragment of plant product or its decoction.

»Determination of minerals, lead and cadmium from Portulaca oleracea:

Identification of the main components are obtained by the technique GFAAS-atomic absorption spectrometry with graphite fumace and FAAS - flame atomic absorption spectrometry.

Flame atomic absorption spectrometry method (FAAS).

In FAAS, the sample is atomized and withdrawn into a burner, so that the elements for analysis can be decomposed in a flame of a fuel gas such as acetylene, and an oxidant gas, usually air. The burner is arranged in such a way that the flame region with the highest concentration of atoms is in the range path of the spectrometer.

Atomic absorption spectrometry with graphite furnace method (GFAAS)

In GFAAS atomization device is used a graphite tube which can heat. The tube is located in the beam path. A small drop of sample is pipetted into the graphite tube, where it is dried by electric heating and and the residues are burnt. In the next step, they are burnt at a very high temperature, the elements present in the residue being atomized. During this phase, the attenuation of the radiation lamp by atomization of the thin volume of the graphite tube can be measured with great accuracy. As a result, the very low detection limits make the GFAAS, a high performance method for the analysis of element behind [15].

The measurements of leaves, stems and roots of Portulaca oleracea were made at the National Institute of Research and Development for Food Bioresources - Bucharest (IBA), during in June 2013.

### ${\it R}$ esults and discussions

Following the analysis performed by spectrometric techniques, at National Institute of Research and Development for Food Bioresources - Bucharest (IBA) resulted the following values of minerals (Fe, Cu, Zn, Cr, Mn, Ca, Mg, Na), of lead



and candiu, expressed ppm or equivalent mg / kg (Table 1, Figure 3, Table 2, Figure 4).

# » The main groups of active substances of Portulaca oleracea

Portulaca oleracea has substantial medicinal and nutritional properties. It has a high content of l-norepinephrine (l-noradrenaline, 0.25% in fresh plant), a neurohormone with pressor action and antihypotensive properties, reducing bleeding of tissue. The raw plant contains vitamins A, B1, B2, C, niacinamide, nicotinic acid (vitamin PP),  $\alpha$ -tocopherol,  $\beta$ -carotene, minerals (Ca, Mg, Fe, Mn, P, K), melatonin,fatty acids especially omega 3 (having the largest amount of all green vegetables) [12], glutathione, glutamic acid, and aspartic acid. The plant also contains cellulos, tannins, mucilaginous substances, calcium oxalate, malic and citric acid, dopamine, coumarins, flavonoids, alkalosis. [Leung & Foster, 1996]. Have been identified in plant composition and portulozide three monoterpenes glycosides.[14].

Flavonoids and related compounds form a group of active substances that after extensive pharmacological investigation have gained importance in phytotherapy.

Tab 1: Values of minerals, Pb and Cd in *Portulaca oleracea* (leaves, stems, roots)

Fe	Cu	Zn	Cr	Mn		Pb	Cd	Ca	Mg	Na
(ppm)	(ppm)	(ppm)	(ppm)	(ppm)		(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
F-	F-	F-	GF-	F-	GF-	GF-	GF-	FAAS	FAAS	FAAS
AAS	AAS	AAS	AAS	AAS	AAS	AAS	AAS			
318.5	32.34	175.40	1.69	30.62	21.51	1.84	0.050	25407.07	17964.7	1095.54

GFAAS - Graphite furnace atomic absorption spectrometry FAAS - Flame Atomic Absorption Spectrometry



Fig. 3 Comparative values of minerals in plant



Various flavonoids inhibit enzymes involved in inflammatory processes. The antiinflammatory properties of various plants recommended by traditional medicine are explained by their bioflavonoids action on the enzyme system. [10]

Polyunsaturated fatty acids have antiaterogenic potential. These acids are known as essential fatty acids because their deficiency leads to ailments such as growth retardation, skin lesions , hair loss and susceptibility to infections.

Vitamin B1 (thiamine) is involved in enzyme systems of carbohydrate metabolism.

Vitamin B2 (riboflavin) enzyme systems is involved in the transport of hydrogen and in protein metabolism.

Vitamin PP (nicotinic acid) is involved in bioenergetic reactions and synthesis reactions, helping the smooth functioning of the digestive system, nervous system, the maintenance of teeth and skin.

Vitamin C helps to form collagen, forming the vascular walls, helps restore bone tissue the formation of hemoglobin, the nonspecific defense against infection.

Vitamin E participates in the structure of certain enzymes involved in the processes that regulate embryogenesis.

Vitamin A participates in the chemical processes underlying of seeing.[5]

Minerals are essential for proper functioning of the body, they have multiple roles and are absolutely necessary for life. They are of two types: macro and micronutrients.

Macronutrients such as sodium, chloride, potassium, calcium, phosphorus, magnesium and sulfur, are mainly plastic part.

Micro-nutrients are found in the body in very small quantities and primarily have the

Tab.2.

Mineral values expressed in mg, obtained from100 g plant material (leaves, stems, roots)

Fe	Cu	Zn	Cr	Mn	Ca	Mg	Na
mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g
31.85	3.234	17.54	0.169	3.062	2540.707	1796.47	109.554



Fig. 4. Comparative value of minerals produced from 100 g of plant material



role of metabolic coordinator.

They either enter the structure of metabolic enzymes (respiratory or digestive), or activate various metabolic enzymes. The main micro-nutrients are iron, copper, cobalt, manganese, zinc, fluorine, iodine, molybdenum, selenium.[6]

## **C**onclusions

From the analysis, we found that the highest values of minerals recorded from 100 g of plant material were in Ca (2540.707 mg/100g), Mg (17964.7 mg/100g) and Na (1095.54 mg/100g) and the lowest values in Cr (0.169 mg/100g), Zn (17.54 mg/100g) and Fe (31.85 mg/100g). Thus, we can conclude that Portulaca oleracea, having a high content of essential minerals such as Ca, Mg



Fig. 5 Powder



Fig. 6 Oily macerate



Fig. 7 Ash

and Na can be used in curative, therapeutic and food, in various forms: fresh, powder or oil.(Fig. 5). Due to the high content of unsaturated fatty acids, Portulaca oleracea oily macerate (Fig. 6) can be used in the food industry, with a pleasant taste, having aroma of hazelnuts. This oil can successfully replace oil with saturated fatty acids and it is similar with safflower oil, olive, sesame, etc. From the ashes of the plant (Fig.7) can be extracted a salt plant particularly valuable for food. The importance for agriculture is special, because Portulaca oleracea extract a significant amount of salt in the soil, thus a reasonable ground to salinity. It can be grown both between cultures and lands that no other plants can be grown.

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