SUSTAINABLE EXPLOITATION OF BIOACTIVE COMPONENTS FROM THE BLACK SEA AREA TRADITIONAL FOODS

BaSeFood

NEWSLETTER

Issue 4, March 2011

Individual Highlights

The period from October 2010 to March 2011 did not see any plenary BaSeFood meetings. However, project management had an intense work for the preparation of mid term reporting and review, that took place in Brussels at beginning of January.
With respect to previous issues, in which substantial parts were devoted to project's life, the contents of this Newsletter mainly report R&D and dissemination activities.

Organisation and training

Armenian NGO Eco-Globe contributes to BaSeFood research

L. Filippo D'Antuono (UNIBO) and Nune Darbinyan (Eco-Globe)

Geographically, Armenia is not properly a Black Sea area country, but is an integral part of the Black Sea Area Economic organisation, and represents a fundamental part of the European / Caucasian continuum, that is presently at the centre of several international issues.
In two previous issues of BaSeFood Newsletters (issue 2, April 2010, and issue 3, September 2010) the relevance of Armenia in the food picture of the area has been pointed out.
The Armenian environmental NGO Eco-Globe and BaSeFood coordinating institution, University of Bologna, signed a sub-contract in September 2010. Eco-Globe is a non-governmental organisation based in Erevan, the capital of Armenia. This NGO has been established in 2001 and has a mission to promote sustainable development via environmental protection in conjunction with the economic growth.
The NGO is active nationally, regionally and internationally. One of the main objectives is to transfer know-how to the national and local levels. The organization implements many projects, capacity building initiatives, provides consultancy, contributes to the development of legislation and alliances with the partners in many countries worldwide.
The collaboration of Eco-Globe with BaSeFood was initially focused on drawing an overall picture of Armenian plant-based traditional foods, starting from a raw material approach, and to contribute at specific monographs on plant based foods of the area.
In this perspective, documentation activities are in course about traditional wheat crops of Armenia, and will be extended at least to herbs and fruit crops and products. In these activities Eco-Globe will collaborate with local experts and stakeholders.
Eco-Globe however also gave a contribution to the experimental part, connected to the characterisation of primitive wheat populations. Four *Triticum dicoccum* seed samples were supplied, and included in an experiment carried out in Italy. A sampling was carried out in a local processing plant in Central Armenia; samples were sent to Italy for analyses and the data are being put together with similar data registered in Italy and Turkey, aimed at the determination of yield and retention factors of some bioactive components in traditional hulled wheat processing.

Eco-Globe president, Mrs. Nune Darbinyan, visiting emmer wheat fields, Fantan, Armenia, August 2009.

Additional Food Index Training Course for BaseFood partners from Romania

Marija Djekic Ivankovic, Marija Ranic and Mirjana Gurinovic (IMR)

In the frame of Work package 1 the additional Food Index Training Course for BaseFood was organized for partners from Romania who could not attend the 2009 training session, due to the flu epidemic. The additional training was organized on November 18-19, 2010 in Belgrade at the Institute for Medical Research (IMR) and was hosted by Dr Maria Glibetic and her team. Participants from Romania were Professor Carmen Costea and PhD student Adrian Vasile. During the training session selected traditional foods from Romania (i.e. polenta, nettle soup, plumbs jam, herbal dish, socata, and cabbage leaf rolls with dehydrated plums and apples) were indexed in Langual. Participants were very satisfied with the content of the training and with the applicability of the acquired knowledge.
Moreover, the participants faced some challenging questions that were later added by the team to the Lanced dictionary of inquiries (a sort of FAQ), which may be useful for future training.

Dr Maria Glibetic and Dr Mirjana Gurinovic with Dr Carmen Costea at IMR.

Adrian Vasile from Romania with Jasmina Debeljak Martacic and Marija Djekic Ivankovic during the BaseFood Food Indexing Training in Belgrade 2010.

Analytical determinations on the selected traditional foods

Nutritional composition of BaSeFood samples at National Health Institute Dr. Ricardo Jorge (INSA), Lisbon, Portugal and Institute of Food Research (IFR), Norwich, UK

Ana Sanches Silva, Tânia Gonçalves Albuquerque, Helena Soares Costa (INSA) and Paul Finglas (IFR)

The National Health Institute Dr. Ricardo Jorge (INSA) is responsible for carrying out the nutritional composition of the 33 selected traditional foods within the frame of BaSeFood Project. INSA has received 26 (out of 33) traditional foods from Black Sea Area countries (BSAC). The Institute of Food Research (IFR) has already performed the folate analyses in the samples. Nutrients are the constituents in food that should be supplied to the body in suitable amounts. Among other nutrients, water, protein, fat, minerals, and dietary fibre are being determined in BaSeFood samples, since July 2010. Most of the methods used for nutritional composition are accredited ISO/IEC 17025 and for others the laboratory has successfully participated in Proficiency testing.

Moisture is being determined by gravimetric method, using a dry air oven at 101 °C ± 2 °C for 2 h, until constant weight. Total nitrogen for protein content is being determined by the Kjeldahl method in combination with a copper catalyst using a block digestion system. The protein content was calculated using an appropriate conversion factor.

Samples in a dry air oven for moisture content determination.

Traditional food samples from BSAC.

Acid hydrolysis for total fat determination.

A - Preparation of FAME

B – Gas chromatograph used to carried out FAME analyses

Total fat is being performed with an acid hydrolysis method followed by extraction using a Soxhlet with petroleum ether, as the extraction solvent. The obtained residue is dried for 1 h 30 min at 101 °C ± 2 °C, until constant weight, according to the acid hydrolysis method.

The determination of fatty acids profile is being carried out after the conversion into their correspondent fatty acids methyl esters (FAME). Preparation of FAME was performed by a combined method of methylation and transesterification (A). Analysis of FAME was performed on gas chromatograph (Agilent 6890 N) coupled with a mass selective (Agilent MS-5975 inert XL) detector (B). Identification of chromatographic peaks was achieved by comparing their retention times with those of a commercial mix standard. Confirmation of the chromatographic peaks was performed by comparison of samples spectra with those of standards from NIST and Wiley spectral libraries.

Total dietary fibre is being determined using an enzymatic–gravimetric method. For mineral and trace elements analyses: Calcium, Magnesium, and Zinc are being determined by flame atomic absorption spectroscopy; potassium by flame photometry; phosphorous by spectrophotometry and for selenium determination, graphite furnace-atomic absorption spectroscopy was used.

Carotenoids are also being analysed in the selected traditional foods due to their importance as bioactive compounds. They play an important role in health by helping to minimize the impact of oxidative damage caused by free radicals. Carotenoids are natural fat-soluble pigments with antioxidant activity. They are responsible for the bright red, orange and yellow colours of many vegetables, and can be a source for vitamin A activity. There are more than 600 known natural carotenoids and they may be classified as carotenes or xantophylls, which are oxygenated derivatives of carotenes.

Preliminary assays to determine carotenoids in BaSeFood traditional foods were carried out in order to evaluate the best extraction conditions (extraction solvent; volume of solvent; extraction time) as well as chromatographic conditions (four pictures, from left). An Ultra Performance Liquid Chromatography (UPLC®) equipment was used to develop a method which allows the determination of several carotenoids and two vitamins (vitamin A and E) simultaneously (right side picture).

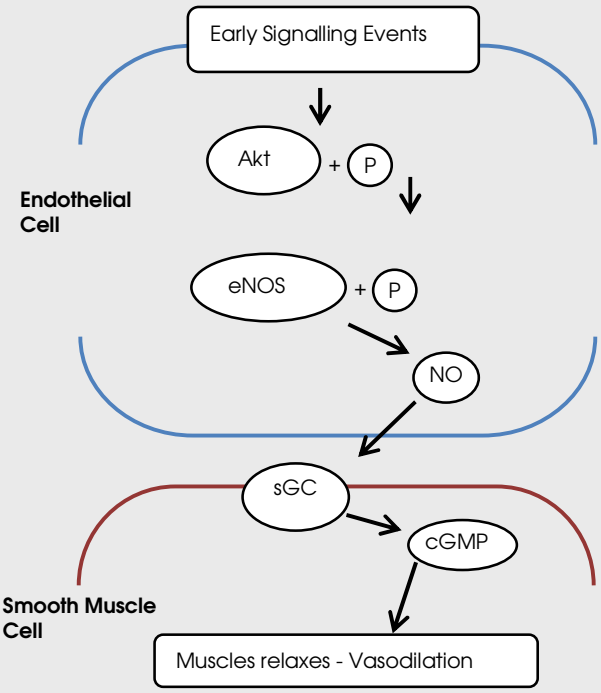
Some steps of the carotenoids analytical method, and, right-hand picture, an Ultra Performance Liquid Chromatograph.

Determining bioactivity of the Black sea area plants and foods

Black Sea area plant food bioactives and endothelial function

Mark Woodcock and Paul Kroon (IFR)

What is endothelial function?
One of the objectives of Work-package 3 is to assess the ability of Black Sea area food plant extracts, and of individual bioactives within these plants, to modulate vascular function. The research at IFR is focussed on establishing the potential for plant extracts and bioactives to affect endothelial function. Endothelial function is a good measure of the 'healthiness' of the arteries. Physiological measurement of endothelial function is normally done by measuring flow-mediated dilatation of the brachial artery, in which ultrasound is used to image the lower forearm brachial artery before and after the release of an inflated ischemia-causing cuff applied to the upper forearm. Arteries respond to the blood flow by dilating, and whereas healthy arteries respond by dilating quite a lot, older, less healthy arteries dilate less well and we would consider the arteries as stiffer (less flexible). Poor endothelial function is predictive of cardiovascular disease risk (Libby et al., 2002).
Endothelial function is the result of a balance between vasodilating factors such as nitric oxide which induce arterial smooth muscle relaxation, and vasoconstrictor factors such as endothelin-1 which induce smooth muscle contraction. These factors are produced by the endothelial cells that form the single cell thick layer of cells lining the inside of arteries and veins. Nitric oxide functions as a potent activator of the enzyme guanyl cyclase; this enzyme makes cyclic GMP which is the endogenous signalling molecule that causes smooth muscle to relax.



P)hosphorylation; NO – nitric oxide; GC – soluble guanyl cyclase.

Signalling events involved with the release of nitric oxide

How will effects on endothelial cell function be assessed?
At IFR, we are measuring vascular endothelial responses in cultured cells. The cells primary human umbilical vein endothelial cells and they are widely used to study the effects of drugs, bioactives, and flow / sheer stress on the endothelium. Nitric oxide bioavailability is a consequence of the balance between the production of nitric oxide by enzymes such as endothelial nitric oxide synthase (eNOS) and processes which increase the rate of breakdown of nitric oxide including the activity of the enzyme NADPH oxidase. The IFR researchers plan to measure the following:

- eNOS protein and mRNA
- Phosphorylated eNOS (phosphory-
- lation activates eNOS→ peNOS)
- Phosphorylation of Akt (=activated
- Akt) induces activation of eNOS
- Rate of cyclic GMP production
- Endothelin-1 (ET1) protein and mRNA
- NADPH oxidase activity.

Sideritis scardica

What BSAC plants will be assessed?
In Work-package 1, a large quantity of data concerning a large number (>300) traditional foods of the Black Sea area countries was compiled into a report. This information included the recipes and cooking methods, but also some information on the composition in terms of bioactive compounds such as flavonoids, phenolics, glucosinolates, phytosterols, carotenoids, etc.... Based on a number of criteria, a priority list of 30 traditional dishes was selected for further study within the project. The IFR researchers used this information and identified plants that were likely to be of particular interest, for example because they are widely consumed, or particularly rich sources of bioactives, or sources of bioactives that were likely to be beneficial for endothelial function. Plants that were not widely consumed in other parts of Europe were given higher priority, as were polyphenol-rich plant because that is the expertise of the IFR Kroon group. The main ingredients were determined using information obtained from partners and systematic internet searches. The bioactive contents of plant ingredients were established based on searches through the literature and the polyphenol database website http://www.phenol-explorer.eu. Eventually, six plant ingredients – pomegranate, kale, dill, nettle, Sideritis and persimmon – were selected for further study at IFR.

Pomegranate: not as widely consumed in other parts of Europe; very high content of polyphenols including ellagitannins and ellagic acid; emerging data of health benefits; significant commercial interest.
Kale: widely consumed In Northern Turkey; high content of polyphenols and glucosinolates. Glucosinolates are highly bioactive anti-cancer agents, and there is increasing interest in their potential cardiovascular protective properties.
Dill: used in a wide variety of BSAC foods. High consumption of both fresh and dried dill in certain countries (e.g. Georgia) is rather unique. Very high content of bioactive polyphenols (especially quercetin).
Nettle: consumption of Urtica urens L. (the 'small nettle' or 'annual nettle') is common in the BSAC but not elsewhere. Nettle is a component of several of the BaSeFood prioritised foods; high content of polyphenols especially quercetin glycosides.
Sideritis: *Sideritis scardica* is used to make a hot water infusion which is called 'mountain tea' and is also added to dishes as an herb. Its consumption is typical of BSAC but not elsewhere; high content of polyphenols; wide variety of bioactive compounds including phenylethanoids such as verbascoside, and flavones such as isoscutellarein.

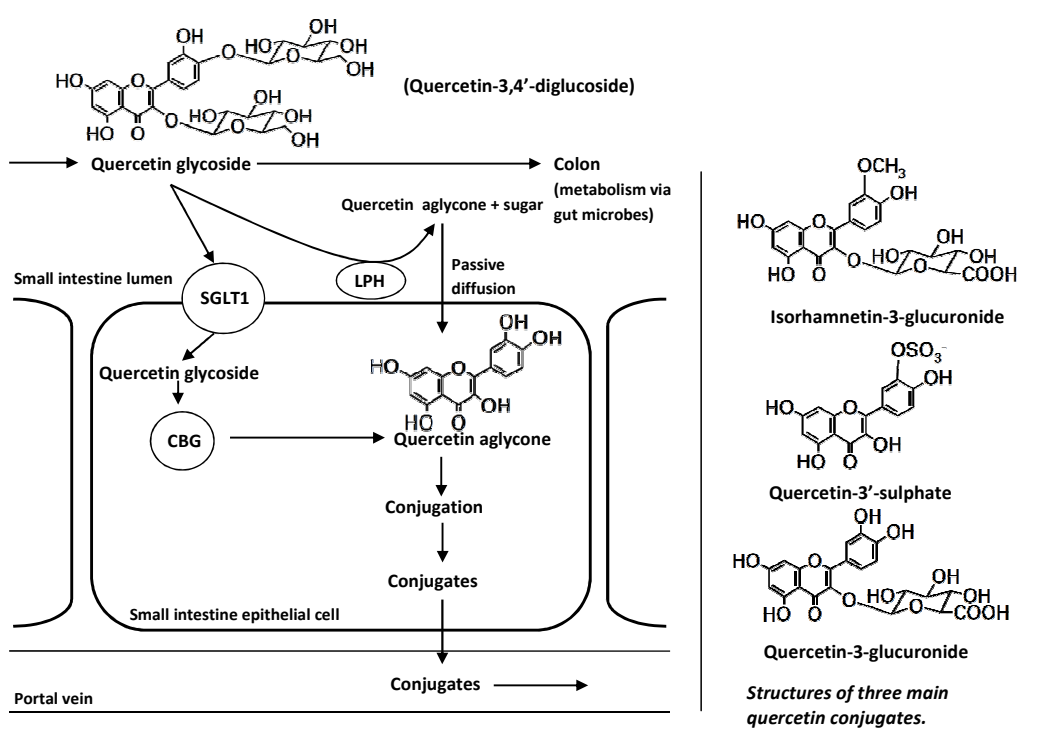

Pomegranate

Persimmon: also called a Sharon fruit. High content of polyphenols, especially flavanols including tannins; Contains a wide variety of bioactives including hydroxycinnamic acids and procyanidins. Not as widely consumed in other parts of Europe.

Currently, the content and composition of polyphenols and glucosinolates in methanolic extracts of these plants are being determined using chemical analytical methods.

Do the major bioactives in these plants cause similar responses to the crude extracts?
The main bioactives in the plant extracts are expected to be similar to those that have been described in the literature. A small selection of these will be chosen for use in the nitric oxide bioavailability assays, as an assessment of the bioactivity of pure compounds will go some way as to help explain the effects produced by the extracts. The literature review to determine the bioactive content of the plants to be extracted revealed that kale, nettle and dill, contained high concentrations of the bioactive flavonol quercetin. In these plants, quercetin is present in various glycosidic forms. Kale is a rich source of glucosinolates (e.g. glucobrassicin, sinigrin). Therefore, we will measure the bioactivity of quercetin aglycone, a quercetin glycoside, and a glucosinolate such as glucobrassicin in the assays listed above.

Do the human metabolites of these bioactives have pro-endothelial function activities?
When quercetin and its glycosides are consumed, they undergo extensive metabolism before reaching the blood stream. A human intervention study where volunteers were fed onions showed that 3 of the main metabolites produced were quercetin-3'-sulphate (Q3'S), quercetin-3-glucuronide (Q3GlcA) and isorhamnetin-3-glucuronide (IR3GlcA) in a ratio of 6:3:1 (Mullen et al., 2006).

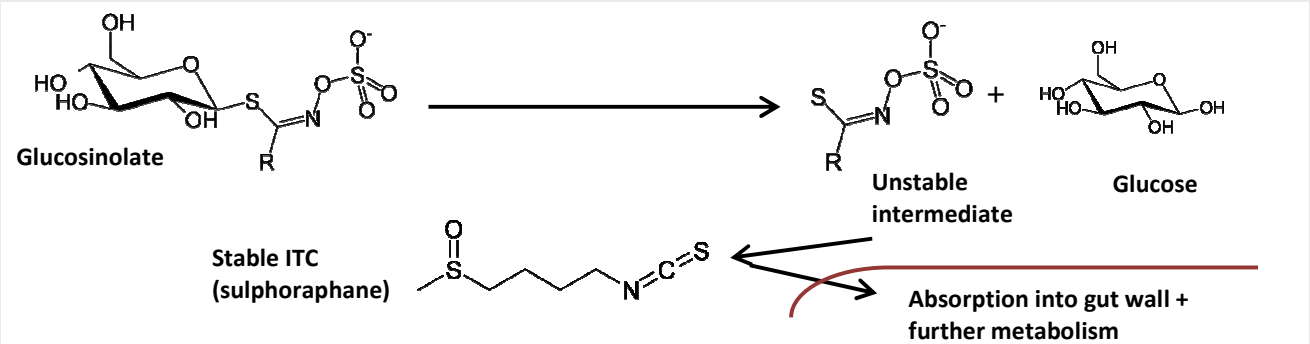


Structures of three main quercetin conjugates.

Quercetin metabolism. LPH - lactase-phlorizin hydrolase; SGLT1 - sodium-dependent glucose co-transporter; CBG - cytosolic ß-glucosidase

The quercetin metabolites mentioned above have been previously synthesized in our laboratory (Needs & Kroon, 2006) and we will examine the potential bioactivity of these human metabolites (alongside a quercetin glycoside and quercetin aglycone) with respect to endothelial cell signalling and NO bioavailability.

Glucosinolates themselves have poor bioavailability, but when plant tissue is broken, such as through chewing or slicing, they come into contact with the enzyme myrosinase, and are broken down to form isothiocyanates (ITCs). These are much more readily absorbed in the gastrointestinal tract. As well as this, microbes in the colon have been shown to have thioglucosidase activity, and can also produce ITCs.



Glucosinolate metabolism. ITC - Isothiocyanate

The ITC mentioned above, sulphoraphane, is available to the IFR Kroon group, along with its human metabolites (sulforaphane-glutathione, -cysteine, -N-acetylcysteine conjugates). However its glucosinolate, glucoraphinin, is not very prevalent in kale, so we will use SFN and SFN conjugates to examine how human metabolism affects the activity of an isothiocyanate. The main isothiocyanate in kale is 2-propenyl-glucosinolate which yields allyl-isothiocyanate upon deglycosylation by myrosinase, so this compound will also be tested.
Once completed, we will have collected data that indicates the vascular bioactivity of crude plant extracts, individual food plant bioactive compounds, and of a series of human metabolites of these bioactive compounds. These data will indicate the potential of BSAC food plants and the foods they contain to influence vascular function by modulating the bioavailability of nitric oxide and the secretion of endothelin-1, which control vascular tone.

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Belgrade researchers start their search for Black Sea area herbs that can thin the blood

Aleksandra Konic-Ristic, Dunja Kardum, Filip Stojanovic and Maria Glibetic (IMR)

In March 2011, the IMR team in Belgrade, Serbia started the human intervention study entitled "Effect of traditional dietary plants of Black Sea region on platelet function and other risk factors of cardiovascular disease" (acronym: PLATAN). This followed approval of the study protocol and associated paperwork by the BaSeFood Ethics Committee and by a local ethics committee (Ethical Board of the University Hospital Zemun, Belgrade).
According to the protocol, the study consists of three consecutive phases.
During the first phase of the study, effects of a single dose of water extracts of selected traditional herbs (nettle, dill and Sideritis scardica ('mursal tea') on platelet function (platelet activation, aggregation and conjugation with neutrophils and monocytes) will be investigated in 80 patients with metabolic disease who will be randomly allocated to one of four intervention groups (one of the three plant water extracts or the water control). In addition, during this phase all participants will be screened for platelet hyper-reactivity (based on platelet responses to suboptimal concentrations of agonists). These data will be used to select fifty participants with more reactive platelets for the habitual intervention.
For the second phase, the herb that will be investigated will be selected from the three tested in the acute study based on the results obtained during the acute study and from ongoing in vitro and ex vivo experiments. Ex vivo and in vitro investigations are based on evaluation of antiplatelet, anti-inflammatory and anti-oxidative effects of selected extracts, their major bioactives and metabolites in blood cells of healthy people and patients with metabolic disease, and endothelial cells in culture.
Once the second phase is complete, the data will be evaluated and used to select subjects for the chronic study which is phase 3.
After the approval of the study protocol by the Ethical Board of the University Hospital Zemun, Belgrade, the IMR team started the recruitment of patients with metabolic disease as part of phase 1.
The recruitment process includes clinical measurements (blood sugar, cholesterol and triglycerides levels), blood pressure and anthropometric parameters (BMI and waist circumference). After providing informed consent, the subjects were randomly allocated to one of the four treatment groups. A series of measurements were made and blood samples taken before and after consumption of the randomly allocated treatment or control. The blood samples were used to test the effects of a single treatment dose versus control on platelet function and to determine each subject's platelet hyper-reactivity.
To date, 20 subjects have completed the acute phase of the study (see images provided). At this stage we are unable to determine the potential effects of consuming the three plant water extracts because the study is being undertaken with blinding in place, so the researchers do not know which treatment has been allocated to each subject. The allocation key, including all patient's data, is being retained in a locked cabinet by a third party who performed the randomisation until the completion of this phase of the study.

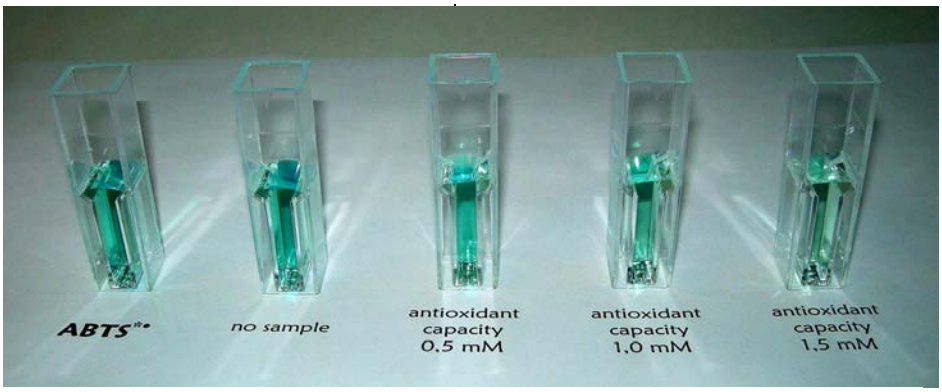
Subjects undergoing screening and having a blood sample taken on a study day.

The overall purpose of the PLATAN study is to determine the efficacy of selected foods or plant ingredients in terms of their ability to reduce platelet reactivity. When platelets are too reactive, we call them 'sticky'. Although platelets need to have some sticky function in order to clot blood (e.g. to prevent bleeding to death when the skin is cut), platelets that are too sticky are likely to cause internal clots (thrombi) and to decrease the stability of atherosclerotic plaques. Thrombus formation reduces blood flow through arteries, and if plaques break up, they can cause heart attacks, strokes and other very serious events. The most well known anti-platelet drug is aspirin (acetyl-salicylic acid).

Black Sea area traditional foods: a valuable source of antioxidants

Francesca Danesi and Alessandra Bordoni (UNIBO)

Plant-based foods, such as vegetables, fruits, and whole grains, contain significant amounts of bioactive phytochemicals. When plant-based foods dietary intake is significant, these compounds have a significant role in the maintenance of human health. Vegetable food bioactive compounds have many relevant biological properties, among which antioxidant activity. The knowledge of total antioxidant capacity of plant foods, which is the cumulative capacity of food components to counteract the oxidative stress, could be useful for setting up an healthy diet.
Antioxidant capacity of commonly consumed fruits and vegetables is well-documented, while traditional foods are less characterized. Despite they are extraordinary sources of bioactives, particularly antioxidants, data on their antioxidant capacity have not been reported yet.
In the BaSeFood project, we are testing the antioxidant capacity of more than 30 traditional foods of the Black Sea area. These foods have been neglected from scientific literature and this project aims to revaluate them. Traditional dishes were prepared in different Countries of the Black Sea area (Bulgaria, Georgia, Romania, Russia, Turkey, and Ukraine) by local Partners, according to the typical recipes and following the seasonal availability of vegetables and fruits. After preparation, foods were freeze-dried and sent to the University of Bologna to evaluate the antioxidant capacity. The samples were prepared using a water extraction procedure followed by an ethanolic extraction. The measurement of the antioxidant capacity was carried-out with one of the most widely used assay, the ABTS+• method, and compared to the activity of a well known antioxidant (an analogue of vitamin E).



Cuvettes for antioxidant testing, at different antioxidant concentration

Our preliminary results evidence that the antioxidant capacity of many vegetable foods and beverages commonly used in the Mediterranean area.
For the first time, the antioxidant properties of traditional foods of the Black Sea area were studied. Although in this area these traditional foods are largely consumed with the daily diet, less is known about their overall potential health benefits.
The revaluation of traditional foods will be interesting for both consumers, who can discover or rediscover typical dishes, and food industry, that can exploit these scientific data for producing "new" traditional foods.

Information from Uzhhorod National University, Ukraine

Nadya Boyko and Mariia Mudryk (UZHNU)

Start of the quantitative pro/antibacterial experiment
Some edible plants, e.g. garlic and onion, are widely known for their antibacterial properties.

The well plate with titrated in PBS and BHI plant extracts and control wells

The idea to investigate the pro- and antibacterial properties of different plant extracts were accomplished by the UzhNU team in the frame of specific BaSeFood task. The quantitative experiments, which examined the influence of the different plant and some traditional dishes extracts on the beneficial and pathogenic bacteria growth, using co-cultivation techniques, were conducted in September-October 2010. During the first stage, the UzhNU team studied 28 extracts and selected few of them, which showed stimulatory and/or inhibitory effects on 26 bacteria.

Within the second stage, started in March, 2011, an experiment with extracts able to affect bacterial growth (quantitative analysis) is being carried out. The results of this experiment will be used in the development of a database of in vitro bioactivity data for typical BSAC foods, as well as the for functional products development and implementation. The most promising plants/dishes which can be used as functional food are sauerkraut, green and red Georgian plum sauces, cabbage and pumpkin, which show their antibacterial effect on the pathogenic species and stimulatory effects on the beneficial bacteria growth.

Samples with the pomazanka, green borsch, green dill and nettle leaves after the lyophilization procedure. Bottles are covered with hermetic rubber and iron caps for long-term preservation.

Different ways of sample preparation
The UzhNU team has prepared different kinds of samples for the next following shipping to the partners (IFR, INSA and IMR) for the further specific investigations.

Different types of the samples preparation and packing: freeze dried samples in glass bottles; vacuum-packed freeze dried samples in plastic bags; sun-dried dill and nettle.

Borsch green transcarpathian, pomazanka with dill and garlic were prepared by the volunteers in home kitchen and in a restaurant, transported to the lab, homogenized, poured in to the special glass containers and lyophilized. Lyophilization was prepared in collaboration with the Transcarpathian regional blood transfusion station. Freeze dried samples was packed under vacuum (in the supermarket "Dastor"). Besides the fresh leaves of dill, parsley, nettle and homogenized blueberries were lyophilized. Green dill and nettle were sun-dried, also packed and sent to the partners.

Plant and food bioactive components characterisation and retention

A comparison of traditional processing of hulled wheats

L. Filippo D'Antuono, Manuela Manco (UNIBO), Bike Koçaoglu, Osman Hayran (YEDITEPE), Nune Darbinyan (Eco-Globe)

Emmer wheat (*Triticum turgidum* L., subsp. diccocum (Schrank ex Shubler) Thell) and einkorn wheat (*Triticum monococcum* L. subsp. *monococcum*) are the two hulled wheat species being considered in detail within BaSeFood. Einkorn wheat is cultivated in some areas in Turkey, mainly to prepare bulgur. Emmer wheat is still extensively grown in Armenia, as human food. In Italy it is presently a valuable niche product.

Emmer wheat sampling, Monteleone di Spoleto, Italy, November 2010.

Glume removal represents the preliminary, essential step to human consumption of hulled wheat kernels. The traditional process for glume removal is strikingly similar in the three countries. The nature of the final product is affected by the available technology: in fact, traditionally, the glumes are removed by crushing the grain in stone mills; as a result, the resulting main product is broken grain, that is subsequently sorted by sieving. In Italy, where the traditional processing scheme was exactly the same until about 30 years ago, the technology of the process has been recently updated, to respond to an increasing demand for intact grain. As a first step the glumes are removed by means of special machines, that minimize kernel breaking. After this, the kernels are mostly pearled by abrasive discs. Intact grains are more rarely milled, or crushed in a hammer mill, an operation generally reserved to the breakings from glume removal.
An experiment have been carried out to compare the yield of the traditional process, still used in Turkey and Armenia, and the updated procedure, used in Italy.
The on-plant sampling were jointly carried out by YEDIEPE and UNIBO in Turkey, August 2010, on einkorn wheat bulgur; by UNIBO in Italy, November 2010 and by ECOGLOBE, with protocols agreed with UNIBO, in Armenia, November 2010, on emmer wheat.

The individual fractions of processing were accurately weighted. Each fraction was also sampled and is being used to determine the retention of some bioactive components (phenolics and lipid associated components), since there is evidence that processing selectively separated some parts of the kernels.
The calculated total yield of the preliminary, fundamental step of glume removal did not vary a lot between the different plants, ranging from about 71 to 78%; anyway, a quite high figure with respect to the lab-measured maximum value of 73 to 81%. However, the analysis of wastes and the figure of individual processing fractions are quite different and will be the subject of specific contributions.

Emmer wheat sampling, Kaputan, Armenia, November 2010.

The kale experiments in Italy

Filippo D'Antuono, Federico Ferioli, Manuela Manco and Elisa Giambanelli (UNIBO)

Within the experimental activities on kales, several tasks are being carried out.

Palm tree kale crop Italy, December 2010

Characterisation of plant material.
As illustrated in other issues of this newsletter (issue 2, April 2010, issue 3, September 2010), kales have being targeted as relevant food plants for BaSeFood, because of their importance in traditional food systems and putative health-promoting properties, connected to their content of specific phytochemical components.

Kale harvest, for experiment, Cesena, December 2010

Variability of these characters may exist depending on environmental and genetic factors.
A field trial was established in Italy, including 17 local populations: 8 from Italy, 7 from Turkey and 2 from Portugal. The plant material was harvested on December 9, 2010, and prepared for analyses of glucosinolates, phenolics and carotenoids. The same determinations are being carried out on local material, collected on place in several areas during winter 2010.

The cooking experiments

Glucosinolates are key components of the Brassica family vegetables, to which kales belong. Glucosinolates are water soluble components, so they can be lost in cooking water.

Cooking experiment: sample preparation.

Glucosinolates are also enzymatically transformed into degradation components (thiocyanates, isothiocyanets and nitriles) when cells are damaged. Like all enzymes, myrosinase, catalysing the start of these degradation chain reactions, is inactivated by heath. Intact glucosinolates, however, can also be enzymatically hydrolysed at the level of human gut.

Cooking can therefore affect in various ways the availability of glucosinolates and their degradation products, some of which are recognized as the major responsible of some potential health promoting properties. It is therefore evident how cooking is a critical step for the valorisation of health promoting properties of kales.
Traditionally kales are used to prepare soups, with cooking time ranging from few minutes, as reported for Portuguese Caldo-verde, to quite long times (the Italian word Ribollita, that is the name of a traditional kale soup, means "re-boiled several times"). The cooking duration adopted in home practice is amply empirical.
Kales are sometimes also used boiled, as side-dishes, so in a form where the cooking water is drained before consumption.

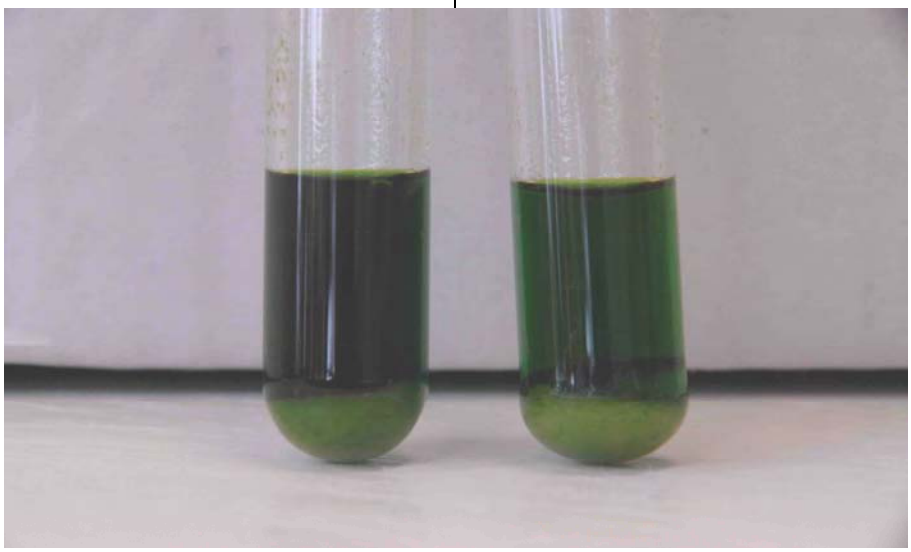
Vapour cooked Turkish kale.

A limited number of experiments aimed at measuring the retention of glucosinolates and other components during cooking have been carried out on cabbages. So kales are still largely un-explored.

Samples of cooking water: from vapor cooking (left); from water cooking (right).

A first experiment on kales has been carried out in Italy, within BaSeFood, including:
- three kale populations (Italian, Turkish an Portuguese)
- two ways of cooking: with water, with vapor
- four cooking times: 5, 10, 15 and 20 minutes.

Preliminary results indicate that almost no leaking occurs when cooking with vapour, whereas up to 30% of plant dry matter is found in the cooking water, when the leaves are boiled.
Exact yield factors, and retention factors of glucosinolates, phenolics and carotenoids are being analytically determined.



Non-polar kale extracts, containing chlorophylls and carotenoids.

Nature colour and food colours

The 33 selected foods plus some additional materials are being analysed at UNBO for antiodidant properties and the content of phenolics substances, connected to these properties.
Food extracts show an amazing difference in colour, depending on the main class of phenolics, and other components. Nature diversity is reproduced in foods !

Colour of food extracts, ready for total phenolics determination.

An amazing array of different tahin (oilseed paste) and halva was received at UNIBO from UFT, in the frame of a collaborative experience aimed at characterizing these products, determining bioactive substances retention during processing and shelf life: these were from sunflower, sesame, groundnuts, almond, hazelnuts, walnuts and pumpkin seeds.
Also in this case, it is expected that analytical results will reflect colour variation.

Different colours of oilseed tahin (oilseed pastes)

Tkhemali sauce is typical of Georgia. Tkhemali was prepared in the traditional way during a visit of the coordinator in Georgia, in August 2010. The sauce was simply bottled, while still hot, the bottles were closed by pressure plastic caps, and were not subject to any further sterilization.

Preparation of Tkhemali sauce, Aspindza, Georgia, August 2010, with plenty of colourful ingredients

Bottling fresh Tkhemali, Aspindza, Georgia, August 2010.

A bottle of Tkhemali sauce, left open for 9 month, at ambient temperature!

A bottle was opened in Italy, to taste it , and then forgot open at ambient temperature until March 2011.
A little change of colour occurred, by there was not any decay due to mould development. Tkhemali has lot of coloured ingredients: a kind of fantastic synergy !

Activities in the frame of consumer and stakeholders surveys

A on-line questionnaire

SPES-GEIE, in collaboration with UNIBO, has implemented the questionnaire addressed at surveying the attitude on Italians towards Black Sea area traditional food in a on-line platform.
The questionnaire can be found at: http://www.kwiksurveys.com/online-survey.php?surveyID=HLOMJF_e1d68938

Questionnaire submission in course at UzhNU

The team of the Uzhhorod National University has already started to work on Task 5.1., has translated all Questionnaires and started interview the respondents in following locations: in 3 shopping areas, in working and school area (respondents working in insurance company, business owners, hospital and bank staff, students of the medical department) and on the street on the Wine festival in Mukachevo, Ukraine (13-14 January 2011).
Consumers' questionnaire compilation on the street fair "The red wine", Mukachevo, Ukraine, (13 January 2011) and in the city supermarket "Dastor", Uzhhorod (5 January 2011), by Nelya Koval, UzhNU.

A collaboration to questionnaire submission to migrant communities in Italy

Olha Ivaniv was born in Cernivtsi, Western Ukraine and is presently living in Cesena, Italy. She graduated with honours at the Ivan Franko state University in L'viv, in foreign literatures and languages, with specialisation in English language and literature. She has been lecturer of English language at the same University from 2002 to 2007. While in Italy, she took a master degree in foreign languages and literatures at the University of Bologna. Presently she is collaborating with BaSeFood in the administration and processing of questionnaires to the Ukrainian communities of the Emilia Romagna region.

BaSeFood dissemination

A contribution about garlic and its uses in Romanian cuisine

Garlic is one of the most largely employed culinary plants, worldwide. It also has important putative health-promoting properties.
Prof. Stroia and colleagues from ASE released an interesting contribution about the use of garlic in Romanian cuisine. The entire material is available at the Foods Plants and People section of the BaSeFood web site. Foods, plants and people contains reports about all aspects of plant foods and edible plants.
This section presently contains three contributions:

- Hulled wheats: a coming back within BaSeFood.
- Plums: versatile fruits for traditional foods.
- Garlic sauce.

A new project in the area of traditional foods

Antonietta Branni (SPES-GEIE)

In September 2010 a new European project started. AFTER project (*African Food Tradition Revisited by Research*) involves seven African countries: Benin, Cameroon, Egypt, Madagascar, Senegal, Ghana and South Africa and four EU countries:

France, Portugal, Italy and the United Kingdom. SPES-GEIE is participating for Italy.
In AFTER project, ten traditional products, which represent three main categories of products are studied: Fermented cereal-based products (*Akpan, Gowe, Kenkey, Kishk Sa'eedi*), Fermented salted fish and meat (*Lanhouin, Kitoza, Kong*), Traditional plant based extracts for functional foods (*Bissap, Buy, Jaabi*). By generating and sharing knowledge on food technology AFTER intends, for each product group, to improve, develop and create a product or a technology representing an interest shared between European and African food companies.
The evident common background of BaSeFood and AFTER may suggest the seek for future networks in the area of traditional foods and local food resources.

Presentation at the 6th European forum "Let's Liberate Diversity!"

Mariam Jorjadze, Elene Shatberashvili (ELKANA)

On February 24-26, 2011 representatives of the Biological Farming Association Elkana Mariam Jorjadze and Elene Shatberashvili participated in the 6thEuropean forum "Let's Liberate Diversity!" in Szeged, Hungary.
The "Let's Liberate Diversity!" forums are the annual gatherings of farmers' organizations and associations working on agricultural biodiversity in Europe. The forum is organized by European Coordination on Farmers' Seeds, which is a European network committed to the conservation and renewal of crop biodiversity in farms and gardens. This year the central themes of the Forum ware Farmers' Rights and on farm conservation, development and sustainable use of agricultural biodiversity (for more information please visit: http://www.liberate-diversity-hungary2011.org).
In the frame of the forum several workshops were conducted on the problems like: collective management of agricultural biodiversity on farms and in gardens, seed legislation in the European Union, animal biodiversity, wheat and bread traditions etc.; Also poster exhibition of the activities of the participating organizations, a seed exchange and an exhibition of local apple varieties took place.
In the frame of the Poster Exhibition, Association Elkana presented its work regarding on farm conservation of the local wheat varieties, as well as public awareness and promotional campaigns for the promotion of traditional bread baked from Tsiteli Doli variety. Besides this the poster presented the core activities of the BaSeFood project, including the on-going laboratory studies conducted by BaseFood partner organizations on Tsiteli Doli bread.

In the frame of the workshop – "Restoring Our Heritage of Wheat and Bread Traditions" Elkana representative Elene Shatberashvili made a presentation on Elkana agricultural biodiversity conservation activities as well on on-going research on traditional foods in the frame of the BaSe Food Project. It should be mentioned that BaSeFood Project activities gained the interest of the workshop participants from various counties of the European Union (farmers, gardeners, seed artisans, bakers and processors of products derived from agricultural biodiversity, civil society organizations, researchers, breeders and representatives of gene banks) and some asked additional questions on the project implementation and outcomes so far. The workshop aimed to build a network and share the knowledge and skills for conservation, exchange and restoration of the landrace grains, and traditions of baking, culinary arts, seasonal grain festivals and folk arts.

Keeping the faith in education

Note from the project management and coordination

This contribution was received from ASE, as a witnessing of the concepts and involvement underlying a book published by ASE. The book is freely available, so this piece by no way can be considered an advertisement, nor have any commercial implication

Simona Buso and Carmen Costea (ASE)

Before being a European project, Base FOOD represents a great asset and opportunity for both members and the other potential participants, especially the youngest ones. Getting closer to such research they become aware of their own capabilities of self development and the market opportunities helping to set up a better connection with the Society. Eighteen months of hard work within the project topics gave us, periodically the opening to get together with students and stakeholders, with academics and business people in our familiar, by now, *idea-shops*. It is there we love *cooking* not only the prioritized BCAS foods but our development together as the traditional food can't be analyzed beyond searching and celebrating traditions, knowing the historical meanings and pass them to the youngest of us. The book *Keep learning* represents the core of our workshops of the first half of the practical research display, a book wrote in Romanian, by the members of BaSeFood together with our students who never missed any of our meetings.

Coordinated with dedication by Professors Carmen Costea and Alexandru Stroia the book edited by the ASE Publishing House proved itself the chance to wake up minds and move souls in formation on the way to discover the hidden researches of the old Romanian knowledge of culinary art. That was the way to go to monasteries and farthest villages of the country to look for old recipes and traditional treasures. Generally speaking, all those recipes were mainly transmitted from mouth to mouth, from mother to daughter, from generation to generation, but also other aspects of Romanian tradition with lot of other details linked to the way of preserving pots, cooking tools, folkloric issues and movements.

The students looked for those old recipes, learnt to cook them and both their new knowledge in our meeting sharing this new knowledge together with specialists and their colleagues. In the same time their competitive behaviors bought up to our surprise native qualities of singers, story tellers, painters and decorators. The young people put together recipes brought from the most famous regions of Romania, proud of themselves and of their trophy and contribution to the society benefit. They looked to feel keeping the final competition fresh for ever, in their minds. It was there where each of participants tried to convince the two juries of specialists and of community about the values of their country sides as portraitures in the presentation lessons and those to cook and the dishes exhibitions. These thoughts will remain for ever in their mind and soul helping them to better evaluate from now on that side of civilian and citizenship that was ignored for some time. Enriched in this fortunate way, they will be capable to teach others what they have learned. And the enthusiasm born from their experiences will lead them certainly to want to share with others what they have learned. Now they know, without any doubt, what is the true Romanian "magiun", (a Romanian traditional product made exclusively from ripe plums without any added sugar) what is the difference between it, plum jam and the marmalade than we usually find at the store, how and where it is prepared and which are its properties beneficial to the human health. Once entered into the human soul, the red-copper or bronze colored "magiun", bearing in its perfection the fragrances of the lea and of the sun and hiding with finesse in its sourish-sweet depth bitter and sometime the bold nut cores can not be forgotten anymore. Kept in traditional pots in order to stay cold, this soft essence of ripe plums approaches to the culinary perfection and remains always tempting in its wonderful simplicity. Preparing it, knowing it with all their senses, young people learn at the same time several wonders: the wonder of the "magiun" itself, the wonderful of the people who managed to invent something like this, the divine perfection of the nature that created the plums and the pleasure, perhaps unexpected, of working along with friends in a pleasant harmony. So they come to know themselves better, to marvel that their hands, which they didn't know to be so skillful, were capable to make such special dishes. They know themselves better because finding out witch are the roots of their people, their traditions, learning where they really come from, they can discern where they are going. Getting so close to the spiritual essence of the Romanian people, essence made from the Romanian folklore and Romanian traditions, generally unknown to the young people, largely overwhelmed by the imported consumer society, they gain more balance, more stability and confidence in themselves and discover their true identity.

The recipes were many, because the traditional Romanian cuisine is a treasure of flavors. But the real gain is to enrich young participant people with pieces of Romanian soul, discovered during the project. After each infusion of authentic tradition, after each spiritual journey in the orchard, in the garden of vegetables, or in the kitchen with aromatic herbs, but also in visited monasteries, after each bath in the clear water of the pure love for our ancestral lands and after every encounter with the divine essence contained in all these, young people have found themselves more fulfilled, richer, cleaner, wiser. That is why we say that the project BaSeFood represents a series of opportunities.

A part of the things that the young travelers around the country have gathered in their spiritual bags can be found in the book "Learn everything" that ASE Publishing House recently published. Between its pages is hidden a priceless treasure: bits from the minds and hearts, now richer, of the participants in the project - thoughts about education and about the fact that it should increase and stimulate the curiosity of young people, memories, knowledge, feelings shared with others, and last but not least, delicious and healthy recipes. We find in the book, as said in the preface by the coordinator, a succession of lessons learned directly from life, for the lessons "must be first lived, in order to be understood." Pieces of folklore - verses of Romanian folk songs filled with spirit - thoughts and lyrics, essays about tradition in Romania, all reflect the activities from the "ideashops" born from souls and managed with passion - where participants have learned by doing several things, all in the BaSeFood project. In the rush of nowadays global area in which we all are caught up with better or less good issues, defining, cultivation and preservation of national identity is certainly a goal. If we forget the past, if the nationality is forgotten or ignored, the European Union can't be built as its roots are national roots uniquely developed together, in the same garden. No roots, no purpose policy can easily transform us into an amorphous mass, sad and vulnerable population, forgetting to remain people. We need to keep ourselves reunited by roots, traditions and natural diversity, to better know, honor, appreciate and preserve both the local assets and the European values. For the world natural order sake we need to never forget our belonging, our soul's treasures as spiritual rings, closely placed side by side, as in the secular tree stems. We carry through life these rings, each loaded with the spiritual messages of our ancestors. It is our duty to keep alive the light that shows the path and this project is a step towards this goal and a guiding torch. The authors of the book "Learn everything" managed to convey some of the riches of spiritual essence gathered in the workshops. Thus, after a preface that refers to the very essence of the project, we are carried to the lands of Moldova and Bucovina, though the multicultural Danube Delta, both so young and so old at the same time, through the bold Oltenia, we dress in Romanian shirts, adorned with traditional stitches, we feel and learn some of what the students, traveling through the regions filled with Romanian culture and tradition, felt and learned. For these young people dressed in traditional costumes, met the peasants at home, in the country side, picked herbs from the garden and fruits from the orchard, mixed with a wooden spoon the big pot put over the fire in the yard to make "magiun", walked on the fresh grass and felt God and the Romanian, Ukrainian soles in all this simple things, that we managed to keep clean and healthy, that were carefully preserved in some areas of Romania that remained, magically, untouched. These "points of view" that form the first part of the book are some gems from the great chain of lessons lived and learned by the participants to the project BaSeFood.

The second part of the book refers to Romanian culinary traditions, to the wisdom contained in them, and to the fact that the food habits of our ancestors were formed through the experience gathered by generations of people, which is why traditional recipes are all delicious lessons about how to stay healthy. Surely cooking these recipes represent lessons of health for the young people threatened by the aggressive culture of the fast-food.

ASE Publishing House has succeeded, we believe, to emphasize the spiritual essence contained in this book, essence resulting from years of exercises and facts of love, harmony, knowledge, faith and work, not necessarily in this order that constituted BaSeFood project. Speaking mainly about fastening dishes, the Easter time will be the precious moment to offer the book, or free, to other young people, and contribute to their cultural and spiritual enrichment. Such dissemination is an appropriate joy of the coming spring, a deep fulfillment for those who are going on to work with passion, with skill and high devotion to the BaSeFood project.

Dissemination events

- ***Finglas P***. *Recent achievements in Europe through EuroFIR and BaSeFood projects. Project overview. International scientific symposium: Biodiversity and Sustainable Diets, November 3-5, 2010. FAO Rome, Italy.*
- ***D'Antuono L.F., Costa H., Sanches-Silva A. Koçaoglu B., Hayran O., Jorjadze M., Boyko N., Buhyna L., Fedosov S., Alexieva I., Costea C., Stroia A., Karpenko D*** *. Plant and food diversity in the Black Sea Area: preliminary case-studies outline. International scientific symposium: Biodiversity and Sustainable Diets, November 3-5, 2010. FAO Rome, Italy.*
- ***Costa H.S., Albuquerque T.G., Sanches-Silva A***. *Comparison of fatty acids profile and total fat content in five types of oilseeds. 8th Euro Fed Lipid Congress, November 21-24, 2010, Munich, Germany.*
- ***Boyko N***. *"BaSeFood": Strategy (Lessons) to Learn. InfoDay "The EU Seventh Framework Programme (FP7) on Research & Technology Development: Opportunities for Ukraine's Research Communities in Biotechnology", Odessa, Ukraine, November 4, 2010.*
- ***Mudryk M., Bugyna L***. *Quality of the plant-originated traditional food products. The 64th students' and young scientists' conference "Actual problems of the modern medicine",*

Seminars carried out by ASE in Romania

- **5-17 October 2010** Fall traditional dishes in the Carpathian cuisine Simon, Bran Cluster workshop participants: researchers, facilitators in disseminating food recipes, general public Romanian traditional foods keeping and producing
- **June 11-13, 2010** Spiritualization of Health through Tradition, Love and Knowledge Braditel, Piatra Neamt, Romania,Workshop Participants : food scientists, social science scientists, general public. Project's overview, Romanian traditional foods
- **April 30-May 2, 2010** Tradition, Health, Responsibility Hobana, Gureni, Tg. Jiu Romania Itinerant Cluster workshop for traditional recipes recording and networking with stakeholders and facilitators Presentations Applications stakeholders , producers, consumers, traditions keepers Project's overview, Romanian traditional foods
- **Every Thursday of April 2010m** Business Club - Branding Romania through its culinary habits
  Bucharest, Romania, Training Idelier, Project team, researchers, students Changing into good the local consumption habits Project's overview, Romanian traditional foods
- **December 10-17, 2009**, Bridging Spirituality and Health from Traditional Cuisine during Christmas Fasting Bucharest, Romania Workshop Food scientist, social science scientists, general public, Project's overview, Romanian traditional foods.

Editing
Lorenzo Cerretani

E-mail
basefood@gmail.com

We're on the Web!
See us at:
http://www.basefood-fp7.eu/