



PROJECT FINAL REPORT

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Executive summary

BaSeFood (Sustainable exploitation of bioactive components from the Black Sea Area traditional foods) was a 43 month EU funded project, aimed at investigating, documenting and describing the traditional foods of the Black Sea region, and their content of health-promoting substances, in order to create the knowledge base for the production of tradition-based healthy foods, in a context of sustainability, including issues of cultural and biological diversity, and consumers' facts.

The Consortium was built of 13 beneficiaries. Seven are from all the coastal Black Sea area countries. Six represent Western European entities, with consolidated European research experiences. Strong links were established with two FP6 projects: EuroFIR, from which methodologies were shared, along with some relevant research lines, TRUEFOOD, with the aim of enforcing links with industrial stakeholders.

BaSeFood was organised in 5 R&D work packages (WPs), plus Dissemination and Management.

From the beginning, a considerable effort has been devoted to retrieve information from the basic pool of knowledge of traditional foods of the Black Sea area.

WP1 produced preliminary traditional food archives, containing more than 250 documented foods. 33 of them have been selected and represented the base for further activities namely:

- a) the documentation of recipes, flow charts and accessory technical and cultural information, and food preparation, finalised at their nutritional analyses
- b) chemical analyses (WP2) aimed at the nutritional characterisation of the 33 selected foods
- c) the integration of these activities lead to formulate preliminary hypotheses of possible nutritional claims.
- d) the 33 selected foods were also microbiologically characterised and used as references in some consumers analyses.

WP2 carried out the chemical analyses to determine the nutritional composition and also performed the microbiological characterisation of the selected traditional foods

WP3 carried out several *in vitro*, *in vivo* experiments, and two intervention studies aimed at the determination of potential effects on the protection of chronic-degenerative diseases, with special respect for cardiovascular risk factors. These activities were carried out at different levels of detail, either on some foods or specific raw materials or active components, with special respect for phenolics and glucosinolates. The results were able to evidence some mechanism involved in possible protective actions

WP4 was articulated in a series of activities. It investigated the variability of bioactive compounds in local resources of raw materials, with special respect for kale, emmer and einkorn wheat. Retention factors of key compounds (carotenoids, tocols, phenolics, glucosinolates) were determined in water cooking of kale and emmer wheat. Traditional flow charts of some foods (bread, fermented beverages such as ljuto and kvass, oilseed products, such as tahin and halva) were studied and subject to experiments for their updating or modification with the use of new ingredients, also with sensory evaluation of final products. The results allowed to quantitatively determine retention factors of key bioactives and to draw productive hypotheses potentially exploitable by food processors.

WP5 carried out consumers and stakeholders surveys. Joint consumers and expert ratings of binary combination of foods, health claims and active compounds for specific characters lead to the definition of the more promising combinations to be potentially exploited in the perspective of the manufacturing of traditional foods with attached health claims. Consumers surveys allowed to define the perception of traditional foods by the Black Sea area populations and migrants and by the Italians, allowing a preliminary, although not fully quantitative indication about the perceptual characters that may have the highest acceptance impact.

Finally large scale on site surveys (WP1) allowed the comparative documentation of over 800 foods in their local context, bringing out important information about the diversity, variability and role of traditional foods in local systems. These results are strictly connected with possible social, sustainability and conservation issues. These activities also indicated the need of further documentation and suggested that some integration in the current efforts of traditional food definitions aimed at registration may be useful. BaSeFood results were amply disseminated by attending several meetings, writing scientific and divulgation papers, and by the organisation of an international final event called Traditional Food International, in which over 120 oral and poster contributions were presented.

Summary description of project context and objectives

BaSeFood responded to the FP7 call : KBBE-2008-2-2-02: Bioactive compounds in traditional food products (SICA Black Sea Region).

The three topics mentioned in the call title: bioactive compounds, traditional foods, and the Black sea region were carefully taken into account as leading points from the very early stages the development of the project's idea and structure.

With respect to bioactive components, it was acknowledged that there is an increased concern, especially in Western industrialised countries world, for chronic-degenerative diseases connected to non-optimal nutritional habits. Several plant origin compounds (bioactives) are presently being studied for their potential positive effects on human health. This interest produced some thousands of scientific papers, yet with still several uncertainties with respect to health claim substantiation.

Also for BaSeFood it was decided to focus on traditional foods plant with main ingredient, or substantial part of composition, coming from plant raw materials.

The health promoting concept is considered a potent tool to add values to food and, as such, is highly considered by the European Food industry. From the available literature and documentation, however, some evidence indicated that both the science approach to the study of bioactives, and the industrial approach to product development, are often lead by a top-down technology or science push, not taking sufficiently into account the chain perspective and the opinion of end users. This caused a high rate of failure in new products, waste of resources and, finally, the risk of generating distrust of people on the original message of health promotion.

At the time of project's planning, the EFSA regulations about nutritional and health claims was not yet implemented; however, the guidelines of this new regulatory approach were already known, indication the need of a consistent evidence of high quality experimental data, including human intervention cases, to support health food health claims.

At the level of consumers' perception, in any case, it was clear that the message of health promoting properties of foods was a typical credence character, needing communication from third parties to be understood.

The second point was based on the fact that traditional foods are, often emotionally, considered as a concentrate of positive traits, including spontaneous relation with healthy properties, yet at a different level of perception.

The analysis of available theoretical and research documentation, suggested that the perception of traditional food value spontaneously occurs in local communities, through the association of generally positive cues to a complex of perceived immaterial traits. Traditional food production and consumption chains approach a closed system, with abundant diffuse knowledge, but scarce flow of information from and to outside, and low opportunities of creating added value. Since traditional consumers maintain a degree of independency in their perception and choices, "traditionality" is perceived among positive traits for food choice. In local contexts, the "traditional character" of foods approaches the perception of an experience character, although subject to supply constraints. On the contrary, urban western consumers largely lost the contact with the direct knowledge of food production chains. Therefore, for them, the "traditional character" largely became a credence quality trait, which intrinsic nature requires the communication from third parties. Also in this case, a regulatory approach to the matter does exist at EU level, implemented in the different categories of registered denomination of origin products.

A first challenge, to be well considered for the general strategy of the work, and especially in view of the exploitation of its foreground, was that combining two different credence attributes (health promotion and "traditionality") in a same food may present some problems.

The Black sea region was a third, central, focus of the call. The countries surrounding the Black sea share, except for Turkey, a recent socialist experience, and almost non-existent economic communication with western Europe, until about 20 years ago. During the previous period, historical and political conditions caused a certain persistence of local knowledge. Industrial food production was neither effective nor sufficient, and often the modest standard of living did determine a flourishing of local production for self consumption, enforced when the political changes caused shortage of food availability. The current transition to more prosperous economies results in local resources being considered of less interest, and there is evidence that dietary habits reminding difficult periods are not fully appreciated. However, some disaffection and reaction is reported against the rush towards low quality mass industrial foods, with a return to foods of local culture. On the other hand, a part of the population still suffers of unbalanced diets. There could be therefore opportunities for combination of traditional and health promoting foods.

The different cultural starting point should have been however taken into account, to correctly address industrial developments in the area. With respect to the interest of West European SMEs, most foods of the Black sea area, except perhaps some Turkish ones, are still largely unknown.

Besides that, the cooperation between western and eastern enterprises and institutions was in the background of this SICA collaboration project. Therefore, a substantial partnership of the area was a requisite on the Consortium building strategy, to be integrated with a western European partnership. The inclusion of at least one partner from each of the Black sea countries was an asset for the project. Black sea area partnerships had the function of retrieving the local primary pool of information, besides bringing individual specific expertise in the project. Seven partners from all six coastal Black sea region countries (Bulgaria, Romania, Ukraine, Russia, Georgia and Turkey) participated in the Consortium. A partner from Belgrade, Serbia, participated for its expertise in clinical intervention assays and previous experience within the EuroFIR project. The western European partnership obeyed to the need of establishing a connection with previous experience in traditional food research, and acquire the project expertise tuned to specific parts of the work. Indeed the previous experience in traditional foods of EuroFIR (partners IFR, from UK, INSA, from Portugal, HHF, from Greece) and TRUEFOOD projects (SPES-GEIE, from Italy) were included in the work plan. UNIBO (Italy), the project's coordinating structure, covering almost all parts of the project, had expertise from 30 year research on local food plants.

Apart from this theoretical background and strategies in Consortium building, operatively the foreground of the above mentioned two previous EU funded project represented a reference for many BaSeFood activities. EuroFIR was taken as a reference for aspects of traditional food definitions, guidelines for traditional food documentation and sampling, in relation to a part of the work, guidelines for robust analytical data collection, concepts of recipe calculation and retention factor determination, food indexing. TRUEFOOD was considered in relation to approaches connected to consumers' issues and the point of view of industrial stakeholders.

The synthesis was a project's strategy aimed at scientifically studying bioactives of traditional foods by means of rigorous analytical and biological assays, but also considering a vast array of characteristics of traditional foods and consumers' issues, in order to put health promotion concepts in a favourable context, to be understood by people and exploited by processors.

The two somewhat strategic main challenges of BaSeFood, already presented in several occasions, were the following:

To create opportunities, by generating a base of knowledge, through research and development activities, from which SMEs and other stakeholders could derive transferable information for product development in a European regulatory context.

To create trust, especially in consumers, in order to enforce the synergy between the health promoting and the traditional food characteristics, and support direct perception of food value, also by addressing environmental, sustainability and equity issues.

The background idea has been, therefore, to produce high quality scientific data regarding phytochemicals contained in foods and their action, in a frame of promotion of general knowledge, awareness and enhancement of the local traditional foods in their context.

With this structure, the traditional foods of the Black sea region have represented the primary pool of information, that have been explored with the following aims, largely corresponding to project's work packages (WPs), although some activities were cross cutting and a strong interactivity among topics was in many cases established.

1. To investigate in detail a defined number of selected and representative traditional foods, for aspects connected to documentation, recipe preparation, flow charts, nutritional composition, phytochemical content.

This first line part included

- the documentation and description of traditional foods on the basis of already available information, in order to compile national traditional food files, and prioritise specific foods, for each country, to be further documented, prepared, sampled and analysed (WP1).
- the nutritional characterisation of the selected traditional foods (WP2)
- the microbiological profiling of the selected traditional foods (WP2)
- the investigation of a more limited sub-set of foods for potential health promoting properties by means of in vitro, in vivo and human intervention studies (WP3)
- the study of technological chain of traditional foods, including the characterisation of some raw materials for their content of bioactive compounds, the determination of their retention factors following basic unit operations, the study of traditional flow charts of selected traditional foods and the drawing of hypotheses for their updating, including consumers' evaluations of final products (WP4)

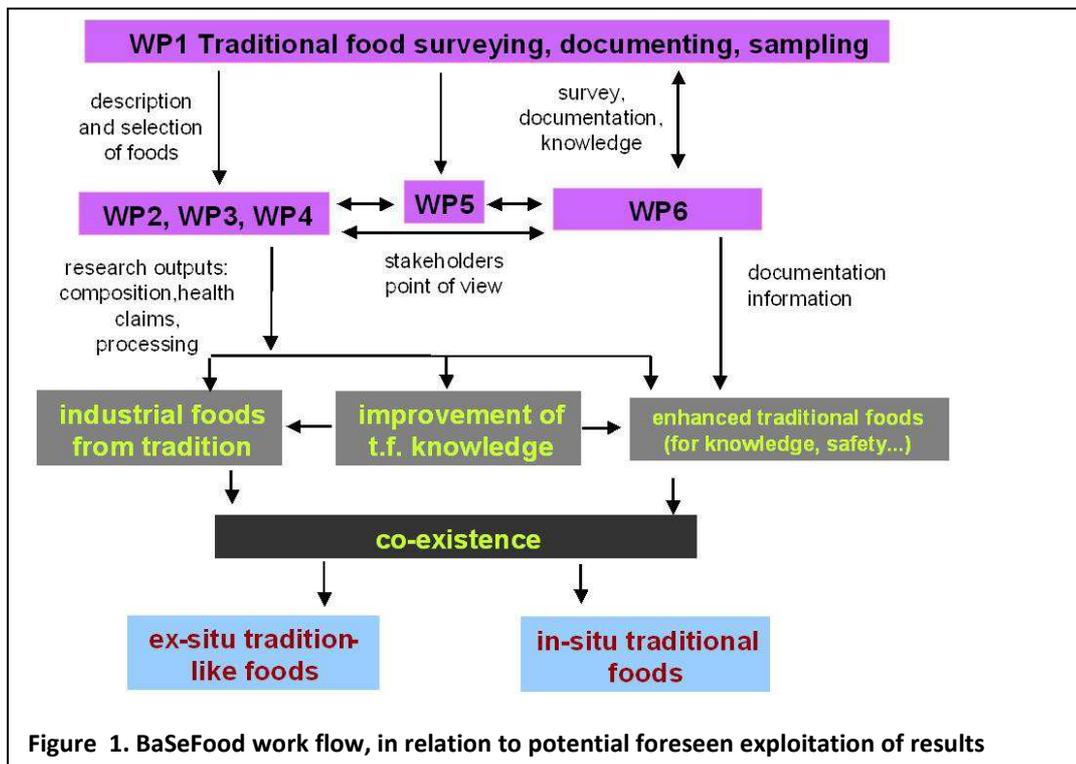
2. To carry out broader perspective surveys on traditional foods at regional and geographic scale, aimed at documenting traditional foods in connection to local plant resources, knowledge, ethnicity, geographic and social constraints, consumers perceptions and establish cross-cultural comparisons.

This second main line included

- qualitative broad range investigations aimed at documenting groups of foods of specific areas, carried out in a cross-national perspective; this line presented some common features with the above mentioned parts connected to the analysis of raw materials and the flow charts of selected traditional foods (WP1)
- preliminary consumers surveys and combined consumers/stakeholders surveys, carried out by means of targeted questionnaires, aimed at investigating the perceptions of traditional foods of the area and focussing of the perspective of traditional/health promoting foods (WP5).

Coordination and management, as well as dissemination activities, were aimed at assuring an overall unitary approach, enhancing the efficacy and global impact of the project.

Globally, a scheme of the foreseen interaction between the primary pool of information (traditional foods of the Black sea region), BaSeFood activities and potential foreground potential exploitation, is represented in Fig. 1.



Description of the main S&T results/foregrounds

1. Traditional food description on the basis of already available information, and selected traditional foods for further documentation and preparation (WP1)

Main activities

This part of the work was carried out by means of the description of traditional foods on the basis of information available on documented existing sources, in the six participant Black sea Area countries. The EuroFIR guidelines for traditional food documentation and description were used in this phase. The foods were categorised on the basis of the main plant ingredient into the following groups: cereal or cereal based foods, vegetable or vegetable based foods, fruit or fruit based foods, oilseeds or oilseed products, herbs, spices and aromatic plants, low or non alcoholic fermented foods and beverages of plant origin.

The work consisted in the compilation of excel spreadsheets, collating the relevant information on each food; the spreadsheet contained the following data:

Main sheet: Description (including name, ingredients and synthetic way of preparation) / origination of the traditional foods

Accessory sheets:

Traditionality of the food according to the EuroFIR definition; Consumption of the food or its wider food group; Availability of composition data; References supporting all the collated information.

The documented foods were prioritised on the basis of the accessory information, with special respect to consumption estimation and availability of composition data. A subset of foods (roughly one per each group, for each country) were then selected for further study and documentation.

The selected foods were:

- further documented for historic and folkloric traits
- a representative way of preparation was chosen, and its preparation flow chart registered
- the food was locally prepared and sampled, to be analysed

Main results

A total of 281 traditional foods were included in the Excel spreadsheets, representing the National Documented traditional food files: south Ukraine:26; west Ukraine: 53; Russian Federation: 37; Romania: 30; Georgia: 37; Bulgaria n:82; Turkey: 16.

After prioritisation, the 33 selected foods to be further studied were the following:

Cereal or cereal based foods

- Sour rye bread - Ukraine
- Buckwheat porridge crumby - Russian Federation
- Cornmeal mush (Mămăligă) - Romania
- Tsiteli Doli Bread - Georgia
- Baked layers of pastry stuffed with pumpkin (Tikvenik) - Bulgaria
- Bulgur pilaf - Turkey

Vegetable or vegetable based foods

- Ukrainian borsch - Ukraine
- Transcarpathian green borshch - Ukraine
- Vegetable okroshka (soup) - Russian Federation
- Nettle sour soup (ciorba) - Romania
- Nettles with walnut sauce - Georgia
- Rodopian dried beans soup- Bulgaria
- Kale soup -Turkey

Fruit or fruit based foods

- Uzvar (Compote) - Ukraine
- Watermelon juice - Russian Federation

- Plums jam (Magiun) - Romania
- Churchkhela - Georgia
- Rose jam - Bulgaria
- Fruit of the evergreen cherry laurel - Turkey

Oilseeds or oilseed products

- Roasted sunflower seeds - Ukraine
- Mustard oil - Russian Federation
- Flax oil - Georgia
- Tahan Halva - Bulgaria

Herbs, spices and aromatic plants foods

- Pomazanka - Ukraine
- Herbal dish - Romania
- Wild plum sauce - Georgia
- Mursal tea - Bulgaria
- Black tea - Turkey

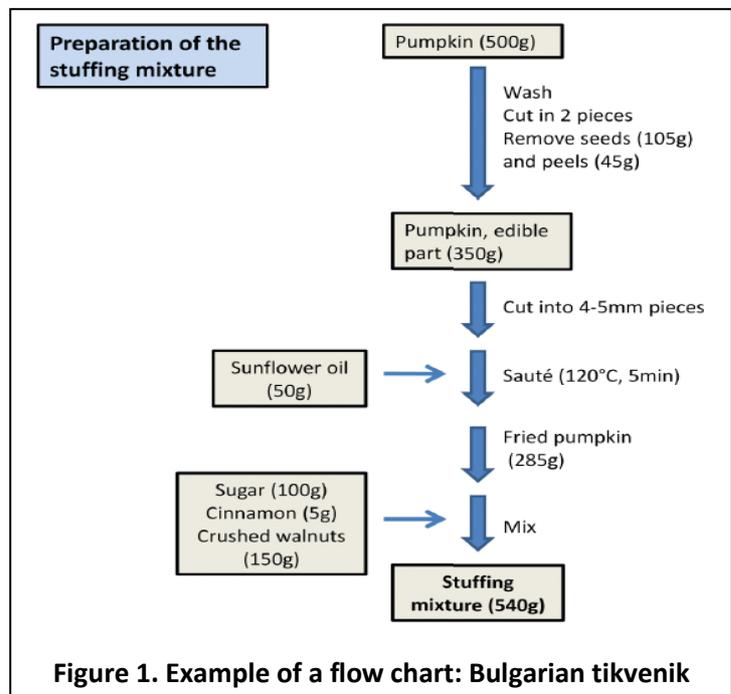
Low or non alcoholic fermented foods and beverages of plant origin

- Sauerkraut - Ukraine
- Kvass southern - Russian Federation
- Elderberry soft drink (Socata) - Romania
- Millet ale (Boza) - Bulgaria
- Sautéed pickled green beans - Turkey

A representative preparation scheme of each of the above mentioned foods was recorded and the flow chart represented (example in Fig. 1). This was the basis for the preparation of each food and its subsequent nutritional analysis (see the next point). The flow charts of preparation were collated in a specific project's deliverable.

Accessory historic and folkloric features for each selected foods were collected, and integrated in a report, together with the nutritional data and comments about the potential nutritional claims.

All this material represented a sample basis for possible actions towards proprietary claims of the specific foods at a national or European level (e.g. Protected Designation of Origin-PDO, Protected Geographical Indications-PGI, and Traditional Specialty Guaranteed-TSG) or health and nutritional claims.



2. Nutritional characterisation of the selected traditional foods (WP2)

Main activities

The 33 selected foods were prepared on place by local partners and sampled according to an agreed scheme. To assure the analytical quality of data, a form was prepared to harmonise the information collected by the partners in different countries, containing information about Food description (including name in English and in the original language and the scientific name in the case of primary foods); sampling plan (including date and time of collection, sampling point, process and preservation method and description of the ingredients) and sample handling (including mixing/homogenization method, storage/container; information about pool preparation, storage and transport to the laboratory).

Deep frozen samples were sent to the centralised lab in charge of nutritional analyses (INSA, Portugal).

Lyophilised or frozen samples were also sent to UNIBO lab for antioxidant analyses and phenolic profiling.

The following proximate and nutritional analytical determinations were carried out at INSA: water, ash, Total N - protein, total fat, individual fatty acids, starch, total dietary fibre and total sugars. Vitamins: vitamin B₂ (riboflavin), vitamin C (L-ascorbic acid), vitamin A, vitamin E (α-tocopherol), total folate (at IFR lab), mineral elements: sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, selenium. Additional analyses of carotenoids were performed (α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, violaxanthin, neoxanthin and lycopene). In all cases, officially recognised methods were employed, when available. Total phenolics and antioxidant capacity, by means of two methods, were determined at UNIBO. Antioxidant capacity was also normalised on average serving size.

Main results

All the 33 selected foods were analysed for their nutritional composition. The analytical data of each food from each country were represented by a series of four column charts (macronutrients, mineral, vitamins and the other components), as in the example in Fig. 2, For each compound, a summary chart was also drawn including all the analysed foods, in order to allow an easy comparison among foods for a specific nutrient, as in the example in Fig. 3,

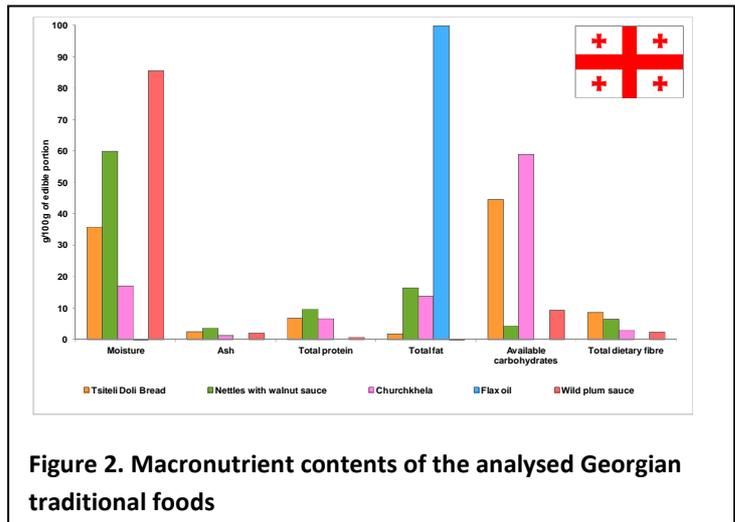


Figure 2. Macronutrient contents of the analysed Georgian traditional foods

Finally, Fig. 4 presents an example of the antioxidant power of some foods normalised on serving size.

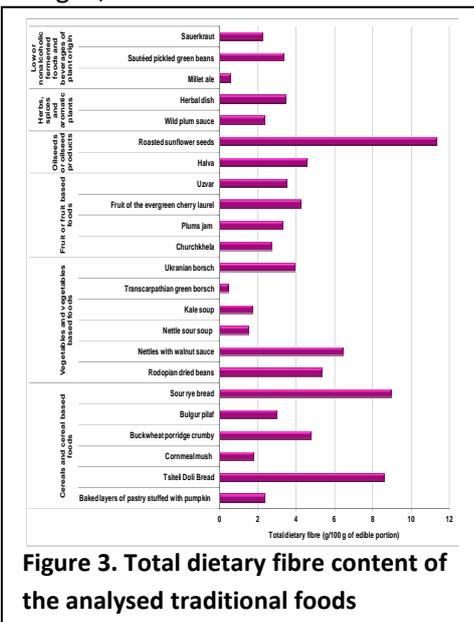


Figure 3. Total dietary fibre content of the analysed traditional foods

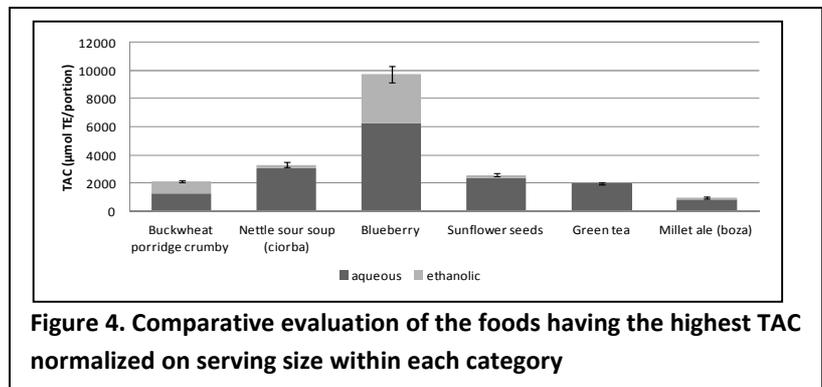


Figure 4. Comparative evaluation of the foods having the highest TAC normalized on serving size within each category

The analytical data allowed to detecting the ample variability of nutritional characteristics of the considered traditional foods, indicating that many of them could contribute at enhancing the diversity of diets. The analytical data were also considered, together with the estimation of the average portion size, to calculate the amount of each macro or micronutrient. This, as reported in the previous paragraph, was used to draw hypotheses of possible nutritional claims, taking however into account the intrinsic variability of traditional foods and the fact that the samples used were representative of a specific recipe.

3. Microbiological profiling of the selected traditional foods (WP2)

Main activities

The microbiological characterisation of foods and raw materials represented a preliminary exploration of foods otherwise little explored for this important character.

A total of 65 foods samples and 157 plants (ingredients), from the Black Sea area countries were sampled. A further distinction was made between the sources of the samples collected in Western Ukraine: street markets (mainly for raw materials), city markets, private households. The samples were taken according to product availability.

The microorganisms were identified and quantified according appropriate methodologies, and divided into the four groups: beneficial bacteria (for their role on gut function or antagonists of dangerous microorganisms), saprophytic or epiphytic of environmental origin (generally neutral for human health), contaminants of human origin (potentially pathogenic) and food borne pathogens.

Main results

The microbiological assays revealed differences in the distribution of microorganisms according to food types and locations. Of course, these results are preliminary, since a systematic sampling plan was not carried out.

As an example, Fig. 5 represents the percentage of the four micro-organism groups detected in Romanian samples.

As a whole, the amount of beneficial bacteria and microscopic fungi was usually less than that of tested groups; the most frequent organisms found were potentially pathogenic bacteria, followed by typical plant epiphytic bacteria; foodborne pathogens (B2) were only rarely found

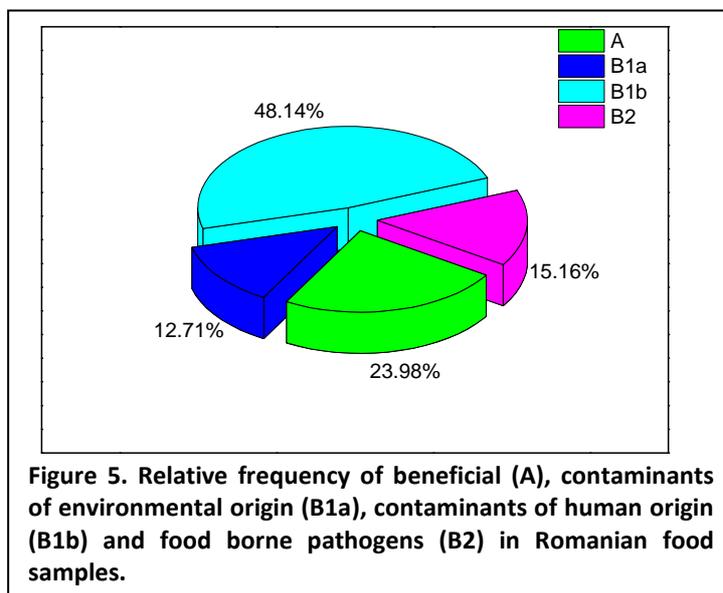


Figure 5. Relative frequency of beneficial (A), contaminants of environmental origin (B1a), contaminants of human origin (B1b) and food borne pathogens (B2) in Romanian food samples.

Samples collected from street markets were more contaminated with microorganisms

than the samples collected from city markets or taken from private yards/farms. The biological diversity of the microbial species was inherently characteristic of samples from street markets. Only a limited number of species was found in samples from city markets but these were isolated in significant titers.

The highest level of microorganisms was observed in green parts of plants and in roots (parsley, sorrel, dill) and the lowest was measured in fresh fruits (apples, pears) and vegetables (tomatoes, peppers, beans).

Some plants and fruits with reported endogenous antimicrobial effects were little not contaminated: wild plum, pomegranate, onion, garlic, basil leaves, sage leaves, and spices: poppy seeds, coriander, chili, fennel, mint, black sesame, saffron, bay leaf.

Among human origin micro-organisms, more frequently detected were different species of *Staphylococcus*, *Streptococcus* genera and various strains of *Enterococcus (faecalis)*, *Aerococcus viridans*, *Pantoea (agglomerans)*, *Serratia*, *E. coli*, *Klebsiella (pneumoniae)*, *Proteus (vulgaris)*, *Enterobacter (cloacae)*, *Acinetobacter baumannii*, *Bacillus cereus*, *Clostridium (carnis)*, *Candida albicans*.

Representatives of the B2 group (classical food-borne pathogens) included *Salmonella enterica*, identified in sorrel (Ukraine), *Shigella flexneri* ABC from kale, crop and green beans (Turkey), and *Listeria monocytogenes* from bread (Georgia).

The prepared foods were either not contaminated with any of the microorganisms or were contaminated with very low amounts (up to 10^2 CFU/ml) of *Bacillus subtilis*, *Staphylococcus epidermidis*, *S. aureus*, *Enterobacter cloacae*, *Enterobacter faecalis*, *Pantoea agglomerans* and *Trichosporon spp*.

There is a difference between microbial species isolated from industrial (street markets, restaurants) and homemade products. It was shown that only homemade or original recipes of *unfermented* foods - corba, herbal dish, original okroshka, pomazanka, and melon juice contained beneficial bacteria.

The richest source of beneficial bacteria is the fermented products. Some isolated strains proved to be interesting for further testing in order to be certified as potentially valuable industrial strains.

4. Investigation of a sub-set of foods for potential health promoting properties by means of in vitro, in vivo and human intervention studies (WP3)

This part of the work was devoted to assay, at different detail level, the potential or actual activity of some plant metabolites or plant extracts on human physiology functions, with special respect to oxidative stress, indicators of cardiovascular diseases risk and gut function, either directly or mediated by the effects of gut microflora.

Only in two cases, the 33 selected foods were directly used as matrices to be analysed. For all the other studies, a preliminary step was the identification of priority compounds and putative effects, by means of a combination of criteria, including : the list of main plant derived food ingredients of relevant Black Sea region traditional foods, the supposed concentration of some bioactive compounds, the available expertise in their analytical determinations, the supposed amount and prevalence in Black sea area traditional dishes, and their supposed effect on human vascular functions, that were considered as the priority field of investigation. Additional criteria were adopted from the selection of plants to be used in the tests connected to microbiological activity, including knowing effects of some micro-organism groups. On the basis of these criteria, specific foods, plants extracts or pure compounds were used in the different experiments carried out.

A synthesis of the experiment carried out and main results is hereafter illustrated.

4.a. Relations between in vitro antioxidant power and phenolic content

All the 33 selected prioritised foods, plus 6 additional plant raw materials, were tested for their antioxidant activity using 2 different assays, for total phenolic, hydroxycinnamic acid and ortopdihenol content. In each groups, some foods had quite high phenolic concentration and antioxidant activities.

Antioxidant activity was strongly related to total phenolic content, with phenolics being however not the only determinants of this activity. On the contrary, no clear effect of the two individual classes was detected

4.b. Platelet aggregation tests using the PFA-100 platelet function analyser

Activated platelets play a key role in the development of atherosclerosis by contributing to plaque formation within blood vessels during the early stages of atherogenesis. Platelet hyperactivity and hyper-aggregation are known to be apparent in disease states such as CVD as well as healthy people (middle aged and older) and people under stress. Optimal platelet function is therefore, an important tool in the prevention of CVD.

Six plant food extracts (dill, sideritis, kale, nettle, pomegranate and persimmon fruit) and human plasma conjugated metabolites of some of their bioactive components: quercetin, sulforaphane and sulforaphane cysteine glyceine were used. Whole blood samples were obtained from recruited donors.

The experiments were carried out according to appropriate protocols using, for the first time in this kind of studies a PFA -100 instrumentation, that simulates in-vitro platelet adhesion and aggregation, using whole blood in a functional assay , in standard conditions. A prolongation in PFA-100 closure time compared with a control indicates a lower threshold of risk. The results were expressed as percentage variation with respect to the control sample.

The control blood samples were in the range of closure time considered as normal. Overall, none of the tested plant extracts significantly increased the platelet closure time; a slight decrease was detected for

kale and dill. Plasma metabolites did not have significant effects and no differences were detected between specific plant extracts and their correspondent metabolites.

The main finding of this *in vitro* assessment was that none of the bioactive-rich plant extracts, or a series of human metabolites of selected major bioactives present in these extracts, exhibited relevant effects on platelet aggregation assessed using closure time as a functional measure of platelet activity (propensity to form aggregates) measured with a platelet function analyser (PFA-100).

4.c. Analysis of plant material

The selected plants to be used in further bioactivity assays (pomegranate, persimmon, *Sideritis scardica*, dill, nettles and kale) were sourced and analysed to characterise and quantify the content of phenolic compounds and glucosinolates (in kale).

The following bioactive compound types were identified for each respective plant extract: Dill - two phenolic acids and 9 flavonols; nettle - 6 phenolic acids, 6 flavonols and 3 anthocyanins; persimmon - 4 phenolic acids, 5 flavonols, 2 flavan-3-ols and 3 flavan-3-ol oligomeric derivatives; *Sideritis scardica* - 3 phenolic acids, 5 phenylethanoid glucosides and 16 flavonoids; kale - 9 phenolic acids, 21 flavonols and 8 glucosinolates; pomegranate - 2 ellagitannins, 2 ellagitannins derivatives 6 anthocyanins, 1 procyanidin, 1 flavan-3-ol.

4d. Effects of plant extracts on cholesterol metabolism and oxidative stress

This part of the experiments were carried out, as a general methodology, on cell systems, by means of the supplementation of cell cultures with selected extracts, in comparison to appropriate controls. Specific functions, on the basis of the target effect to be monitored, were measured. Cytotoxicity assays of the products employed were also carried out.

Cholesterol metabolism

As it is well known, the level of haematic cholesterol is commonly considered as an indication of potential cardiovascular risk. The control of cholesterol level by diet is considered as an effective prevention method, before reaching the need of using specific drugs (statins).

Effects of plant sterols

The effects supplementation by plant sterols (pure compounds), together with a control, a cholesterol and mevastatin supplemented were tested on rat cardiomyocytes cell model systems. Although dietary intervention with PS has been reported to have a lower effect than statin on cholesterolemia, in this study the effect of the bioactives on the reduction of intracellular cholesterol concentration was comparable to the drug (example in Fig. 6)

In cardiomyocytes, the mechanism of action for PS cholesterol lowering effect seems not related

to a modulation of key genes of cholesterol metabolism (SREBP1, SREBP2, HMG-CoAR and LDLR), but to a displacement of cholesterol from membranes. This leads to a reduced cell proliferative activity, as we already observed in a previous work that evidenced that the reduced activity was not accomplished to an increased apoptosis or to modification in the cell cycle. The data represent therefore a positive starting point for future investigation.

Effects of kale and dill extracts

Kale and dill are two plant ingredients often used in several Black Sea region foods. They have been sometimes indicated as potential

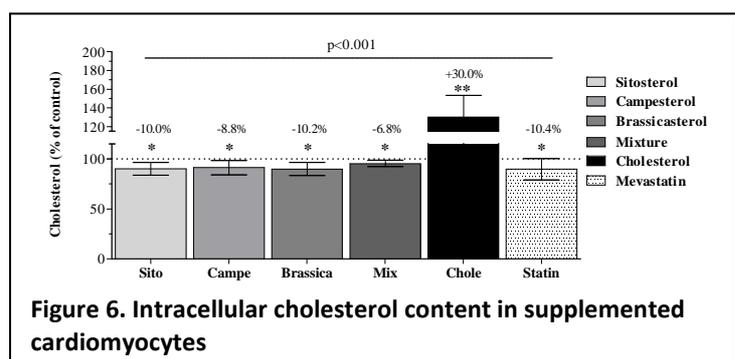


Figure 6. Intracellular cholesterol content in supplemented cardiomyocytes

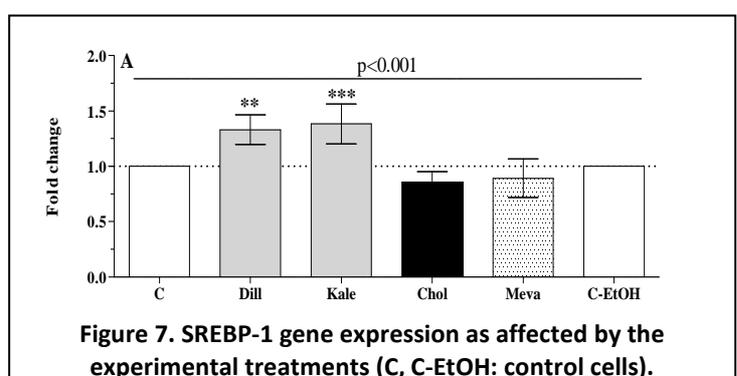


Figure 7. SREBP-1 gene expression as affected by the experimental treatments (C, C-EtOH: control cells).

regulators of cholesterol synthesis and metabolism.

Hepatocytes (HEPG2) cells were supplemented with kale and dill extracts. The gene and protein expression of four main genes related to cholesterol synthesis and metabolism (SREBP1, SREBP2, HMG-CoAR and LDLR) were monitored by comparison to control un-supplemented cells, also including cholesterol and mevastatin treatments.

An effect of both kale and dill extracts was detected in the expression of SREBP1 gene (Figure 7, as an example). Dill extract increased the HMG-CoAR protein expression, and kale extracts significantly increased LDLR protein expression.

In general, in our experiments cells were supplemented with relatively low amounts of the extracts, in order to mimic a nutritional effect and not a pharmacological one. With the intrinsic limitations of a model system study, it can be said that both plants revealed some effects on cholesterol metabolism, although the ones of dill are somewhat contrasting to literature data.

Counteraction of the oxidative stress

Most cardiovascular diseases (CVDs), as well as age-related cardiovascular alterations, are accompanied by increases in oxidative stress. For many plant food rich in bioactive phytochemicals, particularly flavonoids, there is increasing evidence of benefits on cardiometabolic risk that are linked to their potent antioxidant power.

Sideritis experiments

Sideritis scardica, a herb used to prepare herbal teas in the Balkans and pomegranate, a widely used fruit in the Caucasian food tradition, were used in this study.

In a first study, cultured liver cells (HepG2 cells) were submitted to an exogenous oxidative stress by adding *tert*-butyl hydroperoxide (*t*-BOOH) either un-supplemented or supplemented with *Sideritis* extracts or green tea or alpha tocopherol controls.

The data indicate a similar effect of *Sideritis*, green tea and tocopherol on indicators of oxidative stress counteracting mechanisms in stressed conditions. (see, as example Fig. 8).

Although *Sideritis* extracts had a 5-fold lower total phenolic concentration than green tea, it seems however to exert promising antioxidative effects of cell systems, likely connected to its peculiar flavonoid composition.

Pomegranate experiments

The experiments with pomegranate extracts were carried out with similar methodologies as reported for *Sideritis*, also including a range of concentrations. The experiments were carried out with rather low concentrations, since higher concentrations revealed some cytotoxic effects, in the chosen model system. In these conditions, pomegranate extracts did not show any protection against oxidative stress.

4e. Effects of plant extracts on TNF α -induced ICAM and VCAM

Atherosclerosis, a major cause of cardiovascular disease (CVD), is regarded as a chronic inflammatory disease and can lead to a number of vascular complications such as myocardial infarction and stroke. Risk factors may lead to an increased expression of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) contributing to the possible formation of vascular plaques.

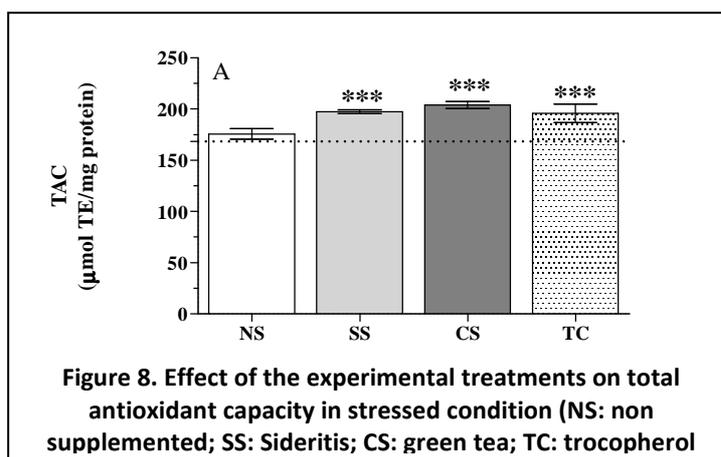


Figure 8. Effect of the experimental treatments on total antioxidant capacity in stressed condition (NS: non supplemented; SS: Sideritis; CS: green tea; TC: tocopherol)

Plant polyphenols have been often indicated as potential protective factors. In this part of the experiment, the ability of six extracts (dill, kale, nettle, persimmon, pomegranate and sideritis), the anthocyanins cyanidin-3-glucoside (Cyn-3-Glc) and delphinidin-3-glycoside (Del-3-Glc), the anthocyanidins cyanidin (Cyn) and delphinidin (Del), found in pomegranate, and their metabolites, protocatechuic acid (PA) and gallic acid (GA), and quercetin, as a control, to suppress the increase in surface expression of ICAM-1 and VCAM-1 induced by TNF α in human umbilical vein endothelial cells (HUVEC).

Preliminary experiments indicated that the nettle and persimmon extracts had detrimental effects on the HUVECs when incubated for the time period required, and this precluded us from assessing their effects on ICAM and VCAM. Therefore, only four of the six extracts (dill, kale, pomegranate and sideritis) were finally tested.

The effect of the four plant extracts was affected by substantial variability that did not allow to evidence significant effects, despite a trend to a linear positive effect of kale extracts. Quercetin had a substantial and significant effect in suppressing TNF α -induced ICAM and VCAM expression (see Fig. 9). None of the tested pure plant compounds had any effect.

It seems therefore unlikely that any cardiovascular benefit associated with consumption of these plants is due to modulation of adhesion molecule expressed by endothelial cells in response to inflammatory mediators.

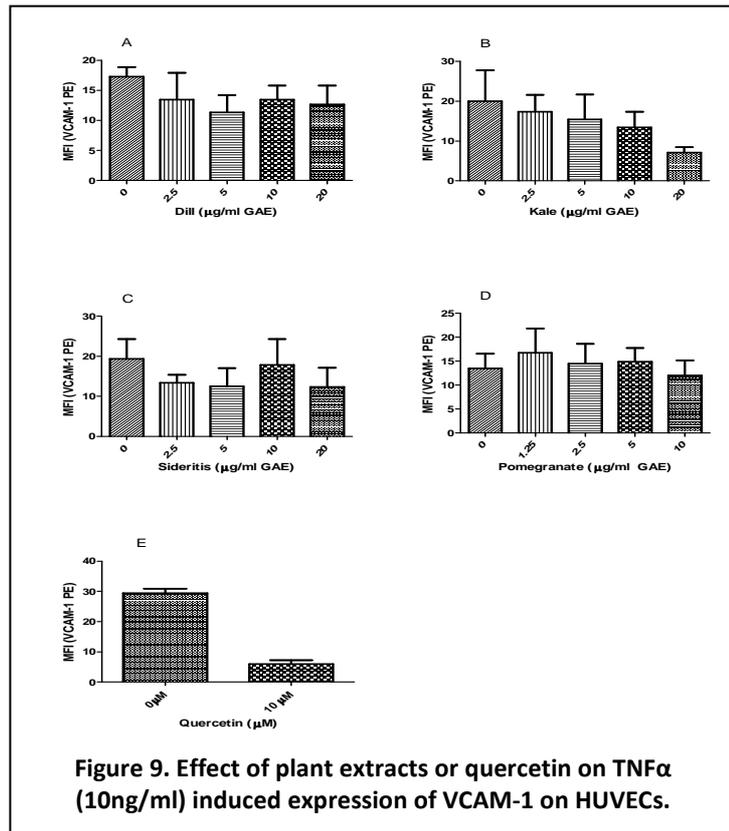
4f. Effects of plant extracts on vasomodulatory factors and investigation of the underlying mechanisms

The control of arterial and venous blood flow is likely to play an important role in the development or control of atherosclerosis and cardiovascular diseases. Blood flow is directly affected by constriction (tone) and dilation of veins and arteries, which, at an intracellular molecular level, is controlled by the nitric oxide (NO) signalling pathway. Endothelial nitric oxide synthase (eNOS) is responsible for the production of NO. An important regulator of eNOS activity is the kinase Akt, which phosphorylates eNOS (p-eNOS) at Ser1177, increasing NO production. Akt itself becomes activated (p-Akt) through phosphorylation of a serine residue, Ser473.

There are already numerous studies showing increases to endothelium-dependant vasodilation after treatment of cells or tissues with extracts of polyphenol-rich plants.

The effects of some plant extracts and pure compounds on the genic and related enzymatic mechanisms controlling vasomodulatory factors were carried out in this part of the research.

Cultured human umbilical vein cells were supplemented with extracts of Sideritis, kale, nettles, dill, persimmon, pomegranate extracts, quercetin and quercetin metabolites, sulforaphane and sulforaphane metabolites, sinigrin and allyl-isothiocyanate. Key enzymes and their phosphorylated forms were detected by appropriate methodologies.



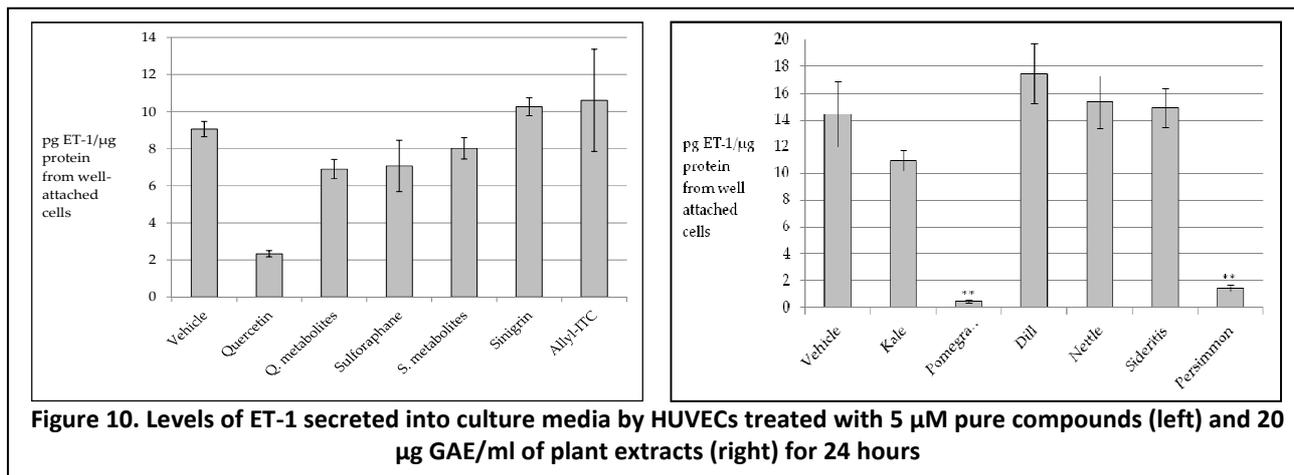


Figure 10. Levels of ET-1 secreted into culture media by HUVECs treated with 5 μM pure compounds (left) and 20 μg GAE/ml of plant extracts (right) for 24 hours

In terms of the effects on vasomodulatory factors, our results indicate that both quercetin and a mix of quercetin metabolites and persimmon and pomegranate extract, reduced levels of ET-1 secreted into cell culture media (Fig. 10). In addition, the pomegranate and persimmon extract increased AKT and eNOS phosphorylation. The pomegranate extract also caused a decrease in intracellular eNOS protein over 24 hours. It is clear that pomegranate and persimmon both contain compounds able to affect nitric oxide signalling.

Further investigations were carried out to elucidate the possible mechanisms of action of quercetin and pomegranate extracts. Although further evidence is needed to determine the exact molecular mechanisms determining these effects, it seemed rather clear that procyanidins were the main compounds involved in the bioactivity of pomegranate extracts on the investigated functions.

4.g. Pro / antimicrobial activity and effect of BSAC foods / extracts on microbiota and gut host immune function

The scope of this part of the work has been the investigation of the effects of food or plant extracts on micro-organism groups and the analysis of the effect of selected plant extracts on potentially positive physiological functions, studied on human cells and animal models.

Pro- and anti-microbial properties of traditional foods (TF) and plants ingredients

A total of over 100 foods, most of the 33 selected foods, and their main ingredients, and additional plants extracts were tested for their inhibitory or stimulatory properties on pathogenic or opportunistically pathogenic bacteria and beneficial bacterial species to human host.

Eleven beneficial or commensal, ten pathogenic and six food borne pathogens micro organisms were selected for testing.

In a second stage the dose-response effect of extracts that revealed some effect were tested.

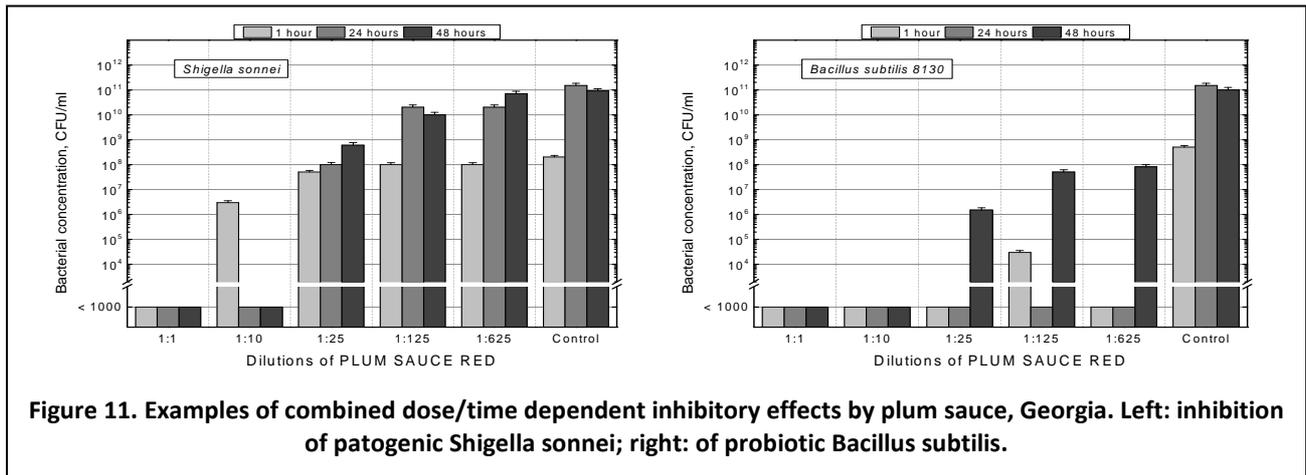
All the plants or food extracts were classified on the basis of their inhibitory or stimulatory effects on different micro-organism categories, with a prevalence of inhibitory effects.

Positive effects (only stimulation of useful microorganisms) were observed from fresh prepared plant juices and some foods.

In other cases, combined positive and negative effects were detected. As examples: a) stimulation of *Saccharomyces cerevisiae* by fresh cherries and buckwheat porridge, however coupled with stimulation of *Candida* and *Morganella morganii*; b) strong inhibition of several harmful bacteria by The Georgian plum dressing (green and red), sauerkraut (a traditional Ukrainian fermented food), Socata and Boza (Bulgarian and Romanian fermented drinks), but accompanies by a similarly strong inhibition of gut microbiota.

Other effects were less evident or somewhat contrasting

All the detected effects were plant-specific, dose-specific, strain-specific, and time of exposure dependent (see Fig. 11, as an example).



As a final result, a preliminary database of pro- and antimicrobial properties of typical BSAC foods and its major plants components was prepared.

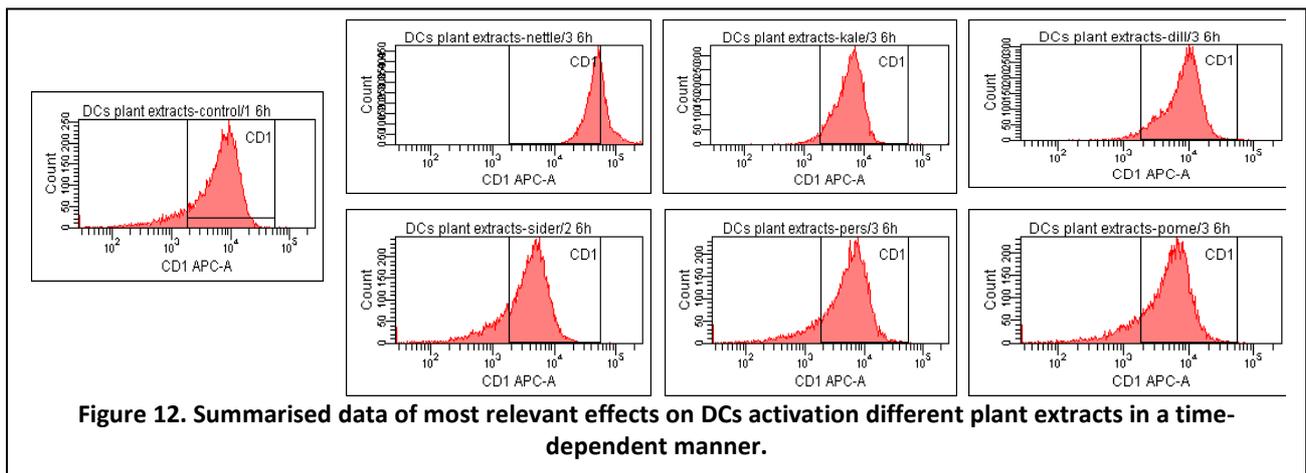
Analysis of ability of plant extracts to activate human dendritic cells DCs in vitro

There is a lot of interest to the activation of human DCs issue, for the role they have either directly or via the produced cytokinines, as pro or anti inflammatory factors.

This part of the work was investigation of the direct effect (immunomodulatory effect) of food and plant extracts (acting as food antigens, Ags) on functions of human DCs (acting as Ag presenting cells) via detecting their phenotype changes (cells markers CD 83 and CD1a+) and indirect stimuli measurements by estimating the levels of produced cytokines by activated DCs after different exposure (6h and 24h). The targeted extracts were from: nettle sour soup, herbal dish, Sideritis mountain tea and plants: kale leaves, persimmon, pomegranate, dill and nettle.

Human dendritic cells were obtained from monocytes from blood of appositely recruited donors, by means of a consolidated protocol.

Two functionally and phenotypically distinct subsets of moDCs: pro-inflammatory CD1a⁺ and anti-inflammatory CD1a⁻ cells had been detected by. The results showed that the tested plant extracts affected the differentiation and activation of DCs in a plant-specific and time-dependent manner. A summary of the obtained patterns is reported in Fig. 12.



Nettle was able to stimulate significantly the expression of CD1a⁺ (10 times and more high amount of cells compare to the control); CD1a⁻ cells were not detected at all. On the same time it is lowering the expression of CD80. Kale is acting similar but had not demonstrated so strong influence. Exposure DCs to extract from dill led to increasing pro-inflammatory CD1a⁺ and decreasing of anti-inflammatory CD1a⁻ cells, when kale act opposite – lowering the amount of pro-inflammatory CD1a⁺ and stimulates developing of anti-inflammatory CD1a⁻ cells. Pomegranate and persimmon down regulating both subsets of moDCs: pro-

inflammatory CD1a⁺ and anti-inflammatory CD1a⁻ cells. Finally Sideritis rather not influencing at all on the DCs *in vitro*, but still had trend to act similar to the pomegranate and persimmon extracts.

On DCs model we have clearly indicate - which plants extracts and when can induce pro or anti-inflammatory response detected by FACS via expression of CD1 + CD80+ markers nettle significantly affected on expression of pro-inflammatory CD1a+ cells compare to the control. Dill and kale demonstrated similar but less strong influence while Sideritis lowered the expression of CD1a+ on 6h but not on 24 h of incubation.

Our results suggests that dill (6 and 24 h), nettle (6 h) and kale induced IFN-g secretion. IL-4 was stimulated mainly on 6th hours of exposure and mainly by nettle and kale, and less significant but more stable effect was caused by Sideritis while pomegranate is effecting on this cytokine production only a little. IL-2 was selectively stimulated by persimmon and on 24 hours after DCs exposure. And TNF- α was dramatically increased after DCs exposure to kale, dill and nettle (6 h) and less altered by exposure of pomegranate, persimmon and Sideritis. IL-b pro-inflammatory cytokine also known as catabolin and as precursor for caspase 1 was selectively stimulated by dill and nettle and persimmon and Sideritis, but not by pomegranate and kale.

As as whole, interesting anti-inflammatory effects were detected for some plants extracts in this model system.

Potential of plant extracts to modulate host immune defense and regulate the key representatives of gut microbiota in animal models

This part of the work was devoted: a) at testing the effect of plant extracts on the equilibrium of gut microbiota in mice, by means of the monitoring of bacterial strains in the feces; b) testing the regulation of immunoresponse, by means of analysis the immunomodulatory cell types and cytochines in blood samples.

The research was carried out by adopting a series of approaches and appropriate methodologies.

Particularities of systemic vs. local, humoral vs cellular immune responses in mice under influence of plants' extracts were determined by means of Fragment culture (FC) and Fluorescent activated cells sorting (FACS). The effect of plants extracts on gut microbial homeostasis after their 14th day oral administration has been clarified by routine time points examination of key microbial representatives in mice feces and colon content. All the cytokines in mice fragment culture of Small intestine – SI - (Duodenum, Jejunum, and Ileum), Peyers' Patches (PPs) and mesenteric lymph nodes (MLNs), serum and supernatant of DCs were detected by Enzyme-linked immunosorbent assay (ELISA).

Lactobacilli were not significantly affected by dill and Sideritis; and were stimulated by pomegranate, inhibited by kale and persimmon at different time points.

Klebsiella pneumoniae and *Enterobacter cloacae* were eliminated from mice colon by feeding of extracts of Sideritis, dill, nettle and persimmon but not pomegranate and kale.

Enterococcus faecium and *E. faecalis* were stimulated significantly by pomegranate. *Bifidobacterium bifidum* and *B. longum* were increased only in colon of mice fed with extracts of Sideritis, pomegranate and kale.

Interestingly, commensal *E. coli* was not affected by any of tested plants' extracts.

All plant extracts promoted *Candida albicans* persistence in mice colon; only kale lowered on 7-14 d.

Plants' extracts differently regulated IgA secretion by splenic and peritoneal cavity B1/B2 cells and changed the ratio of CD45/CD45RB, T4/T8/NK and CD4(+)CD25(+) regulatory T cells (Treg cells) in peritoneal cavity, mesenteric lymph nodes, Payers' patches and spleen.

Various extracts had different effects on cells of peripheral blood of mice. From the first day it was noticed that persimmon and pomegranate increased of amount of granulocytes compared to other tested extracts and control samples. All investigated extracts increased lymphocytes and monocytes, and causing so called "shift of blood formula to right". The most active in this respect are extracts of Sideritis, nettle and kale.

Among cytokines, TNF-a, and IL-2 increased both systemically and locally in the different gut compartments by the effect of dill, nettle and Sideritis only at mucosal sites. IL-2, but also IL-10 and IL-12, IFN-g, and IL-17 but not TNF-a were stimulated at different levels by pomegranate, persimmon and kale, both systemically and locally.

No harmful influence of tested plants was observed. On the basis of the investigations, most beneficial properties appeared to be linked to persimmon and then pomegranate and kale. *Sideritis* rather show no significant influence on all the studied indices while the nettle and dill are acting as pro-inflammatory.

4.h. Effects of bioactive-rich BSAC traditional foods on CVD risk

This part of the research work was organised in two groups of studies carried out respectively in Serbia and Ukraine

Serbian research

The study was performed within three consecutive phases including preclinical screening of anti-platelet effects of methanol extracts of dill, nettle, *Sideritis*, kale, persimmon and pomegranate and four bioactive metabolites of their major constituents; investigation of acute anti-platelet effects of hot-water infusions of nettle, dill and mountain tea (*Sideritis scardica*) in the second study and investigation of chronic effects of pomegranate juice consumption in the third study.

Preclinical screening of anti-platelet effects

The aim of the study was to investigate anti-platelet effects of bioactive-rich extracts of selected dietary plants and isolated bioactive metabolites in *in vitro* settings. The purpose was to select the most promising candidates for potential use in the human intervention trials described further on.

Blood was collected from appositely recruited donors either healthy or at CVD risk. Platelet surface P-selectin and GPIIb/IIIa expression and platelet-leukocyte aggregates were measured by full-blood flow cytometry according to a previously published protocol. Whole blood samples were treated with methanol extracts of dill, nettle, *Sideritis*, kale, persimmon and pomegranate and four bioactive metabolites of some of these extracts before stimulation with the platelet agonist, arachidonic acid

The experiments showed that the examined plants could be effective in modulation of platelet function. Prioritizing P-selectin expression as the main outcome of the study measured in subjects with metabolic syndrome, we narrowed the list to nettle and dill. Additionally, *Sideritis scardica* extract was also prioritized.

Investigation of acute anti-platelet effects of hot-water infusions of nettle, dill and mountain tea (Sideritis scardica)

The main aim of this study was to investigate the effects of acute consumption of dill, nettle and mountain tea hot water infusions on platelet function using flow cytometry.

The experiment was designed as randomized parallel single-blinded controlled intervention study. Hot water infusions of *Sideritis*, nettle and dill were consumed by selected volunteers. Blood samples were collected before and two hours after consumption. Platelet activation markers and platelet aggregates were determined in basal conditions and under the effect of platelet agonists

Rather complex effects of extracts were detected. The results of an acute study showed that all three infusions had significant effects on platelet activation compared to the control and that dill extract exerted the most profound effect shown in different experimental conditions. Adenosine-diphosphate induced *ex vivo* activation was more susceptible to inhibitory effects of investigated plants, than activation induced by arachidonic acid. Platelet aggregation was not influenced by the consumption of either of three investigated extracts in defined experimental setting.

Effects of 6-week of pomegranate juice consumption on platelet function in subjects with metabolic syndrome

The aim of study was to investigate the effects of one selected food (pomegranate juice) on markers of platelet function by flow cytometry, after the long term consumption.

The experiment was designed as randomized parallel single-blinded controlled intervention study.

Total number of 50 subjects meeting the study criteria (subjects with indicators of metabolic syndrome) was randomly assigned to one out of the two treatment groups:

A: Pomegranate juice study group ; B: Control study group

During the 6 week intervention period, subjects in the PJ group consumed 300 ml of pomegranate juice daily.

At the start and the end of the period, blood and urine samples were taken.

The long term consumption of pomegranate juice significantly decreased both activation markers in all experimental conditions, simultaneously inhibiting platelet-monocyte aggregation. Platelet neutrophil aggregation was not influenced by the long term consumption of investigated juice.

As a general conclusion, inhibition of platelet activation shown for most of the investigated extracts suggests that this could be one of numerous mechanisms of beneficial effects of bioactive-rich plants and traditional foods in prevention of cardiovascular diseases. Based on the results of both studies consumption of these plants within regular diet should be an optimal approach targeted to the primary or secondary rather than tertiary prevention of CVD.

Ukrainian research

The study conducted at the Zakarpattya Regional Clinical Cardiology Dispensary (UZHNU, Ukraine) investigated the effects of long-term consumption of mountain tea (*Sideritis scardica*) and nettle hot water infusions on endothelial dependant flow mediated vasodilatation and other risk markers in subjects at high risk of CVD.

The study was a randomized, parallel design (3 groups) clinical trial investigating the effects of mountain tea (*Sideritis scardica*) and nettle (*Urtica dioica*) on endothelial dependant flow mediated vasodilatation and other risk markers for CVD. Subjects were randomized to receive either a) 2 g dried mountain tea infused in 200 ml hot water b) 3 g dried nettle infused in 200 ml hot water or c) 200 ml hot water as a placebo control. After 6 weeks ingestion of nettle and mountain tea by subjects at high risk of CVD and with manifested CVD, neither treatment beneficially affected flow- mediated vasodilatation. Furthermore, there were no beneficial effects of the interventions on blood pressure and lipid profile.

5. Study of technological chain of traditional foods (WP4)

This part of the study was centred on two of the main concepts emerged during the building of BaSeFood research strategy:

- the evaluation and promotion of the values of food plant biodiversity;
- the determination of retention factors of key bioactive components, in simple traditionally used unit operations or production flow charts
- the analysis and study of traditional flow charts of selected traditional foods and the drawing of hypotheses for their updating, including consumers' evaluations of final products

Preliminarily to all phases of the experimental research, literature analysis, integrated with previous knowledge and expertise of participants, and opportunities to interact with local stakeholders, allowed the choice of plants, unit operations and flow charts to be studied in detail. Some choices about the topics to be investigated, also derived from inputs from the broad range on-field surveys, referred to in section 6, with which this part of the work had close interactions.

5.a. Evaluation of food plant biodiversity

Kales and primitive forms of wheat (especially emmer and einkorn, but also other Triticum species), were targeted as subject for analyses, since variability of types and uses were detected during on field surveys.

Kales

Kale populations have been sampled on place in Italy, Portugal and Turkey (25 samples). Seeds were also retrieved and a comparative experimental trial was planted in Italy (15 samples). Glucosinolates, phenolics, carotenoids and chlorophylls were determined. Interesting variability was detected, with samples from the experimental trial richer in carotenoid (see Fig. 13, as an example) and chlorophylls, as a result of the higher soil fertility. Glucosinolates, on the contrary, were rather more abundant in samples retrieved on field. Differences between origin were detected, with Portuguese kales containing relevant amounts of aliphatic sinigrin, and Turkish an Italian samples characterised almost entirely by indolic glucobrassicin.

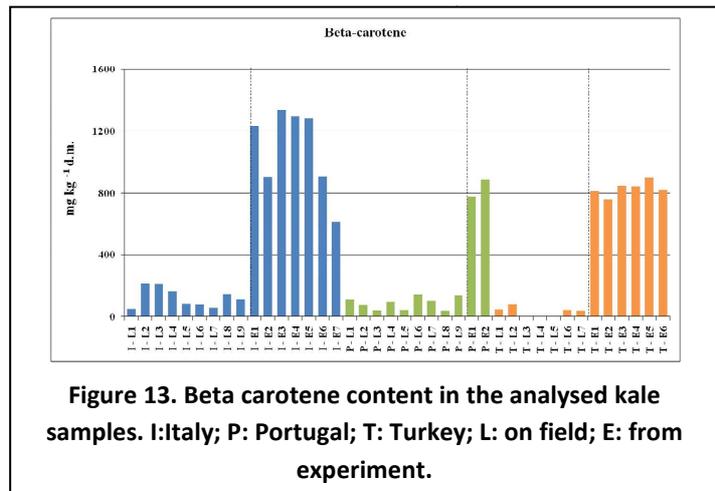


Figure 13. Beta carotene content in the analysed kale samples. I:Italy; P: Portugal; T: Turkey; L: on field; E: from experiment.

Primitive wheats

Seeds of 27 accessions of the genus *Triticum* were sampled, including: 7 from Georgia (*Triticum monococcum*, *T. turgidum* subsp. *dicoccum*, *T. timopheevi*, *T. palaeo-colchicum*, *T. macha*, *T. turgidum* subsp. *durum*); 7 from Italy: (*T. monococcum*, *T. turgidum* subsp. *dicoccum*); 4 from Turkey (*T. monococcum*, *T. turgidum* subsp. *dicoccum*); 4 from Armenia (*T. turgidum* subsp. *dicoccum*); 1 from Bulgaria (*T. turgidum* subsp. *dicoccum*); 4 commercial durum and bread wheat varieties, used as controls.

The seeds were planted in Italy bon late fall and spring sowing sates. the kernels were harvested and subject to the analysis of phenolics, carotenoids, lipids, phytosterols, tocols.

Between and within species variability was detected for most the analytical characters, Einkorn wheat generally resulted richer in carotenoids, whereas distinct sterol and phenolic patterns were detected. This variability could be exploited for the isolation of specific raw materials. The species were effectively discriminated by the complex of analytical traits (see Fig. 14).

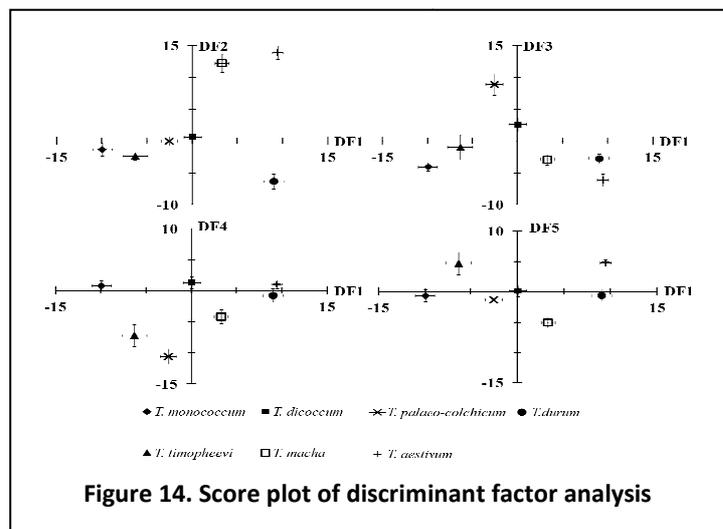


Figure 14. Score plot of discriminant factor analysis

5.b. Determination of retention factors of key bioactive components

Water cooking of kales. Three kale populations were investigated by water and vapour cooking, with cooking times of 5, 10, 15, and 20 min. Carotenoids retention was almost unaffected, whereas chlorophyll content was affected by thermal and hydrolytic degradation. The main results showed: a) a consistent migration of phenolics and GLS from kale leaves to boiling water; b) phenolics were more resistant than other compounds to

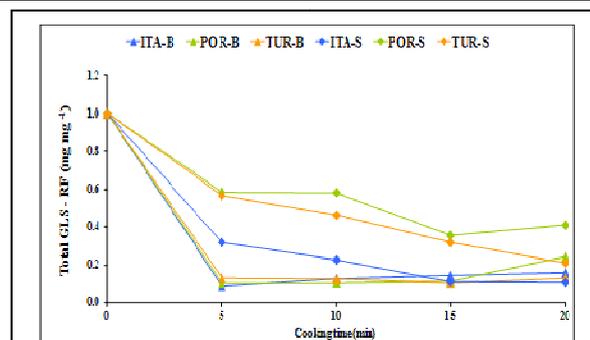


Figure 15 .Pattern of retention factors (RF) for total glucosinolates (GLS). IITA, POR, and TUR: Italian, Portuguese, and Turkish samples. B and S: boiled and steamed samples.

chemical degradation during cooking; c) steaming led to a faster chemical degradation of GLS (see Fig. 15, as example) and chlorophylls in comparison to boiling owing to leaf exposure to oxygen. The experiment allowed for the first time the determination of retention factors of the examined bioactives in kale cooking.

Yield and retention factors in traditional processing of emmer and einkorn wheat

Traditional glume removing process was compared by on-site sampling of einkorn wheat bulgur, in Turkey and emmer wheat grains in Armenia and Italy. The traditional processing flow charts were described (see Fig. 16, as an example). All the processing fractions were sampled and subsequently analysed to determine the content and retention of some bioactive compounds (phenolics, lipid associated compounds: tocopherols and sterols, and carotenoids), with respect to whole grain.

Specific yield factors were determined, revealing some differences between processing schemes. The retention of specific bioactives was linked to the part of the kernel that was lost, with particularly high losses of the lipid connected components (sterol, tocopherols), in processed grains, in which most part of the embryo was lost. Schematic flow chart of a traditional processing still used in Turkey for hulled wheats

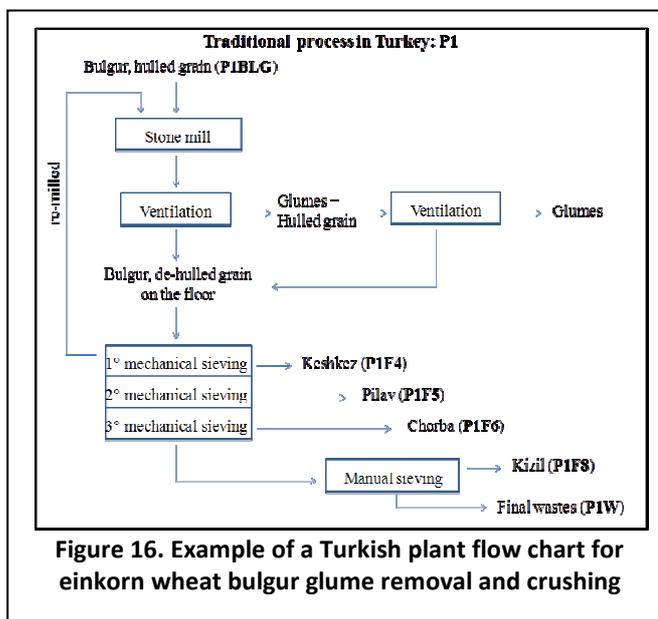


Figure 16. Example of a Turkish plant flow chart for einkorn wheat bulgur glume removal and crushing

Retention factors in emmer water cooking

Two landraces of *Triticum dicoccum* from Italy were selected: a winter type from Garfagnana (Toscana region), for which we considered whole and pearled grain, and a spring type from Monteleone di Spoleto (Umbria region), for which we had whole, pearled and crushed grain. The samples were subject to boiling in water and four cooking times were employed: 20, 40, 80 and 120 minutes for the whole grain; 20, 40, 60 and 80 minutes for the pearled grain; 20, 30, 40 and 60 minutes for the crushed grain.

Phenolics, lipid associated compounds: tocopherols and sterols, and carotenoids were determined in raw and cooked material. Carotenoids (Fig. 17, as an example) and tocopherols were partially affected by boiling in water, especially considering the whole and the crushed fractions, while for sterols a decrease was found only in crushed grain. Free phenolic compounds decreased rapidly showing the lowest retention factors in pearled wheat. Non-polar components were not found in cooking water, whereas we could identify various phenolic acids belonging to the bound fraction. Retention factor ranges for each category of compounds were the following: tocopherols: 0.61-1.08; carotenoids: 0.61-1.00; sterols: 0.72-1.09; free phenolics: 0.36-1.00; bound phenolics: 0.73-1.13.

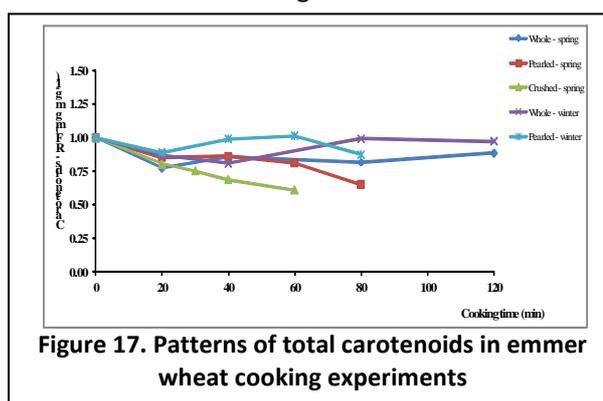


Figure 17. Patterns of total carotenoids in emmer wheat cooking experiments

Oxidative reactions, and bioactive compounds evolution and retention in oilseeds during the traditional processing from raw materials to halva

Seven raw oilseeds (peanuts, almonds, walnuts, hazelnuts, sesame seeds, sunflower seeds and pumpkin seeds), the same roasted seeds and tahin paste and halva obtained by them were considered. Tahin and halva samples were followed during shelf life after (0, 1.5, 3, 6, 9, 12 months). All samples were characterised for their lipid and phenolic bioactive compounds (fatty acids, sterols, tocopherols and

polyphenols), oxidation (peroxide value, conjugated dienes and trienes) and antioxidant capacity (ABTS^{•+} assay).

The results showed big variability depending on the kind of seed; in general fatty acid composition did not present particular differences among products, whereas for many oilseeds sterols and phenols (Fig 18, as an example) it showed slight increase in total amount during processing, probably due to the liberation of bound compounds during some unit operations. Also the oxidation evolution differs from seed to seed and in particular most of the samples showed more peroxides after roasting and a decrease of them in halva.

With respect to the shelf life evaluation phenols content and antioxidant capacity showed a big variability depending on the kind of raw material and probably due also to the presence of interferences. Sometimes, mainly in tahin samples, phenols increased during storage and this result could be due to a liberation of bound compounds or to the breaking of complex phenols. Peroxide value (PV) reflects the typical gaussian trend of these primary oxidation products and, it was high despite the elevated phenolic content and antioxidant activity due to a non interaction between antioxidant compounds and lipids. The same trend was reported by the conjugated dienes (K232) with a slight increase during storage; whereas conjugated trienes (K268) showed different trends with also higher values in halva samples than their corresponding tahin for all the shelf life steps.

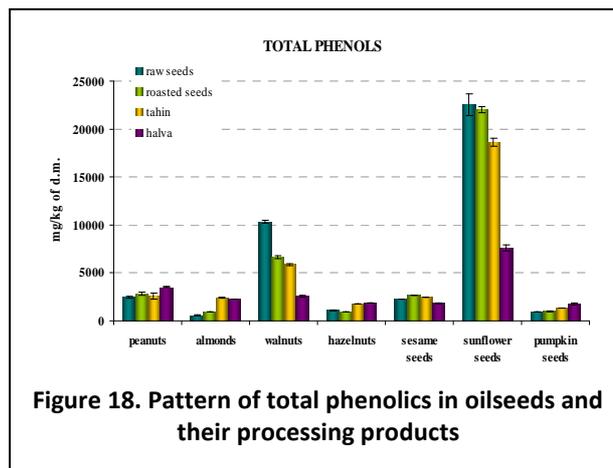


Figure 18. Pattern of total phenolics in oilseeds and their processing products

Buckwheat bioactive retention as a function of grain type and cooking temperature.

Unsteamed high quality buckwheat and buckwheat pre-processed were used to prepare kasha by ONAFT Ukrainian partner in two cooking regimes: low flame and with high heat. Dietary fibre content was similar and almost unchanged by cooking. Phenolic content increased by cooking in unsteamed buckwheat. Carotenoids were in very low amounts and decreased with cooking, in unsteamed buckwheat.

Other determination of bioactive retention were done in the technological experiment dealing with flow chart modifications and are dealt with in the next section.

5c. Analysis and study of selected traditional food flow charts and drawing of hypotheses for their updating, including consumers' evaluations of final products

This part of the work was the more typically technology addressed and was organised in different parts of specific subjects

Studies on the technology of tahin halva, including the possibilities of enrich it with new ingredients.

Tahin Halva is a traditional product, from Middle East to the Balkans, made of oilseed pastes and textured sugar. Sesame and sunflower are the most commonly used oilseeds. A series of experiments of tahin halva production were carried out by the Bulgarian BaSeFood partner.

Effects of roasting on tahin characteristics

Two sunflower, one peanut and one sesame sample were subject to roasting at different times, up to 120 minutes. Humidity, free acidity, phospholipids and carotene contents were determined.

the patterns were very similar in all seeds, and

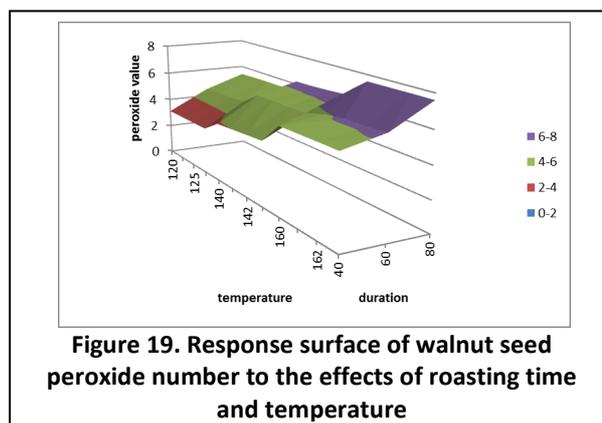


Figure 19. Response surface of walnut seed peroxide number to the effects of roasting time and temperature

allowed to determine the kinetics of the components evaluated as a function of roasting duration.

A further experiment was carried out with walnuts, that were roasted at three temperatures: 100, 140, 180 °C, for 40,60 and 80 minutes. Both factors linearly increased the peroxide values of seeds (see Fig. 19, as an example).

Comparison of sensory characteristics of traditional and new tahini halva

Traditional sesame and sunflower halva sensory character were compared to those of walnut (WH), hazelnut, pumpkin seeds, peanuts and almonds by trained sensory panellists. Colour, by means of a colorimeter and texture, by means of a texture analyser, were determined as well.

Differences among samples were detected for most characters, with pumpkin halva being more different from traditional sesame and sunflower products for less perceived sweetness and characteristic tahin flavour (Fig. 20, as an example). The hardness of halva decreased in the order: pumpkin>almond>sunflower>peanut>sesame> hazelnut>walnut, and sensory panellist perceptions of hardness and instrumental analysis were correlated.

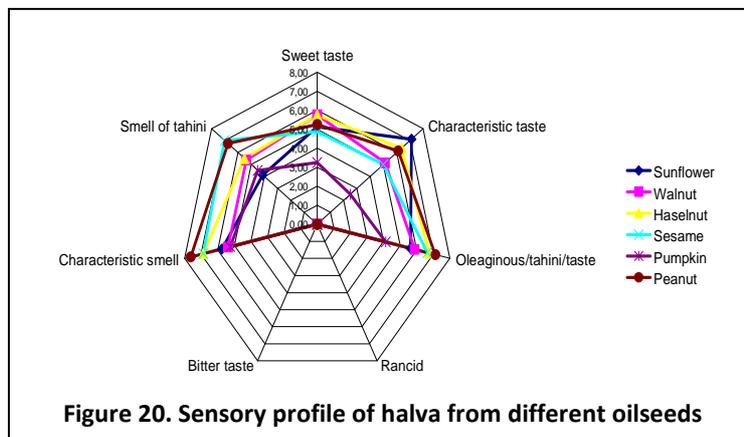


Figure 20. Sensory profile of halva from different oilseeds

Halva added with other ingredients

Traditional sunflower halva was prepared by adding rose elixir, rose petal jam and pumpkin treacle. The samples were subject to sensory, colour and texture evaluations. Product hardness increased with all additions, especially with rose elixir. A higher toughness during chewing of the latter was perceived by panellists. Pumpkin treacle enriched halva was perceived as sweetest. The taste and smell of the additional product prevails and is stronger felt with the sample of pumpkin. It gives specific flavor and after taste. All additions, with special respect to rose jam, determined more intense chromatic coordinates. Consumers showed preference for the sample with pumpkin and most respondents identified halva with rose jam and with pumpkin treacle as different from the traditional sunflower halva.

Traditional Bulgarian beverage Ljuto in relation to technology and raw materials.

Ljuto is a traditional beverage produced in the autumn, in the area of Rodopi mountain from lingo berries (*Vaccinium vitis-idaea*), wild apples, pears, plums, rosehips or a mixture of berries. In some regions it is called "cokazi" if produced by bilberries (*Vaccinium myrtillus* L). The basic technological process is a spontaneous fermentation. Mixed lactic acid and alcoholic fermentation caused by the epiphytic bacteria and yeasts takes place in the substrate at ambient temperature of 15-20°C.

This beverage was chosen as a subject of study by the Bulgarian partner, according to some lines.

Hypotheses of preparing ljuto with a varieties of berries, with different bioactive contents

Samples of strawberries, lingo berries, blueberries and raspberries of different origin were characterised for their antocyanins, flavonoids, total phenolics content and antioxidant capacity.

Dynamics of biologically active substances in traditional ljuto preparation

The extraction rate and fate on several phenolics substances were monitored during 21 days of the traditional fermentation process of lingo berry ljuto. Most compounds were extracted during the first 7 days, after which their concentration substantially decreased. This facts suggested that some technologies could be introduced for a better retention of these biologically active components.

Hypotheses of modifying the traditional ljuto preparation process.

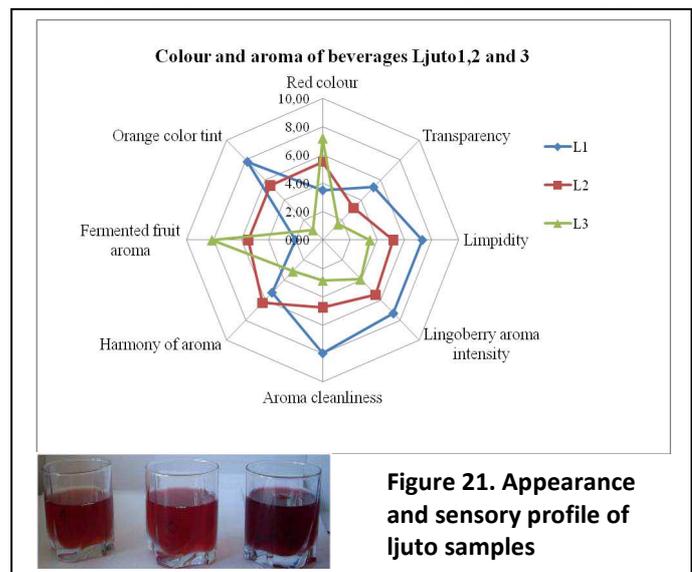
On the basis of the previous observations, two alternative flowcharts were implemented and tested, in comparison with the traditional one (L1): addition of 8% sugar (L2) and addition of 8% sugar + bakery yeast (L2), before fermentation.

The beverages were analysed for phenolics, vitamin C, sugars during fermentation and compared when ready. They were also subject to sensory analysis by a trained panel and to consumers analysis to detect the deviation of the new products from the "ideal" ljuto concept.

Generally L3 was characterized by higher levels of flavonoids and phenolic acids than L1 and L2. The application of sugar and yeasts to the substrate leads to better retention of the antioxidant and total polyphenols. The colour of L3 was more intense and dark than the other two samples (Fig. 21, as an example).

L2 and L3 were rate as sweeter and L1 the least sour. All samples were rated as astringent. L2 was rated highest in relation to aroma harmony.

In L3 astringent and bitter tasted were significantly higher. L1 and L2 were not perceived as very different by consumers, whereas differences were perceived for L3, that was generally rate lower. The higher phenol content of L3, even if potentially beneficial for health, seemed to negatively impact on acceptance.

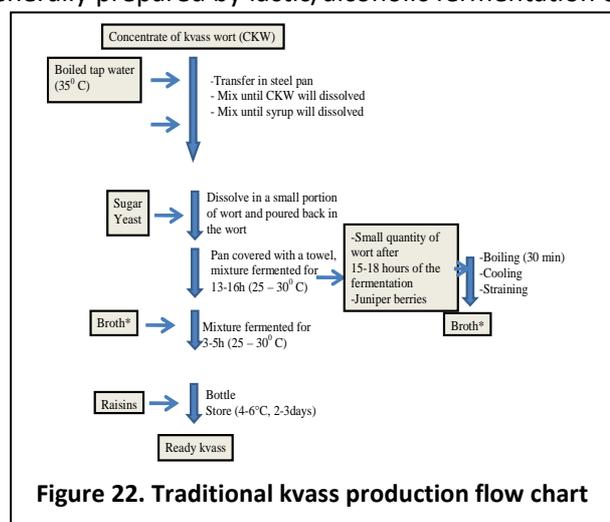


Production of kvass "Southern" according to traditional and modified flow charts

Kvass is a traditional beverage of Russia and Ukraine, generally prepared by lactic/alcoholic fermentation of a wide range of carbohydrate containing substrates, from cereals to fruits, honey beets and others. On of the more classic kvass is prepared use rye bread crusts as a fermentation substrate.

The traditional schemes of kvass "South" preparation (Fig. 22) was compared with tow modified schemes by the Russian partner: a) use of kvass wort concentrate instead of rye bread; b) use of kvass wort concentrate instead of rye bread and addition of baker's yeast. The products were compared fro vitamin C, lactic acid and dietary fibre contents, and were sensorially evaluated.

Both modified flow charts allowed to achieve higher acidity and slightly higher content of ethanol. Kvass produced according to the modified flow chart has higher coloration and lower content of dry matter. Consumer's characteristics of both sorts of kvass are similar, but new sort of kvass has more mild taste and could be better accepted. However, expert evaluations rate higher the kvass produced according to the traditional technology.



New flow charts for traditional bread production

On the basis of a detailed literature review, as well as preliminary experiments and experimental results, the following two hypothetic ideas have been proposed on how to improve content of bioactives, dietary fibres, and nutrients in traditional bread:

- 1) By adding outer parts of grains to flour of the first grade;
- 2) By mixing wheat flour with rye, buckwheat, barley, oat, corn, and triticale flour.

Enriching flour by outer parts of grains

Improvement of the quality of rye-wheat bread is based on increasing the content of bioactives and dietary fibres. It has been found that some important bioactives and dietary fibres go to bran during the rye and wheat grains milling. Therefore, flour with high content of bran was formed by the following three mixing options: the first grade flour (80%) with bran of 11th reduction system (20%); the first grade flour (80%) was mixed with reduction bran (10%) and breaking bran (10%); the second grade flour (94%) was mixed with reduction bran (6.0%). Weighted flour and bran were sent to a mixer and mixed for 2-3 min. After mixing flour was sent to the bunker, then to the weighing machine, the gateway feeder and by pneumatic transport it was transported to the finished product storage area.

It has been found that wheat flour of the extra class and produced traditional bread contained only 0.56% of dietary fibres and did not contain any bioactives. At the same time, in flour enriched by outer parts of grains and in produced bread the following content of the important substances were found: 0.34 – 1.05 mg/g of total phenolics, 0.02 – 0.06 mg/100g of carotenoids, and 1.34 – 6.68% of dietary fibres.

Production of a composite from various grain flours

After the controlled screening, wheat flour and flour of different crops (according to the recipe of the composition mixture) were sent to overdosing hoppers (1, 2), dosed with the required accuracy (3,4) and mixed (5). The finished composite flour mixture with increased nutritional value was supplied to the section of the finished product of the flour mill (Fig. 23).

Bread produced either from flour with the high content of bran, or from composite flour belongs to the bakery products of reduced calorie having, at the same time, high content of protein, vitamins, minerals, bioactive compounds and dietary fibres.

Enriching traditional rye bread by replacing wheat by buckwheat flour and using lactic acid bacteria leaven

Traditional rye-wheat bread is produced from a mixture of rye and wheat flours with the usage of mixed cultures of lactic acid bacteria and yeast. Modifications of the traditional flow chart were the following:

1) Usage of pure culture of homofermentative thermophilic lactic acid bacteria instead of mixed cultures of lactic acid bacteria and yeast.

2) Usage of buckwheat flour instead of wheat flour.

Contents of vitamin C, D- and L-lactic acids, starch and dietary fiber in the products were compared.

Both types of bread were evaluated by using the sensory analysis.

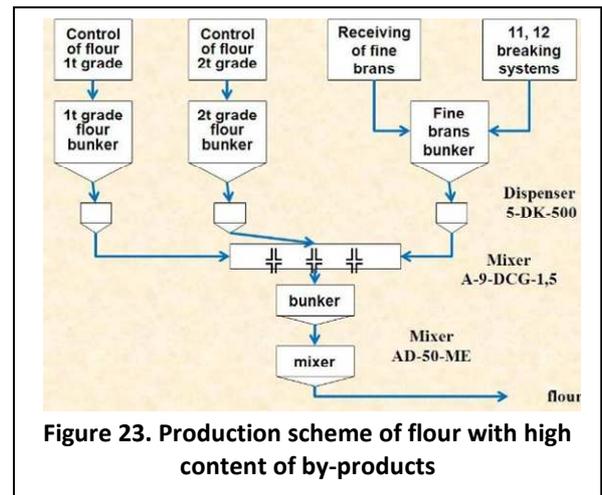


Figure 23. Production scheme of flour with high content of by-products

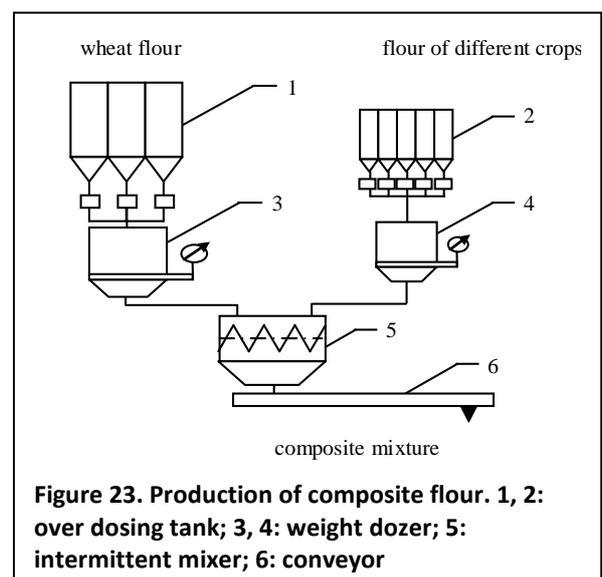


Figure 23. Production of composite flour. 1, 2: overdosing tank; 3, 4: weight dozer; 5: intermittent mixer; 6: conveyor

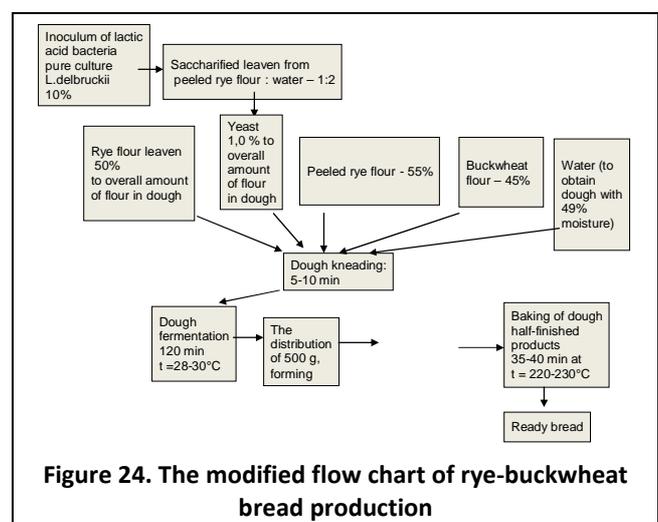


Figure 24. The modified flow chart of rye-buckwheat bread production

The modified flow chart allowed achieving the higher contents of lactic acid, starch and dietary fiber. Physical, chemical and organoleptic indicators of new bread were the same or slightly better than those of traditional bread.

6. Broad range, intercultural, on site, qualitative documentation of traditional foods (WP1)

Main activities

A fundamental goal of BaSeFood was to give a contribution at the study of traditional foods in their natural contexts, in order to improve the knowledge of their real function in local food systems, stimulate the interest and the awareness of consumers and open further possibilities for traditional food potential enhancement and exploitation.

The background concepts that inspired this part of the work were: a) the available definitions of traditional foods; b) the theoretical background of food quality perceptions; c) the different levels of food functions in human evolution.

The scope of this part of the work was to carry out a broad scale, on-field research, in order to document a sufficient number of traditional foods, grouped by categories, depending on the main raw materials, to generate a base of knowledge exploitable to formulate hypotheses related to the three background points and to represent a starting point for further, in deep investigations and intercultural comparisons.

The original scheme of the work forecasted to carry out on field investigations, by means of descriptive qualitative methodologies. In this procedure, the following steps were applied: definition of the investigation subjects, definition of the boundaries, definition of the checklist of concepts to be used during interviews, finding the respondents, field surveys and interviews, data organisation, analysis and report writing, drawing conclusions. The approach adopted was to investigate traditional food not as individual recipes, but starting from the main raw materials, analysis as much as possible their role in local systems. Selected investigation areas were chosen in each country; overall, over 150 documentation missions were carried out, interviewing over 300 informants (Fig. 25).



Figure 25. Moments of on site documentation of traditional food systems

Main results

Overall, the investigation carried out allowed to document and describe 848 foods, 455 of which were reported as "main" foods and 393 as variants, and to start their critical comparison.

The individual foods were described in the specific sections of a specific report this report.

The preliminary analysis of results brought out the following points that deserve further attention in future debates on traditional foods:

- in local contexts traditional food value is still well and directly perceived as an experience character
- traditional foods were generally a combination of an energetic staple (cereal or potatoes) with other available ingredients
- diversity, and the variability of ingredients and preparations is a key point, making the definition of "standard" recipes little more than a convenience artefact
- traditional foods evolve in relation to the availability of raw materials or new soft technologies

- striking convergences of traditional food types do occur across countries (example in Fig. 26), in dependence on basis of available ingredients, social conditions and nutritional needs
- the availability of new technologies and legislation rules may stimulate the evolutions of traditional foods but, especially the second, may also represent a difficulty for small scale stakeholders
- despite variability is a clear characterising trait of traditional foods, small scale stakeholders require a degree of standardisation of raw materials and procedures
- in this perspective products registration and certification revealed to be perceived in very different ways, not necessarily corresponding to an enthusiastic adherence
- a positive trend to work in chain by local stakeholders was detected in several contexts
- the health promoting values was almost never perceived as a fundamental character in local food systems. The study carried out centred to objective of documenting a wide number of traditional foods and analyse their role in local systems. It also opened the way for further investigation of role of traditional foods in local and modern production systems. It indicated the possibilities of collaboration between stakeholders of western and eastern Europe.

Above all, the results seem to indicate that a real priority is to further document what is still left of traditional food systems and initiatives, as a starting point to stimulate the appreciation of diversity, show living example of sustainability, enhance consumers awareness and capability of self appreciation of food quality, accept and stimulate the co-existence of different scale exploitation schemes.

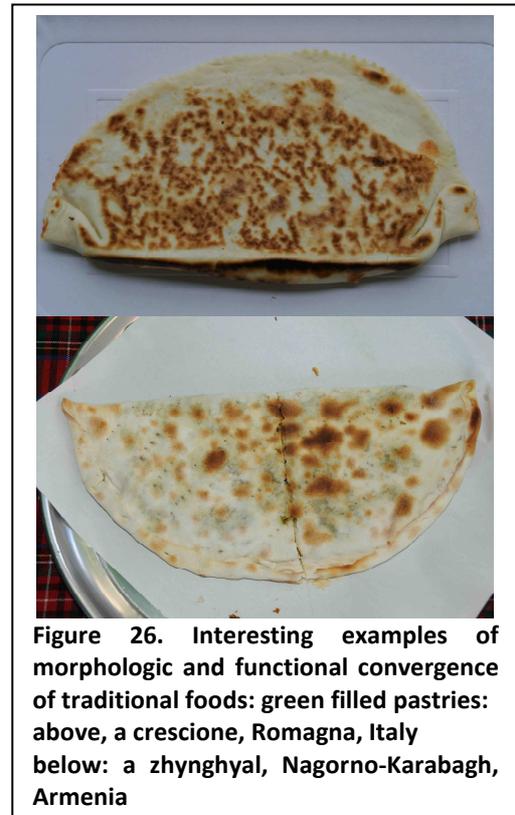


Figure 26. Interesting examples of morphologic and functional convergence of traditional foods: green filled pastries: above, a crescione, Romagna, Italy below: a zhynghyal, Nagorno-Karabagh, Armenia

7. Preliminary consumers and combined consumers/stakeholders surveys (WP5)

This part of the project was addressed at retrieving information about:

the opinions of stakeholders and consumers about the possibilities of combining traditional foods with health promoting concepts

- the perception of traditional foods of the Black sea area by native or west European consumers
- the opinion of stakeholders about the application of innovation to traditional food production chains.

In all cases, the investigations were carried out by ad hoc built questionnaires

7a. Elaboration of consumers / expert healthy food concepts from traditional foods

In this task a questionnaire was built, in which the binary combinations: food / active components; food / health claim, and health claim / active component (a total of 165 combinations), were independently rated for specific characters by consumers and experts (food technologists, marketers and nutritionists). The questionnaire was submitted in Black sea area countries and Italy, The responses were subsequently analysed in order to bring out correlations between traits and the more interesting combinations for the potential development of traditional foods with attached health claims.

A strong effect of the specific carrier (food), with the same health claim attached was detected on consumers' acceptance (Fig. 27, as an example). Food experts ratings were rather sensitive to consumer preference, especially for marketers, even if these results were affected by the participant partners

Food groups	Carriers	Health claims	I opportunity agreement	II opportunity losses	III non-opportunity agreement	IV unjustified investment
Cereal	Whole meal bread	Helps maintaining healthy cholesterol levels	7		1	
	Whole meal bread	Reduces the risk of certain types of cancer	7	1		
	Whole meal bread	Reduces the risk of heart diseases	7	1		
	Whole meal bread	Keeps your arteries healthy	7		1	
	Whole meal bread	Helps control your weight	7			1
	Cereal gruel	Helps maintaining healthy cholesterol levels	5	1		2
	Cereal gruel	Reduces the risk of certain types of cancer	5		2	1
	Cereal gruel	Reduces the risk of heart diseases	4		1	3
	Cereal gruel	Keeps your arteries healthy	5		1	2
	Cereal gruel	Helps control your weight	5		1	2

Figure 27. An example of the effect of the carrier (food) on the potential success of food/health claim binary

“Spice or herb” in combination with health claims “strengthens the natural defence of body”, “reduce certain types of cancer” and in combination with functional properties “rich in antioxidants” and “rich in vitamins” were the most potential carriers-health claims and carriers- functional ingredients mini-concepts.

For the combinations functional ingredients-health claims, high potentiality was observed for “foods rich in antioxidants” in combination with “reduces the risk of certain types of cancer”, “reduces the risk of heart diseases” and “keeps your arteries healthy” and “foods rich in vitamin” in combination with “strengthens the natural defence of the body against frequently occurring diseases like a cold”, “reduces the risk of certain types of cancer” and “reduces the risk of heart diseases”.

7b. Analysis of traditional food perceptions of populations of the Black sea area and western European consumers

This part of the work was built of three different tasks

Perception of traditional food by Black sea Area populations

A specific questionnaire was developed. The 33 prioritised foods were rated for 25 attributes belonging to the spheres of sensory characters, convenience and health promotion. The concept of a generic traditional foods was also rated for these same characters. The questionnaires were submitted in all the participant countries: each country rated only their own foods. Ukrainian migrants in Italy rated a specific subset of foods as well. Over 1000 responses were analysed.

Older people were generally more identifying a traditional food with familiarity, convenience but also sensorial characters and environmentally friendly ways of production.

Educated people tended to give more importance to credence attributes like those connected to health promotion, and ways of production, but also to characters rather deviating from a classical traditional food concepts, like the possibility of being prepared quickly, and being available in supermarkets.

Some other tendencies were revealed, more related to ethnic determinants. As an example, the more northern respondents give less importance to the "spicy" nature of a traditional food.

The data of the partner specific traditional foods indicated that an important dimension of these

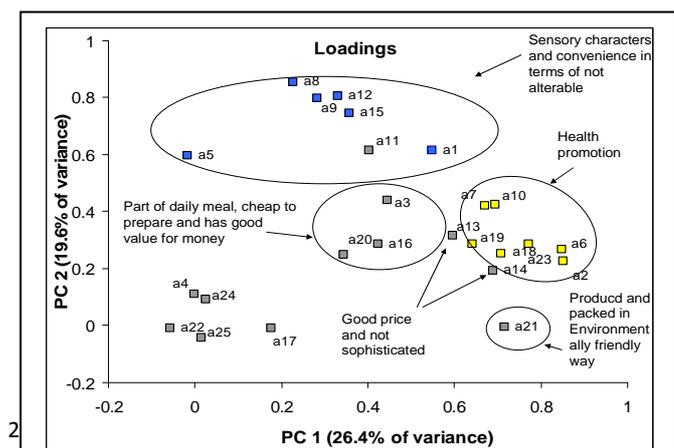


Figure 28. An example of categorisation into perceptual groups of traditional food attributes

foods is represented by some convenience traits related to price, easiness to prepare and sensory character, as well as daily consumption (Fig. 28). The rating of the same or similar food by all the Russian speaking communities was very consistent, although the questionnaires were carried out in completely independent way.

A good correspondence was detected between the directly perceivable health promoting and sensory traits of generic and specific foods, whereas some aspects of a generic traditional food more linked to a modern way of life seemed to be in contrast with the perceptions of specific traditional foods.

Perception of the Black sea area traditional food by the Italians

The work was carried out by means of a questionnaire with three sections: a) general questions about personal feelings of traditional food products and on Black sea area traditional foods; b) ratings of specific traditional foods of the Black Sea region; c) a food neophobia section. The questionnaire was submitted to a convenience sample of Italian population.

A positive attitude to traditional foods was detected; however, about 60% of the respondents did never consume traditional foods from Black sea area and 54% were also not willing to try. The remaining 41 % consumed traditional foods mainly from Romania at friends' home. The respondents rated Black Sea area foods substantially equal to Italian foods in terms of healthiness, safety and taste. A majority declared a willingness to pay lower price of 10-20% for these foods. Food neophobia appeared to significantly increase with the age and to significantly decrease with of respondents without any effect of gender.

About the specific interest on the selected traditional food, males revealed a slight better knowledge and lower trust than females, whereas trust and intention to try were higher for the younger age classes. The interest in tasting these products increased from male to female and from elderly to young classes.

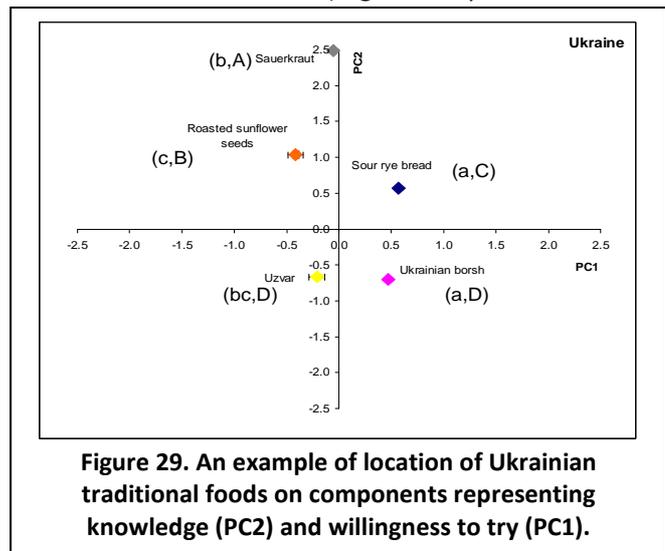
The knowledge level was higher for foods resembling foods of the Italian tradition, whereas trust and intention to try were slightly higher in foods with ingredient commonly recognised as health promoting, like wholemeal breads and vegetables. Males revealed a higher tendency to accept some of the foods with lower intention to try ratings with respect to females.

Romanian migrants and western consumers in Romania.

Slightly modified questionnaire with respect to the general Black sea area consumers was submitted in Romania and to Romanian migrants. Products that are very well known, frequently consumed and form the favourite foods of a large percentage of migrants in Western Europe are: cornmeal mush polenta (Mămăligă), eggplant salad, Nut horns. Other important traditional foods, when it comes to the awareness of migrants are: elderberry soft drink (Socata), plum jam, vegetables soup/cream, green beans dish, pumpkin pie. The "problem products" that require special attention and marketing measures are: vegetables soup with caraway and semolina, spinach with dill and mint, onion stuffed with rice, onion pie. The most important characteristic for traditional food (according to migrants) are: taste, healthiness, lack of alteration. The less important characteristics are: availability in daily diet, availability in restaurants, spicy flavour, lack of sophistication, green packing, and organic, the capacity to be stored, ability to be prepared with the microwave oven.

7c. Black sea area stakeholders' opinion about innovation applied to traditional food production

A questionnaire of 36 items, was submitted to over 60 stakeholders in Greece, Turkey, Bulgaria and Romania.



The results indicated that manufacturers are open to innovation, but believe that external support is important. They still innovate mainly on the process side. They believe that customers care of sensory properties but do not innovate so much in this respect. A slightly different situation seemed to occur with respect to nutritional and health perception, even if nutritional facts and health promotion are not exactly the same.

The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

Potential impacts of project's foreground

BaSeFood was a typical pre-competitive project. It was not aimed at generating products directly exploitable by the participant beneficiaries during or immediately after the project's development, but rather at generating knowledge of potential use for a wide range of potential stakeholders.

This general objective was pursued according to three lines:

- the creation of a specific knowledge base of the Black Sea area traditional foods, in which characters of chemical composition, health promoting properties, traditional and improved flow charts could be used by stakeholders for the possible development of products, including potential regulatory or proprietary claims for origin or health promotion.
- the establishment of context of general trust around the Black Sea area traditional foods, by means of an enhancement of knowledge, aimed at promoting the self awareness and interest of consumers, including issues connected to sustainable utilisation of local knowledge and resources.
- the establishment of exchange of experiences, at scientific and societal level, among the EU member and Black sea area participants.

These three points are synthesised in the two goals illustrated at the beginning of this report: "Create opportunities" and "Create trust".

The following is a list of attained and potential impacts

Harmonisation, at the level of European Union and neighbouring countries, of food registration systems, the use of food databases, food indexing

- A part of BaSeFood activities were carried out on the background of EuroFIR, in which unifying methodologies and concepts in relation to the above mentioned point were set. The EuroFIR concepts have been disseminated to the Black Sea area beneficiaries, throughout the project, and the relations between BaSeFood and EuroFIR were illustrated in several meetings (see the list of dissemination events).
- An important tool, related to this point, has been has been a dedicated training n course, held in Belgrade, November 2009, in which young scientists from BaSeFood beneficiary institutions have been trained in food indexing, the use of food databases and recipe calculations. An important step towards the extension to ICPC countries of standardised systems for food indexing was carried out.
- The goal of training scientists to operate according to common methodologies have been then further attained, by means of operative procedures, during the phase of traditional food preparation, sampling and shipping for nutritional analyses.

Food description and documentation, aimed at possible support of regulatory registration, and possible nutritional claims.

- The systematic description, documentation and nutritional analysis of 33 selected traditional foods gave origin at individual food sheets that can be an example of procedures for traditional food documentation aimed at registration or nutritional claims, to the potential benefit of stakeholders.
- Besides their value as worked out examples, these documents may also be of direct uses for the same purposes.

Health promoting, safety and nutritional effects of traditional foods

- The extensive analytical data on nutrients and bioactive components of traditional foods can be used, once the data are published in peer reviewed papers, to integrate the currently available food composition databases.
- The experiments on bioactive components carried out by means of in vitro, in vivo experiments and intervention studies contributed to clarify some mechanisms of action on bioactive compounds on the control of CVD risk factors, also by excluding the effect on some commonly considered control pathways.
- These data could represent a tassel in possible documentation attached to potential health claims.
- The understanding of beneficial and harmful dietary factors as well as the specific needs and habits of population groups as a major controllable factor in the development and reduction of occurrence of diet-related diseases and disorders was addressed by investigation on the Food Balance Sheets (FBS) and the Household Budget Surveys (HBS), also coupled with the mortality data from the World Health Organization (WHO) of the Black Sea area countries. This part of the work can have also interesting societal implications, given the socio-economic transition phase of these countries.
- the results of the microbiological characterisation of traditional foods in different geographical and supply contexts can represent useful preliminary information in view of the harmonisation of the safety issues between EU and non member countries.

Bioactive retention, processing and improved flow charts of traditional foods

- The analytical data on the fate of some bioactive components during cooking and simple unit operations, once published in peer reviewed journals, will add information about retention factors of these compounds, a field in which information is still amply lacking.
- The specific studies carried out on the traditional flow charts of some specific foods (bread, halva, fermented beverages) clarified the current procedure of preparation and can contribute in the better understanding of critical points for processing with respect to product quality, safety and bioactive retention.
- The study of improved flow charts, implemented by specific experiments, with respect to the modification of unit operations, the addition of components, the use of new raw materials, can be exploited by stakeholders for the production of different sorts of foods, targeted to specific consumer segments not contrasting with the promotion and of the original recipes.
- Some of the above mentioned activities have been carried out by involved beneficiaries, in close contact with local stakeholders

Consumers and stakeholders issues

- The results of a specific survey may represent an important tool for stakeholders to understand which are the better opportunities to combine health claims and traditional food concepts, therefore reducing the risk of failure dependent on evaluation errors leading to opportunity losses or unjustified investments
- The extensive surveys carried out on Black sea area consumers and migrants allowed to identify general trend and peculiarities in the perception of traditional foods, also important for the targeting of actions enteprired by processors or other food production stakeholders
- The latter investigations also have a social value, since they allowed to identify some trends that may arise from the recently history of the Black se area countries.
- Finally, the survey carried out with the Italian consumers brought out which can be the perceptual constraints in promoting Black sea area traditional foods in a western country

Enhancement of knowledge, societal, sustainability, diversity issues

- The complex of the activities carried out by the compilation of national traditional food files and the on-site surveys allowed the documentation of over 1000 traditional food, that will contribute at the enhancement of the knowledge of food and food habits of the Black sea area countries in western Europe. This fact will have a positive potential impact on their exploitation, because of the role that food has in intercultural communication

- On site surveys clearly revealed a latent aspect of traditional foods, connected to their role and functions in local societies of fully exploiting the available resources, that has been somewhat neglected in the recent effort towards a traditional food definition. Together with the other key point that diversity characterises traditional foods, this fact indicates that the recovery of consumers awareness towards these aspects will open the way to an exploitation of traditional foods well beyond the interest of individual industrial stakeholders, to the benefit of local systems.
- The detection of possible points in which the alternative commodities / local raw materials is possible in traditional food chains, may enhance the role of local primary productions, with potential benefits to small scale economic food production systems and the EU common agricultural policy
- The survey and documentation of uses of local plant types, and the comparative situation of Italy and Black sea area countries may open the way for an active conservation of local plant genetic resources

Main dissemination activities

Dissemination and communication have been a characterising part of BaSeFood strategy.

All the classical tools of dissemination have been exploited: web communication, participation at conferences, papers and contributions on information media, participation at stakeholders events, scientific publications.

Dissemination have been carried out at the following levels:

Consortium members

At this level, the relevant event has been:

- The ***BaseFood Food Indexing Training Course***, 9-11th November 2009, Hotel Palace, Belgrade, Jointly organised by BaSeFood and EuroFIR, in which participants were trained in food indexing, food database use and recipe calculations.

Communication within the Consortium was systematically carried out :

- via the restricted area of the BaSeFood web site, in which all the relevant project's technical and administrative documentation have been uploaded
- at BaSeFood periodic meetings (seven, during the project's development).

The public

For the communication of BaSeFood activities and results a fundamental tool has been

The project's web site (www.basefood-fp7.eu), that has been punctually updated all around the project's life. In the public part several documents reporting of project's activities are uploaded:

- The newsletters. The newsletters have been published each six months, and report all the relevant steps of project organisation and activities and illustration of intermediate results of research and surveys. Particular space was given to results of on place survey jointly carried out in several participant countries.
- Some public project's deliverables
- The list if dissemination activities
- The program and oral contributions presented at the final BaSeFood congress: TFI2012

The scientific community and stakeholders

BaSeFood beneficiaries individually or jointly attended with specific contributions at several international, national and local meetings, as reported in the specific table.

Presentation of the project have been given at various EU or internationally addressed stakeholders meeting, among which relevant has been:

- The 3rd International EuroFIR Congress: "European Food Composition Data for Better Diet, Nutrition and Food Quality". Vienna, Austria, 8-11 September 2009.
- The 5th Meeting of the UNU/SCN Network for Capacity Development in Nutrition in Central and Eastern Europe (NCDNCEE). Belgrade, Serbia, 11-12th November, 2009.
- The EuroFIR stakeholder event. Brussels, 25 March 2010.

- International scientific symposium: Biodiversity and Sustainable Diets, FAO, Rome, 3-5 November 2010. Among the scientific meetings to which BaSeFood beneficiaries contributed , some were particularly relevant for the topics and audience:
 - 8th Pangborn sensory science symposium, July 27-30, 2009, Florence, Italy.
 - 28th International Horticultural Congress, August 22-27, 2010, Lisbon, Portugal.
 - 14th International Biotechnology Symposium and Exhibition IBS-2010. September 14-18, 2010, Rimini, Italy.
 - 8th Euro Fed Lipid Congress, Munich, Germany, 21-24 November 2010
 - International scientific conference of Probiotics and Prebiotics, 14-16 June 2011, Kosice, Slovakia
 - 9th International food data Conference, 14-16 September 2011, Norwich, UK
 - 4th International Congress on Food and Nutrition and 3rd Safe Consortium International Congress on Food Safety, 12-14 October 2011, Istanbul, Turkey
 - 5th International Conference on Polyphenols and Health, 17-20 October 2011, Sitges, Spain
 - 11th European Nutrition Conference, 26-29 October 2011, Madrid, Spain
 - 2nd Kiel Food Science Symposium, 19-24 May, 2012, Kiel, Germany
 - X International Symposium on Vaccinium and Other Superfruits, 18-21 June 2012, Maastricht, Holland
 - 26th International Conference on Polyphenols, 22-26 July 2012, Florence, Italy
- Several experimental papers are in preparation, following BaSeFood activities

Final dissemination event

BaSeFood ended with an international dissemination event: Traditional food International - TFI2012 (www.tfi-2012.com) , held in the Teatro Verdi, in the Cesena city centre, Italy, on October 4-5, 2012.

The Congress was aimed at being a forum, making the point of the present knowledge and research about traditional foods, including the public presentation of the highlights of BaSeFood results.

The congress was attended by over 100 participants.

Main oral presentation sessions

Main oral presentations were given by:

Internationally recognised invited speakers, giving plenary lectures on specific topics connected to several aspects of traditional foods research and exploitation.

the coordinators of recently funded or ongoing international traditional food research projects

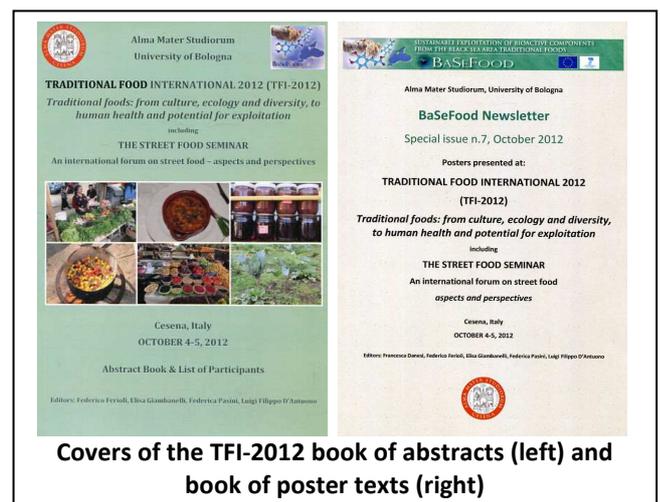
BaSeFood coordinator, work package and task leaders, publicly illustrating the highlight of BaSeFood R&D achievements.

Poster session

In the poster session, open to both BaSeFood and external contributions, 101 posters were presented, representing over 170 authors . 8 selected posters were shortly presented orally.

Street Food seminar session

The Street Food Seminar was held as an integral part of TFI-2012, organised in collaboration with Confesercenti di Cesena, the organisers of the 7th Street Food Festival. It was aimed to be a scientific link between the TFI-2102 event and the Street food festival itself, to be a first international forum about street food aspects, and to find the relations between traditional and street foods. Both the book of abstracts and the book of posters text have been published with ISBN codes:



Covers of the TFI-2012 book of abstracts (left) and book of poster texts (right)

Book of abstracts: ISBN 978-88-902152-1-6

Book of poster texts (special edition of BaSeFood newsletters): ISBN 978-88-902152-6-1

Besides these documents, the International peer review journal ***Journal of the Science of Food and Agriculture*** (Wiley) will publish a selection of about 30 papers, either from oral and poster contributions, in a special issues that is forecasted to be published by the second half of 2013.

BaSeFood logo

The BaSeFood logo represents:

- the Black Sea
- the stars, symbol of the European Union
- rye bread: one of the more characterising traditional food staples in Russia and Ukraine
- molecules of phenolics, among the more relevant health promoting plant compounds



Traditional Food International logo



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