



**COST863 WG4 Joint Meeting with**

***The 4<sup>th</sup> International Conference on Polyphenols and Health (ICPH2009)***

**Bioactive compounds in berry fruits and human health benefits**

***Harrogate International Centre, Harrogate, UK***

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**ABSTRACTS**

***Organised by the School of Food Science and Nutrition, University of Leeds and the COST863 "Euroberry Research: from Genomics to Sustainable Production, Quality and Health"***

**Organised by**

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## **Invited lectures**

### **POLYPHENOLS AND HEALTH: INTRODUCTION AND PERSPECTIVES**

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The recent surge in research interest in polyphenols has led to major advances in understanding of chemistry, biology, and cell biology of this large class of compounds, and progress has been made in the fields of nutritional sciences and clinical medicine. Current focus is on methods and strategies on how to translate results from nutrition studies into perspectives on human health. The issues involved seem straightforward at first sight, but upon scrutiny it turns out that a number of major research topics are to be tackled in the future. These include establishing appropriate methods for studying bioavailability, biokinetics, metabolism and toxicology. Identification of the molecular points of action as well as establishing concepts for validation at the level of the human organism are further challenges. Emphasis will be on such problems and on pointing out ways for their solution in the future.

### **BLUEBERRY BENEFITS IN NORMAL VISION AND AFTER RETINAL STRESS**

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*antioxidant; anti-inflammatory; retina; anthocyanin; brain*

Although it has been suggested that anthocyanins benefit dark vision a review of clinical research concluded that results of rigorous placebo-controlled trials do not support this effect. The effects of blueberry juice (BBJ) consumption on dark vision was recently examined in a randomized placebo-controlled, double-blind, cross-over trial (n=59). BBJ or placebo was consumed daily for 12 weeks. Vision was tested at prescribed intervals using PC-based tests to determine: (1) rate of dark adaptation (2) dark-adapted visual acuity and (3) contrast sensitivity and (4) recovery of visual acuity after exposure to bright light. No BBJ-related difference was found in any test except in the rate of recovery of visual acuity after bright light exposure. This improved recovery was still observed after 4, but not 8, weeks of washout. In a second clinical trial (n=72) BBJ similarly reduced recovery times after bright light exposure after only a 3 week intervention. BBJ consumption may benefit individuals who operate in low light with interruptions by bright light (e.g. drivers, pilots).

Light-induced retinopathy (LIR), which employs prescribed exposure to bright light to induce damage, apoptosis and remodeling in retinal photoreceptors was used as an *in vivo* model to examine possible BBJ effects. For 6 weeks Wistar rats (n=9) received BBJ while placebo-fed rats (n=8) received a solution containing glucose, fructose, malic, citric and chlorogenic acid to the level found in BBJ. Retinal function was objectively measured using electroretinograms (ERG) to determine the capacity (V<sub>max</sub>) and the sensitivity (k<sub>1/2</sub>) of photoreceptors. Prior to

LIR the ERG parameter  $V_{max}$  was  $475(\pm 185)$   $\mu V$  and  $k_{1/2}$  was  $8.6(\pm 7.3)$   $cd \cdot s \cdot m^{-2}$ . Eleven days after LIR the placebo group  $V_{max}$  had fallen to  $183(\pm 98)$   $\mu V$  with  $k_{1/2}$  at  $8.1(\pm 6.6)$  while the BBJ-fed rats had a  $V_{max}$  of  $386(\pm 121)$ , and a  $k_{1/2}$  of  $4.6(\pm 0.8)$ . BBJ protected rat retinae against LIR-induced damage.

## **BERRYFRUIT RESEARCH AT PLANT & FOOD RESEARCH, NEW ZEALAND; FROM CHEMISTRY TO HEALTH**

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Plant & Food Research, New Zealand, is a world-leading plant and fruit science company, recognised as providing high quality and high impact research and innovation outcomes for New Zealand's \$4 billion dollar horticultural sector. Intrinsic 'healthfulness' is one of the biggest strategies in the marketing of functional foods. Fruits have an inherent natural 'health halo' and the new 'superfruits' are emerging with specific perceived and validated health benefits, which enable them to be priced at a premium. Plant & Food Research has a large database of fruits and their compounds and has an active interest in evaluating fruits for health benefits and for use in functional foods. Plant & Food Research has developed a research platform to evaluate physical health, performance and recovery to position its new fruit varieties in this arena. Regular exercise has health benefits believed to be derived from adaptive responses to moderate oxidative stress. However, following exhaustive or unaccustomed exercise, excessive and prolonged oxidative stress can be detrimental and the right balance of antioxidant modulation from nutritional support with fruit phytochemicals (and vitamins) may prevent damage/injury, aid recovery, and/or enhance muscular and immune function. Utilising muscle cell-based assay screening, electrically stimulated whole muscle tissue *ex vivo* experiments, and animal and human intervention and exercise trials, we are evaluating the physical health-promoting effects of phytochemicals derived from some fruits. Analysis demonstrates that certain fruits may both complement the benefits of regular exercise and modulate the excessive oxidative stress and inflammation mediated by over- or unaccustomed exercise. Nutritional support with fruits or fruit-derived functional foods may therefore be beneficial for maintaining physical health, supporting the potential positioning of new fruit varieties into this global value-added market.

This research was funded by the New Zealand Foundation for Research Science and Technology with Blackcurrants New Zealand, contract C06X0807.

## **NUTRITIONAL MODULATION OF BRAIN AGING VIA BERRYFRUIT OR WALNUT SUPPLEMENTATION**

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*Blueberries, strawberries, raspberries, walnuts, brain aging, inflammation, oxidative stress, stress signaling*

The onset of age-related neurodegenerative diseases superimposed on a declining nervous system could enhance the motor and cognitive behavioral deficits that normally occur in senescence. It is likely that, in cases of severe deficits in memory or motor function, hospitalization and/or custodial care would be a likely outcome. This means that unless some way is found to reduce these age-related decrements in neuronal function, healthcare costs will continue to rise exponentially. Thus, it is extremely important to explore methods to retard or reverse the age-related neuronal deficits as well as their subsequent, behavioral manifestations. Applying molecular biological approaches to slow aging in the human condition may be years away. So it is important to determine what methods can be used today to increase healthy aging, forestall the onset of these diseases, and create conditions favorable to obtaining a “longevity dividend” in both financial and human terms. In this regard, epidemiological studies indicate that consumption of diets rich in antioxidants and anti-inflammatory (anti-INF) compounds, such as those found in fruits and vegetables, may lower the risk of developing age-related neurodegenerative diseases, such as Alzheimer’s or Parkinson’s diseases (AD and PD). Research from our laboratory, as well as others suggests that the polyphenolic compounds found in fruits, such as berries, may exert their beneficial effects by altering oxidative stress (OS) or INF signaling and neuronal communication, suggesting that interventions may exert protection against age-related deficits in cognitive and motor function. Our studies showed old rats maintained for 2 months on diets containing high antioxidant strawberry, blackberry, blueberry (BB) or raspberry extracts, or walnuts, exhibited reversals of the age-related deficits in neuronal function or cognitive behavior. Subsequent experiments in both cell and animal models have indicated that polyphenols contained in berries, and perhaps the poly-unsaturated fatty acids in walnuts, may have anti INF/OS properties, as well as direct effects on improving neuronal signaling by reducing stress signaling and increasing neurogenesis. Recent findings from our cell models suggest that berries may prevent the deleterious effects of INF/OS by blocking the stress signals (e.g., nuclear factor kappa B). Their generation results “downstream” production of cytokines and cyclooxygenases I and II which ultimately produce negative effects and invoke further OS/INF in the brain. Importantly, this information can then be utilized to show that the addition of berries (e.g., BB, strawberries, blackberries, raspberries) and walnuts to the diet may increase “health span” in aging. Extending these findings to humans, a collaborative study with Dr. Krikorian has indicated that elderly people with some memory loss, given blueberry juice twice a day (up to 12 ounces/day for 12 wks) showed significant improvements in cognition in on several verbal tests such as list recall. Taken together, these findings suggest that the berries and walnut may be important tools that could be used to forestall or prevent the deleterious of aging on cognitive and motor function.

## **THE INTEGRATION OF HUMAN HEALTH AND SOFT FRUIT BREEDING**

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*Metabolomics, Soft Fruit, Cardiovascular, Cancer*

The agricultural policies of the developed world have meant that, for these countries at least, food is plentiful but changing eating patterns have seen an increase in the consumption of ready-made meals and food elevated with respect to sugar and fat. The knock-on effect of this is evident in the rapidly increasing level of obesity in the western world with 20% of

males and 25% of females now classified as obese in the United States. Associated with this and the related dietary shift are increases in the incidence of degenerative diseases such as atherosclerosis, some cancers etc. Fundamental, clinical and epidemiological research into the basal causes and consequences of these diseases has highlighted the gross and specific benefits of including a significant level of fruit and vegetable within the diet. Polyphenols are ubiquitous in plants and, with respect to dietary plant sources, particularly prevalent in many fruit species and in particular in the common soft fruit such as strawberries, blackcurrant, raspberries etc. As a result of the putative potent health benefits of these compounds, crop (including fruit) breeding has undergone a paradigm shift with the adoption of global metabolite screening rapidly becoming an integral part of the process. Evidence will be presented to show that soft fruit extracts and components exhibit beneficial health effects in model and real systems. In addition a stratagem for integrating these targets into new and ongoing breeding programmes will be outlined using a joint molecular genetic and metabolomics approach.

### **Oral presentations**

#### **BERRY CONSUMPTION IN HUMANS: BIOAVAILABILITY OF POLYPHENOLS AND EFFECTS ON CARDIOVASCULAR RISK FACTORS**

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*Berries, human intervention trial, polyphenols, health effects, bioavailability*

Berries are a rich source of various polyphenols and other potentially bioactive compounds. However, few studies have investigated the bioavailability of the compounds from berries or the effects of berry consumption on cardiovascular risk factors.

The above-mentioned issues were investigated in a single-blind, randomized, placebo-controlled intervention trial. During the trial, middle-aged, unmedicated subjects (n=72) with cardiovascular risk factors consumed moderate amounts of berry or control products for eight weeks. The subjects in the berry group (n=36) consumed bilberries, lingonberries, blackcurrants and chokeberries (as well as small amounts of strawberry and raspberry) as whole berries, puree, nectar and juice. The mean amount consumed daily was 160 g/day. The subjects in the control group (n=36) consumed sugar water, jelly sweets and porridges prepared from semolina and rice. Blood samples were collected at baseline and weeks 2, 5 and 8. Urine samples were collected at baseline and week 8. The intake of polyphenols from berries was 826 mg per day, as measured by HPLC.

The concentrations of various polyphenols increased in the plasma and urine samples collected from the berry group (compared to the control group). Plasma vitamin C concentrations increased also. Plasma folate and tocopherols, as well as urinary sodium and potassium, were unaffected by the intervention.

Favorable changes were observed in some cardiovascular risk factors after berry consumption. Serum HDL cholesterol concentrations increased, but other lipids were unaffected. Systolic blood pressure decreased and the decrease mostly occurred in subjects with high blood pressure at baseline. Furthermore, berry consumption inhibited platelet function as measured with a platelet function analyzer. Plasma biomarkers of platelet activation, coagulation and fibrinolysis did not change during the intervention.

In conclusion, moderate daily consumption of berries may be beneficial for cardiovascular health. Polyphenols (and vitamin C) are the most likely compounds to mediate the effects. More studies are now warranted to explore the effects of berry consumption on human health.

## **STRAWBERRY PHYTOCHEMICALS: ABSORPTION, BIOAVAILABILITY AND METABOLISM IN HUMANS**

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*Strawberry, domestic storage, bioavailability, anthocyanins*

Strawberries (*Fragaria×ananassa* Duch.) contain high amount of antioxidant micronutrients and phytochemicals such as vitamin C, phenolic compounds, carotenoids and anthocyanins mainly pelargonidin-3-glucoside (Pel-glc). The bioactivity of all dietary compounds is mediated by their recovery in blood and tissues, thus bioavailability represents a fundamental issue. In this study antioxidant composition of fresh and stored strawberries and the bioavailability of the main strawberry bioactive compounds, including Pel-glc, were determined in humans. On 13 healthy volunteers, blood and urine were sampled before and after acute ingestion of fresh and stored strawberries, 300 g respectively. After consuming fresh strawberries, plasma levels of  $\alpha$ -carotene increased significantly ( $P<0.05$ ) respect to stored ones. Consumption of fresh and stored strawberries resulted in a significant increase of vitamin C at 2, 3 and 5 h ( $P<0.05$ ). No plasma anthocyanin levels were found. Higher plasma levels of protocatechuic acid, 4-hydroxybenzoic acid and coumaric acid were detected after consuming both fresh and stored strawberries. The peaks of urinary excretion of pelargonidin-3-O-glucuronide and pelargonidin-3-O-glucoside were found between 0-2 h. The bioavailable amount of strawberries antioxidant compounds reflects the variations observed in fresh and stored fruits. Our results show a correlation between Pel-glc content in fruits and pelargonidin urinary excretion as parent compounds or metabolites.

In conclusion we could summarize that the global food quality is related to both native quantity of bioactive compounds and storage treatments. Moreover our results contribute to elucidate the absorption, the metabolism and the excretion of pelargonidin.

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## **ABSORPTION, METABOLISM AND POTENTIAL PROTECTIVE EFFECTS OF BERRY-DERIVED ANTHOCYANINS AND ELLAGITANNINS**

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Berries are a rich source of a number of flavonoids and phenolic compounds. They are an important part of a healthy diet, which should comprise five 80 g portions of fruit and vegetables per day according to recommendations in the UK. There are a number of diverse health claims for berries. They range from protection of neurological tissue thereby maintaining cognitive function [1] to cancer prevention in the gastrointestinal tract [2]. This array of potential health benefits may reflect the diverse range of phytochemicals present in berries which are perhaps best known for their anthocyanin content.

There have been numerous human studies on the bioavailability of anthocyanins. They are rapidly absorbed, typically with a plasma  $T_{max}$  of ca. 1 h and a half-life of ca. 2 h [3]. However, plasma  $C_{max}$  and urinary excretion both indicated that anthocyanins they are poorly absorbed, with excretion ranging from as low as 0.01% of intake for cyanidin-3-glucoside to 1-2% in the case of pelargonidin-3-glucoside [4,5]. Little is known about anthocyanins or their metabolites being sequestered in body tissues and this is likely to remain so until animal studies are undertaken with  $^{14}\text{C}$ -labelled anthocyanins.

Rats are often used in feeding studies with anthocyanin-rich berry extracts. However, unlike rats, berries are part of the normal diet of many birds and as such represent a useful model to investigate if they play a role in improving cognitive function of birds. Our results showed that blackberry anthocyanins, fed to greenfinches, cross the blood brain barrier. With improvement in mass spectrometry technology it should be possible to determine the area of the brain the anthocyanins are located in.

Another area of interest in berry phenolics is the ellagitannins. These are high molecular weight compounds made up of esters of hexahydroxydiphenic acid and a polyol, usually, glucose. Ellagitannins are powerful antioxidants and *ex vivo* have strong vasodilatory properties [6]. However, their bioavailability is very low and there are no reports of ellagitannins being detected in either the circulatory system or urine. Ellagic acid, released by the breakdown of ellagitannins has been detected in plasma extracts, though at low nmol/L concentrations, that are unlikely to elicit any biological effects [7]. However, ellagic acid is not the only compound that is found after ellagitannin ingestion. Urolithins, formed by the action of colonic microflora on ellagic acid are absorbed and remain in the body, measured by urinary excretion, for over 56 hours [8]. Urinary excretion of urolithins after ingestion of raspberries is currently being investigated and results obtained from this study will be reported.

## **CHARACTERIZATION AND FREE RADICAL SCAVENGING ACTIVITY OF BILBERRY (*Vaccinium myrtillus* L.) ANTIOXIDANTS**

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*Vaccinium myrtillus* L., phenolic acids, flavonoids, ESR, HPLC

Epidemiological studies have related the dietary consumption of fruits and vegetables, with a decrease in the incidence of cancer and cardiovascular disease mortality. This has been associated to the content of phytochemicals as one of the key factors. Phytochemicals are substances found

in fruits and vegetables that can be ingested daily (in grams) and those that exhibit a potential to modulate human metabolism by favoring prevention of cancer and other diseases.

Berries have scientifically been examined in the last few years extensively, and are recommended diet due to their nutritious characteristics. Phytochemicals present in berries are a potential rich source of health-promoting food constituents, possessing antioxidant activities. Berries are mostly dominated by the family *Ericaceae*, and bilberry (*Vaccinium myrtillus* L.), originated from this family, was selected for this study. A wide variety of phytochemicals are being proposed as antioxidants. Of these, the phenolic compounds such as simple phenolic acids and flavonoids are receiving the most attention.

Extraction and separation of typical biologically active compounds, phenolics and vitamin C, from berry extracts was conducted using solid phase extraction (SPE). The complete characterization of phytochemicals in separated fractions of bilberry by high performance liquid chromatography (HPLC) revealed high content of vitamin C, quercetin and coumaric acid. Evaluation of free radical scavenging activity on superoxide anion radicals and detection of antioxidant-derived radicals during reactions of fractions of bilberry with  $O_2^{\bullet-}$  radicals was conducted using electron spin resonance (ESR) spectroscopy. Investigation of antiproliferative activity of separated fractions of selected bilberry extracts on human breast adenocarcinoma (MCF-7) showed very prominent antiproliferative activity of fractions containing neutral and acidic phenolics, but no activity for the fraction containing polar compounds. On the basis of these results it can be concluded that bilberry is a rich source of naturally occurring antioxidants and these findings are of importance since natural antioxidant mixtures could be suitable for the treatment of diseases which are characterised by an overproduction of free radicals.

## **ARONIA JUICE, A BERRY JUICE RICH IN POLYPHENOLS, INDUCES REDOX-SENSITIVE ACTIVATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE AND RELAXATION IN PORCINE CORONARY ARTERIES**

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### **Objectives:**

Several rich sources of polyphenols strongly increase the endothelial formation of nitric oxide (NO), a potent vasoprotecting factor, via the redox-sensitive activation of the Src/PI3-kinase/Akt pathway leading to the phosphorylation of endothelial NO synthase (eNOS). The present study examined the potency of Aronia juice to induce redox-sensitive activation of eNOS and relaxation.

### **Methods:**

Vascular reactivity was assessed using porcine coronary artery rings in the presence of indomethacin and charybdotoxin plus apamin. The phosphorylation level of Akt and eNOS was assessed in cultured coronary artery endothelial cells by Western blot analysis.

### **Results:**

Polyphenols rich Aronia juice caused endothelium-dependent relaxations in coronary artery rings and the phosphorylation of Akt and eNOS in cultured endothelial cells. Both of these responses were inhibited by the membrane permeant analogue of SOD and Catalase, MnTMPyP and PEG-Catalase, whereas native SOD or Catalase were without effect. They were also affected by inhibitors of Src (pp2) and PI3 kinase (wortmannin). Finally, they were inhibited by calmidazolium, suggesting the implication of calmoduline. In addition, aronia juice up-regulated the expression of eNOS in a time dependent manner.

### **Conclusions:**

Aronia juice, a berry juice rich in polyphenols, causes endothelium-dependent relaxations of coronary arteries and activation of eNOS via the redox-sensitive Src/PI3-kinase/Akt pathway. In addition, calmodulin plays a role in the Aronia-induced relaxations and eNOS phosphorylation.

## **THE MOLECULAR EVENTS UNDERLYING BLUEBERRY-INDUCED IMPROVEMENTS IN SPATIAL WORKING MEMORY IN YOUNG AND AGED RATS**

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*Blueberry; Spatial; Memory; Learning; Flavonoids*

Phytochemical-rich foods have been shown to be effective at reversing age-related deficits in memory and learning in both animals and humans. Evidence suggests that these effects are underpinned by the ability of flavonoids to modulation neuronal signalling and to inhibit neuroinflammation. We have investigated the effects of a chronic blueberry (BB) intervention on spatial working memory in both young and aged animals, and have determined the molecular changes in the brain underlying these behavioural changes. In two independent experiments, young (1 month) and aged (18 month) rats were fed a placebo control or a control + BB (2% w/w) diet for a total of 6 weeks. During feeding, spatial memory was assessed in an 8-Radial Arm Radial Maze (young) or T-maze (old) model. The results indicated that blueberry intervention significantly increased spatial working memory task performance in both young ( $p=0.023$ ) and aged animals ( $p=0.001$ ), although the effect observed in the young animals was less pronounced. Furthermore, chronic supplementation of aged animals with pure flavanols (catechin and (-)-epicatechin) or a purified BB anthocyanin extract, revealed that flavanol intervention resulted in a pattern of cognitive improvement very similar to that observed following blueberry intervention ( $p=0.001$ ). Whilst anthocyanin intervention also significantly improved cognitive performance over the 6 weeks ( $p=0.004$ ), the overall improvement was less pronounced relative to both the blueberry and flavanol groups. In order to investigate the mechanisms underlying flavonoids actions in the brain, we have assessed widespread changes in hippocampal gene expression in response to the various interventions. In particular, using RT<sup>2</sup> Profiler PCR arrays we show how behavioural changes relate to the expression profile of 83 genes related to neuronal cell growth, differentiation, neuronal regeneration, apoptosis and survival. Our data provide further weight to the growing body of evidence linking flavonoid-rich foods with improvements in both memory and cognition.

## **CULTIVAR AND PRODUCTION SYSTEM: POSSIBILITIES TO AFFECT THE CONTENT OF BIOACTIVE COMPOUNDS IN STRAWBERRY**

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*Fragaria x ananassa, antioxidant capacity, phenols, cultivation technique, vitamin C*

Bioactive compounds in strawberry fruits include mainly ascorbic acid and polyphenols. These health promoting components vary in their content affected primarily by genotype and environmental factors.

In the present study the variability in bioactives of the three June-bearer ‘Elsanta’, ‘Korona’ and ‘Clery’ and the ever-bearer ‘Everest’ were compared. As expected, the contents of total anthocyanins, total phenols and vitamin C were significantly different between the cultivars. Additionally, the four cultivars distinguished by their polyphenol profile. However, the health beneficial compounds were exceedingly influenced by harvest date and year.

Nowadays there are several possibilities to cultivate strawberries like tunnel cultivation, hill plastic culture or fertilization. These cultivation practices affect the environmental parameters e.g. temperature, radiation or relative humidity. Beside different production systems the environmental factors are dependent on the growing location according to the climate. The aim of the trials was to explain changes of the bioactive compounds in strawberry by external factors. In general, the antioxidant capacity (TEAC, total phenols) of the fruits was only slightly affected by different production systems. Whereas, diverse growing conditions affected the polyphenol profiles of the fruits. Generally, cultivars reacted similar on environmental changes. Particularly the formation of anthocyanins seems to be affected by temperature. Nevertheless, the response on the content of several polyphenol fractions like quercetins was different between the cultivars. The interaction between genotype and growing conditions on health beneficial compounds will be discussed.

## **QUALITY, NUTRITIONAL QUALITY AND NUTRACEUTICAL VALUE AS A NEW TASK FOR STRAWBERRY BREEDING**

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*Fragaria, germplasm, seedlings, fruit quality, antioxidant capacity, phenols*

The quality of fruit is considered an extremely complex matter because it is submitted by the subjectivity of the consumers. Recently, research has focused on improving fruit nutritional value. In the last few years the nutritional value of fruit has been widely studied and requested by the consumers, especially for the general health benefits it can provide. These benefits can be described mainly to the antioxidant capacity of fruit. Both quality and antioxidant attributes are good tools to describe the nutritional quality of fruit.

During our research we have noticed that progenies from strawberry crosses having *F. virginiana glauca* (FVG) as a common parent showed a significant increase in their fruit

quality and nutritional features confirming the interest of the wild species to improve cultivated strawberry for several characters. For those reason our work is more focused on the study of inter-specific crossing to evaluate new genotypes that shown the best compromise between quality and nutritional characteristics.

In this work, fruit quality, nutritional quality and nutraceutical was evaluated by taking in account the following parameters: Firmness, Color, Soluble Solid content, Titratable Acidity, Total Antioxidant Capacity, Anthocyanin Content, Total Phenolic Content and vitamin C. 81 strawberry offspring, originated from 8 *Fragaria spp.* inter and intra-specific crosses, were screened for identifying and selecting new genetic material with an improved combination of quality, nutritional quality and nutraceutical value.

## **METABOLIC ACTIVITY STIMULATION OF THE YEASTS BY POLYPHENOLS FROM BLACK CURRANT BERRIES (*Ribes nigrum L.*)**

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*Ribes nigrum L., polyphenols, Saccharomyces cerevisiae, cells multiplication, fermentation ability*

The most important group of phenolics in berry fruits are the flavonoids which consist mainly of flavonols, anthocyanidins, proanthocyanidins, catechins, flavons, and their glycosides. The content of phenolics and antioxidant capacity in berries is affected by genetic differences (genotypes), the degree of maturity at harvest, preharvest environmental conditions, and post-harvest treatments and storage conditions.

To study different aspects of oxidative stress on biochemical, molecular-biological and cellular level useful models are microorganisms. Oxidative damages to proteins, lipids, nucleic acids and other cell components as well as defence systems against oxidative stress are basically almost similar to all levels of cell organization.

However, *in vivo* assays are also necessary to have a more accurate evaluation of the effect of red grape polyphenols on metabolic activity stimulation potential. Preferred model to study the response to stress in eukaryotic cells is *Saccharomyces cerevisiae*.

In this study two aqueous of 1% tartaric and citric acid solutions were used for polyphenols extraction from black currant skins berries (*Ribes nigrum L.*).

The effect of polyphenols extracted from black currant skins on the *Saccharomyces cerevisiae* strain as a model organism was investigated. Yeast was cultivated in aerobic and facultative anaerobic conditions on grape must medium enriched with different concentrations of polyphenol extracts in variable ratios. Yeasts growth kinetics was evaluated by direct counting of the cells. Cells multiplication was monitored at different moments using kinetic parameters, like number of generations, rate of multiplication and generation time. Cell viability and stability in time were estimated through microscopic observation of cell suspended in methylene blue, used as redox indicator.

Results showed that polyphenols from black currant stimulated the cell multiplication and metabolic activity and decreased the intracellular oxidation with positive effects on cell stability and viability. The maximum of fermentation intensity was reached for polyphenols concentrations of 0,82 mg/mL extract in tartaric acid and 0,65 mg/mL extract in citric acid, at levels of 98.42% and 92.37% respectively.

## POSTERS

### P1

#### ANTIOXIDATIVE EFFICIENCY OF BILBERRIES: INTESTINAL DEGRADATION AND ANTIOXIDATIVE EFFECTIVENESS OF ANTHOCYANINS FROM BILBERRIES

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*Vaccinium, bilberries, anthocyanins, intestine, antioxidants*

Bilberries (*Vaccinium myrtillus L.*) and its major polyphenolic constituents, the anthocyanins, are discussed to be preventive against disease associated with oxidative stress, like inflammatory processes and cancer. The gastrointestinal tract (GIT) might be a target for prevention. In this study antioxidative efficiency of bilberry anthocyanins was investigated *in vitro* in the human adenocarcinoma cell lines (Caco-2 and HT-29). Modulation of intracellular generated reactive oxygen species (ROS) levels were investigated by DCF (dichlorofluorescein) assay. Oxidative DNA damage was monitored by single cell gel electrophoresis (comet assay) with additional treatment of the DNA with formamido-pyrimidinglycosylase (FPG) to enhance sensitivity towards ROS induced DNA lesions. Therefore, in a two step protocol cells were first treated with a bilberry extract (5-500 µg/ml; 1 and 24 h) and then with the redox-cycler menadione.

Data on the microbial degradation and stability of the bilberry anthocyanins in the small intestine were generated using an anaerobic chamber to simulate GIT conditions. Bilberry anthocyanins were incubated under anaerobic conditions for 24 h using ileal content from ileostomy patients. Degradation time courses of anthocyanins were monitored by HPLC-DAD analysis.

The data achieved revealed that anthocyanins from bilberries decrease intracellular ROS levels in HT-29 and Caco-2 cells in a dose dependent manner. Slight decreases of induced DNA damages were detected in anthocyanin treated cells by comet assay.

The results evidenced that the anthocyanins reveal antioxidant efficiency under the conditions used. At a concentration of 10 µg/ml the used anthocyanin rich extract significantly reduces ROS. The anaerobic incubations using ileal content indicate that all anthocyanins under study are unstable. Therefore, techniques to enhance stability of anthocyanins in the upper parts of the GIT like encapsulations might be promising to optimize preventive efficiency of these compounds.

The study was supported by the FEI, AIF cluster; project no. 15614 N

### P2

#### THE EFFECTS OF BLUEBERRY FLAVONOIDS ON MEMORY AND COGNITION: BEHAVIOURAL AND MOLECULAR ACTIONS

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*Flavonoid, brain, cognition, blueberry, memory*

Emerging evidence suggests that dietary phytochemicals, in particular flavonoids, may exert beneficial effects on the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation and by improving cognitive function. We have recently shown that a acute intervention with 200g of blueberry improves memory and cognitive performance in both young and aged human subjects. In addition, we have shown that a 2% blueberry intervention in old animals induces a significant improvement in spatial working memory after 3 weeks. Historically, flavonoids were believed to do this via an ability to express classical antioxidant activity in the brain. However, their poor brain bioavailability and extensive metabolism means that this is unlikely. Instead, we show that their actions on the brain appear to be mediated by two separate mechanisms. Firstly, they interact with critical protein and lipid kinase signalling cascades in the brain, leading to an inhibition of neurotoxin-induced apoptosis and the promotion of synaptic plasticity. For example, their ability to activate the extracellular signal-regulated kinase (ERK1/2) and the protein kinase B (PKB/Akt) signalling pathways leads to the activation of the cAMP response element-binding protein (CREB), a transcription factor responsible for increasing the expression of a number of neurotrophins critical in memory processing. Secondly, they induce effects on the vascular system which lead to changes in cerebrovascular blood flow capable of causing angiogenesis, neurogenesis and changes in neuronal morphology. Through these mechanisms, the consumption of flavonoid-rich foods, including berries, throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent losses in cognitive performance.

### **P3**

#### **EFFECTS OF CRANBERRY FRUITS ON PROSTATE HEALTH**

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*Vaccinium macrocarpon*, prostatitis, clinical trial, PSA

Benign prostatic hyperplasia and chronic prostatitis, two of the most common medical conditions affecting older men, are associated with lower urinary tract symptoms (LUTS) which can have a negative impact on quality of life. The objective of this study was to evaluate the efficacy and tolerability of cranberry fruit powder (CFP) in men at risk of prostate diseases with LUTS, elevated prostate specific antigen (PSA), negative prostate biopsy and clinically confirmed chronic nonbacterial prostatitis. Therefore, we performed a pilot randomized clinical study in a cohort of 42 volunteers, which received either 1500 mg/day of CFP containing (% w/w) 14.85 organic acids, 15.5 sugars, 0.11 anthocyanins, 1.95 tannins, 3.49 phenols for six months or no treatment. They were evaluated using physical examination, the International Prostate Symptom Score, Quality of Life Score, basic laboratory parameters, hematology, selenium, testosterone, PSA, antioxidant status, transrectal ultrasound prostate volume, urinary flow rate, ultrasound estimated postvoid residual urine volume at baseline and at 3 and 6 months. Urine *ex vivo* anti-adherence activity was determined on the last day of study. In the CFP-treated arm (n=21) were found statistically significant improvement in IPSS, QoL, urination parameters including voiding parameters: rate of urine flow, average flow, total volume and postvoid residual urine volume, lower PSA<sub>tot</sub> level on day 180 of the study. There was no influence on blood testosterone levels. No improvement was found in the control arm (n=21). These results are the first firm

evidence that cranberries may improve the prostate health of men at risk of prostate diseases leading to a number of medical complications including prostate cancer. This study was supported by the grant (MSM 6198959216).

#### **P4**

### **PLASMA KINETICS AND URINARY EXCRETION OF THE POLYPHENOLS IN HUMANS AFTER INGESTION OF BERRIES AND RYE PORRIDGE**

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*Berries, polyphenols, bioavailability, human*

Berries are a rich source of various polyphenols. It is well established that polyphenols are metabolized prior to absorption and that the molecular forms reaching the peripheral circulation differ from those present in foods. The objective of the study was to characterize and compare the bioavailability of several polyphenols from berries and rye. In this diet-controlled, two-period cross-over study, 14 healthy nonsmoking volunteers received either berries or rye porridge. The experiment consisted of two 2-day treatment periods and a one-week wash-out period between them. During the study, the subjects followed a low-polyphenol diet. Blood samples were obtained between 0 h and 32 h post-dose. Urine samples (24h) were collected before and during each treatment period. Plasma and urine polyphenols were analyzed by GC-MS and HPLC and berry polyphenols by HPLC. According to the chemical analyses of the study products, the total intake of polyphenols were 1000 mg and 76 mg from berries and rye porridge, respectively. The main constituents were anthocyanins and soluble procyanidins in the berry products and phenolic acids in the rye porridge. The data presented describes the absorption and pharmacokinetics of polyphenols from berries and rye. Various polyphenols were absorbed in humans, but there were large inter-individual variations in the plasma concentrations of the compounds.

#### **P5**

### **THE ANTIOXIDANT RESPONSE INDUCED BY LONICERA CAERULAEA BERRY EXTRACTS ON HEALTHY ANIMALS**

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*Lonicera caerulea, berry extracts, antioxidant response, polyphenols*

*Lonicera caerulea* is a species of bushes originating from Kamchatka peninsula with berries that are extensively studied due to their antioxidant potential. Previous investigations on the *Lonicera caerulea* berries revealed the presence of ascorbic acid, carotenoids and polyphenols.

The aim of our work was to investigate the *in vivo* antioxidant action of *Lonicera caerulea* berry extracts on healthy rats that were fed on normal diet, supplemented or not with berry extracts. Tests were carried out on lots consisting of 10 animals each. The total antioxidant capacity, the lipid hydroperoxides, thiobarbituric-reactive substances, uric acid, albumin, and ceruloplasmin were assayed in plasma drawn from the animals.

Our data indicated that the *Lonicera caerulea* berry extracts induced a decrease in certain oxidative stress markers: lipid hydroperoxides, thiobarbituric-reactive substances, and ceruloplasmin.

## P6

### STRAWBERRY NUTRITIONAL QUALITY & HUMAN HEALTH IMPACT

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*Fruit nutritional quality, antioxidant capacity, phenols, flavonoids, erythrocytes.*

Formerly, we evaluated and compared the nutritional quality (NQ) of strawberry fruits of eight different commercially available varieties and advanced selections in order to assess the influence of the well-known genetic backgrounds on the strawberry NQ. All genotypes were evaluated for their standard NQ parameters, by measuring the total antioxidant capacity (TAC), the total phenolic, the total flavonoid and the total anthocyanin contents of the strawberry extracts. Also vitamin C and folate contents were quantified, and the individual antioxidant metabolites in the extracts were separated and characterized by HPLC analyses coupled to an on-line postcolumn antioxidant detection system, in order to simultaneously compare the phytochemical and antioxidant profiles among the studied genotypes. Analysis were carried out during 3 consecutive fruiting seasons (2006-08) to evaluate the seasonal and ripening influence on the genotype-to-genotype NQ. Significant genotype-to-genotype differences were outlined confirming that the genetic background may strongly affect all the NQ parameters. In particular, the F1 and F2 crosses of *F. x ananassa* x *F. virginiana glauca* resulted among the richest sources of antioxidants, phenolic compounds and vitamin C. In addition, the fruits resulting from these interspecific crosses were by far the richest source of folate, and the findings confirmed how a wild species can contribute to the introgression of interesting nutritional features in cultivated strawberries. Among the strawberry varieties studied, the cultivar Sveva showed the highest nutritional attributes, except only for the anthocyanin content. The cultivar showed a relevant and well-balanced antioxidant, micronutrient and phytochemical composition to be eligible as fruit material for pilot human studies. The latter assessed the effects of strawberry consumption on biomarkers of antioxidant status, in healthy subjects. Three pilot studies were carried out, and the screened participants were involved in an acute and two medium-term strawberry consumption tests. The potential changes in both plasma and cellular markers of antioxidant status were evaluated, by measuring the strawberry-dependent variation in plasma TAC, in the serum concentrations of hydrophilic and lipophilic antioxidants, and in circulating cells responsiveness to oxidative damage. Significant increases in plasma TAC and in vitamin C concentration in serum were observed, both after acute strawberry intake and during the medium-term consumption of the fruits. During the protracted strawberry supplementation, significant improvements of the erythrocyte and lymphocyte resistance to induced oxidative damage were also outlined. The preliminary results obtained in this work provided an interesting starting point, thus further dietary intervention studies are strongly hoped. The

current investigation regards the cumulative and possible synergic effects on healthy subjects of a protracted strawberry intake during 3 periods of 15 days each in which 24 subjects consumed 3 different strawberry cultivars according to their different ripening period.

## **P7**

### **THE INHIBITORY EFFECTS OF BERRY-DERIVED FLAVONOIDS AGAINST NEURODEGENERATIVE PROCESSES**

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*Brain, neuroinflammation, flavonoids, Parkinson disease, neurotoxins*

Abundant evidence exists to suggest that increased oxidative stress may contribute to the neuropathology of age-related brain disorders such as Alzheimer's and Parkinson diseases. The autoxidation of catecholamines has been shown to lead to the formation of reactive oxygen species (ROS), and to the generation of neurotoxic cysteinyl-dopamine conjugates. Furthermore, the activation of glial cells may also contribute to neuronal degeneration via the release of nitric oxide, ROS, peroxynitrite and cytokines, such as TNF- $\alpha$ . Recently there has been an increased interest in the potential of dietary-derived flavonoids to protect against neuronal damage associated with aging and neurodegenerative disorders. The major objective of this project was to investigate the ability of the berry-derived flavonoids to protect against neuronal damage by attenuating neuroinflammatory processes and by protecting against 5-S-cysteinyl-dopamine-induced neuronal injury. In the present study, we found that the flavanols (+)-catechin and (-)-epicatechin, but not the anthocyanidins cyanidin and pelargonidin, attenuated LPS/IFN- $\gamma$ -induced TNF- $\alpha$  production in glial cells. In neurons, peroxynitrite was observed to generate both 2-S- and 5-S-cysteinyl-dopamine and a dihydrobenzothiazine species, DHBT-1, following the reaction of dopamine with L-cysteine. The formation of 5-S-cysteinyl-dopamine and DHBT-1 in the presence of peroxynitrite induced significant neuronal injury. However, pre-treatment of primary cortical neurons with pelargonidin and the 4-O-Me derivatives of catechin and epicatechin (0.1–3.0  $\mu$ M) resulted in concentration dependant protection against 5-S-cysteinyl-dopamine-induced neurotoxicity. Taken together, these data suggest that berry-derived flavonoids may protect against neuronal injury relevant to the aetiology of the Parkinson and Alzheimer's diseases.

## **P8**

### **EVALUATING RASPBERRY ANTHOCYANIN METABOLIC DIVERSITY: BREEDING A BETTER BERRY**

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An ever increasing number of studies have attributed health-promoting effects to anthocyanins, those red-blue-purple pigments found in abundance in small berry fruits, including raspberries.

While berry breeders have traditionally focused their efforts towards agronomic traits influencing resistance to abiotic stresses and diseases, yield potentials, ease of harvestability and product shelf life, it is becoming increasingly evident that the varying bioactivity and

bioavailability of the component phytochemicals is also of critical importance for future fruit breeding programs. Increasing the health-promoting effects of berry crops not only benefits the public good, but also increases their value and marketability in a health-obsessed consumer market.

Anthocyanins show remarkable diversity in their chemical structures, with varying hydroxylation, methoxylation, glycosylation and acylation patterns. These factors have been demonstrated to critically influence the relative bioavailability and membrane transport kinetics of anthocyanins by bilitranslocase (TC 2.A.65.1.1), altering the specific molecular targets and cellular-signalling cascades (bioactivities), and determine their chemical stability kinetics within the GI system.

For fruit breeders to apply the results of anthocyanin bioactives research and develop improved functional foods, the genetic/metabolic potential available within the germplasm must first be evaluated. In the summer of 2009, a pilot study was conducted to examine the metabolite profile differences within a raspberry germplasm collection maintained at AAFC-Kentville. The anthocyanins and other related polyphenolics were isolated, fractionated and separated via novel chromatographic methods, and were then characterized via HPLC-MS and NMR, with preliminary results presented here.

## **P9**

### **DEVELOPMENT OF A DISPOSABLE ELECTROCHEMICAL SENSOR FOR THE AMPEROMETRIC DETERMINATION OF ASCORBIC ACID CONTENT IN SELECTED BERRIES**

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*Blackcurrant, strawberry, screen-printed electrodes*

Ascorbic acid (AsA) is among the principle compounds responsible for the health-promoting properties of blackcurrant and strawberry fruits, with reported concentrations ranging from 0.5 to 5.4 mg per g of fresh fruit. Due to its key role in human nutrition, a diverse range of analytical techniques to measure AsA in foodstuffs have been developed; most of these being relatively time-consuming and costly, and hence not appropriate for routine analysis. The present study describes a simple, rapid and low cost sensor based on enzyme-less amperometric determination (+300 mV versus Ag/AgCl) of AsA in blackcurrants and strawberries using bespoke disposable platinised-screen printed electrodes. A quasi-perfect linear correlation was observed when the sensor was tested with standard AsA solutions ranging from 0 to 75 mM (sensor response ( $\mu\text{As}$ ) =  $96.82 \times \text{AsA (mM)}$ ;  $r^2 = 0.999$ ). When applied to real juice samples from different cultivars, an excellent correlation was observed when comparing AsA concentrations as determined using the newly developed sensor to that measured by standard HPLC coupled to UV detection ( $r^2 = 0.90$ ). In addition, time of analysis was considerably reduced, taking no more than 200s per sample. The proposed sensor could be suitable for assisting in breeding programmes where large sample sets need to be analysed or even be used as a routine and generic quality control technique in the soft fruit industry where AsA determination should be of paramount importance. Technic

## P10

### EUROPEAN SMALL BERRIES GENETIC RESOURCES, GENBERRY: IDENTIFYING NEW SOURCES OF FRUIT NUTRITIONAL QUALITY AND NUTRACEUTICAL VALUE IN STRAWBERRY

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*Fragaria, germplasm, seedlings, fruit quality, antioxidant capacity, phenols*

GENBERRY project promotes conservation and characterization of genetic diversity of small berries, particularly strawberry and raspberry. This project is supported by the European Commission (DG-AGRI). The objective of this project was to ensure that agricultural biodiversity of small berries, strawberry and red raspberry, is preserved, characterized for using them in further breeding programs. In fact, breeding for new objectives need genetic resources possessing the new demanded agronomical traits as resistances or as high content in health compounds. This project is based on the notion of networking, considering that maximum added-value can only be reached by bringing together, otherwise rather scattered, competences on techniques of cultivation, phenotypic description, molecular biology, as well as evaluation for health value compounds and disease resistances. One important task of the project is the development of analytical protocols to study fruit nutritional quality of strawberry genetic resources. The fruit nutritional quality and nutraceutical value was studied by using the following analytical methods: Total Antioxidant Capacity (TAC) by Trolox Equivalent Antioxidant Capacity method (TEAC) (Re *et al.*, 1999); Total Phenolics (TP) by Folin Ciocalteu's reagent method (Slinkard *et al.*, 1997); Total Anthocyanin Content (ACY) (Giusti and Wrolstad, 2001); Vitamin C Content (VitC) (Tulipani *et al.*, 2008).

In a first year of evaluation (2008), these analytical methods were used to analyze 40 cultivars originated from 4 different collections:

- 21 from Italy Ancona collection: Anita, Roxana, Corona, Elsanta, Clery, Ciflorette, Evangeline, Antea, Daroryal, Candonga, Darsilette, Asia, Record, Darselect, Patty, Queen Elisa, Sveva, Dora, Alba, Adria, Irma, Onda (new varieties).
- 14 from Italy CRA-Forlì collection: Candonga, Ciflorette, Clery, Darlisette, Daroyal, Darselect, Onda, Queen Elisa (new varieties), Annalie, Regina, Tardiva di Romagna, Louis Gauthier, Avallon Classic, Rabunda (old accessions).
- 11 from France CIREF collection : Charlotte, Ciflorette, Cirafine, Clery, Darselect (new varieties), Docteur Morere, Louis Gauthier, Madame Moutot, Mara des bois, Ostara, Rabunda (old accessions).

For most of these genotypes, originated from Italy and France, this set of analyses was repeated also for a second year of production (2009). The results evidenced an large variability among genotypes and the highest value of fruit nutritional quality and nutraceuticals was detected for some old accession. This variability was also influenced by the different cultivation locations and year of cultivation.

## P11

### THE EFFECT OF GENOTYPE ON BLACK CURRANT CHEMICAL CONTENT GROWN IN LATVIA

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*Ribes nigrum*, cultivars, chemical content, polyphenols

Black currant is one of the most important berry crops in Latvia. One of the tasks of the black currant breeding program is to find varieties richest in biologically active compounds. The aim of the study was to evaluate the biochemical composition of different black currant varieties and hybrids grown in Latvia State Institute of Fruit-Growing during years 2007–2009. The studies included 59 black currant varieties and hybrids. Berries were tested for the content of soluble solids, organic acids, vitamin C, phenolic compounds and anthocyanins.

The current collection consists of varieties which are mainly of Russian and Scandinavian origin. These countries have widely utilized *R.nigrum ssp. scandinavica* in their breeding programs, which is a mildew resistance donor. However, the berries of many varieties used for breeding from this subspecies are characterized by relatively low content of ascorbic acid. There are known some forms of black currant whose berries contain over than 300 mg 100g<sup>-1</sup> of ascorbic acid. Three samples of black currant varieties and hybrids included in the study contained vitamin C slightly above 200 mg 100g<sup>-1</sup> and three forms were with total phenol content more than 500 mg 100g<sup>-1</sup>, as well as one was selected with more than 600 mg 100g<sup>-1</sup>. The highest content of polyphenols was found in the hybrids in whose breeding were used Scandinavian origin varieties, such as ‘Intercontinental’, ‘Titania’, ‘Selechenskaya’ (Sejanets Golubki x 32-77 (Brödtorp x Brodorp)), directly or indirectly. Six hybrids with the total content of anthocyanins more than 240 mg 100g<sup>-1</sup> were found in the study. Low content of organic acids and high content of soluble solids in berries is an important provision for fresh consumption. Two varieties ‘Izyumnaya’ and ‘Selechenskaya’ with the total content of organic acid less than 2% and nine black currant varieties and hybrid berries with the content of soluble solids 19-20% in average were selected.

## P12

### EVALUATION OF THE CHEMICAL COMPOSITION OF VACCINIUM SPP. FRUITS IN LATVIA

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*highbush blueberry*, cultivars, chemical compounds

The objective of the research was to assess the biochemical content of blueberry fruits grown in Latvia. Different species of *Vaccinium* (bilberry – *V.myrtillum* L., lowbush blueberry – *V.angustifolium* Ait., highbush blueberry – *V.corymbosum* L. ‘Patriot’ and half-highbush blueberry *V.corymbosum* x *V. angustifolium* ‘Northblue’) were analyzed for phenolic, anthocyanin, vitamin C, soluble solids and total acid content. The study was carried out in three fruit-growing areas of Latvia (western part: region of Jelgava, eastern part: region of Aluksne and central part: region of Riga) in treatments with or without fertilization, and fruits from 16 natural habitats were also tested. The berries of *V.angustifolium* were collected from

one area - the plantation was located on peat soil. The chemical composition analyses were carried out at the Latvia State Institute of Fruit-Growing in 2008-2009. Fertilization positively affected the total phenolic content in berries. In cultivars 'Patriot' and 'Northblue' total phenol content in average was 240 mg 100g<sup>-1</sup>, the total anthocyanin content was 79 mg 100g<sup>-1</sup> (including 2.6 mg 100g<sup>-1</sup> cyanidin 3-O-galactoside chloride and 2.5 mg 100g<sup>-1</sup> cyanidin 3-O-glucoside chloride), chlorogenic acid content was 38.6 mg 100g<sup>-1</sup>. Fruits of *V.myrtillus* contain in average: phenols 643.5 mg 100g<sup>-1</sup>, anthocyanins 371 mg 100g<sup>-1</sup> (including 50.4 mg 100g<sup>-1</sup> cyanidin 3-O-galactoside chloride and 43.4 mg 100g<sup>-1</sup> cyanidin 3-O-glucoside chloride), chlorogenic acid 18.1 mg 100g<sup>-1</sup>. In fruits of *V.angustifolia* the highest content of chlorogenic acid was found (on average 88.5 mg 100g<sup>-1</sup>).

## P13

### PORTUGUESE CROWBERRY (*COREMA ALBUM*), A INTERESTING ANTIOXIDANT WHITE BERRY

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*Portuguese crowberry., antioxidant activity, neurodegeneration*

Portuguese crowberry (*Corema album*), is an uncharacterized typical fruit of coastal habitats of Iberian Peninsula and the rocky sites of Azores Islands. The fruit is a small spherical berry white or pinkish when ripe.

Berry fruits are natural functional foods since they are a particularly rich source of antioxidants, activity mainly attributed to compounds represented by vitamin C and polyphenols such as anthocyanins, phenolic acids, flavanols, flavonols and tannins.

A hydroethanolic extraction of *C. album* fruits was performed. Total phenolic content and the *in vitro* antioxidant properties of extracts were then evaluated by the Oxygen Radical Absorbance Assay. The ratio ORAC/total polyphenols is much higher for *C. album* fruits (20,70 µmol TE/mg GAE) in comparison with *R idaeus* fruit (11,60 µmol TE/mg GAE), a well know antioxidant berry. Portuguese crowberry has small amounts of anthocyanins and its HPLC-MS profile reveals flavonol derivatives, chlorogenic acid derivatives and phenolic acids while raspberry is rich in tannins, anthocyanins and also flavonol derivatives.

Since a wide array of plant phenolic substances have been reported to have substantial neuroprotective activity we decided to test the intracellular antioxidant activity for both fruits extracts in a neuroblastoma cell line (SK-N-MC).

For a previous defined nontoxic range of concentrations, the intracellular radical scavenging activity of the fruits extracts in an oxidative stress-induced model of neurodegeneration in SK-N-MC cells was evaluated using Cell titer blue. The pre-treatments with the extracts of *C. album* fruits protect the cells from the oxidative stress injury as detected by an increase in cell viability up to 75% with 175 µg GAE.mL<sup>-1</sup> a much higher effect when compared to the maximum reached for *R idaeus* fruit, 36% for 50 µgGAE.mL<sup>-1</sup>.

These results are very interesting since clearly reveal the importance other polyphenols than anthocyanins, ellagitannins and protoanthocyanidins, normally the more representative in

berries. *Corema album* white berry presents higher antioxidant activities both in vitro and intracellular in a neurodegeneration cell model. These uncharacterized fruit revealed to be a promising source of a new combination of natural antioxidants. Further studies will confirm their possible future use as neuroprotective compounds

#### **P14**

### **VARIABILITY OF THE ANTHOCYANINS AND TANNIDS BERRIES CONTENT IN SOME *LONICERA CAERULEA*, *ARONIA MELANOCARPA* AND *BERBERIS VULGARIS* GENOTYPES**

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*Honeysuckle, chokeberry, barberry, variety, selection*

The blue honeysuckle, chokeberry and barberry are fruit species of cultural and ornamental interest for their richness in biochemical berries composition, yield and plant habit. Of these, the honeysuckle is outstanding for earliness in ripening (before or together with strawberries) and high antioxidant activity (Oprea et al., 2002). *Aronia melanocarpa* is a shrubby type important as natural colouring matter for the food industry. *Berberis vulgaris* var. *atropurpurea* has the richest fruits in organic acids, more than 5,0 % (Mladin Gh. et al., 1996). Anthocyanins and tannoids are phenolic compounds playing a major role in the berries color, taste and antioxidant activity. In this study, during 3 years, anthocyanins and tannoids composition of 25 varieties and selections honeysuckle, two selections of barberry and one variety of chokeberry was investigated. The berries anthocyanins, tannoids and vitamins content is one of the most important criterion for the selection of the genotypes. The total anthocyanins in honeysuckle genotypes ranged in large limits, from 101,83 mg% to 611,00 mg%. There were differences between the genotypes and the climatic years. The richest in these compounds were the following selections: SL-6 (611,0 mg%), SL-17 (553,97%), SL-18 (513,24 mg%), SL-20 (512,30 mg%), SL-15 (500,52 mg%), SL-22 (456,21 mg%). Nero chokeberry variety was also rich in anthocyanins (509,16 mg%) and barberry selections B-V-5 and B-V-6/95 accumulated between 59,06 and 79,43 mg%. The blue honeysuckle genotypes contained relatively low and medium tannoids, from 0,178 % to 376,78 %. The barberry selections and chokeberry Nero contained high tannoids (0,640 - 0,673% and 0,762 %, respectively). Correlations between the biochemical content, genotypes and climatic years will be presented.

#### **P15**

### **ANTHOCYANIN AND POLYPHENOL CONTENT OF WILD GROWN EUROPEAN ELDERBERRY (*Sambucus nigra* L.) GENOTYPES**

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*European elderberry, Sambucus nigra, polyphenols*

Black elder or European elderberry (*Sambucus nigra* L.), is one of the most widespread species among *Sambucus*, that grows particularly sunlight-exposed locations in most parts of Europe, Asia, North Africa and the USA. The fruits has been using mostly medicinal purposes so a long time in Europe including Turkey. Elderberry plants mostly grow wild in Europe and are cultivated on a small scale in some northern European countries. The industrial processing of elderberries mainly takes place in Northern Europe where the berries are mostly processed into juice and juice concentrate. They have gained interest as potent agents against oxidative stress and reducing oxidative damage to the human body. The elderberry fruits are very dark purple and the mild-flavored fruits ripen in mild to late summer. For the commercial harvest, the entire cluster is picked and the entire crop is processed. More recently, elderberries have received increased attention due to their high contents of anthocyanins, that are widely used as colour ingredients in various beverages, and which may also provide nutritional benefits. In this study we determined anthocyanin, total phenolic and antioxidant activity (FRAP assay) of three wild grown European elderberry. There were differences among genotypes in terms of total anthocyanins (556-859 mg/100 g FW). The genotypes with the highest total antioxidant capacity and total phenolic content were BSEA3 (7.01 mmol/100 g fw and 463 mg GAE/100 g fw). The average major cyanidin based anthocyanins determined in the wild grown black elderberry genotypes were cyanidin 3-sambubioside and followed by cyaniding 3-glucoside, cyaniding 3-sambubioside-5-glucoside, cyaniding 3,5-diglucoside and cyanidin 3-rutinoside. The most abundant anthocyanin in elderberry fruit was cyanidin 3-sambubioside.

## P16

### COMPARISON OF PHYTONUTRIENT PROPERTIES OF WILD AND CULTIVATED BLACKBERRIES (*RUBUS FRUTICOSUS* L.)

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*Anthocyanins, FRAP, TEAC, total phenolic, wild*

Antioxidant compounds absorbed from our diet are thought to have an important role in preventing degenerative chronic diseases that result from oxidative damage. Compared with most fruit, berries, especially blackberries, are high in antioxidant properties. Blackberry is consumed by both wild and cultivated forms in Turkey. We investigated antioxidant capacity and some of the chemical properties of 58 wild blackberry (*Rubus fruticosus* L.) accessions sampled from Northern Turkey between the altitudes 8-1796 m. In addition, seven commercial blackberry cultivars with foreign (“Jumbo”, “Chester”, “Navaho”, “Waldo”) and local origins (“Bartın”, “Bursa1” and “Bursa2”) were included in the study to compare the variation of antioxidant capacity and chemical properties between wild and cultivated blackberries. Fruit total phenolic (TP), total monomeric anthocyanins (TMA), soluble solids (TSS), acidity and some of the fruit physical characteristics were examined. Antioxidant capacity of fruits were determined by both FRAP (ferric reducing antioxidant power) and TEAC (trolox equivalent antioxidant capacity) assays. We recovered significant differences from all of the characteristics tested for the accessions and the groups. Principle component (PC) analysis clearly separated wild blackberries from the cultivated ones. The first three PC explained the 53, 20 and 10% of the variation making a total of 83%. Among the variables tested, the phytonutrient-related parameters (TP, TMA, FRAP and TEAC) were highly correlated with PC1; soluble solids, acidity and their ratios were highly correlated with PC2.

The antioxidant capacity among wild blackberries averaged 23.6 and 30  $\mu\text{mol TE/gfw}$  compare to cultivated blackberries displayed 9.2 and 14.1  $\mu\text{mol TE/gfw}$  by the FRAP and TEAC methods, respectively. TP mean of wild blackberries was 6475 mg GAE/gfw compare to 4046 mg GAE/gfw in cultivated ones. Also, wild berries displayed 34% more anthocyanin content when compared to cultivated ones. Results from our study indicated that wild blackberries may have higher phytonutrient content than the cultivated blackberries.

## **P17**

### **BIOACTIVE COMPOUNDS AND NUTRITIONAL QUALITY OF BERRIES IN BLACKCURRANT CULTIVARS EVALUATED IN ESTONIA**

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*Blackcurrant, seedlings, fruit quality, poly phenol composition, leaves*

Blackcurrants are highly valued as healthy berries but can also be used as raw material for production of bioactive compounds. Breeding of blackcurrants and evaluating of cultivars at Polli Horticultural Research Centre has been focused on selecting cultivars possessing high productivity and suitable for the use in local climatic conditions including screening for winter hardiness and resistance to the most dangerous pests and diseases. Besides important agronomic characteristics more attention has been recently paid on fruit quality aspects and content of various bioactive compounds.

Results on biochemical analysis of 4 prospective selections (10B, 2-96-51, 1-96-16, 4-96-1) and 4 new cultivars ('Karri', 'Almo', 'Ats', 'Elo') from our own breeding program and 8 introduced cultivars ('Öjebyn', 'Zagadka', 'Ben Sarek', 'Intercontinental', 'Pamyati Vavilova', 'Titania' and 'Pilenai') are presented.

In addition to analysis of main biochemical characteristics (sugar content, sugar-acid ratio, dry matter content, ascorbic acid content, titratable acids) polyphenol content of berries was determined using high performance liquid chromatography (HPLC). Anthocyanidins in blackcurrants are mainly present as cyanidin and delphinidine glycosides. Total polyphenol content of the berries varied in a wide range. The highest anthocyanin content was found in cultivar 'Almo' (1046,1  $\mu\text{g/kg}$ ) and the lowest in 'Ben Sarek' (212,7  $\mu\text{g/kg}$ ). The analysis also revealed differences in anthocyanin content of berries of different stage of ripening which might influence the results. Ascorbic acid content varied from 80 mg/100g with 'Ben Sarek' to 209mg/100g with elite selection 4-96-1.

Polyphenol composition of blackcurrant leaves was also determined by HPLC, the compounds were identified using polyphenol standards and/or compound's molecular weight in mass spectrometry.

More detailed biochemical investigations of the selections are in progress. Further investigations will include fatty acid content of blackcurrant seeds.

## **P18**

### **ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES OF STRAWBERRY TREE TISSUES**

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*Arbutus unedo*, polyphenols, antioxidant activity, MMP9, HPLC-MS

Strawberry tree (*Arbutus unedo*) is a Mediterranean endemic plant that belongs to *Ericaceae* family. Its fruits are edible and its tissues are used in folk medicine for diverse purposes. Some studies have shown the fruits are rich in flavonoids responsible for their antioxidant properties and it was also isolated some compounds from the entire plant that seems to be promising in cancer chemopreventive therapy.

The aim of this work is to study the antioxidant and metalloproteinases inhibitory activities of polyphenol enriched fractions of fruits and leaves from this species. Using this approach instead of a pure compound or a crude extract we expect to maximize these activities through possible positive synergisms between the different polyphenols.

Fruits and leaves were harvested in Arrábida Mountain (Portugal) and were extracted using a hydroethanolic solution. The extracts were fractionated using a SPE column C18-E. The obtained fractions were tested for total phenol content, antioxidant activity by ORAC and MMP9 inhibitory activity. Also the phenolic profiles of both tissues were determined by HPLC-MS.

Concerning the fruits, after fractioning by SPE the polyphenols yield was less than 20%. However, the obtained fraction presented 200% of the antioxidant activity compared with the crude extract. This result seems to indicate that the interfering substances excluded by the SPE were acting as antagonist, resulting as a negative synergism. For leaves extract, it was recovered from the SPE more than 50% of the phenols added to the column, being the antioxidant activity maintained after fractioning. Antioxidant activity of fruits and leaves enriched fractions is higher when compared with other species already described as highly antioxidants as blackberry and green tea leaves. Regarding the results obtained for MMP9 inhibitory activity, the IC<sub>50</sub> for fruits and leaves enriched fractions were 1,681 and 1,308 mg GAE. mL<sup>-1</sup> respectively. These values are quite similar to the ones determined for blackberry and green tea leaves, already described as MMP9 activity inhibitors. In the phenolic profile of fruits, it was detected gallic acid and quercetin derivatives as well as some proanthocyanidins. Leaves phenolic profile revealed also gallic acid derivatives as well as flavonol derivatives and some tannins.

The fractions obtained from both strawberry tree tissues seem to be quite promising as antioxidants and antiproliferatives. Additional cell based assays are under going to study these possible outcomes.

## P19

### ANTHOCYANIN CONTENTS OF COMMERCIAL BLACKCURRANT JUICES PURCHASED FROM VARIOUS EUROPEAN COUNTRIES

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*Ribes nigrum*, juice, polyphenols

Blackcurrants are economically significant berries in many European countries, and they are primarily grown for juice. In addition to vitamin C, polyphenol content is a very important quality factor of black currant. Freshly pressed blackcurrant juice is very rich in anthocyanins but the processing to commercial juice can affect their stability. The aim of this study was to evaluate the variation in the anthocyanin content among commercial juices purchased from Finland, Poland, Germany and the United Kingdom. Popular blackcurrant juice (or juice concentrate) trademarks were selected in each country. Three subsamples were purchased for each trademark and these were purchased from three different retail stores in each country. After purchasing, the juices were delivered to the laboratory and stored in the freezer until analyzed for anthocyanins. Anthocyanins (cyanidin-3-rutinoside, delphinidin-3-rutinoside, cyanidin-3-glucoside, and delphinidin-3-glucoside) were analyzed by HPLC using external standard method. The results were calculated per recommended serving (250 ml) using the instructions of the producers. The data were tested statistically by ANOVA (general linear model). There was 14-fold variation in the contents of anthocyanins in the 12 analyzed European commercial blackcurrant juice trademarks (from 4.3 to 58 mg/2.5 dl), and the mean contents of anthocyanins varied widely between Finnish, British, Polish and German juices (11.9, 7.45, 32.0 and 38.6 mg/2.5 dl, respectively,  $p < 0.0001$ ). In addition, there was statistically significant within-country variation ( $p = 0.0202$ ). The variation within trademarks was also significant in many cases. Generally, the juice samples with shorter sell-by dates contained much lower anthocyanin levels than fresher ones. Hence, the within-trademark variation was probably more directly influenced by different storage stages of the juices rather than different batches

## P20

### STABILITY OF ANTHOCYANINS IN BLACK CURRANT JUICE DURING STORAGE

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*Ribes nigrum*, anthocyanins, HPLC

Anthocyanins are responsible for the red, blue and purple colour of many fruits and berries. Besides their colour attributes, the interest in anthocyanins has increased because of their possible health promoting properties. Anthocyanins are not very stable and their contents can change significantly during processing and storage. Black currant juice is a common anthocyanin rich product on European market. The shelf-life of commercial black currant juice is normally from several months up to a year. The aim of this study was to evaluate changes in black currant juice anthocyanins during storage at fridge and at room temperature. 'Ready to drink' black currant juices were stored at 5 °C and at room temperature (approx. 25 °C) for one month (31 days). The major anthocyanins (delphinidin-rutinoside, cyanidin-rutinoside, delphinidin-glucoside and cyanidin-glucoside) were analyzed by HPLC at selected time points.

The contents of all analyzed anthocyanins decreased during storage at both temperatures. Delphinidin-derived anthocyanins tended to degrade slightly faster than those based on cyanidin. Furthermore, anthocyanidin disaccharides proved to be more stable than their corresponding monosaccharides. The decrease in anthocyanin contents was remarkably higher at room temperature than in the fridge. After one month storage in the fridge the juice had still approx. 80 % of the initial anthocyanins while only 40 % was left in the juice stored at room temperature. A first-order reaction equation was applied to evaluate the reaction rate constants

for the degradation of anthocyanins. In the juices stored at room temperature the half-lives of anthocyanins varied from 18 to 28 days for delphinidin-3-glucoside and cyanidin-3-rutinoside, respectively. The parallel half-life values were 102 days (delphinidin-3-glucoside) and 126 days (cyanidin-3-rutinoside) in the juices stored at fridge. The results showed that a storage temperature has a great impact on the stability of anthocyanins in black currant juice.

## **P21**

### **MAXIMISING BLACKCURRANT ANTHOCYANINS: TEMPORAL CHANGES DURING RIPENING AND STORAGE IN DIFFERENT GENOTYPES**

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*Maturity, Ribes nigrum L., storage temperature*

Increasing evidence suggests that anthocyanins, as natural antioxidants, exert anti-carcinogenic, anti-inflammatory, vaso-protective and anti-obesity effects when tested *in vitro* or *in vivo*. Blackcurrant (*Ribes nigrum* L.) berries are a rich source of anthocyanins with concentrations up to 4-fold greater than that of other common berry fruits. However, there seems to be paucity of information on how blackcurrant anthocyanins change during ripening on the bush or during postharvest storage, such that an improved understanding could lead to better selection of fruits with enhanced health-promoting properties. Accordingly, the present study describes the temporal changes in anthocyanins in different blackcurrant genotypes during the latter stages of development or as a result of postharvest storage at different temperatures. In all cases, four major anthocyanins (*viz.* cyanidin-3-glucoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and delphinidin-3-rutinoside) were identified accounting for over 80% of the total anthocyanin concentration. Three different development stages (*viz.* early ripe (ER), fully ripe (FR) or over ripe (OR)) were chosen when fruit was at commercial maturity. Anthocyanin concentration was nearly double in OR berries than ER; however the temporal changes during ripening were genotype dependent, with some cultivars reaching maximum concentrations by the FR stage. Similarly to the changes observed during ripening on the bush, postharvest storage led to enhanced synthesis of anthocyanins; this said, ER berries never reached the total anthocyanin concentrations of those harvested at FR stage. Temperature during storage crucially affected anthocyanin concentrations in FR, but not in ER berries. The results from this study may assist in selecting blackcurrant berries at optimum maturity as well as provide optimum storage conditions that could maximise anthocyanin concentrations in blackcurrant berries.

## **P22**

### **CULTIVAR AND STORAGE EFFECTS ON STRAWBERRY BIOACTIVE COMPONENTS.**

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*Fragaria, antioxidant capacity, phenols, anthocyanins.*

The strawberry is an important dietary source of bioactive compounds, including phytochemicals. Strawberry is a potentially relevant dietary source of bioactive phytochemicals (phenolic compounds, ascorbic acid, vitamins), with putative beneficial effects on human health. The concentration of these nutritional components, like the majority of qualitative traits, is strongly influenced by genotype (some varieties are richer in antioxidants than others), environmental factors and growing systems.

The aim of this work was to compare quality traits and nutraceutical compounds in fresh and stored strawberry fruits. The one-year study (2008) was carried out using five Italian strawberry genotypes: Onda, Unica, Zeta, and selections 148.5 and 136.7, grown in the Cesena area (Emilia-Romagna region, Italy), using cold-stored plants, planted on fumigated soil. Ripe fruits from the third picking were used for all analyses. Fresh quality traits analysis (TSS, titratable acidity, firmness, color) were carried out on a sub-sample, immediately after harvest; another sub-sample of ripe fruits was freeze-dried, and used for analysis of health-promoting components (total polyphenols, antioxidant capacity, total anthocyanins and vitamin C). The same analyses were repeated on samples kept for 3 days in cold storage (+4°C), plus 1 day at room temperature.

The results showed a lower antioxidant capacity of the stored fruits, with respect to harvest, probably connected due to a decrease of total anthocyanins, although further understanding of the involved components and mechanisms is needed.

## **P23**

### **APPLICATION OF SILICEOUS LIGNIN AT BERRIES CULTIVATION**

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*Lignin, silicon, plant development, activation, seedlings transplantation*

Siliceous lignin (Si-lignin) was synthesized on the basis of technical lignin (multi-tonnage solid residue from lignocellulosic bioethanol production) as bio-mimetic complex “Si-containing clay minerals – soil organic matter”. The new product was tested as cultivation agent on various crops (vegetables, tuberos, cereals, legumes) in glass house and field conditions. Si-lignin revealed positive influence on plants growth and development, increased harvest, improved its quality (increasing carotenoids, ascorbic acid, proteins and sugars content etc.) and provided plants resistance towards biotic and abiotic stresses. The aim of the present work was assessment of Si-lignin efficiency at cultivation of different berries.

The positive influence of Si-lignin application was observed at growing of strawberry and coffee from seeds, grape and currants propagation by cuttings, and transplantation of strawberry and raspberry meristem seedlings from *in vitro* to *in vivo*.

On the background of Si-lignin, the activation of plant root system development began on the early vegetation stages for all crops studied. In 4.5 months after strawberry meristem seedlings transplantation into a substrate containing 0.5 g Si-lignin per 1 litre weights of the plant aboveground and underground parts exceeded those for control by twofold and threefold, respectively. The leaf-rosette of the experimental strawberry plants was better

developed and content of chlorophylls in leaves was higher by 15% in comparison with control.

For the strawberry plants grown from the seeds in the outdoor conditions on the background of 5 g Si-lignin per 1 m<sup>2</sup> the initial blossom and fruiting was observed by 3 weeks earlier than in control. The experimental plants were more resistant against temperature variations and diseases.

The application of Si-lignin at the rooting of grape meristem seedlings led to acceleration of the process by 2-3 weeks. The plants grown from seedlings treated with Si-lignin were cytogenetically stable.

At the growing of coffee plants on the background of Si-lignin (4 g/l substrate), the significant difference in plants development in comparison with control was observed in 4 months after seeds sowing. For the experimental plants, the height was higher than that for control by 30%, leaf numbers exceeded this index for control by 15%, the area of leaf plates was significantly bigger and the root system was better developed.

The positive effects of Si-lignin observed was explained by its elicitor properties, auxine-like activity as well as activation of agronomically useful soil microbiota and enhancing mobility of soil nutrition elements.